Volume 90 (2, Supplement) August 2008

Journal of Plant Pathology

Formerly *Rivista di patologia vegetale* established in 1892

An International Journal of the Italian Society for Plant Pathology

ICPP 2008
9th International Congress of Plant Pathology

Abstracts of invited and offered papers

Edited by
A. Porta-Puglia and P. Gonthier

Torino, Italy, 24-29 August 2008

EDIZIONI ETS, Pisa, Italy
ISSN 1125-4653
Poste Italiane S.p.A. - Spedizione in Abbonamento Postale - D.L. 353/2003 (conv. in L. 27/02/2004 n° 46) art. 1, comma 1 - DCB AREA CENTRO 1/Pisa.

The **Società Italiana di Patologia Vegetale, SIPaV, (Italian Society for Plant Pathology)** was established in 1992 following the dissolution of the Italian Society for Crop Protection (SIF) and the Italian Phytopathological Association (AFI). Its main aims are to promote research into different branches of plant pathology, to disseminate knowledge about plant diseases and their aetiological agents and to promote cooperation among experts working in the field of plant pathology, and partnership in fundamental and applied reasearch. The Society organizes meetings, gathers and distributes information about plant diseases, and maintains cooperation with other national and international scientific organizations and with national and local administrative authorities on problems involving plant health management.

The Society publishes a journal (Journal of Plant Pathology), which hosts articles by members and external contributors, a bulletin and other bibliographic material to exchange information among members.

The SIPaV is affiliated to the International Society for Plant Pathology (ISPP) and to the European Foundation for Plant Pathology (EFPP).

COUNCIL (2008-2010)

President
Gaetano Magnano di San Lio
Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea,
Località Feo di Vito, 89061 Reggio Calabria

Past-President
Felice Scala
Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli,
Via Università 100, 80055 Portici (NA)

Secretary/Treasurer
Leonardo Schena
Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea,
Località Feo di Vito, 89061 Reggio Calabria

Elected Members
Roberto Buonaurio (**Vice-President**)
Dipartimento di Scienze Agrarie ed Ambientali, Università degli Studi,
Via Borgo XX Giugno 74, 06121 Perugia

Paola Battilani
Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore,
Via Emilia Parmense 84, 29100 Piacenza

Donato Boscia
Istituto di Virologia Vegetale del CNR, sezione di Bari,
Via Amendola 165/A, 70126 Bari

Corrado Fanelli
Dipartimento di Biologia Vegetale, Università "La Sapienza",
Largo Cristina di Svezia 24, 00165 Roma

Quirico Migheli
Dipartimento di Protezione delle Piante, Università degli Studi,
Via E. De Nicola 9, 07100 Sassari

Alessandro Ragazzi
Dipartimento di Biotecnologie Agrarie, Università degli Studi,
Piazzale delle Cascine 28, 50144 Firenze

Membership and Administration

SIPaV, Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea.
Località Feo di Vito, I-89061 Reggio Calabria, Italy
Tel: +39.0965.801284 - Fax: +39.0965.312827 - E-mail: lschena@unirc.it

ICPP 2008
Organising Committee

President
Giovanni P. Martelli, University of Bari

Vice Presidents
Peter Scott, UK
Wenhua Tang, P.R. China
Cleora J. D'Arcy, USA
Jaacov Katan, Israel

Chairperson
M. Lodovica Gullino, University of Torino

International Advisory Committee
Khaled M. Makkouk, Arab Society for Plant Protection
Gert. H.J. Kema, Royal Netherlands Society of Plant Pathology
Eliane Dumas-Gaudot, French Phytopathological Society
José M. Melero Vara, Spanish Society for Plant Pathology
Georg F. Backhaus, The German Phytomedical Society

Scientific Programme Committee
Angelo Garibaldi, University of Torino and
Giuseppe Surico, University of Firenze - Chairpersons
Naldo Anselmi, Tuscia University, Viterbo
Paola Bonfante, University of Torino
Giorgio Calabrese, University of Torino
Antonino Catara, University of Catania
Maurizio Conti, National Research Council, Torino
Francesco Faretra, University of Bari
Paolo Gonthier, University of Torino
Franco Lamberti, National Research Council, Bari †
Cristina Marzachì, National Research Council, Torino
Giancarlo Pratella, University of Bologna †
Felice Scala, University of Napoli

Proceedings and Posters Committee
Angelo Porta-Puglia, Ministry for Agricultural, Food and Forestry Policies, Roma - Chairperson Proceedings
Giovanni Vannacci, University of Pisa - Chairperson Posters
Matteo Lorito, University of Napoli
Paolo Cortesi, University of Milano

Finance Committee
Angelo Garibaldi, University of Torino - Chairperson
Donato Gallitelli, University of Bari
Marina Barba, Agricultural Research Council, Roma

Local Arrangements Committee
M. Lodovica Gullino, University of Torino - Chairperson
Cristina Marzachì, National Research Council, Torino
Paolo Gonthier, University of Torino
Piero Roggero, National Research Council, Torino †
Davide Spadaro, University of Torino
Federico Tinivella, University of Torino
Massimo Pugliese, University of Torino

Congress Secretariat
Valentina Communication
Via Cibrario 27, 10143 Torino (Italy)
ph. +39 011 4374250 - Fax +39 011 4374318
e-mail: info@icpp2008.org

ISPP Executive Committee 2003-2008

President
Richard E. Falloon, New Zealand

Immediate Past President
Peter R. Scott, United Kingdom

Vice President
Tang Wenhua, China

Vice President
M. Lodovica Gullino, Italy

Secretary-General
Greg Johnson, Australia

Treasurer
Chuji Hiruki, Canada

ISPP Newsletter Editor
Brian Deverall, Australia

ISPP Executive Committee 2008-2013

President
M. Lodovica Gullino, Italy

Immediate Past President
Richard E. Falloon, New Zealand

Vice President
Wafa Khoury, Lebanon

Vice President
You-Liang Peng, China

Secretary-General
Greg Johnson, Australia

Treasurer
Thomas Evans, USA

ISPP Newsletter Editor
Brian Deverall, Australia

FOREWORD

The role of plant pathology in our rapidly changing world is of increasing importance. Our discipline is connected with very relevant social and economic issues: environmental protection and conservation, food safety and security, and climate change, to name a few. Yet plant pathology is continuously challenged to maintain its identity and reaffirm an existence based on the needs of those who grow food and fiber. As the ever-increasing world population demands more to consume, we must respond with improved methods of disease control that are less destructive to the environment. Improvements in agricultural technology require attention to basic science with applications that can be quickly focused to solve specific crop production problems. Plant pathology is both a basic and an applied science. We are unique and indispensable because we represent an integrated science, a discipline that brings together components of many sciences such as botany, plant physiology, and microbiology. Striving to achieve the right balance of disciplinary and mission-oriented research, and striving to achieve fundamental advances in knowledge to apply problem/solving approaches, we must constantly adapt and effectively implement our research findings.

I believe that all those aspects are covered in the invited and offered papers that you find in this Special issue of the *Journal of Plant Pathology*, hosting the abstracts of the 9th International Congress of Plant Pathology (ICPP 2008).

The Organising Committee has adopted the theme *Healthy and safe food for everybody* for the Congress, thus underlining the role of plant pathology in food safety and food security as well as emphasizing the effort made by the International Society for Plant Pathology in the field of food security.

The Scientific Programme Committee of ICPP 2008 developed a comprehensive programme of Keynote and Concurrent sessions with an impressive list of presenters to address a broad range of contemporary plant pathology issues and topics. Five Keynote Sessions (Role of plant pathology in food safety and food security; Host-pathogen interactions and molecular plant pathology; Diseases of Mediterranean crops and forests; Recent developments in disease management; Knowledge and technology transfer), two special Plenary Sessions, one on Global food security, the other one devoted to the Centennial celebration of the American Phytopathological Society, will take place. Two Special Sessions, sponsored by the European Food Safety Authority and by the European Commission, together with more than fifty Concurrent Sessions will allow coverage and discussion of a broad range of topics, from airborne plant diseases to the future for plant pathology.

This volume contains more than 1800 abstracts, with over 1600 offered papers that are to be presented at ICPP 2008. All contributors of offered papers have played an important part in the success of the Congress.

The very considerable efforts of people involved in developing the ICPP 2008 Programme and the ICPP 2008 abstract publication are commended and acknowledged. The ICPP 2008 Scientific Programme Committee planned the programme with the help of all Session organisers. The Organising Secretariat Valentina Communication – with Mrs. Flavia Antonino and Dr Chiara Demaria – received and handled the abstracts, Dr Robert Milne did the language editing, while Dr Angelo Porta-Puglia and Dr Paolo Gonthier compiled ICPP 2008 abstracts for publication and did the final editing with incredible patience. The Valentina Communication Group (led by Dr Valentina Parenti) and the Scientific Secretariat of AGROINNOVA (with Pietro Piazzolla *in primis*) have provided essential assistance with the communication required to develop the ICPP 2008 programme. Everybody worked with competence and passion.

ICPP 2008 promises to be a very successful and worthy successor to previous International Congresses of Plant Pathology. I extend my warmest wishes to all ICPP 2008 delegates for a very successful and worthwhile time in Torino.

M. Lodovica Gullino
Chairperson, ICPP 2008
Vice-President, International Society for Plant Pathology
Past President, Italian Association for Crop Protection (AIPP)

PROFILES OF PLENARY AND KEYNOTE SPEAKERS

Plenary Session PS 1

Congress opening

Dr Timothy J. Hall

T.J. Hall worked as a research scientist in the UK before joining the European Commission services in 1983, becoming Head of Unit for S&T Cooperation with Developing Countries in 1994. He has also been responsible for units dealing with general aspects of the life sciences and health research.

His current position is Head of Unit for Agriculture, Forestry, Fisheries and Aquaculture with primary responsibilities for overseeing the management of projects in these areas supported by the EU RTD Framework Programmes.

Since 1 September 2007, he is also Acting Director for Biotechnologies, Agriculture and Food.

Plenary Session PS 2

Public discussion forum: plant pathology and global food security

Dr Peter R. Scott

P. Scott is a plant pathologist and an information scientist. He is Consultant to CAB International, where he has been responsible for new developments in knowledge management in the applied biosciences. He has led the development of CABI's Compendium Programme, and the formation of its international Development Consortia that now include more than 60 participating organizations in the public, private and development assistance sectors.

Previously he was a plant pathologist at the former Plant Breeding Institute, Cambridge, UK, where he was responsible for research on the genetics of resistance to facultative parasites of cereals.

He is the Immediate Past President of the International Society for Plant Pathology.

Prof. Richard Strange

R. Strange is currently Honorary Professor of Biology at the University College London and Honorary Research Fellow in the School of Biology and Chemistry, Birkbeck College, University of London. He has published over 90 scientific papers, mainly concerned with the mechanisms of resistance and susceptibility of plants to disease, and two books: *Plant Disease Control, Towards Environmentally Acceptable Methods* (1992) and *Introduction to Plant Pathology* (2003). His current interests lie in the promotion of global food security through the initiation of a new journal, *Food Security: the Science and Sociology of Food Production and Access to Food* with Peter Scott (see http://www.isppweb.org/nljul07.asp#1) and, at the bench, in the production of toxin-minus mutants of the chickpea pathogen, *Ascochyta rabiei.*

Dr Gurdev S. Khush

G.S. Khush was raised on a small farm in Punjab, India. He received BSc degree from Punjab Agricultural University in 1955 and a PhD in 1960 from the University of California, Davis. After serving as a faculty member of the University of California for seven years, he joined the International Rice Research Institute (IRRI) in the Philippines as a Plant Breeder, and was appointed as Head of Plant Breeding Department in 1972. He retired in February 2002 as Principal Plant Breeder and Head of Division of Plant Breeding Genetics and Biochemistry. During his 35 year career at IRRI he spearheaded the programme for developing high yielding and disease and insect resistant varieties of rice which ushered in green revolution in rice farming. More than 300 rice varieties developed under his leadership have been released in Asia, Africa and Latin America. IRRI bred varieties or their progenies are grown on 60% of world's rice land. Rice production increased from 257 million tons in 1966 to 626 million tons in 2006.

Dr Cantrell, Director General of IRRI summed up Khush's contributions by saying, "while his name may have passed the lips of many, his life's work passed the lips of almost half the mankind".

Dr Khush had made outstanding contributions to advancing the frontiers of rice genetics. He has written 3 books, and numerous papers in scientific journals. He has trained numerous plant breeders and served as consultant to several national rice improvement programs.

For his contribution to food security he received the Japan Prize in 1987, the World Food Prize in 1996, the Rank Prize in 1998 and the Wolf Prize in Agriculture in 2000. He received honorary doctorate degrees from eleven universities, the latest being from Guru Nanak Dev University. G.S. Khush was elected to the Indian National Science Academy, Third World Academy of Sciences, US National Academy of Sciences and Royal Society of London.

Dr Harry Evans

Nearly 40 years experience in disease identification and management in tropical agriculture and forestry. Over 160 scientific papers published on plant and insect pathology, including a standard reference book. Currently responsible for initiating and coordinating research projects on the use of fungi for the biological control of weeds and plant diseases.

Dr James M. Waller

Over 40 years experience in research and consultancy work on diseases of tropical crops, in particular coffee, with much experience as technical advisor to development projects in crop protection in Africa, Asia and Latin America. Research on coffee and sugarcane diseases in particular coffee berry disease in Kenya and in the development of integrated crop management systems. Former Head of Plant Protection Services at CABI Bioscience. Former plant pathology adviser to DFID crop protection programme. Extensive work experience as advisor and consultant and in research projects. Author of 6 books and about 90 publications in books, articles and papers.

Dr James Brown

J. Brown leads a research programme at the John Innes Centre, Norwich, where he has worked since 1989. His main interest is in strategies of developing durable resistance to crop diseases. Most of his work has concerned fungal diseases of cereals, particularly powdery mildew and *Septoria tritici* blotch, and his programme covers both the genetics of plant resistance and the population genetics of pathogens. His most important current goal is to devise ways of breeding for durable disease resistance in crop varieties with excellent yield and quality.

Dr Florence M. Wambugu

F.M. Wambugu, a renowned agricultural plant pathologist with specialization in virology and genetic engineering, has a BSc Botany, University of Nairobi; MSc in Plant Pathology, North Dakota State University; PhD in Plant virology, Biotechnology, University of Bath, England; and a Post-Doctoral Research Associate – Biotechnology, Plant Science Monsanto, St. Louis, USA. F.M. Wambugu who has over 25 years experience in agricultural crop research. She is the Founder, Director and the Chief Executive Officer of Africa Harvest Biotech Foundation International (AHBFI) since 2002. AHBFI is a nonprofit foundation with offices in Nairobi, Kenya, Johannesburg, South Africa, and Washington, D.C. Previously, she founded, established and worked as Africa Region Director, ISAAA – Afri-center, in Nairobi; she also worked as Plant Biotechnology Research Scientist, Kenya Agricultural Research Institute (KARI), Kenya. F.M. Wambugu has made significant contributions in research, development and improved production in maize, pyrethrum, banana, sweet potato and forestry in Kenya. She has published over 100 articles and co-authored various papers. She is also the author and publisher of *Modifying Africa: How Biotechnology Can Benefit the Poor* (www.modifyingafrica.com). Dr Wambugu is a strong believer in the power of biotechnology and has participated in many international forums in support of biotechnology in Africa to increase food production. Under her leadership, the Biotech Tissue Culture Banana Project has positively impacted thousands of small-scale farmers in Kenya and Eastern Africa. She is the recipient of numerous local and international honours, awards, and grants. KARI's 1989 Crop Science Award for outstanding scientist of the year; International Potato Center's (CIP) Regional Research Award/Grant, 1989; Noble Prize of the United Cultural Convention 2002; World Bank Global Development Network Award in 2000 for successful introduction of the tissue-culture banana in Kenya; Woman of the Year 2001 by the American Biographical Institute; and Woman of the Year Award by Eve Magazine. Under the leadership of F.M. Wambugu, Africa Harvest (and consortium collaborating institutions) has been awarded US$ 18.6 million from a global competitive grant from Bill & Melinda Gates Foundation, Global Health Challenge for Health and Nutrition. F.M. Wambugu has served as a board member and in other capacities for both local and international institutions. Previously she served in several board of directors which include Private Sector Committee of CGIAR, United Nations Millennium Development goals Hunger task force; Executive Committee member of Forum for Agricultural Research in Africa (FARA); DuPont Company Biotech Advisory Panel, USA; International Plant Genetics Research Institute (IPGRI now called Bioversity) and African Biotechnology Stakeholders Forum (ABSF). Currently, she is serving as a Council Member of the Japan Science and Technology in Society (STS) Forum, a Steering Committee Member of the European Action on Global Life Sciences (EAGLES) and a Science Board member of Bill and Melinda Gates Foundation Grand Challenge in Global Health.

Prof. Thomas Hohn

T. Hohn is Professor "emeritus" at the Botanical Inistitute, University Basel. He is Austrian, has studied at the Max-Planck Institute of Tübingen and performed postdoctoral studies in Stanford, California. He was junior group

leader at the Bicocenter of the University Basel and group leader at the Friedrich Miescher Institute Basel. His interests are in virology, originally of bacteriophages, where he together with his wife developed DNA-packaging, and later of plant viruses, where he recognized the first plant pararetrovirus (CaMV) and detected special viral translation strategies. Recently he became interested in the topic of RNA-interference and in transgenesis. He has published more than 200 papers during his career. Since several years he is involved in the Indo-Swiss collaboration in Biotechnology project, working together with Indian scientists to apply biotechnology for the improvement of pulses (Leguminosae) and Cassava for the use by subsistence farmers.

Dr Corrado Clini

C. Clini is General Director of the Ministry for the Environment, Land and Sea of Italy since 1990. He received his degree in medicine at Parma University - Departments of Ecology and Occupational Health in 1972, his PhD in Occupational Health at Padua University in 1975 and his PhD in Hygiene and Public Health at Ancona University in 1986. From 1990 to 2001 he had been Chairman of the Environment and Health European Committee. At present C. Clini holds a number of leaderships positions and more specifically Chairman of the Board of the Regional Environment Center of Budapest, Member of the Bureau of the European Environment Agency, Vice Chair of the Hydrogen Economy International Partnership, Chairman of the Global Bioenergy Partnership, Visiting professor at the Department for Environmental Sciences and Engineering at Tshingua University of Beijing, Senior Research Fellow in the Sustainability Science Program at Harvard's Center for International Development.

Plenary Session PS 3

In celebration of 100 years of the American Phytopathological Society

Dr Paul D. Peterson

P.D. Peterson has a diverse background ranging from history to biology. He holds undergraduate and master's degrees in history and a PhD in plant pathology from North Carolina State University. As a postdoctoral fellow at Clemson University, he conducted some of the first research on a newly emerging disease of turfgrass called rapid blight, caused by *Labyrinthula terrestis*. Currently, he holds adjunct faculty appointments at Clemson University and Coker College in South Carolina, where he teaches courses on the history of science and agriculture. He also serves as the E.C. Stakman Visiting Scholar at the University of Minnesota and as the official historian of the American Phytopathological Society.

Prof. Karen-Beth G. Scholthof

K.-B. G. Scholthof is a Professor of Plant Pathology and Microbiology at Texas A&M University. Her research focuses on the molecular biology of satellite panicum mosaic virus. In addition, she has an NSF-funded history of science project underway on the *Development of Plant Virology in the Early 20th Century*. K.-B. Scholthof teaches a graduate course in Plant Virology, a writing-intensive undergraduate course on *Pathogens, the Environment, and Society* and a course on *The History, Literature, and Science of Disease* for the Undergraduate Honors Program. In 2004 she received an *Excellence in Teaching Award* from the American Phytopathological Society.

Dr James D. MacDonald

J.D. MacDonald is a Professor of Plant Pathology at the University of California, Davis. He joined the faculty in 1978 and served as chair of the department from 1995-1999. He has served as executive associate dean of the College of Agricultural and Environmental Sciences since 1999. Over his career, J.D. MacDonald has been very active in APS where, among his many activities, he chaired the Electronic Technology Advisory Committee that conceived and initiated the society's electronic journals Plant Health Progress and Plant Health Instructor. Professor MacDonald was elected vice president of APS in 2004 and served as president from August 2004 to August 2005.

Dr Jacqueline Fletcher

J. Fletcher, Sarkeys Distinguished Professor, Oklahoma State University, earned a BS (Emory University), MS (University of Montana) and PhD (Texas A&M) and was a postdoctoral fellow at the University of Illinois. Her research focuses on niche adaptation of phloem-associated bacteria, the relationships between human pathogens and plants, and microbial forensics.

Service in the American Phytopathological Society included terms as President, Secretary and Councillor-at-Large.

She chaired the APS Bacteriology Committee, was Senior Editor of APS Press and Associate Editor for Plant Disease, and served on the Office of International Programs and the APS Biosecurity Committee. She was named a Fellow of APS (2005) and of AAAS (2007).

J. Fletcher serves currently as Director of the APS Public Policy Board, chairs the APS Plant Pathogen Forensics Group, and is a member of the APS Threatening Pathogens Committee. She serves on the FBI's Scientific Working Group on Microbial Forensics and on a biosecurity advisory committee for the National Intelligence Council. She has participated in EU and NATO sponsored multi-national crop biosecurity initiatives. J. Fletcher was recently appointed Director of OSU's National Institute for Microbial Forensics and Food and Agricultural Biosecurity.

Dr Cristopher C. Mundt

C. Mundt received his PhD in Plant Pathology from North Carolina State University in 1985. He has since been on the faculty of the Department of Botany and Plant Pathology at Oregon State University, where he is currently a Professor. He also was a Visiting Scientist at the International Rice Research Institute from 1992-2001. His research interests are in the epidemiology of plant disease and population genetics of plant pathogens, with a focus on the effects of host diversity. He has served as Editor-in-Chief of Phytopathology, and is a Fellow of the American Phytopathological Society and the American Association for the Advancement of Science.

Dr R. James Cook

R. James Cook is best known for his research at Washington State University (WSU) on biological and ecological approaches to manage root diseases of wheat. Starting in 1998, and before retiring from WSU in 2005, he held the R. J. Cook Chair in Wheat Research, a position endowed with a $1.5-million gift to WSU from the Washington wheat growers. He was elected to the US National Academy of Sciences in 1993 and the US Agricultural Research Service Science Hall of Fame in1997. He holds BSc and MSc degrees from North Dakota State University, a PhD from the University of California, Berkeley, and honorary doctorates from North Dakota State and the University of Turin.

Keynote Session KS 1

The role of plant pathology in food safety and food security

Dr Robert S. Zeigler

Plant pathology, research management. BS 1972 (biological sciences), University of Illinois, USA; MS 1978 (Botany), Oregon State University, USA; PhD 1982 (Plant Pathology), Cornell University, USA. Bob Zeigler was a computer operator (1967-71) and laboratory assistant in Botany (1972) at the University of Illinois; secondary school science teacher as a US Peace Corps volunteer, College Musim, Mokala, Zaire (1972-74); technical assistant, Fred Hutchinson Cancer Research Center, Seattle, Washington (1974); graduate research-teaching assistant in Plant Pathology at Oregon State (1975-77) and at Cornell University (1978-80); visiting research associate, CIAT (1980-81); IDRC technical advisor, Burundi Maize-Pea Program (1982-85); CIAT plant pathologist (1985-86) leader, CIAT Rice Program (1986-92); plant pathologist, IRRI (1992-99); leader of the Rainfed Lowland Rice Research Program, IRRI, (1992-95); leader, Irrigated Rice Research Program, IRRI (1995-1999); professor and head, Department of Plant Pathology, and director, Plant Biotechnology Center, Kansas State University (1999-2003), and director of the CGIAR Generation Challenge Program based in Mexico (2004-2005). Sigma Chi (1982); Gamma Sigma Delta (Honor Society of Agriculture, 2000); International Service Award, American Phytopathological Society (2001); fellow, American Association for the Advancement of Science, 2007.

Dr Emmanuel Moses

E. Moses completed his PhD in Plant Pathology at University College (University of London) in 1997 and joined the Crops Research Institute of its native Ghana in the same year. He has occupied the positions of Head of Root and Tuber Crops Research from 2001 to 2006 in its institute and the Coordinator of Adaptive Research in the Middle Zone of Ghana in the National Root and Tuber Improvement Programme (in the same period). Some of its significant achievements include winning the P.H. Gregory Prize award of the British Society for Plant Pathology in 1996 and receiving the second prize in Scientific Presentations of the International Society of Tropical Root Crops (African Branch) in 2001. He was the recipient of the first Congress Challenge Award of the International Society for Plant Pathology in 2003.

Dr Niek van der Graaff

N. van der Graaff holds an advanced degree in Biology from Leiden University and a doctorate in Agricultural Sciences (Plant pathology) from Wageningen University. He worked from 1973 to 2006 with the Food and Agriculture Organization of the United Nations, starting with five years in Ethiopia, where he worked on resistance of Arabica to Coffee Berry Disease. Subsequently, he was a plant pathologist at FAO's headquarters and the Organization's senior plant pathologist. In 1986, he became the Chief of FAO's Plant Protection Service, responsible for the Organization's programmes on pesticide management, locust control, integrated pest management and plant quarantine. He was responsible for the intergovernmental negotiations that established the International Plant Protection Convention as the treaty that sets International Phytosanitary Standards. He oversaw, together with the Director of UNEP Chemicals, the negotiations that led to the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade. He subsequently was also Secretary for both treaties.

Keynote Session KS 2

Host-pathogen interactions and molecular plant pathology

Prof. Brian Staskawicz

B. Staskawicz, PhD is professor and chair, Department of Plant and Microbial Biology, University of California-Berkeley. B. Staskawicz received his BA in Biology from Bates College in 1974 and his PhD from the University of California-Berkeley in 1980. B. Staskawicz's work has contributed greatly to understanding the molecular interactions between plants and their pathogens. He was elected to the NAS in 1998 for elucidating the mechanisms of disease resistance, as his lab was the first to clone a bacterial effector gene from a pathogen and among the first to clone and characterize plant disease-resistance genes. Staskawicz's research focuses on the interaction of the bacteria, *Pseudomonas* and *Xanthomonas*, with *Arabidopsis*, tomato, and pepper. He has published extensively in this area and is one of the leading scientists in the world working on elucidating the molecular basis of plant innate immunity.

Prof. Roger Innes

R. Innes is Professor and Associate Chair of Biology at Indiana University in Bloomington, Indiana, USA. He has been at Indiana University since 1991. Prior to that he trained with Dr Brian Staskawicz at the University of California at Berkeley and obtained his PhD from the University of Colorado at Boulder, studying under Dr Peter Kuempel. R. Innes's current research focuses on the molecular mechanisms that mediate pathogen recognition and the evolution of these mechanisms. A detailed description of his research program can be found at http://www.bio.indiana.edu /~inneslab/.

Prof. Dr Paul Schulze-Lefert

P. Schulze-Lefert was trained in biochemistry and genetics at Marburg, Freiburg, and Cologne Universities, Germany. After a PhD thesis on cis-and trans-active factors regulating plant gene expression in response to light, he became interested in fundamental processes controlling plant microbe interactions. Major research areas are the innate immune system of plants, mechanisms of fungal pathogenesis, defense suppression, and the molecular basis of biotrophic lifestyle. The research program includes the development of novel quantitative non-invasive imaging technologies and plant chemical genetics as tools for the dissection of dynamic biological processes.

He worked from 1989 to 1990 as postdoctoral fellow in Francesco Salamini's department at the Max-Planck-Institut für Züchtungsforschung (MPIZ) Cologne on the development of DNA marker technologies in plant genomes. In 1991 he started his own research group at the RWTH Aaachen with a focus on plant disease resistance mechanisms to fungal pathogens. From 1995 to 2000, he held a senior research position and supervised a research team in the Sainsbury Laboratory at the John Innes Centre, England. Since 2000 he is head of the Department of Plant Microbe Interactions at the MPIZ, Cologne, and Honorary Professor at the University of Cologne since 2003. Much of his current work is dedicated to bridging traditional research areas like genetics, biochemistry, and cell biology in the endeavor of increasing our understanding of the molecular mechanisms that control plant microbe interactions.

Keynote Session KS 3

Diseases of Mediterranean crops and forests

Prof. Naldo Anselmi

N. Anselmi is currently full Professor of Forest Pathology at University of Tuscia. Scientific Responsible for Italy of EU Forest Project on *Poplar rusts* and FAIR Project on *Root pathogens in forest decline* and PRIN National Projects on *Endophytic fungi in forest decline*. Partecipating to the AIR Project on *Root rots by Armillaria*, and ENVIRONMENT project POPFACE on *The effect of air increasing CO_2 on pathogen aggressiveness*. Scientific responsible of local Research Units in National (IPRA, RAISA, MiPAF) and Regional (DOCUP 5B, POM, PRAL) Projects. Coordinator of the PhD on Plant Protection. Member of FAO Poplar Disease Commission and some IUFRO Working Parties. Member of the Board of Associate Editors of the *Journal of Plant Pathology* (2000-2002) and Councillor of the Steering Committee of the Italian Society for Plant Pathology, SIPaV (2002-2005). Member of Scientific Committees of National and International Conferences. Referee of several scientific national and international journals. Since November 2007 he is head of the Plant Protection Department of the University of Tuscia. Author of a book *Wood Pathology* and of 250 papers on the biology, epidemiology, strain composition and pathogenicity of several fungal disease agents of forest trees (*Abies, Castanea, Juglans, Platanus, Picea, Pinus, Populus, Prunus, Quercus, Salix*, etc.) and on their integrated control. In the last 15 years, he has focused his research on forest decline, carrying out investigations on the role of water deficiency and fungal weakness pathogens; on fungal colonization of tissues exposed with pruning cuts; on the endophytic habitus of weakness pathogens living in the bark in healthy trees; on the characterization of pathogenic fungi with isozyme and molecular markers. At the same time, he has been continuing his studies on rot roots agents, with special emphasis on *Phytophthora* and *Heterobasidion* in the Mediterranean area of central-southern Italy.

Prof. Alessandro Ragazzi

BA in Forestry Sciences in 1973. Full Professor in Forest Plant Pathology. Member of the College for PhD in Forest Plant Pathology. Member of the Executive Board of the National Union of Forestry Institutes. Member of the Italian Forestry Academy. Member of the IUFRO Groups *Rust of Forest trees*, *Complex diseases*, *Phytophthora diseases*. Coordinator of the Work Group *Diseases of Forest and Urban Trees*, within the Italian Phytopathological Society. President of the Curriculum in Tropical Agricultural Sciences. Member of the Inter-departmental Centre for Tropical Studies. Scientific Reviewer of 'NATURA' Scientific Advisory Group. Coordinator of the main theme *Plant Control* in the European Forum Preparatory Committee: *Research in Agricultural for Developing Countries*. Chief Editor of the journal *Micologia Italiana*. Head of the Plant Pathology Section of the Agricultural Biotechnology Department, University of Florence. The main scientific competences are: biology and epidemiology of rusts of hard pines; patterns and evolution of oak decline; role of microrganisms in oak decline, with special reference to fungal endophytes; effects of salinity on the behaviour of soil microrganisms. Author or co-author of 200 scientific papers, included 6 volumes, on Forest Plant Pathology.

Prof. Andrea Vannini

A. Vannini is Associate professor at the Department of Plant Protection of the University of Tuscia (Italy). After the degree in Forestry in 1984 he has been post doc at the Department of Botany and Plant Pathology of Michigan State University and at the College of Environmental Science and Forestry at the New York State University. His main activity is focused on diagnosis, monitoring and epidemiology of forest diseases with particular emphasis in decline syndromes and soil borne pathogens of the genus *Phytophthora*. Most recent research activities are focused on alien invasive species and their centres of origin. Andrea Vannini is coordinator of several national and international research projects, centres of excellence and coordination actions. Of particular relevance are the cooperation research activities, with ICPC countries as Algeria, Nepal, Argentina funded by national and International Institutions. He is author of more that 110 research publications. Andrea Vannini is consultant of the Italian Ministry of Environment and cooperates with the Food Agricolture Organization (FAO) in development projects in Asia. He has been invited speaker at the ICPP 98 in Edinburgh.

Dr Matteo Garbelotto

M. Garbelotto is a Forest Pathology and Mycology Extension Specialist and Adjunct Professor in the Department of Environmental Science, Policy, and Management, Ecosystem Sciences Division, at UC Berkeley (www.cnr.berkeley.edu/garbelotto/). His major area of research is the study of microorganisms, and in particular fungi, in forest systems. He is working to elucidate the effects of ecological modifications, whether man-made or natural, in the ecology

and evolution of various fungi. Ongoing research in Garbelotto's lab seeks to determine the biological and ecological characteristics of important introduced pathogens in California and the impact of such introduced organisms on different or differently managed forests. M. Garbelotto, who has published widely, received his PhD from UC Berkeley in 1996.

Dr Khaled M. Makkouk

K.M. Khaled M. Makkouk received his PhD in Plant Pathology from the University of California at Riverside in 1974. He was researcher in Plant Virology with the Lebanese National Council for Scientific research, and Professor of Plant Pathology at the American University of Beirut. K.M. Makkouk is currently virologist at the International Center of Agricultural Research in the Dry Areas (ICARDA), and President of the Mediterranean Phytopathological Union.

Keynote Session KS 4

Recent developments in disease management

Prof. Matteo Lorito

Professor of Plant Pathology and Biotechnology, Faculty of Agriculture, University of Naples, Italy. Research Scientist (1989) then Professor (2000) at the University of Naples; Visiting Scientist at Cornell University (1990-1994) and Technical University of Vienna (1995); awarded fellowships by the Italian National Research Council (1990), the OECD (Organization for Economic Co-operation and Development) (1995), the Fulbright Research Scholar Programme (1997), the CIES-Occasional Lecturer Program in USA (1997) etc. Author of over 100 publications in refereed journals, inventor in about 20 patents, invited speaker or chairman to many International Congresses, co-founder of four Companies for implementation of new biotechnologies. Main research interests: biological control, new methods for plant protection, molecular basis of plant-microbe interactions.

Dr Richard Broglie

R. Broglie is Manager, Crop Genetics Research, DuPont Central Research & Development. He received his PhD in Microbiology from Rutgers University and served as both Postdoctoral Fellow and Assistant Professor in the Laboratory of Plant Molecular Biology at The Rockefeller University before joining DuPont in 1985. At DuPont, he has led research programs to identify traits for modified soybean and canola oils as well as disease resistance in corn, soybean, wheat and rice. His most recent assignment involves developing and implementing global R&D strategies in India and China to accelerate the discovery of genes for high value agronomic traits such as stress tolerance and efficient nutrient utilization.

Dr Andy Leadbeater

After training as a plant pathologist in the UK, A. Leadbeater entered the plant protection industry in 1980 when he joined Ciba-Geigy in the UK fungicides field trials department. He has now worked in industry for over 25 years and during that time has been involved in the development and introduction and support to the market of many novel active ingredients. He has led the UK development group of the company, has headed the European development department of Syngenta (for all product types) and is now head of the international development team for fungicides for Syngenta. A. Leadbeater has also taken a significant role in industry and non-industry groups and currently is the chair of the FRAC International QoI Working Group, is the industry representative to the EPPO Working Group on Plant Protection Products and a member of the European Crop Protection Association's Efficacy Expert Group.

Keynote Session KS 5

Knowledge and technology transfer for plant pathology

Dr Steve Parker

S. Parker has worked as a Plant Pathologist for nearly twenty years. He began his career with ADAS, at that time the UK's public extension service, working in the plant clinic and servicing field experiments. This whetted his appetite for a career in plant pathology and he joined Long Ashton Research Station in 1988 to study for a PhD in epi-

demiology. After completing his PhD Steve stayed on to work on diseases of cereal and biomass crops, before rejoining ADAS as a cereals specialist in 1997. S. Parker joined CSL in 2003 where he works on a broad range of arable and horticultural crops. Throughout his career, he has had particular interest in providing decision support tools both for both tactical risk management (*e.g.,* to support decisions about the need for fungicide treatments) and more strategic analyses (*e.g.,* designing crop ideotypes). In pursuit of this interest, he has led and provided plant pathology expertise for a number of collaborative projects with biomathematicians and crop physiologists.

Dr Eric Boa

As head of the Global Plant Clinic, E. Boa lead a team of UK scientists who are active on three continents, supporting mobile plant clinics, reporting on new plant diseases and training people to be plant doctors. This is a long way from where he started, as a tree pathologist looking at ash canker. He then worked in Bangladesh on bamboo blight followed by a spell in Indonesia on Sumatra disease of cloves. Since the early 1990s he has enjoyed close collaborations with anthropologists and social scientists, combining the study of plants (and their diseases) with the study of people. The supply of technologies that people need and can benefit from requires the same rigour and attention to detail that scientists apply to their work. Finding new ways to tackle old problems and helping farmers in developing countries directly is proving the most rewarding time of his long career overseas.

Dr James Stack

J. Stack is Director of the Biosecurity Research Institute at Kansas State University, an 113,000 square foot biocontainment research and education facility with BSL-3 and BSL-3Ag capabilities. As Director of the Great Plains Diagnostic Network, J. Stack coordinates a nine-state project for the rapid detection and diagnosis of high consequence pathogens and pests. As Associate Director at the National Agriculture Biosecurity Center, he is the principal investigator for two international projects regarding plant biosecurity. He formerly worked for EcoScience Corporation as the Director of Applied Research, leading the discovery, development and commercialization of microbe-based products to protect fruit from storage decay pathogens.

The Jakob Eriksson Prize for 2008

Dr Laurence V. Madden

L.V. Madden is a leading international authority in plant disease epidemiology who has made numerous research contributions that have substantially increased our understanding of disease development in time and space. He has pioneered the use of many modelling approaches to: analyze, compare, and predict plant disease epidemics; characterize the spatial pattern of disease incidence, and relate spatial heterogeneity to crop, pathogen, and environmental factors; relate disease dynamics to crop losses; relate environment to disease and inoculum dynamics; and evaluate control strategies. Of major significance is his work with colleagues on development of differential-equation models for plant virus diseases with insect vectors. In this major contribution, the basic reproduction number for predicting invasion and persistence of viruses was derived. In other long-term research, he has shown how spread of diseases with rain-dispersed spores is determined by surface topography, plant canopy, and rain intensity.

Madden's research has been extremely productive, with 200 peer-reviewed journal articles and two books on plant disease epidemiology. He has received many honours, including the Ruth Allen Award from the American Phytopathological Society (APS), the Distinguished Scholar Award from the Ohio State University, and the E.C. Stakman Award from the University of Minnesota. He is an elected Fellow of three scientific societies. He served as President of APS in 1996-97.

INVITED PAPERS

Plenary Session PS 1
Congress Opening

PLANT HEALTH RESEARCH IN EUROPEAN PROGRAMMES.

T.J. Hall. *DG Research, European Commission. Email: timothy.hall @ec.europa.eu*

Agriculture and forestry have received increased political and public attention in the context of today global challenges, including climate change, bio-fuels, food security and the potential competition between food and biomass production, globalisation as well as food safety issues and biosecurity – many have implications for plant health research. The European Commission has been supporting trans-European and wider international scientific cooperation, both on pre-defined topics as well as on investigator-driven research activities through its multi-annual Framework Programmes (FPs). Since plant diseases and their control are important for farmers, consumers and the environment, research on plant health, pesticide usage, low-input farming, food chain issues and associated genomics and biotechnology have been important components of recent FPs and will continue to be in the 7th FP. The latter provides increased opportunities for plant health research and crop-related research in general, under the Theme Food, Agriculture and Fisheries, and Biotechnology, but also in other parts. Since European Commission research funding represents only a small fraction of the overall European effort, it is vital that a more coherent approach is developed across Europe to maximise research output. In recent years there has been progress towards realising a more complete European Research Area in the plant sciences but further advances are still needed. Several Technology Platforms are playing an important role in this respect, together with a number of ERANET coordination actions which bring together managers of similar national programmes from different countries with a view to developing complementary priorities, joint calls and possibly, ultimately, joint programming.

Keynote Session

PLANT DISEASES AND WORLD DEPENDENCE ON RICE.

R.S. Zeigler and S. Savary. *International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. Email: r.zeigler@cgiar.org*

This presentation deals with rice, the first food crop of humankind, and with rice diseases. It also deals with our present and future need for managing rice diseases in a sustainable way. Therefore I will also discuss pests other than diseases, with the diversity of contexts farmers, scientists, and policymakers have to face, and with the climatic challenges global agriculture faces in the near term. Rice is, and is likely to remain, the world's main food crop, partly because of the plant's tremendous adaptability, and partly because of the flexibility of rice-based agroecosystems. This diversity of production situations goes hand in hand with a large diversity of diseases and pests: in many ways, the diversity of these pest injury profiles echoes the diversity of production situations. In order to improve the performance of rice-based ecosystems, one has to manage not a particular disease, but a combination of diseases – an injury profile – whose yield-reducing effects must be minimized. The unprecedented changes that ecosystems are facing today have direct impacts on rice farmers, rice production, and rice diseases. New injury profiles are emerging, in new production situations, where new research must invent ways to produce more rice with fewer resources, while improving rice-based ecosystem performances. As an international scientific community, we must rise to the challenge of increaseing the knowledge we have on rice injury profiles and their management, and from the unprecedented development of new tools to understand, breed, and manage rice for future and sustainable food security.

DEVELOPMENT OF APPROPRIATE STRATEGIES TO CONTROL CASSAVA DISEASES IN GHANA.

E. Moses, J.N. Asafu-Agyei, F. Ayueboteng, A. Adusei and K. Adubofour. *CSIR-Crops Research Institute, P.O. Box 3785, Kumasi, Ghana. Email: e.moses@ cropsresearch.org*

Production in Ghana of cassava (the most important staple food crop) is significantly affected by diseases including African cassava mosaic disease (ACMD) and root rot caused by *Polyporus sulphureus* in some of the major production districts. *P. sulphureus*, a parasitic mushroom, alone is capable of causing complete crop failure in susceptible cultivars of cassava. Varieties were tested for resistance to the major diseases of cassava particularly ACMD and *P. sulphureus* root rot in disease hot spot areas in the Kpando district of the Volta region using farmer participatory approaches. Eight varieties showed resistance to ACMD and *Polyporus* root rot while the local farmers' variety succumbed severely to ACMD. Two of the eight varieties gave root yields of 22.0 and 23.0 t/ha compared to 11.0 t/ha obtained from the farmers' local cultivar. Two hundred farmers and agricultural extension agents from four major cassava producing districts were trained in disease identification and control through workshops and field days. Two farmer field schools were established and operated in the Sabadu and Aveme farming communities of Kpando district, and trained local farmers in disease identification and control. A disease identification and control guide was developed, published and disseminated to increase awareness and promote disease control. With improved disease control practices and cultivation of some of the disease-resistant varieties identified, farmers can increase or double yields of edible roots and improve on their incomes and food security.

BIOSECURITY IN THE MOVEMENT OF COMMODITIES AS A COMPONENT OF GLOBAL FOOD SECURITY.

N.A. van der Graaff and W. Khoury. *Former Chief, Plant Protection Service, Food and Agriculture Organization of the United Nations (FAO). Email: vdgraaff@tiscali.it*

Demand for agricultural produce will closely follow the continued growth of the world population and the change in consumption patterns in major developing countries. International agricultural trade, which more than doubled between 1987 and 2003, is vital for access to food. Biosecurity (regulatory regimes for food safety, animal and plant health, and the introduction of genetically modified organisms) results in technical barriers to trade. The WTO Sanitary and Phytosanitary Agreement (SPS Agreement, 1995) seeks to avoid arbitrary or unjustifiable discrimination among countries. Phytosanitary biosecurity addresses the introduction and spread of plant pests. Phytosanitary policies and measures have a dominant effect on market access and many countries have started a regulatory review to ensure a scientific base for phytosanitary measures. The SPS agreement recognizes the international standards for food safety set by the FAO/WHO Codex Alimentarius as well as International Standards for Phytosanitary Measures (ISPMs) concluded within the framework of the International Plant Protection Convention (IPPC), an intergovernmental FAO Treaty. The latter has led to major changes in the international plant health framework: setting of international phytosanitary standards has been taken up and the number of IPPC Contracting State Parties has increased to 164. Future de-

velopments might include the international recognition of pest-free areas. Many countries are seeking synergies among their national regulatory systems for Biosecurity allowing for consistency of decision-making and best use of limited resources. At international level, there is a need for further alignment between the above-mentioned agreements, and the Convention on Biological Diversity and its Cartagena Protocol. While private standards gain importance in food safety, phytosanitary biosecurity remains primarily a government concern.

Concepts in biological control of plant pathogens

REBECA – ACTION TO REVIEW OVERLOADED EU REGULATION POLICY ON BIOLOGICAL CONTROL AGENTS. **R.U. Ehlers.** *Institute for Phytopathology, University Kiel, Dept Biotechnology & Biological Control, Klausdorfer Str. 28-36, D-24223 Raisdorf, Germany. Email: ehlers@biotec.uni-kiel.de*

Understanding the diversity, mutualism and antagonism of biological control agents opens new horizons for their economic exploitation for sustainable agriculture and safe food production. What begins in the laboratories of highly motivated and dedicated scientists very often, however, ends up only on their shelves. Commercial exploitation is often hindered and rapid market introduction delayed because of overloaded regulation requirements. One of the major hurdles for commercial development is requirements needed to register microbial products according to EU directive 91/414. The REBECA action (www.rebeca-net.de) has reviewed existing regulation practice and proposed innovative alternatives to accelerate the introduction of biological control agents into agricultural markets.

BIOCONTROL OF PLANT PATHOGENS AND PLANT GROWTH PROMOTION BY *BACILLUS*. **B. McSpadden Gardener and R. Raudales.** *The Ohio State University – OARDC, Wooster, OH, USA. Email: bbmg+@osu.edu*

Numerous *Bacillus* strains have been investigated for their capacities to protect plants and stimulate their growth. In the past decade, several of these have proven to be commercially viable products. This talk will review how *Bacillus* and their products have been used successfully as crop protectants and biostimulants. Particular emphasis will be given to new observations made on the biochemistry, diversity, distribution, and genomics of these bacteria and how such information is being used to enhance commercial development of biocontrol inoculants. Key gaps in our understanding will be highlighted.

BIOLOGICAL CONTROL OF *MACROPHOMINA PHASEOLINA* IN PAKISTAN. **S. Shahzad.** *Department of Agriculture, University of Karachi, Karachi-75270, Pakistan. Email: saleemshahzad_pk@yahoo.com*

Macrophomina phaseolina is known to infect more than 500 plant species in different parts of the world. In Pakistan, *M. phaseolina* has been found associated with more than 125 species including almost all the important crop plants. A number of microorganisms have been used for the biological control of the pathogen. A review of progress and the prospects for biological control of *M. phaseolina* in Pakistan will be presented.

BIOLOGY, BIOLOGICAL CONTROL AND MOLECULAR GENETICS OF ROOT DISEASES OF WHEAT AND BARLEY. **L. Thomashow, D. Mavrodi, O. Mavrodi, R. Bonsall, T. Paulitz, P. Okubara, K. Schroeder, Y. Quakand and D. Weller.** *USDA-ARS, Washington State University, Pullman, WA 99164-6430, USA. Email: thomashow@wsu.edu*

Root diseases cause billions of dollars annually in losses to cereal growers. Resistance to foliar diseases is common, but resistance to root diseases is rare. Soilborne pathogens of cereals are managed through crop rotation, tillage, and chemical seed treatments. However, plants also defend themselves by supporting rhizosphere microorganisms antagonistic to soilborne pathogens. One of the best examples of natural root defense is the spontaneous decline during monoculture of take-all of wheat or barley caused by *Gaeumannomyces graminis* var. *tritici*. Take-all decline results from the buildup of strains of *Pseudomonas fluorescens* that produce the antibiotic 2,4-diacetylphloroglucinol (DAPG). The robustness of this suppressiveness appears to be modulated by the genotype of the DAPG producer, the wheat variety, and the sensitivity of the pathogen to DAPG. Strains of *Pseudomonas* that produce phenazine antibiotics are abundant in the Pacific Northwest of the USA where cereals are grown under low rainfall, and these strains may be involved in the suppression of root diseases other than take-all. Pseudomonads involved in natural suppression are excellent biocontrol agents and the basis of recombinant strains with enhanced biocontrol activity. The introduction of genes encoding the phenazine biosynthetic pathway into the DAPG producer *P. fluorescens* Q8r1-96, a highly aggressive root colonizer of wheat and barley, resulted in enhanced control of *Rhizoctonia* root rot. Real-time PCR assays have made it possible to rapidly determine the inoculum density of soilborne pathogens and the population densities of natural microbial antagonists, giving growers new tools to manage root diseases.

Tropical plant pathology

FUNCTIONAL GENOMICS FOR IDENTIFICATION OF DISEASE RESISTANCE GENES IN RICE. **H. Leung, R. Mauleon, L. Yan, L. Bin, J. Leach, K. Satoh and S. Kikuchi.** *Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute, DAPO Box 7777, Manila, Philippines. Email: h.leung@cgiar.org*

Achieving durable disease resistance has been an important yet elusive target in rice breeding. In the case of bacterial blight, pyramiding of major *Xa* genes has been successful in countering the emergence of new pathogen races. However, finding the right gene combinations for stable blast resistance remains difficult. Genetic analyses of rice varieties showing durable resistance against blast suggest that most of these varieties have basal quantitative resistance yet overlaid with a number of R genes. With a complete rice genome sequence available, we are in a position to examine genetic variation in parallel with expression patterns on a genome-wide scale. To understand the genetic basis of quantitative resistance against blast, we have taken two approaches: (1) utilize genetic variants that generate contrasting disease expression patterns, and (2) use the whole rice genome structure to develop statistical methods for associating expression profiles with resistance phenotypes. Disease response transcriptomes of two pairs of genotypes (resistant vs susceptible varieties and a gain-of-resistance mutant vs wild type) were generated using the Agilent 22K rice oligoarrays. Relationships between genome structure and expression patterns were determined by defining aggregation of differentially expressed genes (DEGs) along a chromosome and correlated expression within contiguous gene blocks across

experiments. With this approach, nine genome regions of correlated expression and fourteen regions of aggregation of DEGs were located and characterized bioinformatically. Association of these regions with disease resistance QTLs provides convergent evidence for candidate genes contributing to quantitative resistance that can be subsequently validated in the field.

INFORMATION AND COMMUNICATION TECHNOLOGIES CAN DO MORE TO HELP FARMERS IN DEVELOPING COUNTRIES: NEW METHODS FOR EXTENSION AND RESEARCH. **E. Boa.** *Global Plant Clinic, CABI E-UK, Bakeham Lane, Egham, Surrey TW20 9TY, UK. Email: e.boa@cabi.org*

When the Global Plant Clinic (GPC) set out to improve access to plant health services we became aware of the need to improve extension messages. Diagnosis of plant diseases was not enough: extension workers and farmers wanted practical advice on what to do. There was a mistaken belief that the GPC had access to all the necessary expert knowledge, but this was soon dispelled as the nature of the problems were better understood. Working closely with farmer organisations and researchers we reviewed local and scientific knowledge to identify practical solutions to plant health problems. We saw the need to transform and translate scientific knowledge and make it relevant to local circumstances. Access to the internet was also not enough. The same disease in different places does not mean the same advice. New methods were developed for designing extension messages (the snowman) and validating them (farmer peer review). We taught techniques to improve use of widely available tools such as digital cameras, videos, computers and printers. We analysed demand and responded to it in collaboration with extension staff, valuing their responses but also validating their knowledge. Our experiences suggest practical responses to big themes such as 'knowledge and technology transfer' and the use of 'information and communication technologies' that may be of use to plant pathologists wanting to work more closely with extension and poor farmers.

MANAGING BANANA BACTERIAL WILT IN UGANDA. **W.K. Tushemereirwe.** *National Agricultural Research Organization, National Agricultural Research Laboratories Institute - Kawanda (NARLI), P.O. Box 7065, Kampala, Uganda. Email: banana@imul.com*

Banana is the most important food crop in Uganda but its production is threatened by numerous constraints including: diseases (bacterial wilt = Banana *Xanthomonas* wilt BXW or Kiwotoka, black Sigatoka, *Fusarium* wilt and banana streak virus disease), pests (banana weevil, nematodes), declining soil fertility, socioeconomic problems (marketing, high crop management costs and post-harvest handling/utilization). Of all these constraints, BXW is currently ranked the most important by the farming community. The disease was first reported on ensete and banana in Ethiopia in 1968 and in Uganda in 2001. The Uganda Government responded by developing a comprehensive strategy for disease management. The strategy of integrating research and development emphasized massive creation of awareness and use of participatory approaches to deploy control recommendations. It specifically provided for the following activities: establishing the prevailing status of banana bacterial wilt, generating information and appropriate technologies for management of the disease, disseminating the technologies and information and ensuring their application; evaluating impact of the research and development activities on BXW. It also provided for creation of national and local government level platforms bringing together all research and development actors to ensure coordinated intervention. The highest priority was given to establishing the disease's status and generating of information that would facilitate refinement of the tentative control recommendations. The first comprehensive survey conducted in early 2005 revealed that in central Uganda where the disease was fully established, 76-95% of the fields were affected. The disease was more severe in introduced bananas (especially variety Kayinja, 60-70%) compared to indigenous (Matoke) bananas (12-27%). Epidemiological studies revealed that: insects, mainly stingless bees were key agents of disease spread as they fed on the sap that oozed from wounds created when bracts and flowers fell off banana male buds; early removal of the male buds (using a forked stick to break rather than cut) protected the banana plants from infection; contaminated tools, any other wounding agents and moving infected fleshy plant parts around were also confirmed to spread the disease. *Canna* lily and wild bananas were identified as alternate hosts; persistence of the disease-causing bacteria in soil was established to be two to three weeks. A recommendation based on these findings was formulated, evaluated and disseminated. It involved: avoiding introducing the disease into the garden on plant parts or contaminated tools; breaking male buds to reduce chances of insect transmission; clearing diseased plants and alternate hosts to remove sources of inoculum; and cleaning (decontaminating) tools used on diseased plants to avoid spreading the disease on the tools. Within one year 60-90% of farmers in the target areas were able to completely eliminate the disease from plantations.

STRATEGIES TO MITIGATE THE THREAT TO WHEAT PRODUCTION FROM THE UG99 (TTKS) RACE OF STEM RUST. **R.P. Singh, Y. Jin, J. Huerta-Espino, P. Njau, R. Wanyer and R.W. Ward.** *International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600, Mexico, DF. Email: R.Singh@cgiar.org*

The spread of race Ug99 (TTKS) of *Puccinia graminis tritici*, causal organism of stem (or black) rust, from East Africa to the Arabian Peninsula and its predicted migration towards North Africa, the Middle East, Asia and beyond through predominant winds, or other means, poses a major threat to wheat production worldwide due to the susceptibility of a majority of popular cultivars and breeding materials. Although chemical intervention backed by sound surveillance can reduce losses, replacing susceptible cultivars with resistant ones is the best control strategy. Several known race-specific resistance genes, mostly transferred to wheat from alien relatives, confer resistance and should be deployed in combination to enhance their longevity. Greenhouse and field testing has identified wheat materials that carry high to moderate but adequate levels of adult-plant resistance (APR). A common feature of wheats with the most APR is the presence of the slow-rusting gene *Sr2* in combination with other unknown numbers of minor genes. Because complex APR is expected to be durable, a breeding scheme that includes shuttling of segregating populations between Mexico and Kenya is being implemented to enhance the incorporation of APR in a wider range of wheat materials. Identification of APR genes and linked molecular markers is also being pursued to aid selection of APR in the presence of effective race-specific resistance genes or absence of disease. New resistant wheat lines with high yield potential are being tested in various high risk countries for their fast-track release and seed multiplication.

POST-HARVEST PATHOLOGY

MECHANISM OF PATHOGENICITY IN *BOTRYTIS CINEREA*. **M. Hahn, M. Leroch, M. Kretschmer, A. Schamber, A. Mosbach and G. Doehlemann.** *Department of Biology, University of Kaiserslautern, Postbox 3049, 67663 Kaiserslautern, Germany. Email: hahn@rhrk.uni-kl.de*

Botrytis cinerea is one of the major threats for crop production, both as a pre- and a postharvest pathogen. The availability of a complete genome sequence and microarrays, and the application of molecular techniques have increased remarkably our knowledge of the biology and the mechanisms of infection of the grey mold pathogen. In my lecture, I will concentrate on morphological and molecular processes during early stages of development of a conidium on a plant surface. Conidial surface hydrophobicity promotes early attachment to the cuticle. In contrast to other fungi, no evidence was found that hydrophobins are involved in the coating of *Botrytis* conidia. Germination depends on both morphological (hardness, hydrophobicity) and chemical (sugars) signals. Depending on the signals, different modes of germination and penetration are observed. Signal perception occurs by unknown receptor systems and is mediated by either G-protein/ cAMP or MAP kinase signalling components. These components are also required for the expression of virulence factors that are required for penetration, killing and colonization of plant tissue. Amongst those, ABC- and MFS-type transporters that catalyse the energy-dependent efflux of plant defense compounds have been analysed in our group.

MECHANISMS MODULATING FUNGAL ATTACK IN POSTHARVEST PATHOGEN INTERACTIONS. **D. Prusky, I. Miyara, N. Alkan, O. Moskovitch and I. Kobiler.** *Department of Postharvest Science of Fresh Produce, Agircultural Research Organization, Bet Dagan 50250, Israel. Email: dovprusk@agri.gov.il*

As biotrophs, insidious fungal infections of postharvest pathogens remain quiescent during fruit growth while at a particular phase during ripening and senescence the pathogens transform to necrotrophs causing typical decay symptoms. Exposure of unripe hosts to pathogens (hemi-biotrophs or necrotrophs), initiates defensive signal-transduction cascades that limit fungal growth and development. Exposure to the same pathogens during ripening and storage activates a substantially different signaling cascade which facilitates fungal colonization. This presentation will focus on modulation of postharvest host-pathogen interactions by pH and the consequences of these changes. Host pH can be raised or lowered in response to host signals, including alkalization by ammonification of the host tissue as observed in *Colletotrichum* and *Alternaria,* or acidification by secretion of organic acids as observed in *Penicillium* and *Botrytis*. These changes sensitize the host and activate transcription and secretion of fungal hydrolases that promote maceration of the host tissue. This sensitization is further enhanced at various stages by accumulation of fungal ROS that can further weaken host tissue and amplifies fungal development. Several particular examples of coordinated responses which follow this scheme are described, followed by discussion of the means to exploit these mechanisms for establishment of new approaches for postharvest disease control.

MECHANISMS OF INDUCED RESISTANCE AGAINST *BOTRYTIS*. **T. Mengiste.** *Department of Botany and Plant Pathology, Purdue University, Lilly Hall of Life Sciences, 915 W. State Street, West Lafayette, Indiana 47907-2054, USA. Email: mengiste@purdue.edu*

Necrotrophic fungal pathogens constitute a significant problem in agricultural and horticultural crops production worldwide. Many foliar, soil-borne and storage pathogens are necrotrophs surviving by extracting nutrients from killed cells. To acquire nutrients, necrotrophs induce cell death (necrosis) and degrade plant cell wall by releasing toxins and cell wall degrading enzymes into host tissue. Failure to limit necrosis or cell wall degradation by the plant leads to enhanced infections resulting in diseases that culminate in the death and decay of the entire infected plant or its parts. Over the last decades, significant progress has been made towards understanding the genetic control of plant responses to biotrophic pathogens. In contrast, the biological processes underlying host responses to infection by necrotrophic fungi are not understood that well. We are using host responses to *Botrytis cinerea* as a model to understand the genetic components of plant resistance to necrotrophs. *B. cinerea* is a typical necrotrophic pathogen and causes the gray mold disease in diverse crops. Plant responses to *Botrytis* and other necrotrophic pathogens are regulated by a complex network of interacting genetic, molecular and hormonal factors in the plant. In addition, diverse pathogen derived molecules and significant environmental factors contribute to disease development. Thus, identification of genetic resistance to necrotrophic pathogens particularly *Botrytis* has been challenging. Recent efforts by many laboratories have started to shed light on the mechanisms of host responses to necrotrophic pathogens. Comparisons of basal resistance in *Arabidopsis* and tomato reveal resistance mechanisms that show a functional conservation in the two plant systems but also differences in disease development. I will discuss recent progress in the area of host responses to necrotrophic pathogens, cross-talk with plant responses to insect pests and abiotic stress factors.

ROLE OF PRE-FORMED ANTIFUNGAL SUBSTANCES IN THE RESISTANCE OF FRUIT TO POSTHARVEST PATHOGENS. **N. Adikaram, C. Karunanayake and C. Abayasekara.** *Department of Botany, University of Peradeniya, Sri Lanka. Email: nkba@pdn.ac.lk*

Fruits contain secondary metabolites with antifungal properties, called phytoanticipins. They are mostly concentrated in the fruit peel and compartmentalized in vacuoles, organelles, oil bodies or latex canals. Concentration and activity usually decline during ripening in coincidence with fungal rot development. The information on antifungal systems in immature avocado, *Carica papaya*, mango and wood apple (*Limonia acidissima*), reviewed here, suggests that they play a role in natural disease resistance. Immature mangoes have evolved a formidable antifungal system comprising several galloyltannins, resorcinols and chitinases. Galloyltannin and resorcinol levels are generally higher in resistant cultivars than in susceptible ones. Mango latex, distributed in a fine network of canals in the fruit peel, contains resorcinols and chitinases and has the ability to rapidly digest conidia of *Colletotrichum gloeosporioides.* Galloyltannins and resorcinols decline progressively during ripening and the latex disappears, when ripe rot development begins. Retention of latex in the harvested fruit reduces anthracnose and stem-end rot development during ripening. Although unripe mangoes respond to *C. gloeosporioides* infection by inducing superoxides, peroxidase etc. leading to cell death, the phytoanticipins appear to play a major defensive role. Pre- or postharvest treatment with plant inducers, and soil supplement with potassium or silicates enhanced fruit resistance to anthracnose and stem-end rot. *Carica papaya* fruit too

contains chitinases in the latex while avocados and wood apples have strong antifungal systems, each with several phytoanticipins. Fruits with strong phytoanticipin systems in general do not appear to induce phytoalexins, and chitinase seems to be a compromise defence for phytoalexins.

VASCULAR PLANT PATHOGENS

INTERACTIONS OF *VERTICILLIUM* SPP. WITH *BRASSICA*: ROOT INVASION, SYSTEMIC SPREAD AND PLANT RESPONSE. C. Eynck, N. Riediger, B. Koopmann and A. von Tiedemann. *Dept. of Crop Science, University of Göttingen, Grisebachstrasse 6, 37077 Göttingen, Germany. Email: atiedem@gwdg.de*

Verticillium longisporum (VL) is host-specific to *Brassica* species and particularly threatens oilseed rape (OSR) in intense crop rotations. VL infects the roots in the root hair zone by direct penetration of the rhizodermis without formation of appressoria and invades the vascular system, where it spreads by hyphal growth and conidia. This colonization pattern is not found with *V. dahliae* (VD), a species closely related to VL, which does not penetrate the xylem or induce disease symptoms. In the field, detection of VL in infected plants by ELISA or quantitative PCR showed that spread of infection was retarded until early maturity stages of the crop (> GS 85). Similarly, spread of VL was discontinuous in rapid-cycling rapeseed plants inoculated in the greenhouse. After fast initial colonization of the root vessels until the hypocotyl phase (0 to 14 dpi), an expanded phase (14 to 28 dpi) followed in which the fungus was arrested at the hypocotyl zone. After 28 dpi the pathogen readily spread systemically into the shoot xylem. This pattern of colonization was accompanied by a distinct change in salicylic acid levels in tissues and the xylem sap of infected stems, although colonization did not induce oxidative responses as reflected by the level of H_2O_2 or NO. Resistance was due to inhibition of fungal spread in the vascular system rather than through the prevention of root penetration. Reduced pathogen spread in less susceptible genotypes was associated with distinct phenolic responses in the adjacent xylem parenchyma.

GENE EXPRESSION IN *ARABIDOPSIS* FOLLOWING INDUCTION OF RESISTANCE TO VASCULAR WILT FUNGI. S.E. Tjamos, P.P. Antoniou and E.J. Paplomatas. *Plant Pathology Laboratory, Agricultural University of Athens, 75 Iera Odos str., 11855 Athens, Greece. Email: stjamos@yahoo.com*

This study describes the ability of the biocontrol bacterium *Paenibacillus alvei*, strain K165, to protect *Arabidopsis thaliana* against *Verticillium dahliae*. A direct antagonistic action of strain K165 against *V. dahliae* could be ruled out making it likely that K165-mediated protection results from induced systemic resistance (ISR) in the host. K165-mediated protection was tested in various *Arabidopsis* mutants and transgenic plants impaired in defense signaling pathways. These plants include: NahG (transgenic line degrading salicylic acid (SA)), etr1-1 (insensitive to ethylene), jar1-1 (insensitive to jasmonate), npr1-1 (non-expressing NPR1 protein), pad3-1 (phytoalexin deficient), pad4-1 (phytoalexin deficient), eds5/sid1 (enhanced disease susceptibility) and sid2 (SA-induction deficient). ISR was found to be blocked in the mutants npr1-1, eds5/sid1 and sid2 indicating that components of the pathway from isochorismate and a functional NPR1 play a crucial role in K165-mediated ISR. Furthermore, a *V. dahliae* strain was transformed with the EGFP gene to facilitate monitoring of the colonization of *A. thaliana* roots and stem. It was shown that application of strain K165 delayed the entrance and reduced pathogen colonization of the root system and stem, compared to the control plants. In addition, concomitant activation and increased transient accumulation of the PR-1, -2 and -5 genes was observed in the treatment where both the inducing bacterial strain and the challenging pathogen were present in the rhizosphere of the plants.

EVOLUTION OF VASCULAR WILT *FUSARIUM* TAXA INTO PATHOGENIC RACES AND PATHOTYPES: THE *FUSARIUM* WILT PATHOGEN OF CHICKPEA AS A MODEL. M.M. Jimenez-Gasco. *Department of Plant Pathology, The Pennsylvania State University, University Park, PA, USA. Email: jimenez-gasco@psu.edu*

Evolution of virulence traits of plant pathogens has intrigued phytopathologists for over a century. Clonally-reproducing fungal plant pathogens provide ideal model systems for the study of such traits since recombination obscuring evolution is absent. The *Fusarium oxysporum* f. sp. *ciceris–Cicer arietium* is an ideal system for that study. First, the pathogen is distributed worldwide, since *Fusarium* wilt of chickpeas occurs in all chickpea-producing areas of the world. Second, the pathogen is asexually reproducing and it displays a large amount of pathogenic diversity; populations consist of two pathotypes (symptom types, yellowing and wilting) and eight pathogenic races. Unlike most formae speciales of *F. oxysporum*, which are polyphyletic (i.e., host-specific pathogenicity has evolved independently and multiple times), the forma specialis *ciceris* is monophyletic, which postulates that all their races and pathotypes have evolved from a single individual capable of causing vascular infection in chickpeas. By using transposon-based DNA fingerprints, we have inferred an intraspecific phylogeny for races of *F. o. ciceris.* Mapping of specific virulence to chickpea cultivars onto that phylogeny showed that virulence of races was acquired in a simple stepwise pattern, with few parallel gains or losses. Several lines of evidence also indicate that the yellowing race 0 is probably ancestral to the more virulent, wilting races. Although chickpea cultivars resistant to *Fusarium* wilt are available, they have not yet been extensively deployed, so the stepwise acquisition of virulence is still clearly evident. Understanding race evolution in *F. oxysporum* f. sp. *ciceris* now helps in generating specific hypotheses about the dynamics of pathogen populations occurring in chickpea monocultures.

CHALLENGES IN CONTROLLING *VERTICILLIUM* WILT BY THE USE OF NON-CHEMICAL ALTERNATIVES. G. Lazarovits. *Agriculture and Agri-Food Canada, Southern Crop Protection & Food Research Ctr., 1391 Sandford St. London, Ontario, N5V 4T, Canada. Email: Lazarovits@agr.gc.ca*

Verticillium wilt continues to be one of the most serious soilborne diseases worldwide. The loss of methyl bromide, the mostly wide used fumigant for disease control, has stimulated new efforts to control wilt diseases by non-chemical alternatives. We have shown that high nitrogen organic amendments and liquid manures can significantly reduce disease severity and inoculum density. However, optimizing the use of such products needs refinement if we are to obtain the control levels observed with chemical fumigants. Identification of the modes of action for these products has however provided new avenues to improve their efficacy. Green manures, particularly *Brasicca* amendments, have been widely tested and found effective for *Verticllium* wilt control where such crops are grown on a wide scale. Combinations of

green manures and solarization were shown to enhance the efficacy of both control procedures. Biological control is being widely researched and may soon become available as a useable tool for disease management, as commercialization of some potential products nears reality. The most promising avenue for disease control however, is the use of grafting where susceptible cultivars are grafted onto *Verticillium*-resistant root stocks. Eggplants are proving to be an excellent model system for testing this technology and field trials are providing promising results. The potential benefits and limitations of all these technologies will be discussed.

CLIMATE CHANGE AND PLANT DISEASES

FINGERPRINTS OF CLIMATE CHANGE AND DISEASES. **M.W. Shaw.** *School of Biological Sciences, The University of Reading, Lyle Tower, Whiteknights, Reading, RG6 6AS, UK. Email: m.w.shaw@reading.ac.uk*

The fingerprints of climate change involve changes in the average behaviour or range of a species. The effects of climate change on disease are likely to be complex and hard to predict because they involve changes in host distributions and phenology as well as direct biological effects on the pathogen. The long-term dynamics of plant disease have been relatively little studied, due to a lack of empirical data over long time-spans. The long-term prevalence will be a balance between decline in unfavourable parts of the season, and immigration and increase during favourable parts; the latter has been most studied because of its economic importance and ease of study. The worst surprises are likely to arise from changed host ranges; however, for native plants whose ideal ranges have moved or disappeared, stress may produce entirely novel disease problems. Changes in vector-borne virus diseases may be the easiest to predict, because vector activity and range is often related to simple medium-term climatic variables such as temperature sums. For bacterial and fungal diseases the position is more difficult. Short-term forecast criteria form a basis; however, most of these forecasts account for only a moderate proportion of the annual variance in severity and are based on empirical correlations over a decade or so, necessarily ignoring parts of the life-cycle which were not critical in the observation period. Geographic comparisons offer the best hope of predicting which diseases are likely to become more severe; but we cannot expect to avoid surprises.

CHANGES IN HOST-PATHOGEN INTERACTIONS AT HIGH LEVELS OF CO_2. **S. Chakraborty.** *CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, Brisbane, QSL 4067, Australia. Email: sukumar.chakraborty@csiro.au*

Rising atmospheric CO_2, frost, variable and unseasonal rainfall, humidity, drought, cyclones and hurricanes, and warmer temperatures will influence host-pathogen interactions to alter the distribution, abundance and management of endemic pathogens and re-define new and emerging biosecurity threats. The few available host-pathogen models in climate change do not capture impacts of rising CO_2 or their underlying mechanisms. This is due to a lack of empirical knowledge of key influences/relationships essential to develop process-based models. The handful of field studies under free air CO_2 enrichment (FACE) have confirmed changes in leaf surface properties, host physiology and resistance, previously observed from the more abundant growth chamber studies. Increased disease severity at high CO_2 in the majority of host-pathogen interactions, another finding from growth room studies, also holds up in FACE trials on poplar rust, rice blast and rice sheath blight diseases. Two important trends have emerged from growth chamber studies. First, the enlarged plant canopy, which traps more pathogen spores, has more infection sites and a favourable microclimate, shows enhanced resistance. Secondly, the pathogen is slow to invade due to increased resistance but grows faster through host tissue and increase its fecundity by several fold. Mutation, selection and other evolutionary processes act on the massive pathogen population to accelerate evolution of new races. Only long-term FACE studies can realistically determine the interactive and evolutionary changes in host-pathogen interaction and disease epidemiology at high CO_2. As host-pathogen studies in FACE become widespread, plant pathologists can harness synergy through collaboration and exchange of ideas.

ECOLOGICAL GENOMICS FOR UNDERSTANDING AND RESPONDING TO THE EFFECTS OF CLIMATE CHANGE. **K.A. Garrett.** *Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502, USA. Email: kgarrett@ksu.edu*

Ecological responses to global change offer one of the most compelling contexts for genomic analysis. New genomic tools make it easier to study one of the most important factors determining the effects of climate change on species – the ability of species to adapt to the resulting new environments. Climate change predictions for the tallgrass prairie regions of the U.S. Great Plains include shifts to fewer, larger precipitation events, potentially resulting in longer periods of drought stress. Tallgrass prairie shares pathogens with the surrounding grass-based agricultural systems, so changes in land use will also interact with climatic shifts to alter selection pressures on pathogens. Our research group evaluated visually-distinct foliar symptoms in synoptic samples of over twenty of the most common tallgrass prairie species in a long-term field study. The highest disease incidence was observed for rust of big bluestem and rusts were the most common foliar diseases observed for all plant species studied. As a first step in understanding the potential for adaptation in this pathosystem, we have studied the ecological genomics of big bluestem rust. Drought stress led to increased rust severity upon recovery from drought, associated with reduced expression of some genes associated with disease resistance as well as dampened phytohormone responses to rust.

CLIMATE CHANGE AND GEOGRAPHICAL DISTRIBUTION OF PATHOGENS. **B. Marçais, C. Husson, D. Piou, M. Déqué, C. Robin and M.-L. Desprez-Loustau.** *UMR1136 Interactions Arbres-Microorganismes, INRA-Nancy, 54280 Champenoux, France. Email: marcais@nancy.inra.fr*

Climate is a key driver in the dynamics of plant pathogen populations. Continual change, by it magnitude is likely to deeply alter the impact and distribution of many important plant diseases. This may occur directly through impact on pathogen growth or winter survival or indirectly through impact on host phenology or physiology. Expansion in pathogen range may be dramatic when it results in contact with new hosts that did not coevolve with the pathogen, with results similar to introduction of exotic pathogens. However, although climate has already changed over the last century, surprisingly few studies relate changing pattern of disease occurrence with ongoing climate change. This is largely caused by the lack of available data on the severity of plant diseases at large geographical scales and/or long time periods for most of the im-

portant plant pathogens. Cases of emergence of forest disease possibly related to climate change will be illustrated. Our study aimed at simulating the effect of climate change on the distribution and impact of an array of important forest diseases in France, using a regionalised climatic scenario derived from global circulation models. Several approaches were used and compared: i. A mechanistic model focusing on key steps in pathogen life cycles; ii. Empirical models using available data on pathogen occurrence from the forest health survey system; iii. Generic models using the Climex software. The results of the study will be discussed, in particular in relation to recent climate changes. However, one of the main questions asked by managers is on the consequences of climate change on the overall impact of pathogens on a plant species. The pathogen-specific approaches used in our study gives limited insight into this more general question. Studies on determinants of pathogen community richness might be more informative, as for many groups of organisms, species richness increases with latitude, a gradient that appears to be related to increase in temperature. Unfortunately, very few studies document this pattern for pathogens.

SCIENTIFIC PUBLICATIONS

CHALLENGES OF LAUNCHING A NEW JOURNAL IN AN ELECTRONIC AND HIGHLY COMPETITIVE AGE: THE MPP STORY. G.D. Foster. *School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK. Email: Gary.Foster@bristol.ac.uk*

Molecular Plant Pathology On-line (MPPOL), published by the British Society of Plant Pathology (BSPP) was the world's first electronic copy-only, peer-reviewed journal dedicated to plant pathology. MPPOL was a pioneering journal, created in 1996, as a rapid vehicle for publication of papers on a variety of aspects of molecular pathology from scientists around the world. The aim for the journal was to handle all papers and refereeing electronically, and to publish papers via the WEB. In many ways MPPOL was before its time, and suffered from the lack of a traditional paper version of the journal, and the journal ceased to publish papers in December 1999. The advances made in technology and experience gained in creating MPPOL formed an excellent foundation for the launch of the new journal, *Molecular Plant Pathology* (MPP), in January 2000, a joint venture between BSPP and Blackwell Science (Wiley-Blackwell). The journal was published electronically, as well as traditional hard copy version that at the time would allow recognition by ISI. For all journals this is now the norm, but during late 1990s early 2000s, MPP was one of the first journals launched into this rapidly changing publishing world, with online access, open access, shrinking journals, and the importance of impact factors taking on a greater significance in many countries. This talk will cover the early years, as the journal launched into a highly competitive age, and how it progressed to the strong and secure position it holds today.

OPTIMAL ACCESS TO JOURNALS. R. Campbell. *UK PA to Eric Swanson, Wiley-Blackwell 9600 Garsington Road, Oxford OX4 2DQ, UK. Email: linda.harris@oxon.blackwellpublishing.com*

Around 1.6 million peer-reviewed articles were published in 2006 and the number has risen steadily at 3-4% per annum for decades. Driven by rising global spending on R & D, this rate of increase could go up to around 4-5%. Library budgets have not kept pace but through investing in new technology and developing new pricing models, access to the growing research literature has improved hugely in all parts of the world. Almost universal access has been achieved without risking the sustainability of the publication. Sustainability however remains a major problem with Open Access (OA). Hardly any of the pay-to-publish OA journals are profitable or even breaking even and we are now seeing new titles being launched of questionable standard. The other route to OA, self-archiving, is also unproven and the drive for access (demanded by some funding agencies) could corrupt the minutes of science. Users will be accessing different versions of an article often without realising. Funders need to work with publishers to establish international standards for archiving and access.

CHALLENGES FACING ESTABLISHED JOURNALS: "THE PHYTOPATHOLOGY STORY". C.C. Mundt. *Dept. of Botany & Plant Pathology, Oregon State University, Corvallis, OR, 97331-2902, USA. Email: mundtc@science.oregonstate.edu*

Phytopathology has been a dominant plant pathology journal for almost 100 years. Unlike many other modern journals, *Phytopathology* is published by a non-profit scientific society, and this has influenced the income sources and pricing structures used to support the journal. The American Phytopathological Society (APS) has created new publications as the nature and volume of plant pathology research has changed; today APS publishes three print journals, three electronic-only journals, and owns a major book publishing company. Though these ventures have been quite successful, the process has required careful planning to maintain identity and financial support for all publications. Like most journals, *Phytopathology* is facing the challenge of keeping readership high through increasingly open electronic access, while maintaining an adequate income through subscriptions. Additional challenges include responding to the 'electronic revolution', the increasingly international nature of APS and its journals, and to political issues such as biosafety and economic embargoes. Today, there is a large number of high quality, 'competing' plant pathology journals, and the science of plant pathology is increasingly (and appropriately) being published in journals in which plant pathology is not the primary emphasis. Thus, *Phytopathology* cannot depend primarily on its past reputation, but must be responsive to the changing needs of science, rapid changes in science communication, and changing expectations for the editorial and peer review processes. The accomplishments of *Phytopathology* are due in great part to a strong spirit of volunteerism among APS members, and to a highly effective and dedicated professional staff at APS headquarters.

PHYTOPATHOLOGIA MEDITERRANEA. A. Graniti, G. Surico, L. Mugnai and A. Canova. *Dipartimento di Biotecnologie Agrarie, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. Email: giuseppe.surico@unifi.it*

The Mediterranean region is an ecological unit with peculiar characteristics which have created distinctive plant pathology and related problems. In 1960, Prof. A. Ciccarone and Prof. G. Goidanich founded *Phytopathologia Mediterranea*, an international Journal devoted to plant diseases in the Mediterranean region. *Phytopathologia Mediterranea* is a non-profit Journal with a mission to assist the development of agriculture and agricultural research in the region. In view of the economic conditions of several of the countries, subscriptions were kept as low as possible, and for years the Journal received funding from the Italian CNR (National Research Council). Young plant pathologists from the Mediterranean countries were encouraged to publish their papers

in the Journal, and, as long as the results were sound, the Editors preferred to help authors to improve their ability to write scientific papers properly. In 1964, in order to promote cooperation among Mediterranean plant pathologists, a regional society, the *Mediterranean Phytopathological Union* (MPU), affiliated with the ISPP, was established, and *Phytopathologia Mediterranea* became its official organ. As key events of the MPU, many meetings on specific topics and 12 international congresses have been held in various Mediterranean countries. In spite of serious difficulties and a lack of funds, the Journal has published 47 volumes. Many of the papers have contributed substantially to a knowledge of diseases of important Mediterranean crops, such as olive, citrus, grapevine, date palm, fruit, ornamental and forest trees, cereals and vegetables. Several diseases and pathogens new to the region have also been first described here.

THE JOURNAL OF PLANT PATHOLOGY. G.P. Martelli. *Department of Plant Protection and Applied Microbiology, University of Bari, Via Amendola 165/A, 70126 Bari, Italy. Email: martelli@agr.uniba.it*

The *Journal of Plant Pathology* (JPP), an international journal of the Italian Society for Plant Pathology (SIPaV), is the continuation of the *Rivista di Patologia Vegetale* (RPV), the second oldest phytopathological journal in the world. RPV saw the light in 1892, just one year after the birth in Germany of the scientific journal *Zeitschrift für Pflanzenkrankheiten* (now *Journal of Plant Disease and Protection*), founded by P. Sorauer in 1891. RPV was founded by the brothers A.N. Berlese, plant pathology teacher in the Oenological High School of Avellino and A. Berlese, professor of entomology in the Faculty of Agriculture of the University of Naples at Portici. It was intended to host scientific and practical articles in the field of plant pathology at large, contributed by Italian and foreign scientists. The editors that followed the Berlese brothers were L. Montemartini (1905-1943), R. Ciferri (1961-1964), E. Baldacci and O. Ciferri (1965-1990) and G. Belli (1991-1995). In 1997, the newborn SIPaV determined to resume the publication of the once prestigious RPV under the name of JPP. Editors of JPP have been in succession A. Matta (1997-1998), A. Catara (1999-2000) and G.P. Martelli (2001 to date). JPP is a quarterly journal that publishes original research papers, short communications, disease notes and minireviews on fundamental and applied aspects of plant pathology. JPP has been growing steadily (from 150 pages in 1997 to 445 pages plus a 88 page supplement in 2007) and is winning international reputation. Its current impact factor is nearly 0.8.

Molecular diagnostics for plant pathology

BASICS OF REAL-TIME PCR, LABELLING SYSTEMS AND MULTIPLEXING. N.W. Schaad and P. Planchon. *ARS/USDA Foreign Disease-Weed Science Research Unit, Ft. Detrick, MD, USA. Email: norman.schaad@ars.usda.gov*

Unlike classical PCR, with real-time PCR amplification of the DNA product is continuously measured based on fluorescence. Advantages over classical PCR include: 1) the assay is much more rapid, 2) the system is closed, 3) the results are quantified in the log-linear phase of amplification compared to traditional end-point analysis, 4) an unknown sample can be quantified based on extrapolation of a standard curve, and 5) the results can be observed live on-line. The fluorescence is incorporated into PCR by one of the following approaches: 1) intercalating dyes, 2) hydrolysable probes, 3) displaceable probes and, 4) mixed primer-probes. All of these chemistries allow for detection of PCR amplicons via the generation of a fluorescent signal. Intercalating dyes are fluorophores that emit a fluorescent signal when inserted into double-stranded DNA. Assays which incorporate SYBR Green are highly sensitive but can lack specificity due to potential hybridization of the dye to primer-dimers and other non-specific products. Each of the probe methods depend on Fluorescence Resonance Energy Transfer (FRET) to generate the fluorescence signal, using two fluorochromes attached to the same or different oligonucleotide substrates. Since fluorescent dyes with different emission spectra may be attached to different probes and because of the wide range of available dyes, fluorogenic probes allow up to four DNA targets to be measured in the same sample (multiplex PCR). Also, multiplex PCR allows for internal controls to be co-amplified in single-tube. Several examples of multiplexing protocols for the detection of plant pathogens will be discussed.

COMPLETE BACTERIAL SEQUENCE INFORMATICS FOR USE IN DESIGNING PRIMERS AND PROBES. A. Ignatov. *'Bioengineering' RAS, pr. 60-let Oktyabrya, 7/1, Moscow, 117312, Russia. Email: an.ignatov@gmail.com*

Dramatic achievements in complete microbial genome sequencing (558 completed and 835 in progress by 27 July 2007) has created the greatest expectation in microbial diagnostics since the discovery of the polymerase chance reaction (PCR) and development of specific PCR analysis. To find sequences potentially useful for plant-pathogenic bacteria diagnostics and verify them we conducted genome analysis of the available genomes within the genus *Xanthomonas*. Analysis was made by comparison of total nucleotide sequences, gene presence, gene copy number, gene order, amino acid sequence of homologous genes, regulone/operone structure, promoter and terminator regions, repetitive sequence number and location, single nucleotide polymorphism in key genes and intergene regions, and by unique shortest substring locations. The most important conclusion made from comparison of the close bacteria was the high level of DNA and amino acid similarity for most genes and the large share of lost/gained genes and large genomic regions across the whole chromosome. The chosen genes and intergene regions were tested as PCR targets on world-wide collections of strains of *Xanthomonas campestris* pv. *campestris, X. euvesicatoria, X. vesicatoria, X. perforance,* and *X. gardneri.* The variation across genes and DNA regions important for pathogenesis and bacterial adaptation was usually higher and involved considerable duplication of short (2–10 bp) and long (up to 300 bp) DNA fragments. Such target genes were more informative for species and pathovar diagnostics. Major sources of possible error in target sequence identification using bioinformatic tools are described.

REAL-TIME PCR PROTOCOLS FOR DETECTION OF QUARANTINE ORGANISMS. I. Barker, G. Muller, S. Fuentes, R. Mumford and N. Boonham. *International Potato Center, Apartado 1558, Lima 12, Peru. Email: i.barker@cgiar.org*

Real-time PCR offers a generic platform technology for the specific and sensitive detection of a wide range of plant pathogens. The technique fits well into testing schemes for quarantine organisms either as for primary screening or for confirmation. Real-time PCR is arguably a more robust method than conventional PCR and yields results that are easier to interpret. The use of control assays targeted against host plant genes, either in a

single or multiplex format, additionally permits the interpretation of negative test results which predominate in quarantine testing. The ability to dry down the required reagents so that they are stable at room temperature, the lowering cost of equipment and advances in nucleic acid extraction will further stimulate uptake of the technique, including use in smaller laboratories. The advantages of the method will be discussed using the detection of *Potato spindle tuber viroid* in potato and avocado as an example. Challenges still remain in devising suitable validation strategies, reducing the cost of reagents, achieving desired specificities, and sample extraction.

UNIVERSAL DETECTION AND IDENTIFICATION OF PHYTOBACTERIA USING PCR/ELECTROSPRAY IONIZATION-MASS SPECTROMETRY. E. Postnikova, C. Baldwin, C. Whitehouse, A. Sechler, N. Schaad, R. Sampath, V. Harpin, F. Li, R. Melton, L. Blyn, J. Drader, S. Hofstadler and W. Schneider. *United States Army Research Institute for Infectious Diseases, Fort Detrick, MD USA; US Department of Agriculture Foreign Disease/Weed Science Research Unit, Fort Detrick, MD USA. Email: chris.whitehouse@us.army.mil*

PCR-electrospray ionization-mass spectrometry (PCR/ESI-MS, previously known as "TIGER") utilizes PCR with broad-range primers to amplify products from all possible organisms within a taxonomic group, combined with calculation of PCR product mass using mass spectrometry. The precise masses are analyzed by computers to determine base compositions for the broad-range PCR products, which are compared to a database for identification of the organisms in the original sample. PCR/ESI-MS has the high sensitivity and high throughput capacity characteristic of PCR-based assays, but can also detect and identify organisms with no prior characterization or sequence data. PCR/ES-MSI has been used to characterize human bacteria, to type unknown human viruses and to strain-type human bacteria. The technology is universal in approach, and should be applicable to phytobacteria. Existing broad-range PCR primers were tested for their ability to amplify DNA of well-characterized phytobacterial strains, and the data collected was used to populate the existing PCR/ESI-MS bacterial database with base counts from phytobacteria. In a blinded panel study, PCR/ESI-MS identified 93% of bacterial DNAs to the genus level and 73% to the species/subspecies level. PCR/ESI-MS also detected and identified multiple bacteria within a single sample. The sensitivity of PCR/ESI-MS was within the range of typical PCR assays. Preliminary tests with samples from infected and healthy plants showed that the PCR/ESI-MS system was readily capable of distinguishing pathogens from endophytes as well. Additional broad-range primer sets that will improve PCR/ESI-MS for phytobacteria are under development.

Disease models, epidemiology

INTRODUCTION: SCOPE AND IMPORTANCE OF SCALE FOR MODELING AND DISEASE MANAGEMENT. H. Scherm. *Department of Plant Pathology, University of Georgia, Athens, GA, USA. Email: scherm@uga.edu*

During the past quarter century, the concept of scale has emerged as a fundamental paradigm of ecological research. Since system processes or properties are often scale-dependent, various approaches to scaling, i.e., relating or extrapolating measurements made at one scale to those at another (finer or larger) scale, have been developed. Scaling can occur temporally or spatially, or across the molecular, physiological, and ecological attributes of organisms. Most remarkable among the latter types of scaling relationships are the power laws that seek to predict a species' life history characteristics and population interactions based on metabolic theory of the individual organism. In the narrower confines of botanical epidemiology, interest in the concept of scale has increased steadily during the past decade. Traditionally, epidemiological investigations have focused on the field scale, a logical choice given the importance of individual fields as the basic unit for disease management. More recently, however, the scale of analysis has broadened to include both finer and larger scales, the former resulting from increased interest in precision pest management, for example, and the latter as a consequence of the need to understand the continental spread of non-indigenous pathogens. Two examples will be given to illustrate temporal and spatial scaling in botanical epidemiology and to provide context for the presentations in this symposium: 1) a multi-scale analysis of the effects of climate cycles on long-term epidemic dynamics; and 2) a simple model for changes in the rate of advance of epidemics over successively larger spatial scales.

TREE DISEASE MANAGEMENT IN HETEROGENEOUS LANDSCAPES. O. Holdenrieder, M. Pautasso and P.J. Weisberg. *Institute of Integrative Biology, Department of Environmental Sciences, ETH, 8092 Zurich, Switzerland. Email: ottmar.holdenrieder@env.ethz.ch*

Infectious tree diseases can significantly alter ecosystem structure, functions and services, particularly in the face of environmental changes. Recent landscape pathology studies have deepened our understanding of interactions between pathogen, host and environment over broad spatial scales of relevance to forest landscape management. Examples comprise, among others, basidiomycetous root rots, *Phytophthora* diseases, rust epidemics and beech bark disease. Disease patterns and dynamics are at least partly explained by host density and diversity, topographic, edaphic and climatic factors, landscape connectivity and metapopulation dynamics. The effects of forestry on landscape structure influence the host-pathogen system in potentially predictable ways, through fragmentation effects on pathogen spread, and influences of mosaic patterns of host composition on pathogen population dynamics. Recent research also provides quantitative models for how environmental heterogeneity influences forest susceptibility to disease expression. New tools are being developed for spatial risk assessment and monitoring of tree diseases at landscape and regional scales. We will explore the value of these findings for sustainable forest management within a changing world.

PATTERNS OF DISTRIBUTION OF GENETIC VARIATION FOR RESISTANCE AND VIRULENCE. J.J. Burdon and P.H. Thrall. *CSIRO-PI, P.O. Box 1600, Canberra, ACT 2601, Australia. Email: jeremy.burdon@csiro.au*

Understanding the patterns of distribution of genetic variation for resistance and virulence in natural host-pathogen systems depends on recognizing that these interactions occur in heterogeneous environments that vary in space and time. Individual populations are parts of local metapopulations in which mutation, selection, genetic drift, extinction, gene flow and recolonization, all play a complex part in shaping their structure and that of the metapopulation as a whole. Computer simulations of gene-for-gene interactions provide a picture of the importance of dispersal

distance in influencing the rate and direction of evolutionary change through its direct impact on the probability of encounters between host and pathogen. Empirical studies provide strong evidence supporting the stochastic nature of these interactions with marked variability in the size, persistence and structure of populations over time and space. Through time, resistance structures of host populations may change rapidly in response to major disease epidemics. Conversely, host populations may affect the infectivity structure of pathogen populations, with host populations with diverse resistance structures favouring the local dominance of pathotypes with broad infectivity, while more uniformly susceptible host populations favour pathogen populations dominated by simpler pathotypes. At the metapopulation level there is evidence of spatial structure in that more closely adjacent host populations have a more similar resistance structure than more distant populations. For mobile pathogen populations, there is less evidence of spatial structure in infectivity at very local scales, although local adaptation may occur at regional scales, resulting in a mosaic of different population structures among component demes.

MODELLING DISEASE IN LARGER LANDSCAPES: THE CONNECTIVITY OF THE U.S. AGRICULTURAL LANDSCAPE. **K.A. Garrett, M.L. Margosian, J.M.S. Hutchinson and K.A. With.** *Kansas State University, Manhattan, KS, 66506, USA. Email: kgarrett@ksu.edu*

Scaling up models of epidemiological processes to regions and countries is a frontier for plant science. While these models are being developed, the connectivity of landscapes may be studied as a proxy for spatially realistic models of pathogen population processes. We employed a graph theoretic framework in geographic information systems to describe agricultural connectivity for four major crop species across the U.S. We estimated transmission costs, based on crop acreage among adjacent counties, to evaluate landscape connectivity and assess the risk of spread within each crop across a range of scales. For organisms capable of overcoming high transmission costs, owing to long-distance dispersal or high environmental tolerance, maize and soybean present a highly connected landscape at a national scale, compared to the more regional production patterns of wheat and cotton. Determination of the scales at which connectivity becomes disrupted (as indexed by thresholds in transmission costs) may facilitate the identification of regions where rapid response to outbreaks or implementation of quarantine areas may be particularly effective. Such thresholds could also be exploited in implementing policies to strategically enhance heterogeneity of the U.S. agricultural landscape. We conclude by presenting a new decision tree for response to an introduced pathogen or pest that emphasizes the importance of agricultural connectivity.

POST-HARVEST PATHOLOGY

DEVELOPMENT OF POST-HARVEST PATHOGENS AT LOW TEMPERATURE: THE *BOTRYTIS CINEREA*-GRAPES INTERACTION. **A. Lichter, S. Ish-Shalom, T. Kaplunov, Y. Zutchi, S. Lurie, A.K. Pandey and M.R. Davis**. *Department of Postharvest Science, Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan, POB 6, 50250, Israel. Email: vtlicht@agri.gov.il*

Cold storage is the primary means to prevent deterioration of fresh agricultural produce after harvest and development of fungal decay. However, during cold storage there is often a selection process in which postharvest pathogens become dominant and prevent further storage. *Botrytis cinerea* is a prominent example of a pathogen that dominates this niche, attacking a multitude of fresh produce. Table grapes with their fleshy and sweet berry are an ideal substrate for *B. cinerea*. Cold storage at 0°C slows down but does not prevent the fungus from causing decay, necessitating the use of aggressive antifungal treatments. Understanding the processes underlying development of *B. cinerea* at 0°C should enable better control of fungal development in cold stores. Measuring germination and growth of *B. cinerea* at different temperatures and on different media including grape berries enabled determination of the kinetics of development and acclimation of the fungus to low temperature. Molecular enrichment for RNAs highly expressed at low temperature helped to identify several novel genes, and their involvement in low-temperature development was genetically dissected. Availability of the *B. cinerea* genome sequence enabled the systematic study of mechanisms and genes known to be involved in the response and survival of psychrotrophic microrganisms to low temperature. Taken together, our data suggests that *B. cinerea* is highly adopted to pathogenic interactions at low temperature.

GLOBAL REGULATION OF GENES IN CITRUS FRUIT IN RESPONSE TO THE POSTHARVEST PATHOGEN *PENICILLIUM DIGITATUM*. **L. González-Candelas, S. Alamar, A.R. Ballester, P. Sánchez-Torres, J. Forment, J. Gadea, M.T. Lafuente, L. Zacarías and J.F. Marcos.** *Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC). PO Box 73, Burjassot. 46100-Valencia, Spain. Email: lgonzalez@iata.csic.es*

Large-scale EST sequencing projects and microarray hybridization nowadays constitute two major approaches to analyse biological systems at a molecular level. Although the use of these methodologies are becoming commonplace in plant pathology, their application to postharvest pathology has not yet been reported. We will present analyses of the overall response of citrus fruit to *Penicillium digitatum* infection. We have constructed both subtracted and regular cDNA libraries from infected fruits. Analysis of the non-subtracted library gives us a picture of what genes are being transcribed, whereas the subtracted library provides more direct information on what citrus genes are induced upon *P. digitatum* infection. A cDNA macroarray generated from the subtracted library has been used to identify what genes are upregulated in response to pathogen infection, and to determine the influence of ethylene on their expression. Under the framework of the Spanish 'Citrus Functional Genomic Project, CFGP' more than fifty cDNA libraries have been generated encompassing more than 90000 ESTs. These clones have been used to develop a cDNA microarray representing roughly 7000 unigenes. With this tool we have analyzed the differences and commonalities of the citrus fruit responses to ethylene, wounding, *P. digitatum* infection and induced resistance. We will present the results of such analyses emphasizing not only what genes are affected to a larger extent but also describing what are the major changes in biological processes and metabolic pathways.

HOST RESPONSES TO BIOLOGICAL CONTROL AGENTS. **R. Castoria.** *Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università del Molise, Campobasso, Italy. Email: castoria@unimol.it*

Biological control of plant diseases is a three-way interaction in which pathogen, plant tissue and biocontrol agent (BCA) are involved. The plant tissue cannot merely be considered as the battle-

field where pathogen and biocontrol agent confront each other, because it appears to perceive both the presence of the pathogen and the BCA. Perception of the pathogen and/or the BCA can be followed by deployment of an active response that results in localized and/or systemic induced resistance. The localized response is based on rapid cell death, reinforcement of cell walls and accumulation of phytoalexins. Systemic resistance also involves synthesis and accumulation of antifungal depolymerases that attack the fungal wall. Although systemic resistance can be considered a typical response of more metabolically active tissues, some of its mechanisms have also been reported in BCA-treated stored fruits. Especially for stored fruits, the defence capability gets weaker with time. As the physiology of the fruits changes during maturation and senescence, inhibitors of fungal growth decrease and the fruit become more susceptible to pathogens, especially necrotrophic ones. These changes, which affect the fruit in favour of necrotrophic pathogens, such as tissue softening and the physiological shift of the plant cells to a more oxidized state, also negatively influence the success of BCAs. This work attempts to gather and analyse the available information on host responses to BCAs, thereby presenting new possibilities for research in postharvest biocontrol.

PREHARVEST TREATMENTS FOR INDUCTION OF RESISTANCE TO POSTHARVEST DISEASES IN FRUITS. **E.K. Dann and Zainuri.** *Department of Primary Industries and Fisheries, 80 Meiers Rd, Indooroopilly, QLD 4068, Australia. Email: elizabeth.dann@dpi.qld.gov.au*

Resistance to post-harvest diseases has been successfully enhanced in a range of annual and perennial fruit crops, including melon and mango, by preharvest treatment with non-pesticidal activators. One of the most effective treatments has been acibenzolar-S-methyl (Bion®). Three or four sprays or soil drench applications applied to mango trees from early fruit-set reduced post-harvest anthracnose in fruit caused by *Colletotrichum gloeosporioides,* by up to 50%. Application of the activator to well maintained trees resulted in better induction of resistance than in poorly managed orchards. The activities of chitinase and β-1,3-glucanase were enhanced in leaves of mango seedlings treated with Bion, however activities were not significantly enhanced in fruit peel after soil drench applications to trees. Activities of the enzymes and concentrations of two alk(en)ylresorcinol compounds were greatest in developing fruit prior to harvest, then declined to the 'sprung' stage, but did not decline further as ripening progressed. Soil drench or trunk injection treatments with potassium silicate solutions were less consistently effective against the same diseases in mango and avocado. Activation of plant defences for post-harvest disease control should be considered in conjunction with other disease-minimising practices. For example, severity of anthracnose is largely influenced by rootstock or variety in mango and avocado, and can be related to levels of the pre-formed antifungal compounds alk(en)ylresorcinols and 'diene', respectively. As defences are maximally enhanced in healthy plants or trees, other important disease management strategies include balanced and timely fertilisation and irrigation, maintaining good crop hygiene (eg. pruning for inoculum minimisation), and targeted applications of fungicides and other pesticides.

Genomics and proteomics

GENOMIC ANALYSIS OF *HYALOPERONOSPORA PARASITICA*. **J. Beynon, L. Baxter, R. Allen, M. Coates, L. Hughes, S. Lee, S. Hall, B. Tyler, J. Rogers and S. Clifton.** *Warwick University, Warwick HRI, Wellesbourne, CV35 9EF, UK. Email: jim.beynon@warwick.ac.uk*

The interaction between *Arabidopsis thaliana* and the downy mildew parasite *Hyaloperonospora parasitica* has played a major role in the identification of key genes involved in plant resistance. Recent advances have allowed us to identify the pathogen genes that are involved in this interaction. The cloning of avirulence genes has allowed the definition of the RXLR motif that allows targetting of the pathogenicity efectors to the host plant cytoplasm. The *H. parasitica* genome sequence is being assembled in a collaboration between Warwick University and The Sanger Centre in the UK and Virginia Tech and Wash U in the USA. The genome reveals that it is adapted to an obligate biotrophic lifestyle, containing a vast repetoire of potential pathogenicity effector proteins. These genes imply an exquisite and complex relationship with their host plant and represent new tools with which to elucidate this interaction.

MULTIPLE HOST-SPECIFIC TOXINS, LATERAL GENE TRANSFER AND GENE LOSS IN THE EVOLUTION OF CEREAL PLEOSPORALEAN PATHOGENS. **R.P. Oliver, P.S. Solomon, J. Hane, M. Ferguson-Hunt, B.A. McDonald, J. Faris and T.L. Friesen.** *ACNFP, Murdoch University, WA 6150, Australia. Email: roliver@murdoch.edu.au*

We are analysing the wheat pathogen *Stagonospora nodorum* in order to determine the molecular basis of its pathogenicity. The genome sequence of *S. nodorum* has been obtained and compared to the tan spot pathogen *Pyrenophora tritici-repentis*. Combined analyses of genome sequences, specific wheat populations segregating for susceptibility, and pathogen population genetics have revealed a new model of pathogenicity in *S. nodorum*. In this model, the pathogen expresses at least 4 different host-specific toxins (HSTs), each toxin interacting with a different host susceptibility gene. Interaction leads to necrosis which, unlike a classical gene-for-gene system, benefits fungal reproduction. The consequences of this model for disease control will be discussed. The evolutionary history of the toxin genes can be inferred by analysis of the genome sequences and by sampling geographically distinct pathogen populations. We have presented evidence that ToxA has been laterally transferred both into *S. nodorum* and from *S. nodorum* into *P. tritici-repentis*. The fate of the transferred DNA in *P. tritici-repentis* has been examined, and evidence of gene duplication and gene loss was found. The history of twentieth-century emergence of plant disease is littered with examples of fungi related to *S. nodorum* and *P. tritici-repentis,* whose pathogenicity is determined by host-specific toxins. The idea that lateral transfer of the HST genes might be a common theme of these twentieth century diseases will be discussed.

TRANSCRIPTOMICS IN THE *USTILAGO*-MAIZE PATHOSYSTEM. **R. Wahl, M. Vranes, C. Pothiratana, K. Heimel, G. Doehlemann, R. Horst, L. Voll, U. Sonnewald, B. Usadel, F. Poiree, M. Stitt, R. Kahmann and J. Kämper.** *Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse, D-35043 Marburg, Germany. Email: kaemper@mpi-marburg.mpg.de*

We have used comparative transcriptomics of both fungal and plant cells to get insight into the processes occuring during infection of maize by the corn smut fungus *Ustilago maydis*. Our data revealed a complex cascade of fungal transcription factors that coordinate cell cycle and filamentous growth. Specific regulators

orchestrated a complex repertoire of secreted proteins thought to establish the biotrophic interaction, which includes suppression of plant defence, and reprogramming of metabolic resources within the plant. Analysis of the host's gene expression and metabolome during infection revealed that *U. maydis* is recognized initially and elicits plant defence reactions. With the establishment of the biotrophic interaction these responses are repressed by the fungus. Our data indicate further that *U. maydis* interferes with normal leaf development by preventing the transition from sink to source tissue. Our studies provide novel insights into the complexity of a biotrophic interaction.

GENOMIC ANALYSIS OF TRANSCRIPTIONAL REGULATORS OF *FUSARIUM GRAMINEARUM*. **R. Coulson, M. Urban, J. Antoniw and K.E. Hammond-Kosack.** *Rothamsted Research, Herts, AL5 2JQ, UK. Email: kim.hammond-kosack@bbsrc.ac.uk*

The trichothecene mycotoxin producing Ascomycete fungus *Fusarium graminearum* causes ear blight disease of small grain cereals. Infections lower grain quality and safety, and are of increasing global concern. The *F. graminearum* genome was sequenced to a depth of ~10 × coverage by the Broad Institute. This assembly has been completely aligned to the four chromosomes by using an available genetic map (Cuomo *et al.*, 2007, Science 317, 1400-1402). As part of the global initiative to annotate manually the genome, we have explored in depth *F. graminearum* sequences involved with the transcriptional process. Eukaryotic transcription is a highly regulated process involving interactions between large numbers of proteins, exhibiting a high degree of taxon-specificity (Coulson & Ouzounis, 2003, NAR 31, 653-660). To identify transcription-associated proteins (TAPs), TAP protein families within the genome were detected by TAP-TRIBE analyses and by querying with hidden-Markov models of DNA-binding protein domains present in well-characterised transcriptional regulators. We have also explored the physical distribution of each *TAP* gene amongst the four chromosomes, their pattern of expression using the publically available Affymetrix data sets, sequence differences occurring between two field strains collected in the USA and the presence or absence of each *F. graminearum TAP* gene in three additional *Fusarium* species for which full genomic sequence is now available.

Microbial endophytes

ENDOPHYTES: MASTERS OF PHENOTYPIC PLASTICITY. **B. Schulz.** *Institute of Microbiology, Technical Institute of Braunschweig, Spielmannstr. 7, D-38106 Braunschweig, Germany. Email: b.schulz@tu-bs.de*

Fungal endophytes are defined as those fungi that colonize their plant hosts without causing visible disease symptoms. Life history strategies of the individual fungi can vary considerably, e.g. growth as latent pathogens, quiescent colonization, sporulation as saprophytes following senescence. Many factors determine the outcome of the interaction, including the extreme phenotypic plasticity of some endophytes. This is demonstrated by *Phialocephala fortinii*, isolated from the roots of *Larix decidua.* When reinoculated into the roots of its host, the interaction was mutualistic, fungal colonisation improving growth of the host. Inoculated into non-host tomato (*Lycopersicum esculentum*), the interaction was also asymptomatic. However, when the same fungus was inoculated into the roots of the non-hosts *Arabidopsis thaliana* or *Hordeum vulgare,* it was pathogenic. In contrast, the host endophyte, *Leptosphaeria maculans* colonized *A. thaliana* asymptomatically. In endophytic interactions there is an intricate balance between fungal virulence and plant defence, a balance of antagonisms that results in tolerance of the fungal inhabitant. The host reacts to endophytic infection with defence metabolites, the fungi with numerous mechanisms to modulate host defence. For example, when *P. fortinii* is inoculated into tomato, host defence seems to have been overcome by detoxification of the host metabolite, a-tomatine. When colonizing *L. decidua*, *P. fortinii* synthesizes IAA. Endophytes also produce numerous biologically active metabolites. It is the phenotypic plasticity of fungal endophytes that allows them to adapt to their respective hosts, colonizing asymptomatically, colonizing as pathogens and causing disease, or merely growing as saprophytes on a dead host or other organic matter.

***RHIZOBIUM RADIOBACTER* IS A DETERMINANT OF THE SYMBIOSIS BETWEEN *PIRIFORMOSPORA INDICA* AND ITS HOST.** **K.H. Kogel, M. Sharma , M. Schmidt, A. Zuccaro, J. Imani, P. Schäfer and T. Hartmann.** *Research Centre for BioSystems, Land use and Nutrition, Justus Liebig University Giessen, D-35392 Giessen, Germany. Email: Karl-Heinz.Kogel@agrar.uni-giessen.de*

Fungi of the newly defined order Sebacinales are found on all continents in temperate and subtropical climates in mutualistic associations with orchid plants. The canonical form of this order, *Piriformospora indica,* colonizes plant roots with the capability to promote grain yield and to induce resistance against various infectious diseases. Owing to their remarkable beneficial activity in a broad range of plants, including cereals and Brassicaceae, the Sebacinales have attracted considerable interest as potential biocontrol agents. Here we show that at least part of the beneficial activity previously ascribed to the fungus itself, originates from a close association with α-*Proteobacteria* of the species *Rhizobium radiobacter.* Treatment of *Arabidopsis* seedlings with the bacterium results in growth promotion and systemic resistance to the powdery mildew fungus *Golovinomyces orontii*. Moreover, we found an exact match in *Arabidopsis* mutants defective in the jasmonate/ethylene pathway to failure in both *P. indica* and *R. radiobacter*-induced disease resistance response. A screen of Sebacinales fungi closely related to *P. indica* revealed presence of endobacteria in all isolates tested suggesting that *Sebacinales* species take part in complex symbioses comprising host plants and bacteria.

FUNCTIONAL AND STRUCTURAL DIVERSITY OF BACTERIAL ENDOPHYTES. **G. Berg.** *Graz University of Technology, Environmental Biotechnology, Austria. Email: gabriele.berg@tugraz.at*

Endophytes are an interesting group of plant-associated bacteria that live inside plants and show neutral or beneficial interaction with their hosts. The structure of bacterial communities in endophytic microenvironments of important crops (different cultivars of potato, lettuce, and sugar beet) and native plants (different bryophyte species) was analyzed by a multiphasic approach at different field sites in Europe. Interestingly, results of cultivation-independent approaches using single-strand conformation polymorphism (SSCP) and/or terminal restriction fragment length polymorphism (T-RFLP) of 16S rDNA amplified by universal as well as group-specific and functional primers revealed a high diversity and specificity in endophytic bacterial communities. The

antagonistic potential of endophytic bacteria, which was determined by screening for *in vitro* antagonism against different pathogens (bacteria, fungi, protists, and nematodes) ranged from 5 to 43%. An impressive, phylogenetically diverse spectrum of antagonistic strains was found. The indigenous antagonistic potential of endophytic bacteria was influenced by the plant species and developmental stage, the internal microenvironment, and the soil type. Screening for biocontrol strains resulted in the selection of promising candidates. These strains were evaluated in greenhouse and field trials regarding their efficiency to control pathogens under in situ conditions. One product (RhizoStar®) based on *Serratia plymuthica* HRO-C48 to control *Verticillium* wilt on different host plants was developed. For other promising candidates like *Pseudomonas trivialis* 3Re2-7 (B3) and *Serratia plymuthica* 3Re4-18 (B4) a biological control strategy against the soil-borne pathogen *Rhizoctonia solani* will be established.

VISUALISATION OF PLANT-MICROBE INTERACTIONS USING AUTOFLUORESCENT PROTEINS. E. Lagendijk, F. Constantinescu and G.V. Bloemberg. *Institute of Biology, Leiden University, Wassenaarseweg 64, 2333AL Leiden, The Netherlands. Email: G.V.Bloemberg@biology.leidenuniv.nl*

Plant roots are colonized by a large diversity of microorganisms, pathogenic, neutral or beneficial to the plant. We focus on the effects of beneficial plant growth-promoting rhizobacteria (PGPR), which also interact and compete with the endogenous microflora, consisting of other bacteria, fungi and/or mycorrhizal fungi. Specifically we are interested in the use of PGPRs (for example *Pseudomonas* and *Bacillus* species) for protecting plants against pathogens, which is refered to as biocontrol. Usually PGPRs interact with the plant through (i) attraction to (root) exudate compounds (for example amino acids, organic acids and sugars), (ii) attachment, (iii) proliferation and (iv) colonization of e.g. of roots, stem, leaves and flowers. To suppress plant diseases a direct interaction between the biocontrol agent and the pathogen is often observed. A powerful marker tool to study interactions of PGPRs with the plant and the endogenous microflora is the use of auto-fluorescent proteins (AFPs), of which a growing variety is available to the researcher. We will report the latest results in developing tools for tagging microorganisms with autofluorescent proteins and the success we have with visualizing their interactions.

URBAN PLANT PATHOLOGY

SPATIAL ANALYSIS OF OAK WILT IN AN URBAN FOREST. D.N. Appel and K. Camilli. *Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA. Email: appel@ag.tamu.edu*

The impact of tree diseases on urban forests depends on the value of the trees, the structure of the tree populations, and the nature of the disease-causing organisms. The destructive tree disease oak wilt, caused by the vascular pathogen *Ceratocystis fagacearum*, provides an excellent model of how these factors combine, under various circumstances, to cause significant localized epidemics in Texas urban forests. Spatial data analyses of oak wilt in Dallas, TX, were conducted to increase our basic understanding of the epidemic process and provide clues on how to better control the disease. For example, mating types of the pathogen (A and B) appeared to be segregated throughout the 130 km^2 study area. Significant clustering of disease centers, largely in residential neighborhoods, was revealed using Rooks Case Moran's I and single linkage cluster analysis. Spatial autocorrelation, as examined with semivariograms, revealed spatial dependency among the disease centers. Oak wilt centers also exhibited anisotropy, or, a significant directional distribution of disease centers along an axis. Various environmental parameters, such as street orientation, slope, aspect, and soil type, appeared to have little direct influence on the occurrence of infections. Some of the factors responsible for oak wilt epidemiology are fairly well understood, such as relative susceptibilities of *Quercus* spp., insect vectors, spread through root connections, and inoculum sources. These are discussed in relation to the spatial patterns of oak wilt observed in Dallas.

MASSARIA DISEASE (*SPLANCHNONEMA PLATANI*, ASCOMYCETES) OF PLANE TREES IN GERMANY. R. Kehr and D. Dujesiefken. *University of Applied Sciences and Arts, Buesgenweg 1a, D-37077 Goettingen, Germany. Email: kehr@hawk-hhg.de*

Since 2004, Massaria disease, caused by *Splanchnonema platani* (Ces.) Barr (Anamorph: *Macrodiplodiopsis desmazieresii* [Mont.] Petrak), has been recognized as the cause of premature branch death and branch breakage of plane trees (*Platanus* × *hybrida*) in Germany. Reports from urban tree care professionals indicate that the disease, which is widespread in southern Europe and the USA, has been present in warm parts of southern Germany since the late 1990s. The disease is now found throughout Germany. Symptoms consist of elongate bark and cambium necroses on the upper side of branches of all sizes in the lower crown. The necroses are widest at the branch base, encompassing from less than 20% up to 50% of the upper branch circumference, and extend outwards several meters, becoming thinner toward the outer branch parts. The initial necrosis is followed either by death of the whole branch or, more commonly, by breakage of the affected branch. In the wood tissue below the necroses, wood decay bearing resemblance to soft rot/white rot becomes established within only a few months after bark death, making the branch very vulnerable to breakage. Massaria disease of plane trees has become a very important economic problem for German tree owners and municipalities, since the cost of tree safety inspection and tree care has increased dramatically due to the disease. Hot, dry summers apparently predispose plane trees to attack by the weak parasite *Splanchnonema platani*. Therefore, further climatic warming is seen as a problem for plane trees on dry sites.

CONTROL OF PLANE TREE ANTHRACNOSE BY SANITARY PRUNING. M.L. Tello. *Plant Pathology Lab, IMIDRA, Apdo. 127, 28800 Alcalá de Henares, Madrid, Spain. Email: marisa.tello@madrid.org*

Plane trees (*Platanus* spp.) are common in urban areas, representing nearly 15% of the total urban trees in southern Europe. In Spain *Platanus* × *acerifolia* (Ait.)Willd. is the most numerous species. Anthracnose, caused by the fungus *Apiognomonia veneta* (Sacc. & Speg.) Höhn. (anamorph *Discula nervisequa*), is the most important and frequent disease affecting mature planes in the central part of the Iberian Peninsula, and is also widespread throughout Europe and other continents. The fungus affects leaves, buds, shoots, twigs and older branches causing leaf necrosis, bud death, severe defoliation, branch growth in whorls, cankers and eventually tree death. At present, the control of plane anthracnose is restricted to prevention, via fungicide treatments and cultural methods such as removing dead branches and

fallen leaves. In 2000, 33 mature plane trees were selected in two locations of central Spain. A third of them were pruned in February 2001 and another third were trunk-injected with a fungicide in May 2001. The trees have been visited 3 times per year and disease progress recorded following a disease severity rating scale (DSRS). Results show better disease control in the pruned trees than in both the injected and the untreated trees. In plantations already established under the environmental conditions of central Spain, sanitary pruning consisting of the removal of all shoots and branches up to 3 years old is a suitable method to keep the disease under control, whilst for new plantations the best control method would be the use of tolerant *Platanus* spp.

TECHNIQUES FOR THE DETECTION OF AGENTS OF DECAY AND ROT IN STANDING TREES. **G. Nicolotti and P. Gonthier.** *DI.VA.P.R.A. Dept. for Exploitation and Protection of Forestry Resources, University of Torino, Via Leonardo da Vinci 4, I-10095 Grugliasco (TO), Italy. Email: giovanni.nicolotti@unito.it*

Detection of wood decay in standing trees and identification of the causal agents are crucial for assessment of hazardous trees and prediction of severity and development of decay. The VTA instrumental approach represents an advance compared with the use of the increment borer in the past, but penetrometers rarely allow precise evaluation of the extent of rot. Recently there has been an improvement in diagnostic techniques like electrical conductivity meters, ultrasonic detectors, X-rays and γ-rays, densimeters and nuclear magnetic resonance, but these instruments only give local information concerning the material surrounding the sensors. Some of these techniques have recently been developed for tomographic investigation allowing reconstruction of the section investigated, measuring the energy passing through the trunk. Different types of energy give information about the different physical properties of wood: ultrasonic waves describe elasticity, electric fields and radar electromagnetic waves reveal conductivity, γ-rays and X-rays measure density. Although modern technologies are improving the detection of wood decay, identification of the fungal agents is not always feasible without the presence of fruit bodies. Their identification is important to predict the pattern of spread within the tree and the risk of spread to neighbouring trees. Fungal DNA extraction directly from wood is a promising method for specific and sensitive diagnoses. PCR methods, based on taxon-specific nuclear or mitochondrial primers, have proved effective for identification, at different taxonomic levels, of the most important and widespread decay fungi responsible for tree failures.

PLENARY SESSION PS 2
PUBLIC DISCUSSION FORUM: PLANT PATHOLOGY AND GLOBAL FOOD SECURITY

ISPP AND THE CHALLENGE OF FOOD SECURITY. **P. Scott and R. Strange.** *International Society for Plant Pathology, c/o CABI, Wallingford, UK; Birkbeck College, University of London, UK. Email: p.scott@cabi.org ; r.strange@sbc.bbk.ac.uk*

We live in a world in which over 800 million people do not have food security. Through malnourishment they are unable to live healthy lives and many die prematurely. Children are particularly vulnerable, 18,000 dying every day according to one estimate. Although there are many reasons for food insecurity such as inhospitable climate, poor soil, inadequate access to sources of food, trade barriers and political constraints, plant disease is a fundamental limitation. In response to a public challenge at ICPP 1998, the International Society for Plant Pathology (ISPP) established a Task Force on Global Food Security. This has focused on a few priorities where it was considered possible to deliver something tangible with limited resources. Some of the results and prospects will be presented. A 3-year Challenge Project has been supported, "Development of Appropriate Strategies to Control Cassava Diseases in Ghana". Cassava is but one staple crop. Others and their principal diseases have been reviewed in "Plant Disease: A Threat to Global Food Security", published for ISPP in *Annual Review of Phytopathology*. A further Challenge Project is to be supported, aimed at raising the public and political profile of plant disease in developing countries. Also, from an initiative within the Task Force, a new *Journal of Food Security* is proposed which would reflect the multifaceted nature of the subject. ISPP's Task Force welcomes suggestions for new initiatives that have the prospect of delivering practical benefit from limited resources.

WHY PLANT DISEASE MATTERS TO FOOD SECURITY. **G.S. Khush.** *39399 Blackhawk Place, Davis California, 95616, USA. Email: gurdev@khush.org*

World food security depends upon continued productivity of our food crops. However, crop productivity is always under threat from attack by numerous pathogens such as viruses, bacteria, fungi and nematodes. These pathogens may cause mild symptoms but sometimes the losses are catastrophic resulting in food shortages and famines. Examples of such large-scale famines are those caused by potato blight in Ireland which resulted in one million deaths in the 1840s. The great Bengal famine of 1943 resulted from destruction of the rice crop by *Cochliobolus miyabeanus* and two million people died. These examples show that in areas of the world where a large proportion of the population depends upon a single crop, they are at risk if that crop fails owing to a disease epidemic. The most logical defense against damage from plant disease is varietal resistance. However, pathogens are able to overcome such resistance. Therefore it is important to study the biology of the disease-causing organisms and examine the potential for emergence of new virulent races. Crop genetic diversity is an important insurance against sudden breakdown of resistance. Large-scale collection, conservation, evaluation and utilization of crop germplasm is an important component to guard against disease. Fortunately, large germplasm collections of food crops are being maintained by CGIAR centres and National Crop Improvement programs. For ensuring our future food security it is important that national and international crop improvement and plant pathology programs are adequately funded.

GLOBALIZATION AND THE THREAT TO BIOSECURITY. **H.C. Evans and J.M. Waller.** *CAB International, UK-European Centre, Egham, Surrey TW20 9TY, UK. Email: h.evans@cabi.org ; j.waller@cabi.org*

Globalization has led to a dramatic increase in trade and travel, and alien or immigrant species are now being exchanged, either accidentally or deliberately, at unprecedented rates between geographically isolated regions, countries and continents. Thus, the natural barriers which once separated the world's floras and faunas no longer exist and invasive alien species pose a potent and burgeoning threat to both natural and agricultural ecosystems. Due to a combination of luck, in the early days, and judicious selection or quarantine, in later times, man successfully separated many of the coevolved natural enemies (pests and pathogens) from his crop plants to such an extent that most of the major crops are concentrated outside of their centres of origin where they are released from, or have a much reduced, natural-enemy pressure. However, progressively accelerating globalization over the past 150 years, which is now in over-drive, has meant that coevolved natural enemies have been catching up with their plant hosts, often with disastrous consequences. This paper discusses some past and present examples of the accidental, as well as the deliberate (bioterrorism) introduction of plant pathogens; and highlights those pathogens with "restricted" distributions which have the potential to: destabilise world commodity markets; disrupt global food security; and promote natural-habitat destruction. Far-reaching impacts of biosecurity on global warming and climate change are also discussed, as well as measures to improve both pre- and post-entry control of plant diseases.

GENETIC UNIFORMITY OF CROPS AND THE THREAT TO FOOD SECURITY. **J.K.M. Brown.** *John Innes Centre, Norwich, NR4 7UH, UK. Email: james.brown@bbsrc.ac.uk*

The emergence of new crop diseases is nothing new: previously unknown pathogens appear from time to time and forgotten pathogens re-appear. Environmental factors are often important in the emergence of new diseases, while the acceleration of global climate change will make it difficult to predict the most significant future threats to our crops. Plant breeding has been a cornerstone of global food security, especially in providing cultivars resistant to disease. Its success depends upon predictability, with the results of breeders' trials being reflected in the long-term performance of selected varieties. Increasing variability and uncertainty in the prevalence of different diseases presents a tough challenge to breeding and indeed to all agricultural technologies. Robust strategies of breeding will be more vital than ever in a rapidly changing environment. Such a strategy could be based on selection against high susceptibility to a broad spectrum of diseases, because susceptible cultivars not only suffer damage themselves but also spread disease to other, more resistant cultivars. Effective selection against susceptibility needs to proceed in parallel with selection for the key traits of yield and quality. An important recent development in plant breeding is association genetics, in which information about phenotypes, markers and pedigrees of many cultivars is combined. Simultaneous analysis of the genetics of numerous traits in diverse varieties is enabling breeders to achieve the long-desired goal of using diverse genes to control disease in top-class cultivars. It should also enable breeders to contribute to future food security by responding rapidly to new disease threats.

GM AS A NEW TOOL IN THE RESISTANCE TOOLBOX. **F. Wambugu, T. Hohn and J. Onsando.** *Africa Harvest Biotech Foundation International, Nairobi, Kenya; Sainsbury Laboratory, John Innes Centre, Colney, Norwich, UK. Email: fwambugu@ahbfi.or.ke; david.baulcombe@sainsbury-laboratory.ac.uk*

GM technology has developed multiple approaches to disease resistance in plants. There are GM plants with modified or enhanced forms of natural resistance mechanisms and others into which completely novel mechanisms have been engineered. We will describe some examples of both approaches and discuss the issues for their application in African agriculture, with the aim of improving food security. Sorghum is a staple for about 300 million people in Africa but its grain has a number of nutritional deficiencies and poor digestibility. The African Biofortified Sorghum Project aims to develop sorghum varieties that will substantially improve grain nutritional quality. Specific reference will be made to Kenya as a case study of how biotechnology can benefit the poor and hungry.

KEYNOTE SESSION
HOST-PATHOGEN INTERACTIONS AND MOLECULAR PLANT PATHOLOGY

ROLE OF PATHOGEN EFFECTOR PROTEINS IN PLANT INNATE IMMUNITY. **B.J. Staskawicz.** *Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA. Email: stask@berkeley.edu*

It has now been well established that all classes of pathogens are able to deliver effector proteins directly to host plants often via specialized infection structures. Pathogen effector proteins are involved with the suppression or modulation of plant innate immunity and fundamentally control plant pathogenesis. Interestingly, the same proteins that modulate pathogen virulence are also involved in triggering genotype-specific plant disease resistance. In this presentation, I will highlight the original approaches that led to the discovery of pathogen effectors and how this information has shaped our current understanding of the "dual" role of effectors in both plant pathogenesis and the activation of disease resistance signalling pathways. Furthermore, I will provide recent data from my laboratory in our attempts to employ pathogen effector proteins as molecular probes to identify host targets controlling plant innate immunity. Finally, I will present our recent data on elucidating the molecular events that are involved in effector recognition and the activation of plant disease resistance.

MOLECULAR MECHANISMS UNDERLYING THE ACTIVATION OF NB-LRR DISEASE RESISTANCE PROTEINS. **R.W. Innes, J. Ade and B. DeYoung.** *Department of Biology, Indiana University, Bloomington, IN 47405, USA. Email: rinnes@indiana.edu*

This talk will focus on the molecular mechanisms by which plant disease resistance (R) proteins mediate pathogen recognition. In *Arabidopsis*, the *RPS5* gene confers resistance to *Pseudomonas syringae* strains that carry the *avrPphB* gene. *RPS5* encodes a member of the nucleotide binding (NB)-leucine rich repeat (LRR) family of disease resistance proteins, while *avrPphB* encodes a cysteine protease. We have previously shown that AvrPphB specifically targets a protein kinase in *Arabidopsis* called PBS1. RPS5 forms a protein complex with PBS1 and cleavage of PBS1 is required to activate RPS5-mediated resistance (i.e. both *RPS5* and *PBS1* are required for recognition of AvrPphB). We have recently shown that mutations in *RPS5* that abolish nucleotide binding block RPS5 signalling, while mutations that reduce hydrolysis, but not binding, constitutively activate RPS5. In addition, we have shown that removal of the LRR domain also constitutively activates RPS5. We thus propose a model whereby RPS5 is held in an inactive ADP-bound state by intramolecular interactions and association with PBS1. Cleavage of PBS1 would then induce a conformational change in RPS5 that promotes exchange of ADP for ATP, which in turn enables RPS5 to engage downstream activators of resistance. Current work is focused on testing this model using a recently developed *in vitro* system containing purified RPS5, PBS1 and AvrPphB proteins. We are also attempting to identify downstream activating proteins. Progress in both of these areas will be presented.

SIGNAL INTEGRATION IN AND INTRACELLULAR DYNAMICS OF THE PLANT IMMUNE SYSTEM. **P. Schulze-Lefert.** *Carl-von-Linné Weg 10, 50829 Köln, Germany. Email: schlef@mpiz-koeln.mpg.de*

We study fundamental molecular processes underlying interactions between plants and pathogens. Plants and animals have evolved structurally related innate immune sensors inside cells to detect the presence of microbial molecules. Evolutionarily ancient folding machinery becomes engaged for the synthesis of autorepressed receptors in both kingdoms. The receptors act as regulatory signal transducers and are activated upon direct or indirect perception of non-self structures. Our findings indicate that nucleo-cytoplasmic partitioning and nuclear activity is critical for the function of plant immune sensors that trigger disease resistance responses to widespread ascomycete fungal parasites. The activated immune sensor directly associates with WRKY transcriptional repressors and interferes with their activity, thereby linking receptor function to transcriptional reprogramming of host cells for pathogen defence. Plant cells assemble pathogen-inducible machinery at the cell surface that shares several features with the immunological synapse, a specialized junction between a T cell and a target cell in animals. This, in both plants and animals, enables execution of immune responses through focal secretion. Common mechanisms include co-stimulatory non-self/alarm signals as triggers, cell polarization driven by actin cytoskeleton remodeling, protein concentration into ring-shaped assemblies at the cell periphery, and focal SNARE protein-mediated exocytosis. Whilst in plants execution of immune responses by polar secretion appears to be a cell-type independent property, its confinement to T cells in animals might reflect a greater division of labour in the animal immune system.

CONCEPTS IN BIOLOGICAL CONTROL OF PLANT PATHOGENS

IMPROVEMENT OF *TRICHODERMA* BIOCONTROL ACTIVITY BY PROTOPLAST FUSION AND *AGROBACTERIUM TUMEFACIENS*-MEDIATED TRANSFORMATION. **Y. Hetong, M. Ryder, H. Yujie, P. Harvey and T. Wenhua.** *Biology Institute of Shandong Academy of Sciences, Jinan, Shandong Province, P.R. China. Email: yanght@keylab.net*

Protoplast fusion and *Agrobacterium tumefaciens*-mediated transformation (ATMT) methods were adopted in an attempt to improve the ability of *Trichoderma* spp. to control plant pathogens. *Trichoderma harzianum* isolate T9 which is a good

producer of conidia and is naturally resistant to benomyl but is sensitive to hygromycin B, and *T. koningii* isolate 7a (Tk7a) which is a poor producer of conidia and is naturally resistant to hygromycin B but sensitive to benomyl, were used for protoplast fusion to obtain fusants with improved biocontrol activity and conidiation. Protoplasts obtained from the two parental strains after digestion of young hyphae with lysing enzymes were used in the fusion experiment. Most fusants performed better than parental strains in chitinase, glucanase and endoglucanase activity. There were no observable differences between fusants and the parental strains in their ability to parasitise *Rhizoctonia solani* and *Gaeumannymyces graminis* var. *tritici in vitro*.

The *Agrobacterium tumefaciens*-mediated transformation (ATMT) system is an important technical method in the study of many filamentous fungi. We successfully transformed *Trichoderma viride* LTR-2 by *A. tumefaciens* mediated transformation with an efficiency of 30-80 transformants per 10^5 conidia. When compared with the wild type in a dual culture *in vitro* experiment, LTR-2C7 had an increased effect against *Rhizoctonia solani*, with the inhibition percentage increasing by 9.4%. In a pot experiment, LTR-2C7 also had a greater effect on suppression of cotton *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* than the wild parent. The bio-control effect of LTR-2C7 increased by 28% compared with the wild parent as assessed by rating the disease severity.

IMPORTANCE OF MULTITROPHIC INTERACTIONS FOR SUCCESSFUL BIOCONTROL OF PLANT PARASITIC NEMATODES WITH *PAECILOMYCES LILACINUS* STRAIN 251. **S. Kiewnick.** *Agroscope Changins-Waedenswil, Research Station ACW, Plant Protection, Zoology, Schloss P.O. Box 185, CH-8820 Waedenswil, Switzerland. Email: sebastian.kiewnick@acw.admin.ch*

The facultative egg-pathogenic fungus *Paecilomyces lilacinus* is the most widely tested biocontrol agent for control of plant parasitic nematodes. The commercial strain 251 (PL251) is available in several countries and is mainly used for control of nematodes on Banana in the Philippines and root-knot nematodes in southern Europe. In the US, PL251 was recently registered as a bio-nematicide under the trade name MELOCON WG for use on a variety of crops. So far PL251 has demonstrated efficacy in reducing root-knot, cyst and free-living nematodes on a range of crops. To better understand the multitrophic interactions between PL251 and host-plants, target nematodes and other soil antagonists, ecological studies were conducted. The interactions of PL251 with host- or non-host plants, nematodes, mutualistic fungal endophytes, and mycorrhizas were investigated and their importance for biological efficacy and possible unwanted side effects determined. It could be shown that the efficacy of PL251 is not linked to the presence of the target nematode or to the host plant. Furthermore, some plants seemed to provide unsuitable conditions in their rhizosphere. Unlike other nematophagous fungi, rhizosphere competence is not a key factor for the efficacy of PL251, which makes it a control option in production systems where nematode infestation is severe and highly susceptible crops are grown. In none of the studies conducted, did the application of PL251 result in adverse effects on mutualistic fungal endophytes, mycorrhizas, fungal antagonists or entomopathogenic nematodes. Co-application of PL251 with rhizosphere-competent antagonists increased biocontrol efficacy against root-knot nematodes.

INTERACTIONS BETWEEN *GLIOCLADIUM CATENULATUM*, *FUSARIUM OXYSPORUM* AND CUCUMBER ROOTS LEADING TO BIOLOGICAL CONTROL OF *FUSARIUM* ROOT AND STEM ROT. **Z.K. Punja and S. Chatterton.** *Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, V5A 1S6, Canada. Email: punja@sfu.ca*

The biocontrol product Prestop Mix (Kemira Agro Oy) containing *Gliocladium catenulatum* (G.c.) (*Clonostachys rosea* f. *catenulata*) provides effective control of root rot diseases on cucumber caused by *Fusarium* and *Pythium* species. Roots were extensively colonized by G.c. within 7 days following application, especially at the root tips. Survival on the roots was evident for up to 50 days, with 1×10^5 colony-forming-units per gram of root. G.c. was transformed using *Agrobacterium* containing the β-glucuronidase (*uid A*) and hygromycin phosphotransferase (*hph*) genes. Histochemical staining for GUS expression showed hyphae in the underlying epidermal cells of roots and stems, suggesting endophytic colonization. In culture, G.c. produced chitinases and β-1,3-glucanases when grown on *F. oxysporum* cell walls. Several isoforms (20 to 45 kDa in size) of β-1,3-glucanase were observed on SDS-PAGE gels when laminarin or cucumber roots was the sole carbon source. Reverse-transcriptase PCR confirmed the induction of β-1,3-glucanase expression. Cell-free culture filtrates containing glucanases were inhibitory to mycelial growth and spore germination of *F. oxysporum*. Extensive root colonization and production of hydrolytic enzymes contribute to the biological control efficacy of *G. catenulatum.*

SCREENING OF BIOCONTROL AGENTS FOR CONTROL OF FOLIAR DISEASES. **J. Köhl.** *Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands. Email: jurgen.kohl@wur.nl*

Success of biocontrol depends on antagonists with high efficacy and optimum niche adaptation. Success of commercialisation of biocontrol agents depends on more criteria, and many criteria besides efficacy testing have to be considered. Criteria for applications of antagonists in the phyllosphere are cold tolerance, drought tolerance, resistance against high temperatures and UV-irradiation and rain-fastness. These ecological characteristics determine the persistence of inoculum. Other important criteria for commercial exploitation are suitability for mass production, and potential risks which may jeopardise registration. Examples will be presented of testing various characteristics of *Botrytis cinerea* or *Venturia inaequalis* antagonists under controlled conditions and field conditions. Conidia of the antagonist *Ulocladium atrum*, efficient in crops such as grapevine, tomatoes and ornamentals against *B. cinerea*, survive on leaf surfaces during periods with UV irradiation and high temperatures, and are very efficient in germinating during short wetness periods in a broad temperature range. Novel antagonists of *V. inaequalis* (cause of apple scab) were first selected by pre-screening. All candidates obtained from apple leaves were tested for their ability to produce a minimum number of spores on a standard medium, to grow at 5°C (cold tolerance) and to grow at low water potentials (drought tolerance). Candidates growing at 36°C or belonging to fungal genera with known potential to produce mycotoxins such as *Aspergillus, Fusarium* or *Penicillium* were excluded. Approximately 50% of the candidates passed the pre-screening and were subsequently tested in bioassays and in the field for their potential to suppress *V. inaequalis*.

PLANT VIRUS EPIDEMIOLOGY

MOLECULAR EPIDEMIOLOGY OF POTYVIRUSES, AN EXAMPLE: *WATERMELON MOSAIC VIRUS*. **C. Desbiez, C. Rys, B. Joannon, M. Girard, C. Chandeysson and H. Lecoq.** *INRA, Station de Pathologie Végétale, Domaine Saint Maurice, PB94, 43143 Montfavet, France. Email: Cecile.Desbiez@avignon.inra.fr*

Potyviruses cause severe epidemics in crops worldwide and several 'emerging' species or strains have been observed during the last decades. However, very little is known on the dynamics of dispersal and evolution, key factors for understanding potyvirus epidemiology and emergence. *Watermelon mosaic virus* (WMV) is very common in cucurbits. It is widespread in France but had, until recently, a limited agronomic impact on zucchini due to its mild symptoms. Since 1999, severe symptoms on zucchini have been associated with WMV. A molecular analysis was performed to study the origin of severe isolates. We found that worldwide WMV populations cluster in 3 groups without obvious geographic structure. Only one group ('classic' isolates) was observed in France before 1999, whereas since 1999 a second group of 'emerging' isolates, highly divergent molecularly, was also detected. Epidemiological surveys in France (2004 to 2007) showed the presence of 'classic' isolates everywhere, but 'emerging' ones were restricted to South-Eastern France. Four molecular subgroups were defined within the "emerging" group, with a strong population structure. In South-Eastern France, 'classic' and 'emerging' strains were frequently found in the same field or plant, and this raises the question of interactions between isolates (competition, population replacements, and potential emergence of recombinants...). The same distribution patterns were observed from 2004 to 2007, suggesting limited long-distance movement of the virus. However, the proportion of emerging isolates tended to increase. Our results suggest that the 'emerging' strains spread only slowly from their site(s) of introduction but are progressively replacing local ('classic') strains.

BARLEY YELLOW DWARF EPIDEMIOLOGY AS INFLUENCED BY APHID VECTORS AND DISEASE RESISTANCE. **N.A. Bosque-Perez, E.S. Jimenez-Martinez, K.J. Medina-Ortega, E. Ngumbi and S.D. Eigenbrode.** *Department of Plant, Soil and Entomological Sciences, University of Idaho, P.O. Box 442339, Moscow, ID 83844-2339, USA. Email: nbosque@uidaho.edu*

Barley yellow dwarf is a serious cereal disease caused by *Barley yellow dwarf luteovirus* (BYDV). Our research addressed ecological and epidemiological relationships among wheat, BYDV and bird cherry-oat aphid, *Rhopalosiphum padi*, a main BYDV vector. We monitored life history, behavior and virus transmission efficiency of *R. padi* on BYDV-infected or uninfected virus-susceptible Lambert and moderately-resistant 103.1J (Lambert-derived transgenic expressing BYDV-PAV coat-protein gene). Virus-infected transgenic plants were inferior hosts for aphids compared with virus-infected Lambert, but non-infected transgenics were superior hosts than non-infected Lambert. We observed reduced transmission efficiency after acquisition from virus-infected transgenics compared to virus-infected Lambert. Aphids preferentially responded to headspace from virus-infected rather than non-infected Lambert, but showed no preference for virus-infected compared to non-infected transgenics. Further tests indicated aphid responses to virus-infected plants are due to attraction rather than arrestment and that responses are mediated by volatile cues. Aphids preferentially responded to paper strips treated with individual compounds from virus-infected Lambert than to a blank control. Volatile organic compounds (VOCs) released by virus-infected Lambert are higher in total concentration and differ in relative component concentrations compared to virus-infected transgenics, or non-infected plants of either genotype. We conclude that transgenic virus resistance in wheat can indirectly affect vector life history. Such resistance influences production of VOCs by virus-infected plants altering aphid response to transgenics relative to non-transformed plants. The combined effects of reduced vector population growth on, reduced attraction to, and reduced transmission efficiency after acquisition from virus-infected transgenics could reduce secondary virus field spread, influencing virus epidemiology.

EPIDEMIOLOGICAL INTERACTIONS OF TOMATO VIRUSES IN GREENHOUSE COMPLEXES IN SPAIN. **E. Moriones, J. Navas-Castillo, J.A. Díaz-Pendón and C. Cañizares.** *Laboratorio Virología Vegetal, Estación Experimental La Mayora, CSIC, 29750 Algarrobo-Costa, Málaga, Spain. Email: moriones@eelm.csic.es*

Viruses cause great damage to tomato crops worldwide. In Spain, tomatoes are severely affected by insect-transmitted viruses. Notably, outbreaks of the whitefly *Bemisia tabaci* Gen have resulted in the emergence of viruses like the begomoviruses (family *Geminiviridae*) associated with tomato yellow leaf curl disease or the crinivirus *Tomato chlorosis virus* (ToCV; *Closteroviridae*). Also, important losses are caused by the thrips-transmitted tospovirus *Tomato spotted wilt virus* (TSWV; family *Bunyaviridae*), although in this case virus damage could be controlled by using cultivars carrying the *Sw-5* resistance gene. Mixed viral infections are frequent in nature, and result in interactions with unknown epidemiological consequences. We have shown that multiple begomovirus infections occur in commercial tomatoes. Moreover, interactions in single plants can result in the emergence of new recombinant viruses, with novel pathogenic characteristics. The dynamics of recombination and of associated mechanisms was studied, as this knowledge would help to understand the evolution of begomovirus populations and to forestall damage. Interestingly, interaction of viruses can result in synergisms or antagonisms that could compromise virus control. We observed synergistic interactions in tomato plants doubly infected with ToCV and TSWV, resulting in a dramatic increase of disease symptoms in susceptible plants with enhanced ToCV accumulation, although with no evident egress from phloem tissues. More remarkable was the synergism observed in TSWV-resistant plants that resulted in the breakdown of *Sw-5* resistance in the presence of ToCV. Viral products that could be involved in viral synergism have been investigated.

EVOLUTIONARY EPIDEMIOLOGY OF PLANT VIRUSES. **F. Van den Bosch.** *Rothamsted Research, Harpenden, AL5 2JQ, UK. Email: Frank.vandenbosch@bbsrc.ac.uk*

Any method of control of plant viruses inevitably imposes a selection pressure on the virus. This can cause the pathogen to evolve strains that are less prone to the control method. Models of these evolutionary processes help define disease control methods that put a minimal selection pressure on the virus to evolve more harmful strains. In this presentation I discuss two virus traits that can undergo evolutionary change due to selection pressures imposed by disease control. These are the within-cell multiplication rate of the virus, and the evolution of suppressors of gene-silencing. The results show how the direction of selection depends on the effect of the virus on plant traits. The two effects investigated are damage to plant parts increasing necrosis of this plant part, and damage to the plant resulting in reduced plant

growth and development. A range of disease control options will be discussed including plant resistance traits such as (i) acquisition/inoculation resistance, (ii) tolerance, (iii) reduced systemic virus dispersal, as well as control using (iv) sanitation, (v) vector control, and (vi) selection of planting material. The feasibility of disease control without selecting more damaging virus strains will be discussed.

Airborne plant diseases

A RAINFALL CYCLE INVOLVING PLANT PATHOGENS AS INCITANTS OF PRECIPITATION? D.C. Sands. *Dept Plant Science and Plant Pathology, Montana State University, Bozeman, MT 59717-3150, USA. Email: dsands@montana.edu*

A rare trait among microbes is ice nucleation activity. Only three groups of bacteria are able to initiate ice formation at temperatures as high as -1 or -2 °C, and these are the plant-associated *Pseudomonas syringae*, *Xanthomonas campestris* pv. *translucens*, and *Pantoea agglomerans* (formerly *Erwinia herbicola*). The plant-associated fungus *Fusarium avenacearum* is also ice-nucleation active. The primary interest among researchers has been that ice nucleation activity was important in initiating frost injury in plants. A second factor may be that ice nucleation enhances pathogen dissemination by favouring the formation of ice in clouds leading to snow or rainfall thereby bringing bacteria back to the ground. The completion of such a cycle would allow feedback via strong selection for traits leading to such dissemination. Furthermore, there is evidence that some of these organisms can enhance the condensation of water (cloud condensation nuclei) and thus favour the formation of clouds. The global ubiquity of non-host-specific strains of *P. syringae* and *P. agglomerans* may be because of their ability to disseminate via rainfall. They might now be an important component of rainfall initiation, and with a concerted collaborative effort among scientists, selected populations of these ice nucleation bacteria could be increased in the environment, in particular via agricultural practices favourable to their epiphytic development, to enhance precipitation and in the process complete a bioprecipitation cycle.

OUTSIDE THE TRADITIONAL BOUNDARIES OF AGRO-ECOSYSTEMS: SURPRISING NICHES FOR PLANT PATHOGENS. C.E. Morris. *INRA, Plant Pathology Research Unit, B.P. 94, 84140 Montfavet, France. Email: cindy.morris@avignon.inra.fr*

Disease control practices are based on knowledge of the life cycles and ecology of pathogens and on disease epidemiology. Sources of inoculum and conditions favoring pathogen proliferation and disease expression are essential clues for reducing pathogen development and disease severity. Understanding the mechanisms generating genetic diversity in pathogens can also give essential insight into pathogen evolution so as to make disease control practices durable. Knowledge of pathogen life histories can be translated into cultural practices to avoid inoculum or limit its proliferation, and be useful for developing biocides and other chemical or biological control agents, and for strategies to create and deploy resistant plant lines. For human and animal pathogens, it has long been recognized that some can reside and flourish in non-host habitats where they evolve and diversify somewhat independently of their activity as pathogens, whereas the life cycles of plant pathogens have almost exclusively been defined in agricultural contexts *sensu stricto* i.e. in terms of the availability and response of host crop plants. The role of non-agricultural and non-plant niches in the evolution of plant pathogens and in their life history has seemingly been ignored. Recently, evidence has emerged that some strains of *Pseudomonas syringae* inhabit diverse substrates in aquatic and alpine environments, and their life history seems to be intimately linked to the water cycle. The potential for important roles of non-agricultural habitats for this and other plant pathogens, and the impact for their ecology and diversification will be discussed.

WHERE DO ALL THE NEW POWDERY MILDEWS COME FROM? L. Kiss. *Plant Protection Institute of the Hungarian Academy of Sciences, H-1525 Budapest, P.O. Box 102, Hungary. Email: lkiss@nki.hu*

Powdery mildew fungi (Erysiphales) are ubiquitous obligate biotrophic plant pathogens as the symptoms they cause are obvious on leaves and other aerial parts of their host plants. Thus, their occurrence and spread can easily be monitored. Year by year, the highest number of 'new disease reports' published in international journals refers to this group of fungal plant pathogens. In most cases, however, the origins of the powdery mildew fungi reported for the first time on a certain host plant species or in a given geographic area remain unclear. Sometimes it is likely that they originate from other geographic regions, e.g. other continents. Biological invasions caused by North American and Asian powdery mildew fungi in Europe have already been documented using molecular tools. The biological invasion caused by the grapevine powdery mildew pathogen, *Erysiphe necator*, in Europe in the 19th century is a well-known example, and the histories of two recent severe European powdery mildew epidemics, caused by *E. flexuosa* on horse chestnut (*Aesculus* spp.) and *E. elevata* on Indian beam (*Catalpa bignonioides*) trees, seem to be similar to that process. Some other species of the Erysiphales, thought to be native to North America or Asia, have also started to spread in Europe quite recently. Another source of 'new' powdery mildews could be a host range expansion of taxa already known from certain host plant species. Such an event has recently been documented for the first time in powdery mildews. However, a recent genome-wide analysis of a few 'new' powdery mildew fungi showed that these all should be regarded as distinct taxa with restricted host ranges. Other sources of 'new' powdery mildews will also be discussed.

DETECTION AND QUANTIFICATION OF AIRBORNE FUNGI BY FLOW CYTOMETRY. G. Lingua, V. Prigione and V. Filipello Marchisio. *DiSAV, Università del Piemonte Orientale "A. Avogadro", via Bellini 25/G, 15100 Alessandria, Italy / Dipartimento di Biologia Vegetale, Università degli Studi di Torino, viale Mattioli 25, 10125 Torino, Italy. Email: guido.lingua@mfn.unipmn.it / valeria.marchisio@fastwebnet.it*

Fungal propagules prevail in the bioaerosol. Their quality and quantity are relevant to the assessment of air quality in hospitals, sewage areas, composting and industrial plants. In agriculture detection of airborne plant pathogenic fungi can assist in forecasting diseases and assessing pathogen resistance to fungicides and the environmental impact of biological control treatments. Meth-

ods for detecting the mycoaerosol usually rely on microscopy or culture techniques. The first approach is tedious and time consuming, while the precision and efficiency of the second is limited by the inability of media to allow the growth of all species, by the presence of fungal aggregates and by fungistasis. Flow cytometry is a quantitative cell-biology approach, allowing the counting and characterization of single cells or even organelles in liquid suspension. Due to its rapidity, it allows analysis of several thousand cells in a few minutes. A protocol to stain conidia with a fluorescent dye was set up, after microwave pretreatment of the propagules. Conidia of various fungal species from laboratory and environmental samples collected by an impinger were successfully counted by epifluorescence microscopy and flow cytometry. Flow and microscopy results were compared, showing that the former provides a correct assessment of the samples, while the latter was affected by a systematic error, linked to the concentration of conidia. An immunological approach was also introduced, indicating that immuno-labelling of conidia is possible, although specific identification still requires some research. These results disclose appealing and promising applications in the area of environmental monitoring.

MANAGEMENT OF FOREST DISEASES

MANAGEMENT LESSONS LEARNT FROM 90 YEARS OF DUTCH ELM DISEASE RESEARCH. J. Webber. *Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK. Email: joan.webber@forestry.gsi.gov.uk*

Over the 20th century several pathogens have emerged which have been extremely harmful to wild or naturalised plants including long-lived woody perennial species such as trees. One of the best known examples is illustrated by the two pandemics of Dutch elm disease that have swept through the Northern Hemisphere; the first caused by *Ophiostoma ulmi*, the second by *Ophiostoma novo-ulmi*. The biology and epidemiology of the disease caused by these two pathogens has been well documented, and it is now clear that episodes of hybridisation and genetic introgression have produced the highly aggressive elm pathogen *Ophiostoma novo-ulmi*. A further driving force has been the activities of a few species of highly effective bark beetle vectors, mainly within the genus *Scolytus.* The behaviour of each beetle species, and even the behaviour of individual beetles, can markedly influence the transmission of *O. novo-ulmi* and *O. ulmi* at many points in the disease cycle. Beetle behaviour during breeding in the bark of diseased trees affects the quantity and quality of pathogen spores carried by newly emerged beetles. Later on, the behaviour of beetles during dissemination and host seeking also impacts on the successful transfer of the pathogen from vector to feeding groove and, ultimately, the potential for colonisation of a tree's vascular system. Overall, the interaction between host, pathogen and vector is finely tuned, providing many opportunities to interrupt the disease cycle. The challenge has been to use this knowledge as part of an integrated approach to manage and control Dutch elm disease.

***HETEROBASIDION* ROOT ROT IN CONIFERS IN EUROPE. P. Capretti and K. Korhonen.** *DiBA Sez. di Patologia Vegetale, Piazzale delle Cascine 28, 50144 Firenze, Italy; Finnish Forest Research Institute, Vantaa Research Unit, P.O. Box 18, FI-01301 Vantaa, Finland. Email: paolo.capretti@unifi.it*

Root and butt rot caused by *Heterobasidion annosum s.l.*, also known as *Fomes annosus,* is regarded as the most injurious fungal disease of coniferous forests in the Northern Hemisphere. The fungus commonly attacks species of *Pinus, Picea* and *Abies.* The disease agent is a species complex consisting of five taxa. Three Eurasian taxa are regarded as species: *H. annosum s.s*, *H. parviporum* and *H. abietinum*. The taxonomic rank of the two North American taxa is unclear; the 'North American P group' is closely related to the Eurasian *H. annosum s.s.* while the 'S group' is related to both *H. parviporum* and *H. abietinum*. In Europe, *H. annosum s.s.* has a wide distribution and causes mortality in pine forests but is also able to attack several other conifers. *H. parviporum* causes extensive damage only on *Picea abies*, and its distribution follows the natural distribution area of this tree. The known distribution of *H. abietinum* is restricted to southern and central Europe and Asia Minor. It grows on different species of *Abies* but is generally a relatively weak pathogen. The North American P group now also occurs in Europe; it was probably imported by US troops to coastal *Pinus pinea* forests of central Italy during the Second World War. It appears to be well adapted to these forests in terms of pathogenicity and spreading, and it is able to produce hybrids with the European *H. annosum s.s*. This pathogen is a new threat to the pine forests of Europe.

ADAPTIVE MANAGEMENT OF SUDDEN OAK DEATH IN CALIFORNIA WOODLANDS. M. Garbelotto and D. Rizzo. *University of California, 338 Hilgard Hall, Berkeley, CA 94720, USA. Email: matteo@nature.berkeley.edu*

Sudden oak death is an emergent disease caused by the exotic pathogen *Phytophthora ramorum*. This newly described *Phytophthora* is also responsible for a disease of ornamental plants, and multiple lines of evidence indicate its introduction in North America is linked to the trade of infected ornamental plants. In particular, some US nurseries contain all three known lineages and both mating types of the pathogen, while California and Oregon woodlands only contain one lineage and one mating type. *P. ramorum* is predominantly aerially transmitted, but a soil and water phases, not unlike other forest Phytophthoras are also present. One striking feature of the epidemiology of the disease is that the largest amounts of infectious aerial sporangia are produced on bay laurel leaves, while oaks appear to be non-infectious. Our strategy to deal with the disease has been multiple and involves the following aspects: 1)- Understand the mode of introduction of the pathogen, and monitor potential new escapes in the wild; 2)- Understand factors linked to the reproductive and spread potential, e.g. seasonal patterns in the life cycle of the pathogen and distance of spread; 3)- Define the relationship between ecological stand characteristics and severity of the disease, inclusive of predictive modeling; 4)- Qualify precise pathways for infection; 5)- Reduce infection rates by modifying known infection pathways; 6)- Change stands characteristics to make them less favorable to the spread of the disease, e.g. by selective thinnings; 7)- Protect individual trees and some populations of the highly susceptible tanoak using phosphonate chemical treatments. Because our knowledge of the epidemiology and biology of the disease is still rather limited, recommendations are expected to change in time, as our understanding of the disease improves.

MANAGEMENT OF *SCLERODERRIS* CANKER IN NORDIC COUNTRIES. J. Hantula. *Finnish Forest Research Institute, P.O. Box 18, 01301 Vantaa, Finland. Email: jarkko.hantula@metla.fi*

In Finland the last major epidemic of *Scleroderris* canker

caused by *Gremmeniella abietina* occurred in the 1980s. In Sweden an even more serious epidemic caused large-scale destruction in 2001. Both epidemics were due to exceptional weather conditions. In Finland, since the 1980s epidemics, the pine forests have been relatively healthy for two main reasons. Weather conditions suitable for *G. abietina* have not occurred and the legislation on the provenance is relatively tight. In future the major question will be the influence of climate change on the balance between climate and trees, especially as pressures may appear to favour southern provenances against the local ones. No chemical control has been used for *Scleroderris* canker in Finland outside the nurseries. However, recent research has shown that *G. abietina* is infected by a diverse community of mycoviruses, although the usefulness of these is unclear.

Crop biosecurity

IS THERE REALLY A BIO-WAR THREAT TO CROPS? S. Whitby. *Bradford Disarmament Research Centre, Department of Peace Studies, University of Bradford, UK. Email: S.Whitby@Bradford.ac.uk*

In order to address the question "is there really a bio-war threat to crops?", this session seeks to develop an appreciation of state biological warfare programmes is so far as they relate to the development of offensive capabilities to wage biological warfare against food and cash crops. It highlights some of the salient features of such programmes and focuses on agent/pathogen choice, and methods of dispersal. It illuminates the possible linkages and areas of concern between state programmes that have resulted in militarily-significant crop warfare capabilities, and sub-state groups/terrorists, and offers an appraisal of the health of the international legal prohibition which bans this form of warfare. In this connection, legally-binding obligations regarding the implementation of national measures are discussed alongside related emerging national measures concerning deterrence, preparedness and response.

BIOFORENSICS AND PLANT PATHOLOGY: NEEDS, METHODS AND APPLICATIONS. J. Fletcher. *National Institute for Microbial Forensics, Food & Agricultural Biosecurity, Department of Entomology & Plant Pathology, Oklahoma State University, Stillwater, OK 74078, USA. Email: jacqueline.fletcher@okstate.edu*

Because national agriculture and food systems are extensive, open, interconnected, diverse, and complex, they are vulnerable to diseases and pests that occur naturally, are unintentionally introduced, or are intentionally delivered. An attack on a nation's crops, rangelands and/or forests could have catastrophic social and economic effects, such as reduced yield and quality, erosion of consumer confidence, economic impact, and effects on human nutrition. Crop producers, consultants and agricultural scientists, unaccustomed to thinking of intentional pathogen introduction, traditionally focus disease management strategies on prevention, eradication and/or long-term management. Efforts targeted largely towards prevention may reduce the introduction and movement of plant pathogens and pests, but are not sufficient for responding to and mitigating an attack. Preparedness for bioterror events requires a strong security plan that also includes steps for microbial forensics and criminal attribution. Microbial forensic technology is needed to determine the source of the pathogen and provide robust evidence that can lead to prosecution of those responsible. This capability should include strategies for (1) assuring high stringency (validation, confidence, and consistency); (2) tracing pathogen origin and movement; (3) deducing the timing and site of initial pathogen introduction; (4) identification of the perpetrators; (5) evidence for criminal attribution; and (6) links to the law enforcement and security communities. Although much currently available plant pathology information, technologies and resources, developed for peaceful applications, can be used for such purposes, new initiatives and targeted research are needed to support the emerging discipline of plant pathogen forensics.

USDA'S HOMELAND SECURITY ROLES AND RESPONSIBILITIES. S. Maddux. *U.S. Department of Agriculture, Room 12B, 1400 Independence Ave S.W., Washington, DC 20250, USA. Email: Sheryl.Maddux@usda.gov*

Due to its vast nature and open systems, protection of the Food and Agriculture Sector is riddled with challenges. The United States Department of Agriculture (USDA) plays an instrumental role in the protection of this sector. As identified in Homeland Security Presidential Directive 7 (HSPD-7), USDA, along with the Food and Drug Administration, are the Sector Specific Agencies responsible for the coordination of infrastructure protection activities within the Sector. In that role, USDA works closely with our private sector partners, who own or control the majority of the sector's assets. Further, HSPD-9 sets a national policy for defending our food and agriculture system against terrorist attacks, major disasters, and other emergencies. USDA is the lead (or is the lead in conjunction with other Federal agencies) on several tasks under HSPD-9. These activities will be outlined within the presentation.

IMPACTS OF BIOTERRORISM THREAT ON PLANT PATHOLOGY RESEARCH. C. Allen. *Dept Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706, USA. Email: cza@plantpath.wisc.edu*

Plant pathologists depend on open transfer of information, seeds, and strains to understand and control plant diseases. This is especially true now that globalization and free trade agreements have increased movement of plants and their associated microbes, which include many destructive exotic pathogens. Diseases like soybean rust and citrus greening (Huanglongbing) have appeared on new continents, causing serious losses. Plant pathologists struggling to manage outbreaks caused by introduced pathogens need scientific expertise and reference strains from colleagues in the pest's countries of origin. Unhappily, this wave of exotic pathogen movement coincided with anxiety about bioterrorism in the developed world and about biopiracy in the developing world. In the past 10 years, increasingly draconian legislation has restricted scientific exchange and constrained the ability of plant pathologists to address serious threats to agriculture. US anti-bioterrorism laws mandate strict new security and reporting measures for researchers working with selected high-risk pathogens. They also limit or prevent transfer of strains between scientists. Meanwhile several developing countries have tried to protect biological patrimony by forbidding export of native plants or microbes for research purposes. Thus, as globalization increases the risk of new crop epidemics, biosecurity laws reduce ability of plant pathologists to manage them. A further perverse outcome of these laws is that researchers in the developed world are discouraged from studying diseases of crops critical to economic and nutritional well-being in the tropics. Scientists

need to find effective ways of developing reasonable policies that support the research activities needed for continued agricultural productivity.

BIOREMEDIATION

POLLUTION ALLEVIATION OF WATER AND SOIL BY *TRICHODERMA* AND *TRICHODERMA*-PLANT INTERACTIONS.

G.E. Harman, J. Lynch and M. Lorito. *Cornell University, 630 W. North Street, Geneva, NY 14456, USA. Email: geh3@cornell.edu*

Fungi in the genus *Trichoderma* are well known for their plant growth promoting effect and for their abilities to produce extracellular enzymes. They are able to enhance root growth and development; therefore, roots of plant colonized by *Trichoderma* spp. are likely to penetrate more deeply into soil profiles and to extend in the soil more completely than in the absence of these fungi. This is expected to make phytoremediation strategies more rapid and effective. In fact, one plant useful in such processes is crack willow, and root colonization by *Trichoderma* increases both shoot and root mass. In addition, some plants and *Trichoderma* strains are able to degrade toxic compounds such as cyanide. Willows are among the cyanide-degrading plants. Since their growth is substantially enhanced by *Trichoderma* spp., and these fungi also degrade cyanide, *Trichoderma*-colonized willows can be grown on sites polluted by this toxicant or other pollutants and efficiently degrade them. Willows grow rapidly and can be used for energy-producing biomass. Thus, sites can be simultaneously detoxified and used to produce energy. Further, *Trichoderma* spp. may degrade toxic compounds in bioreactors. An example is the complex polyphenol mixtures produced from olive pressing. The byproducts are readily degraded in bioreactors containing *Trichoderma* spp. and thereby the toxicity in the material eliminated or strongly reduced. The abilities of *Trichodema* spp. to produce a wide range of enzymes that can degrade toxicants make them highly attractive for either *in-situ* applications where toxins must be removed from soils or in bioreactor settings.

DEGRADATION OF PETROLEUM AND OTHER SOIL POLLUTANTS WITH PLANTS COLONIZED BY ACC DEAMINASE-PRODUCING BACTERIA.

B.R. Glick, X.D. Huang and B.M. Greenberg. *Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1. Email: glick@sciborg.uwaterloo.ca*

Phytoremediation is defined as the use of plants to remove, destroy or sequester hazardous substances from the environment. Unfortunately, even the growth of plants that are relatively tolerant of various environmental contaminants can be inhibited in the presence of the contaminant so that the plants grown in contaminated soils often remain small and slow growing. To remedy this situation, plant growth-promoting bacteria that facilitate the proliferation of a wide range of plants especially under environmentally stressful conditions may be added to the plant roots. These bacteria typically produce both the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which can lower the level of growth-inhibiting plant stress ethylene levels, and the phytohormone indoleacetic acid (IAA), which can stimulate plant growth. In addition, by synthesizing siderophores, the bacteria can help to provide the plant with iron from the soil. The net result of adding these bacteria to plants is a significant increase in both the number of seeds that germinate and the amount of biomass that the plants are able to attain, making phytoremediation in the presence of plant growth-promoting bacteria much faster and more efficient. The application of plant growth-promoting bacteria, both in the laboratory and in the field, as an adjunct in the phytoremediation of polycyclic aromatic hydrocarbons and persistent total petroleum hydrocarbons will be discussed in detail. In addition, the mechanistic basis for the positive effects observed will be elaborated.

A NOVEL PHYTOREMEDIATION SYSTEM FOR SOILS CONTAMINATED WITH PERSISTENT ORGANIC POLLUTANTS.

M.P.N. Gent, J.C. White, B.D. Eitzer and M.J.I. Mattina. *Connecticut Agricultural Experiment Station, PO Box 1106, New Haven, CT 06504 USA. Email: Martin.Gent@po.state.ct.us*

Contamination of soils by persistent organic pollutants may be remedied by growing certain plant species, such as squash (*Cucurbita pepo),* that take up such chemicals into their stems and leaves. We examined how this species differs from cucumber (*Cucumis sativus*), a species that does not accumulate organic pollutants from soil. Two subspecies within *Cucurbita pepo* differ in phyto-extraction from soil of DDE (a metabolite of DDT). Field-grown zucchini, subspecies *pepo*, phyto-extracted about 1.0% of the DDE, while yellow summer squash, subspecies *ovifera*, removed less than 0.2% of the contaminant in the soil. Organic acids from the roots of these genotypes were collected under hydroponic conditions. Subspecies *pepo* exuded more citric acid than *ovifera* only under phosphorus depletion. Under nutrient-stressed conditions, cucumber seedlings accumulated similar amounts DDE in the roots as zucchini, but it did not move to above-ground tissues. The concentrations of organic acids in rhizosphere soil of nutrient-stressed cucumber were at least as high as those of zucchini. Thus, DDE uptake into roots seemed to be related to organic acid exudation. We also supplied to DDE to plants at a constant concentration in hydroponic solution over two weeks. The concentrations and amounts of DDE were assayed in various tissues of cucumber and zucchini plants over time. These measurements indicated that DDE moved in the transpiration stream, rather than through the air. The apparent fugacity, or tendency of DDE to partition to xylem water, appeared to be 25 fold greater in zucchini than in cucumber.

PHYTOREMEDIATION OF ORGANIC XENOBIOTICS: HOW MIXED POLLUTION INTERFERES WITH DETOXIFICATION CAPACITY.

P. Schröder, L. Lyubenova and A. Golan-Goldhirsh. *Department of Microbe-Plant Interactions, GSF-Research Centre for Environment and Health, Ingolstädter Landstraße 1, D-85764 Neuherberg, Germany. Email: peter.schroeder@gsf.de*

Heavy-metal stress in the environment has become of prime concern in the last decade, because metal contaminants can penetrate into the food chain and cause severe poisoning in humans, cattle and other animals. In low amounts some heavy metal ions such as Cu^{2+}, Zn^{2+}, Co^{2+}, Fe^{3+} and Mn^{2+} may be essential as enzyme cofactors and for interactions with proteins. Others, such as Cd^{2+}, Pb^{2+}, Hg^{2+} and Ag^{2+} are already toxic at low concentrations. They activate the defence mechanisms in cells. Most described effects depend to a large extent on the ability of the metal to induce free radicals which may damage cell components via oxidative stress. As metals are not degradable, reducing their toxicity in plants is best achieved by lowering absorption and uptake or by binding and sequestration to biomolecules. This latter capability to sequester heavy metals is exploited in phytoremediation, where research identifies metal hyperaccumulator plants by

their ability to cope with heavy metal stress. With a view to the use of plants in phytoremediation and the removal of unwanted organic xenobiotics and metals from the environment, the performance of plant detoxification enzymes under multiple pollution is of utmost importance. We present data indicating induction or inhibition of key enzymes of the glutathione pathway after incubation with heavy metals. Multiple pollution must be seen as a risk for the performance and survival of plants in phytoremediation. It is important to obtain data on the actual capacity of plants to metabolize organic pollutants from soil and water.

Molecular diagnostics for plant pathology

PAST AND FUTURE OF IMMUNOLOGICAL ASSAYS FOR THE DETECTION OF PLANT PATHOGENS. R.R. Martin. *USDA-ARS Horticulture Crops Res. Lab., 3420 NW Orchard Ave., Corvallis, OR, 97330, USA. Email: Bob.Martin@ars.usda.gov*

Prior to the 1980's, most serological applications in plant pathology were based on gel diffusion assays used with viruses or immuno-fluorescence used with bacteria. With the development of monoclonal antibody technology in 1975 and the introduction of ELISA in 1977, the application of serology for detection of plant pathogens increased greatly. Since monoclonal antibodies interact with a single epitope it became possible to clearly separate closely related pathogens including viruses, bacteria, fungi and phytoplasmas, or to develop diagnostics that were useful in the identification of pathogens at the species or genus level. These two developments, ELISA and monoclonal antibodies, resulted in standardization of testing for many plant pathogens, the development of mechanized sap extraction devices and robotics for processing large numbers of samples. It also spawned companies based on production and sales of detection related supplies and that also performed assays. These serological assays have become integrated into certification and quarantine programs and are critical in quality control used for international commerce in plants and plant products. Due to the mechanization, standardization and low costs, these serological assays are the method of choice for large scale detection of many pathogens and will continue to be so for the foreseeable future. The use of immuno-capture PCR (or RT-PCR) for the detection of plant pathogens can be used to avoid problems with plant inhibitors often observed with woody plant tissues or to increase the sensitivity of detection by effectively concentrating the pathogen prior to nucleic acid extraction and PCR.

FIELD APPLICATIONS OF DNA ARRAYS FOR DETECTION OF PLANT PATHOGENS. B. Lievens and B.P.H.J. Thomma. *Scientia Terrae Research Institute, Fortsesteenweg 30A, 2860 Sint-Katelijne-Waver, Belgium. Email: bli@scientiaterrae.org*

Failure to adequately identify plant pathogens using culture-based morphological techniques has prompted the development of alternative, culture-independent methods. Increasingly, methods based on detection of nucleic acids are being used for plant pathogen diagnosis, providing reliable identification, sensitive detection, and accurate quantification of plant pathogens. However, since plants or parts thereof can be infected by multiple pathogens, multiplex assays that can detect and quantify different pathogens simultaneously are highly desirable, especially when taking into account efficiency, cost, time, and labour. Currently, polymerase chain reaction (PCR)-based DNA array technology is one of the most suitable techniques to detect multiple pathogens in a single assay, even if they differ in only a single to a few bases in the nucleotide sequence that is targeted. Recently, this technology was made quantitative, rendering DNA arrays highly attractive for several practical applications. Increasingly, diagnostic laboratories are implementing DNA array technology for routine plant pathogen diagnosis. In addition, the ability to get quantitative results has led to the challenge of predicting disease development based on pathogen densities in specific environments, such as plant parts, soils, artificial substrates, water or air. Ultimately, timely and regular analyses using such DNA arrays should allow prescribing and performing preventive treatments, resulting in minimized environmental impact.

EXPLOITING PHYLOGENETIC AND DNA BARCODE DATABASES TO DESIGN BROAD SPECTRUM ASSAYS. C.A. Lévesque. *Agriculture and Agri-Food Canada, Central Experimental Farm, 960 Carling Ave., Ottawa, ON, K2A 2P8, Canada. Email: levesqueca@agr.gc.ca*

The size of the average dataset for phylogenetic studies is getting larger because the cost and effort involved in DNA sequencing is constantly decreasing. Moreover, the advent of large DNA barcoding initiatives contributes to the rapid expansion of databases that can be exploited to design assays for pathogen detection. DNA sequencing is no longer a bottleneck for designing assays but new limitations have emerged. Detection assays must be designed and validated with strains that are well identified and datasets that are as complete as possible. Unfortunately, taxonomic expertise and biological collections have not seen the expansion that has happened to DNA sequencing capacity. For a few rare genera of Eu/Oomycota, the sampling and coverage of the phylogenetic/barcoding work has been extensive and supported by solid taxonomy. In these genera, bioinformatics becomes the next bottleneck to analysis because standard genomics tools are not readily amenable for the design of multiplex detection assays. For instance, we have used datasets of *Penicillium, Fusarium, Pythium* and *Phytophthora* to design DNA array detection systems. In some cases where variation of the selection was rather low, the assays had to be designed on the single nucleotide polymorphisms to provide species specificity. The standardization of the array systems provides an opportunity to multiplex further by combining the arrays of different genera on a single platform. Once such multiplex assays have been designed the next bottleneck is validation, an issue that needs to be discussed further among laboratories interested in these tools.

PADLOCK PROBE TECHNOLOGY, VISION OF A UNIVERSAL, MULTIPLEX AND QUANTITATIVE SYSTEM FOR VERSATILE APPLICATIONS. C.D. Schoen, M. Szemes, R. van Doorn, M. Dieho, O. Mendes, M. van den Berg, T. Prins, J. van Dijk, J. Peters, J. Bergervoet, M. Vasic, H. Zuilhof, M. Slawiak, E. Lojkowska and P.J.M. Bonants. *Plant Research International (PRI) B.V., Department of Biointeractions and Plant Health, Droevendaalsesteeg 1, 6708 P.B. Wageningen, The Netherlands. Email: cor.schoen@wur.nl*

Plant Research International has recently developed a principle for multiplex detection based on padlock probe technology, which offer a means of introducing a universal step into target detection by microarrays and real-time analyses. Padlock probes (PLPs) are long oligonucleotides carrying the target complementary regions at their 5' and 3' ends, which recognize adjacent sequences on the target DNA or RNA molecule. Thus, upon hy-

bridisation, the ends of the probes get into adjacent position, and they can be joined by enzymatic ligation. Ligation occurs and the probes are circularized only when both end segments recognize correctly the target sequences. Subsequently, the target-specific products are detected by micro-array or real-time analyses using special designs of the PLPs. Advantages of the developed PLP based diagnostic applications are: flexible and easily adaptable design, high level of specificity and multiplexing, universal downstream processing after ligation, and high-throughput format with real-time analysis. Potential fields of application are targeting of quarantine pathogens, pathogens on cultivated crop, identification of micro-organisms based on multiple motifs and/or indicator organisms for soil health status. Recently developed padlock probe-based applications are multiplex target detection and genotyping, microbial community analysis and multiplex quantitative target detection. Advantages of different design strategies in different applications of the multiplex detection system will be discussed.

EFSA SPECIAL SESSION

RISK ASSESSMENT AT THE EUROPEAN FOOD SAFETY AUTHORITY.

R.L. Maijala. *European Food Safety Authority, Risk Assessment Directorate, Largo N. Palli 5/A, 4300 Parma, Italy. Email: Riitta.Maijala@efsa.europa.eu*

The European Food Safety Authority is the keystone of EU risk assessment regarding food and feed safety. In close collaboration with scientists, national authorities and in open consultation with its stakeholders, EFSA provides independent scientific advice and communication on existing and emerging risks. Since EFSA's advice serves to inform scientific knowledge for risk managers, a large part of EFSA's work is undertaken in response to specific requests for scientific advice. Requests for scientific assessments are received from the European Commission, the European Parliament and EU Member States. EFSA also undertakes scientific work on its own initiative, so-called self-tasking. The EFSA risk assessment is based on available scientific evidence and is undertaken in independent, objective and transparent manner. The EFSA Scientific Committee and the Scientific Panels provide opinions on issues related to specific biological risk factors for human health, animal and plant health and regulated substances and products. Aiming at strengthening the European food safety system EFSA further develops risk assessment approaches and methodologies. Databases, monitoring and reporting systems are built jointly with the EU Member States to ensure that the risk assessments are supported by comprehensive data. Scientific excellence of EFSA experts in risk assessment matters matched with high scientific standards, independence, transparency and efficiency supports the EU risk managers in their decision making processes.

THE EFSA PANEL ON PLANT HEALTH: ACCOMPLISHMENTS AND CHALLENGES FOR THE EU PEST RISK ASSESSMENT.

J. Schans, E. Ceglarska, S. Cheek and G. Stancanelli. *European Food Safety Authority, Panel on Plant Health, Largo N. Palli 5/A, 4300 Parma, Italy. Email j.schans@minlnv.nl*

Directive 2000/29/EC of the European Community enforces protective measures against the introduction into and spread within the Community of organisms harmful to plants or plant products. The right to take such measures has been established (IPPC, 1997; WTO-SPS, 1995), provided that these are based on scientific principles and are not maintained without sufficient scientific evidence. Internationally endorsed guidelines for 'Pest Risk Analysis' have been developed (ISPM No. 2, No. 5, No. 11, No. 21). The EFSA Plant Health (PLH) Panel provides scientific advice on the risks of plant pests to the safety and security of the food production chain and its environment in the EU, underpinning risk management decisions. The PLH Panel started its activity in June 2006. It is composed of 21 Members with expertise in various fields of risk analysis and plant health. It operates with the support of the EFSA PLH secretariat through four permanent working groups. Since its start, it has published six scientific opinions on the risk posed by invasive plants, weeds, a citrus bacterial disease and a citrus insect pest. The PLH Panel is currently reviewing pest risk assessments of banana and citrus pests for the French overseas departments. The target of the PLH Panel is to contribute to development of a harmonized approach for pest risk assessment in the EU, in cooperation with EU Member States and international organizations. Noteworthy challenges are the analysis of uncertainties relating to pathways and impacts and evaluation of their effects on conclusions of pest risk assessment.

OVERVIEW ON THE WORK OF THE EFSA PANEL ON PLANT PROTECTION PRODUCTS AND THEIR RESIDUES (PPR PANEL) SINCE 2003.

M. Dunier-Thomann. *European Food Safety Authority, PPR Unit, Largo N. Palli 5/A, 4300 Parma, Italy. Email: muriel.dunier-thomann@efsa.europa.eu*

In the EU, the placing of pesticides used to protect plants or plant products on the market is mainly regulated by Directive 91/414/EEC, which states that chemical substances or micro organisms in pesticides are only approved for use after undergoing a peer-reviewed safety assessment. In 1993, the European Commission launched an extensive review programme –including the peer review of active substances used in plant protection products, managed by the Pesticide Risk Assessment Peer Review (PRAPeR) unit of EFSA– to evaluate the safety of active substances used in the EU. The timeline is 2008. The Panel on plant protection products and their residues (PPR) gives scientific advice on issues that cannot be resolved within peer review procedures or when further scientific guidance is needed. Since 2003, the PPR Panel has produced 35 scientific opinions in the fields of toxicology, ecotoxicology, fate and behaviour of pesticides and on residues. The PPR Panel is also in charge of revising/updating existing EU guidance documents (GD) and developing new GD for risk assessment. GDs provide guidance to notifiers (industry) and Member States on how to conduct a risk assessment in the context of the peer review of active substances used in plant protection products. The first GD to be updated is on Risk Assessment for Birds and Mammals, which will be finalised by mid 2008. The second GD under revision is on Persistence in Soil. An open consultation of stakeholders on the EFSA website is launched before and at the end of each revision.

EFSA'S EUROPEAN RISK ASSESSMENT OF AFLATOXINS IN ALMONDS, HAZELNUTS AND PISTACHIOS.

C.W. Heppner, D.J. Benford, J.C. Larsen, J.R. Schlatter, C.P. Wild and S. Fabiansson. *European Food Safety Authority, CONTAM Unit, Largo N. Palli 5/A, 4300 Parma, Italy. Email: claudia.heppner@efsa.europa.eu*

Aflatoxins primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus* are commonly associated with e.g. maize, groundnuts, tree nuts, dried fruit, rice. Aflatoxins are genotoxic and carcinogenic and the European Union introduced regulations for these toxins in 1998. The Codex Alimentarius proposes

the setting of higher maximum levels of total aflatoxins (AFs) in groundnuts and nuts than those currently in place in Europe. Therefore, EFSA's CONTAM Panel was asked by the European Commission to provide an opinion on the potential increased risk to consumer health if the European maximum levels were increased from 4 to 8 or 10 µg/kg for AFs in "ready-to-eat" almonds, hazelnuts and pistachios. In Europe, estimated AF average intakes from all sources were 0.352-1.934 ng kg b.w. per day. The CONTAM Panel derived a BMDL10[1] (ng/kg b.w. per day) of 170 for liver carcinogenicity in rats and of 870 based on human data (China) as well as a BMDL1[1] of 78 using human data (Africa). Margin of exposures (MOEs) were calculated for the general population, vulnerable groups and high level consumers by dividing the BMDL values by the dietary exposure estimates. The CONTAM Panel concluded that raising the maximum levels for AFs from 4 to 8 or 10 µg/kg in almonds, hazelnuts and pistachios would have minor effects on dietary exposure, cancer risk and MOEs because of the low contribution of these commodities to total exposure. However, the Panel also pointed out that exposure to AFs from all sources should be reduced to as low as reasonably achievable, because of the genotoxic and carcinogenic nature of AFs.

[1] 95% lower confidence limit of the benchmark dose for a 10% or 1% increase in cancer.

EVALUATION OF RISK ASSESSMENTS ON FUNGAL AND BACTERIAL PATHOGENS OF BANANA FOR THE FRENCH OVERSEAS DEPARTMENTS. **D. Caffier, J. Choiseul, E. Dormannsné Simon, D.R. Jones, D. Makowski, C. Manceau, L. Manici, A. Oude Lansink, A. Porta Puglia, J. Smith, G. Stancanelli, R. Steffek, E. Stefani, A. Strömberg and I. Vloutoglou.** *European Food Safety Authority, Panel on Plant Health, Working Groups on fungal and bacterial plant pathogens, Largo N. Palli 5/A, 4300 Parma, Italy. Email: david.caffier@agriculture.gouv.fr*

The EFSA Panel on Plant Health has recently evaluated 30 pest risk assessments made by France for the French overseas departments of Martinique, Guadeloupe, French Guiana and Réunion, with the aim of harmonizing their phytosanitary rules with the EU plant health legislation. A total of 15 pest risk assessments regarded organisms (arthropods, viruses fungi and bacteria) harmful to banana. These included three bacteria (*Xanthomonas campestris* pv. *musacearum*, *Ralstonia solanacearum* race 2, *Ralstonia* sp. agent of banana blood disease), four fungi (*Fusarium oxysporum* f. sp. *cubense* races 4 and T4, *Mycosphaerella fijiensis*, *Mycosphaerella eumusae* and *Phyllosticta musarum*), and one oomycete (*Trachysphaera fructigena*). The evaluation was carried out following the International Standard for Phytosanitary Measures No. 11. Pest categorization, assessment of the probability of entry, establishment and spread and assessment of the potential economic consequences, including social and environmental impact, were evaluated for each pathogen. Two main sources of uncertainties were noted: unclear or undefined taxonomical position for some bacterial pathogens; lack of published scientific and technical literature both for new emerging pathogens (*X. campestris* pv. *musacearum* and *M. eumusae*) and for pathogens investigated in the past but recently neglected (*T. fructigena*). Access to data sources regarding entry pathways, host plants and agricultural practices in the PRA area proved to be a limiting factor of the pest risk assessments and a challenge for the Panel in the evaluation procedure. This contribution is based on Scientific Opinions adopted by the EFSA Plant Health Panel (www.efsa.europa.eu/EFSA/ScientificPanels/PLH/), whose Members we wish to thank.

Diseases of Mediterranean crops

CLIMATE AND PLANT DISEASES IN THE MEDITERRANEAN REGION. **G. Surico and M. Bindi.** *Dipartimento di Biotecnologie agrarie sez. Patologia vegetale, Piazzale delle Cascine 28, 50144 Firenze, Italy. Email: giuseppe.surico@unifi.it*

The history of countries bordering on the Mediterranean Sea is integral with the history of man, and the reason for this is in large part the peculiar climate of the region, which has been described as a sort of planetary anomaly, inasmuch as at different seasons it resembles one or other of its two flanking climate zones, in winter the temperate zone, and in summer the tropical zone. Most climates have greater rainfall close to, or within, the summer period, but in the Mediterranean region rainfall is concentrated outside the summer season; as a consequence there are long, hot, dry summers, and mild winters. This climate has led to the formation of a distinctive Mediterranean biome, and distinctive development of a number of plant diseases. In 1960, Israel Reichert, in the first number of the Journal *Phytopathologia Mediterranea,* noted that plant pathologists working in the region would have "to be cautious in accepting the directions and instructions worked out in other geographic latitudes and applying them in their own countries." In addition to differences in the disease cycle of certain diseases there are also plant diseases that are peculiar to the region. This paper will examine the distinctive aspects which some diseases assume in the Mediterranean region, also considering future impacts of climate change.

INTEGRATED MANAGEMENT OF WHITEFLY-TRANSMITTED VIRUSES IN VEGETABLE CROPS. **E. Moriones, D.M. Tomás, E. García-Cano, F. Monci, J. Navas-Castillo, M.J. Rodríguez-López, R. Fernández-Muñoz, R.O. Resende and L.S. Boiteux.** *Plant Virology Laboratory and Plant Breeding Department, Estación Experimental La Mayora, CSIC, 29750 Algarrobo-Costa, Málaga, Spain. Email: moriones@eelm.csic.es*

Epidemics of viral diseases are a major constraint to vegetable production in the Mediterranean area. Notably, insect-transmitted viruses cause severe damage and among them whitefly (Hemiptera: Aleyrodidae)-transmitted viruses are the major cause of concern. Tomato (*Solanum lycopersicum*) is an important vegetable crop in this area and it is severely affected by whitefly (*Bemisia tabaci* Gen.)-transmitted viruses. Emergence of this insect has resulted in outbreaks of viruses like begomoviruses (family *Geminiviridae*) associated with the tomato yellow leaf curl disease (TYLCD) and the *Tomato chlorosis virus* (family *Closteroviridae*). The spread of whitefly-transmitted viruses can only be partially curtailed by spraying insecticides against the vector, which is the major protection measure used by growers. Therefore, integrated management strategies are one of the most viable alternatives to reduce virus incidence. We have evaluated several strategies for integrated management of TYLCD. Induction of systemic acquired resistance and use of UV-absorbing plastics films in protected crops resulted in reduced disease incidence. Moreover, lines/populations with begomovirus and/or whitefly resistance were developed and they can be used as basic breeding materials to control virus infections and to minimize insecticide sprays. Combination of these strategies will be discussed for effective integrated management of TYLCD.

MAJOR AND EMERGING DISEASES OF POME FRUIT IN THE MEDITERRANEAN REGION. **E. Montesinos.** *Institute of*

Food and Agricultural Technology-CIDSAV-CeRTA and Department of Chemistry, University of Girona, Campus Montilivi, 17071 Girona, Spain. Email: emonte@intea.udg.edu

Apple and pear are the main Mediterranean pome fruit crops. Nearly half a million hectares with a production of up to eleven million tons per year are grown in fifteen countries across Europe, but the main production is in Spain, Italy and France. Several diseases affect orchards due to a wide range of agents like viruses, bacteria, fungi and nematodes. Disease susceptibility usually follows a cultivar-dependent pattern, and the severity of outbreaks changes greatly with year and region. However, only a few diseases have been economically important or of serious concern in recent years. Key points and prospects will be given on economically important classical diseases like fire blight caused by the bacterium *Erwinia amylovora* affecting apple, pear and ornamentals, and scab caused by *Venturia* spp. mainly in apple and less so on pear. Some emerging diseases have become of economic importance like necrosis of dormant flower buds of pear, a disease of complex aetiology in which *Pseudomonas syringae* pv. *syringae* has been implicated, and brown spot of pear caused by *Stemphylium vesicarium.* The epidemiological and integrated control aspects of these diseases will be discussed in more detail.

ESCA DISEASE OF GRAPEVINE. L. Mugnai, P. Larignon and W.D. Gubler. *Dipartimento di Biotecnologie Agrarie, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. Email. laura.mugnai@unifi.it*

Trunk diseases of grapevine have recently received wide interest among phytopathologists in many production areas around the world. Of trunk diseases, esca has become increasingly important due to its recently increased incidence and spread worldwide. Research in all grape-growing countries has shown that the disease no longer appears only as a wood rot, leading to disruption of the xylem, but as a complex of different diseases, in which tracheomycotic fungi belonging to recently described genera, *Phaeomoniella* and *Phaeoacremonium*, appear to be most important. New research has allowed better understanding of the aetiology of the disease and its epidemiology, factors involved in symptom development, and disease control, and has revealed the unexpected importance of early infections in propagation material. These findings have opened new research fields in the prevention of infection in the nursery, and on factors promoting symptom development in the field from latent infections. The very complex interaction of microbial activity, including enzymes and metabolites produced in the plant, have prevented the development of effective chemical control methods, but the outlook for development of an economic control strategy is good. Many active research programs and collaborations among scientists continue to increase our understanding of the disease and will ultimately lead to effective control.

Soil disinfestation

BEHAVIOUR OF FUMIGANTS IN SOIL. H. Ajwa and A. Minuto. *Department of Plant Sciences University of California, Davis, 1636 East Alisal St. Salinas, CA 93905, USA. Email: haajwa@ucdavis.edu*

Methyl bromide use for soil disinfestation has been phased out in most developed countries and is becoming restricted in developing countries. Several fumigants (1,3-dichloropropene, chloropicrin, iodomethane, propargyl bromide, sodium azide, dimethyl disulfide, and methyl isothiocyanate generators such as metam sodium and dazomet) have been proposed to replace methyl bromide. Due to its very high vapor pressure and low boiling temperature, methyl bromide converts immediately into gas when shank-injected into the soil. The high volatility and biological reactivity of methyl bromide make it the most versatile fumigant. Iodomethane also has a low boiling point and high vapor pressure and is more biologically reactive than methyl bromide on a molar basis. Iodomethane is not an ozone-depleting chemical because it degrades readily in the atmosphere by photolysis. Except for iodomethane, the other available alternative fumigants have low vapor pressure and high boiling temperatures and do not volatilize and dissipate in soils like methyl bromide. Therefore, effective control of soilborne pathogens and weeds requires more specialized application equipment and methods to achieve good distribution of fumigants across the target soil profile. Concerns over emissions and risk to human and environmental health have prompted the development of new formulations and application techniques such as drip application of combinations of fumigants. This presentation will summarize fumigant physicochemical properties and environmental fate.

ACCELERATED DEGRADATION OF FUMIGANTS IN SOIL. A. Gamliel. *Agricultural Engineering Institute, ARO, Volcani Center, Bet Dagan 50250, Israel . Email: agamliel@agri.gov.il*

Soil fumigation is used to control soilborne diseases worldwide. In moist soil, fumigants undergo decomposition to the active ingredient, for example metam-sodium (MS) (sodium *N*-methyl dithiocarbamate) generates methyl isothiocyanate (MITC). The generation and dissipation curve of fumigants in soil follows first-order degradation kinetics and is controlled mainly by microbial and physico-chemical processes. The rate of MITC dissipation from soils increases with increasing soil organic content, temperature and pH. In some cases, MS fumigation is ineffective in controlling soilborne pests. Accelerated degradation of a pesticide occurs when it undergoes rapid decomposition in the soil, losing biological activity. Accelerated degradation of MS following repeated application has been reported as a possible reason for the ineffective control of such diseases. MITC was rapidly degraded in soils which had been previously treated with either metam sodium or dazomet indicating induced cross-accelerated degradation with both fumigants. Accelerated degradation of MITC was documented in Israel under field conditions. This phenomenon is persistent (the rapid degradation capacity of MITC was still evident in soils for 18 to 30 months after treatment). Accelerated degradation of fumigants is likely to cause poor pathogen control in commercial fields upon repeated application of the fumigants. Thus, there is an urgent need for rational use of pesticides to maintain their efficacy.

SOIL SOLARIZATION – 30 YEARS ON: WHAT LESSONS HAVE BEEN LEARNED? J. Katan and A. Gamliel. *Faculty of Agricultural Food and Environmental Quality Sciences, Rehovot 76100, Israel. Email: Katan@agri.huji.ac.il*

Disinfestation using soil solarization (SH) in its present form was first introduced in Israel in 1976. Shortly thereafter, its potential was explored in the USA and since then, in over 60 countries, both developed and developing, in hot-climate regions, e.g. the Mediterranean and California but to some extent also in more humid and cooler regions. SH is now used by farmers in the

Mediterranean region, some Caribbean and Latin American countries and Japan among others, in both small and large farms. The introduction of SH has involved several stages covering both fundamental and applied aspects: 1. Exploring its effectiveness (the spectrum of pests controlled) in various regions and cropping systems. 2. Studying mechanisms of pathogen control (both physical, and especially biological) and of crop growth improvement, including physical and biological models. 3. Implementing SH, including special machinery. 4. Improving SH and adapting it to various uses, e.g. structural solarization and use with perennial crops, to name a few. 5. Integration with other treatments, non-chemical and chemical (at reduced dosages). 6. Developing extension and training tools. International symposia and sessions at conferences have been dedicated to SH and hundreds of studies have been published. SH is climate-dependent and has its advantages and limitations. It is not tied to commercial companies, making its dissemination more difficult. Multidisciplinary studies, governmental support and economic analyses are essential for introducing non-chemical methods of control. Global crises, such as the phase out of methyl bromide, emphasized the need for alternatives and enhanced SH adoption.

BACK TO THE FUTURE: TOTAL SYSTEM MANAGEMENT (ORGANIC, SUSTAINABLE). D.O. Chellemi. *USDA, ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, Florida, 34945, USA. Email: dan.chellemi@ars.usda.gov*

Many soil disinfestation programs are implemented prior to crop cultivation due to the paucity of therapeutic interventions for controlling soilborne pests. In the 1950's a proliferation of chemical control options ushered in an era of soilborne pest control based upon a single or limited group of chemicals to control target pest organisms. Unfortunately, many chemicals also affected a broad and complex range of nontarget organisms comprising multiple trophic levels. This has necessitated their perpetual use to ensure pest control in agroecosystems where natural pest regulating mechanisms have been compromised. Presently, regulatory issues impact the availability of many chemical pesticides and urbanization of agricultural production regions restrict their use. Future trends further impacting growers include carbon sequestering and trading, demands for renewable energy sources and conservation and stewardship of natural resources. An alternative systems-based approach with multiple economic, environmental and social goals is suggested for future crop production. In this total system approach, mitigation of soilborne pest outbreaks is incorporated into the design of the crop production system. For example, rotation crops providing renewable energy and increased soil carbon sequestering will be selected to provide economic incentives for their inclusion into long-term farm management plans. Concurrently, selection will also be based upon the ability of rotation crops to minimize outbreaks of soilborne pests, thus reducing the need for interventive pest control actions. An example of a total system management approach under development for producers of field grown fresh market vegetables will be discussed.

GLOBAL PHASEOUT OF METHYL BROMIDE UNDER THE MONTREAL PROTOCOL: IMPLICATIONS FOR THE OZONE LAYER, BIOPROTECTION AND BIOSECURITY. I. Porter, J. Banks, S. Mattner and P. Fraser. *Technology and Economic Assessment Panel, Montreal Protocol CA, Australia. Email: ian.j.porter@dpi.vic.gov.au*

The Montreal Protocol has been very effective in reducing the major ozone-depleting product, methyl bromide (MB), and represents an excellent model for future phaseout of environmentally damaging products, such as those involved with climate change. Over a ten year period, 85% of MB (*c.* 45,000 t) used for preplant soil fumigation has been phased out and a wide range of other products and technologies adopted for disease and weed control in agriculture. Restrictions on the use of MB have stimulated new knowledge on the importance and relationships between microbial diversity and crop growth, and improved our understanding of soil health and the development of new products and production systems to produce futures food without the impact of harsh pesticides. This has led to increased use of substrate systems, grafting and plant resistance for disease control thus avoiding the need for soil disinfestation, and the use of alternative products, such as 1,3-dichloropropene/chloropicrin (Pic), metham, methyl iodide/Pic, drip applied fumigants and barrier films as alternatives strategies for soil disinfestation. Implementation of these alternatives has led to a 45% fall in bromine in the troposphere and 30% reduction in effective chlorine load in the stratosphere. Internationally, pressure is mounting to restrict use of all fumigants worldwide (EC Reg 2037 and US Cluster Analysis) and this will further stimulate new technologies for plant disease control. Pressure is also mounting to restrict MB use for quarantine and pre-shipment (QPS) with QPS MB use exceeding 14,000 t in 2005. Industries need to be prepared for adoption of new crop protection, biosecurity and IPM management strategies.

FASTIDIOUS BACTERIA

HUANGLONGBING DISEASE OF CITRUS AND THE GENUS *CANDIDATUS* LIBERIBACTER. S. Eveillard, D.C. Teixeira and J.M. Bové. *UMR 1090, Institut National de la Recherche Agronomique et Université Victor Segalen, Bordeaux 2, B.P. 81, 33883 Villenave d'Ornon Cedex, France. Email: sandrine.eveillard@bordeaux.inra.fr*

Huanglongbing (HLB), an insect-vector-transmitted disease, is destructive and endangers the very existence of commercial citrus. Leaves with blotchy mottle and lopsided fruits with aborted seeds are characteristic of HLB. The causal agents are endogenous bacteria, restricted to the phloem sieve tubes, and members of the genus *Candidatus* Liberibacter, a new genus within the alpha-proteobacteria. The liberibacters have never been obtained in culture. Their phylogenetic and taxonomic characterizations are based on 16SrDNA sequence comparisons. Until 2004, two species of liberibacters causing HLB were known: *Candidatus* Liberibacter africanus and *Ca.* L. asiaticus, responsible for the disease, respectively in Africa and Asia. In 2004, characteristic symptoms of HLB were observed in São Paulo State, Brazil. Some trees were infected with *Ca.* L. asiaticus, but most trees were PCR-negative with the primers specific for the African or the Asian liberibacter. PCR amplification with universal primers for prokaryotic 16SrDNA led to the discovery of a third species of liberibacter, *Ca*. L. americanus, and the development of specific primers for its detection. HLB was noticed in Florida in 2005, and only *Ca.* L. asiaticus was found to be involved. For further characterization, the *nus*G-*rpl*KAJL-*rpo*BC gene cluster was sequenced for the three liberibacter species, and used to determine the percentage sequence identity. An estimation of speciation dating has been attempted. It appears that *Ca*. L. asiaticus and *Ca*. L. africanus diverged some 150 million years ago, while speciation of *Ca*. L. americanus might have started some 300 million years ago.

CONTROL OF CITRUS YELLOW SHOOT DISEASE (HUANGLONGBING) IN SOUTH AND NORTH AMERICA. A.J. Ayres, S.A. Lopes, R.B. Bassanezi, P.T. Yamamoto, J. Belasque Junior, D.C. Teixeira, N.A. Wulff and J.M. Bové. *Fundecitrus, Araraquara, Brazil. Email: ayres@fundecitrus.com.br*

Huanglongbing (HLB) is a destructive disease affecting all citrus species and *Murraya paniculata*. In Brazil, it is caused by *Candidatus* Liberibacter americanus and *Ca.* Liberibacter asiaticus, disseminated by *Diaphorina citri*, and first reported in July 2004 in Araraquara municipality of São Paulo State. In October 2007 HLB had been detected in 149 municipalities with 80% of the estimated 2 million affected trees found in Araraquara and the vicinity. To suppress HLB, Fundecitrus and governmental institutions have implemented a strong campaign to advise growers of the need to frequently inspect and immediately eliminate symptomatic trees (this is required by law), to control the vector, and to use healthy trees for planting (mandatory production of nursery trees under inset-proof conditions since 2003). Today, a team from Fundecitrus trains growers on symptom recognition and disease control and helps the government in farm inspection. Most farmers who rigorously adopted the management practices have succeeded in suppressing the disease. Low disease incidence and absence of other affected farms in the vicinity are factors that decisively contribute to the success of control. In Florida, *Ca.* Liberibacter asiaticus was first discovered in August 2005 in a commercial nursery in Florida City and in the residential communities of Pinecrest and Coral City. By October 2007, HLB had been reported in 28 counties, predominately in the southern half of the Florida peninsula. Florida is moving to certified screenhouse nurseries, but there is no law for HLB eradication. Psyllids are controlled and symptomatic trees eradicated spontaneously by conscientious growers.

AUSTRALIAN STRAWBERRY LETHAL YELLOWS. C. Streten and K.S. Gibb. *Bioscience North Australia, Charles Darwin University, Darwin, NT 0909, Australia. Email: claire.streten@cdu.edu.au*

Commercial strawberry plants are grown in Victoria, Queensland, Western Australia and South Australia. Between 2003 and 2004 these farms produced approximately 40,000 tonnes of strawberries with a farm gate value of 200 million dollars. As a high-value crop, even low-incidence diseases can cause significant economic hardship, particularly in the runner production area. We report studies on diseases that affect the Queensland strawberry industry in particular. These diseases include strawberry lethal yellows (SLY) and strawberry green petal (SGP) diseases, which are associated with "*Candidatus* Phytoplasma australiense" (*Ca*. P. australiense). In addition, a rickettsia-like-organism (RLO) is associated with SLY disease. SLY and SGP diseases can have a significant impact on productivity because flowering and fruit set is inhibited. Diseased strawberry plants and symptomatic alternative host plant species were sampled between 2000 and 2002. Of 363 SLY samples, 117 tested positive for the RLO, 67 tested positive for *Ca*. P. australiense AGY strain and 11 plants tested positive for *Ca*. P. australiense Phormium yellow leaf variant strain. Thirty one other plant species were observed with symptoms during the study and of these, 18 species tested positive using phytoplasma-specific primers and two for the RLO. Findings from this study suggest regional disease management strategies are required in Queensland. Eight plant species were identified as possible reservoirs for the agents associated with these strawberry diseases. For SLY disease control strategies to be effective, they will have to address the sources of inoculum in the areas surrounding strawberry farms.

***XYLELLA FASTIDIOSA* IN GRAPEVINE AND CITRUS. A.H. Purcell.** *Dept. of Environmental Science, Policy and Management, 137 Mulford, University of California, Berkeley, CA 94619-3114, USA. Email: purcell@nature.berkeley.edu*

Various strains of the xylem-limited bacterium *Xylella fastidiosa* cause diseases in numerous crops and forest trees in the subtropical and tropical Americas, but grapevine and sweet orange are the most seriously affected crops. Significant new epidemics of citrus variegated chlorosis disease in sweet orange in Brazil and Pierce's disease of grapevine in California have accelerated research on this bacterium over the past 10-15 years. Disease prevention is the only available disease management strategy. In California control of leafhopper vectors is the main method of management of Pierce's disease. In Brazil, a rigorous sanitation of planting stock and disease removal combined with vector control is used. A citrus strain of *X. fastidiosa* was the first plant pathogenic bacterium to be completely sequenced, and promising new ideas for control of *X. fastidiosa* have emerged from molecular-based studies employing various knockout bacterial mutants deficient for fimbriae, pili, hemaglutinins, cell-cell communication, Type I and Type V secretion systems. For example, over expression of *X. fastidiosa*'s cell-cell signalling molecule within plants suppresses bacterial movement and reduces symptom severity. A dominant gene (*PdR1*) for resistance to Pierce's disease has been identified and mapped from hybrids of *Vitis vinifera* × *Vitis arizonica*, and backcrosses to *V. vinifera* are underway to produce resistant wine and table grape cultivars with commercial quality. A selection of recent research highlights will be presented.

Diseases of ornamentals and turfgrasses

EMERGENCE OF NEW VIRAL DISEASES AFFECTING ORNAMENTALS. A.M. Vaira and A. Gera. *CNR, Istituto di Virologia Vegetale, Strada delle Cacce 73, 10135 Torino, Italy. Email: a.vaira@ivv.cnr.it*

Plant viruses, discovered at the end of the nineteenth century, are nowadays responsible for great economic losses, affecting plant value by reducing vigour and marketability. The growing demand for high-quality, disease-free propagation material, increasing international trade, and the high sanitary standards imposed by many countries for importing propagation material, have highlighted the significance of virus diseases in ornamental crops. The introduction of new crops, climate change, the migration of virus vectors and the inefficient prevention of infection play key roles in spreading new viruses into old crops and both old and new viruses into newly introduced crops. For many highly destructive viruses, such as Cucumo-, Tospo-, Tobamo- and Potyviruses affecting multiple hosts, reliable, robust and sometimes rapid serological tests are available. High-quality group- or genus-specific PCR assays are also available or under study for some viruses, and microarray tests able to detect most ornamental-infecting viruses will likely become available in the near future. Moreover, new viruses are continuously being discovered and generic virus-detection techniques such as electron microscopy, together with bioassay, virus purification, or dsRNA assays remain extremely helpful diagnostic tools. We will present the most compelling viral problems on ornamentals affecting the five continents with contribution of experts locally involved in detection and molecular studies of viruses infecting ornamentals. Thanks are given to the Floral and Nursery Plants Research Unit, USDA-ARS Beltsville, MD, USA, for hosting the presenting author as a visiting scientist.

EMERGING PROBLEMS IN SOILBORNE DISEASES OF ORNAMENTAL PLANTS. **D.M. Benson and G. Magnano di San Lio.** *North Carolina State University, Raleigh, NC, USA, 27695-7629; Università Mediterranea di Reggio Calabria, Facoltà di Agraria, Reggio Calabria, Italy. Email: mike_benson@ncsu.edu, gmagnano@unirc.it*

In both North America and Europe, *Phytophthora ramorum,* cause of ramorum blight in ornamentals and sudden oak death in forest trees, has had a large impact on the nursery industry. The interconnection of the nursery industry across regions within countries and continents has resulted in spread of this pathogen and subsequent regulatory action. In surveys aimed at detecting *P. ramorum*, new species such as *P. asparagi*, *P. niederhauserii*, *P. hedraiandra* and *P. tentaculata* have been isolated, and exotic species such as *P. palmivora* and *P. tropicalis* have been found on new hosts. PCR-amplification and DNA sequencing are key technologies for identifying these species. In floriculture, damping-off pathogens are endemic and new diseases such as *Fusarium* wilt of African daisy, unnoticed in conventional soil cultivation, have appeared. Intensification of cultural practices has favoured the outbreak and spread of recently introduced pathogens such as *Cylindrocladium* spp. In woody ornamentals, *Phytophthora* root rot occurs frequently due to persistent survival on nursery land and in irrigation supplies. Root-infecting nematodes affect health and vigor of field grown ornamentals in sub-tropical and temperate regions since few fumigants or post-plant treatments are available. As urbanization expands to farm lands formerly in agricultural crops, soilborne pathogens and nematode problems become commonplace on ornamentals planted in subsequent landscapes on commercial properties and around new homes. Management options, however, are limited, so planting stock selection is critical. Growers will continue to meet emerging soilborne disease problems in ornamentals with sound IPM practices.

EMERGING PROBLEMS IN FOLIAR AND VASCULAR WILT DISEASES OF ORNAMENTAL PLANTS. **M. Daughtrey and L. Orlikowski.** *Cornell University L.I. Horticultural Research & Extension Center, Riverhead, NY 11901, USA. Email: mld9@cornell.edu*

During the period 2003-2007, foliar and vascular wilt diseases caused major repercussions in the ornamentals industry. The costly introduction of *Ralstonia solanacearum* race 3, biovar 2 into North America in geranium cuttings from Kenya, Guatemala and Costa Rica in 2002-2003 led to increased regulations for offshore production. New downy mildew diseases have been found on floral crops including argyranthemum, coleus, and rudbeckia in North America. Downy mildew has been important on impatiens in both North America and Europe, and on lisianthus and statice in Europe. New *Fusarium* wilts have affected gerbera in Europe and coreopsis in the US. The rust *Uromyces transversalis* was found on gladiolus in the US for the first time in 2006. *Rhodococcus fascians* has recently been recognized as the cause of leafy galls on numerous new hosts. Powdery mildews new to North America have been found on petunia, gaillardia, scabiosa and mahonia, while powdery mildew diseases on deutzia and lilac have been significant recently in Europe. Vegetative propagation in countries with low labor costs, such as areas of Central America and Africa, is now a major factor in the introduction of new diseases into Europe and North America. Microscopic inoculum or latent infections are easily introduced on cuttings. Slowing the rate of new disease emergence will require better screening of new plant introductions by propagation companies, enhanced communication and cooperation among plant pathologists worldwide, and year-round plant inspections on location in the countries where young plants originate.

NEW TRENDS IN BIOCONTROL OF DISEASES OF ORNAMENTAL PLANTS. **H. Hoitink.** *Department of Plant Pathology, The Ohio State University, 1680 Madison Ave., Wooster, OH, USA. Email: hoitink.1@osu.edu*

The production of ornamental plants in containers is a growing industry. Substrates used for such plants typically are prepared from fibrous, light *Sphagnum* peat, recycled organic products such as bark, composted manures, coir, etc. These substrates have high microbial carrying capacity, and biocontrol agents randomly colonize them immediately after formulation. Controlled inoculation with specific biocontrol agents during the formulation process allows this introduced microflora to establish itself and provide effective control of some diseases caused by soilborne plant pathogens whereas inoculum applied later typically fails. Even so, control of damping-off diseases in these fortified substrates typically is weak. Thus, compatible fungicides still are critical for effective control of the latter diseases. Substrates inoculated with ISR-active biocontrol agents can significantly suppress foliar diseases (powdery mildew, *Botrytis*, *Phytophthora*, bacterial blights, etc.) under mild disease pressures. For example, *Trichoderma hamatum* 382 (T382) can be as effective as fungicides under mild but not under severe disease pressures. T382 increases the expression of several disease and stress resistance genes in plants (includes extension genes). This may explain why T382 provides a high degree of control of *Botryosphaeria* dieback. The significance of these developments in the container industry will be discussed.

Keynote Session
Diseases of Mediterranean crops and forests

IMPACT OF FOREST PATHOGENS IN THE MEDITERRANEAN REGION. **N. Anselmi, A. Ragazzi and A. Vannini.** *Dipartimento di Protezione delle Piante - Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy; Dipartimento di Biotecnologie agrarie, Università degli Studi di Firenze, Piazzale delle Cascine, 28, 50144 Firenze, Italy. Email: anselmi@unitus.it, alessandro.ragazzi@unifi.it, vannini@unitus.it*

Forest cenoses of the Mediterranean Basin represent a unique source of biodiversity at risk due to human pressure and effects of global climatic changes. Recently a number of primary forest pathogens have severely affected vitality and survival of some of the most important Mediterranean forest species, including European chestnut, cypress, elm, pine and plane. Most of these pathogens are alien invasive organisms that in the past found the optimal climatic conditions for reproduction and spread on susceptible hosts. Effects of global climatic changes are determining unexpected scenarios of the presence and impact of old and new invasive pathogens in Mediterranean forests. While some pathogens, for example those strictly dependant on seasonal rain for their spread, are facing a notable reduction in impact; other are increasing in incidence and severity, and are expanding in area; others may find suitable conditions for massive spread in case of accidental introduction. The introduction of alien pathogens in Mediterranean forests is an important issue basically associated with the globalization of the plant trading market. The higher frequency of extreme climatic events such as intense flooding and drought may increase the intensity of diseases asso-

ciated to filamentous Oomycota (e.g. the genus *Phytophthora*), and water-stress-mediated tree decline syndromes. Native weakness pathogens in Mediterranean forests heavily impaired by climatic constraints, are determinant in the modification of tree and shrub specific composition and ranking, causing, in extreme conditions, a dramatic decline of the forest and an increase of the risk of desertification.

USING DNA TO TRACK MICROBIAL INVASIONS OF MEDITERRANEAN NATURAL ECOSYSTEMS. **M. Garbelotto, D. Rizzo and P. Gonthier.** *Department of ESPM, University of California, Berkeley, USA. Email: matteo@nature.berkeley.edu*

In the last decade, the number of known introduced plant pathogens has risen dramatically. The rise can be attributed to a) our enhanced ability to detect invasive pathogens, and b) to an actual rise of introductions linked to increased trade and movement of plants or plant parts around the world. Mediterranean ecosystems are particularly at risk because they are normally densely populated, and characterized by mixed economic models inclusive of a significant agricultural or horticultural component. It is becoming increasingly evident that successful introductions are linked to human activities ranging from the obvious agricultural ones, to less obvious ones such as the ornamental plant business and military operations. It is also becoming broadly accepted that not all introduced microbes cause significant obvious damage to an ecosystem. I will discuss the introduction and spread of four *Phytophthora* species in California natural ecosystems, two causing almost no obvious damage, and two, namely *P. ramorum and P. cinnamomi,* causing extremely high damage as exemplified by the well known sudden oak death disease. I will further discuss the ecological and evolutionary impacts of the introduction of the conifer root pathogen *Heterobasidion annosum* from the US into Italy, by the US Army during WW II. DNA analysis can aid in the diagnosis and the understanding of the epidemiology of introduced diseases. The studies described in this talk had a significant impact on the inclusion of DNA-based diagnostic in the world's regulatory policies for plant pathogens, and succeeded in identifying routes of introduction for at least three exotic pathogens, and in clarifying the reason of their successful adaptation to Mediterranean ecosystems.

CURRENT STATUS OF CEREAL AND LEGUME VIRUS DISEASES IN THE MEDITERRANEAN BASIN. **K.M. Makkouk and S.G. Kumari.** *International Center for Agriculture Research in the Dry Areas, ICARDA, Aleppo, P.O. Box 5466, Syria. Email: k.makkouk@cgiar.org*

Small-grain cereals and food legume are widely cultivated field crops in Mediterranean Basin and provide protein and calories for a large part of the population. Small-grain cereals in the region are reported to be affected by 3-4 viruses, among which *Barley yellow dwarf viruses* (BYDVs, family *Luteoviridae*) are the most important. Due to their host range and the widespread occurrence of vectors, BYDVs cause the most economically important and widespread virus disease of cereals. Losses can be very serious but vary with strains, growth stage at infection, varieties and environmental conditions. High infection has been reported in Algeria, Morocco, Tunisia, Italy and Greece with epidemics occurring every 3-5 years, depending on the area. In addition, *Maize dwarf mosaic virus* (MDMV, genus *Potyvirus*, family *Potyviridae*) causes economic losses on maize in Croatia, Egypt, France, Israel, Italy, Morocco, Spain and Turkey. Around twenty viruses have so far been identified as affecting food legumes in the region. Those with major economic importance are: *Faba bean necrotic yellows virus* (FBNYV, genus *Nanovirus*, family *Nanoviridae*), *Bean leafroll virus* (BLRV, genus *Luteovirus*, family *Luteoviridae*), *Beet western yellows virus* (BWYV, genus *Polerovirus*, family *Luteoviridae*), Chickpea chlorotic dwarf virus (CpCDV, genus *Mastrevirus*, family *Geminiviridae*), *Bean yellow mosaic virus* (BYMV, genus *Potyvirus*, family *Potyviridae*), *Broad bean mottle virus* (BBMV, genus *Bromovirus*, family *Bromoviridae*), *Pea enation mosaic virus-1* (PEMV-1, genus *Enamovirus*, family *Luteoviridae*) and *Pea seed-borne mosaic virus* (PSbMV, genus *Potyvirus*, family *Potyviridae*). Other viruses such as *Alfalfa mosaic virus* (AMV, genus *Alfamovirus*, family *Bromoviridae*), *Cucumber mosaic* virus (CMV, genus *Cucumovirus*, family *Bromoviridae*), *Broad bean wilt virus* (BBWV, genus *Fabavirus*, family *Comoviridae*), *Broad bean stain virus* (BBSV, genus *Comovirus*, family *Comoviridae*) and *Pea early browning virus* (PEBV, genus *Tobravirus*) can be important in specific locations. *Tomato spotted wilt virus* (TSWV, genus *Tospovirus*, family *Bunyaviridae*) has been reported on faba bean in Spain. Yield loss could vary from almost none to a complete crop failure. Barley and wheat genotypes resistant to BYDV, lentil genotypes resistant to PSbMV, PEMV-1, BLRV, FBNYV and *Soybean dwarf virus* (SbDV, genus *Luteovirus*, family *Luteoviridae*); and faba bean genotypes resistant to BLRV and BYMV were reported. Less progress was made with chickpea. Losses have been reduced because of progress made over the last two decades in developing resistant varieties or improving management practices. In some countries of the Mediterranean Basin, facilities are lacking and virus identification is based only on field diagnosis and indexing on indicator plants. Development of integrated control measures to control virus diseases in some important crops is urgently needed.

KNOWLEDGE TRANSFER FOR PLANT PATHOLOGY

TECHNOLOGY TRANSFER IN EXTENSION: EXPERIENCE IN THE US. **P. Vincelli.** *University of Kentucky, Department of Plant Pathology, 207 Plant Science Bldg. Lexington, KY, USA. Email: pvincell@uky.edu*

The internet is the most important catalyst of change in the way the science of plant pathology is extended in the U.S. Provision of real-time disease alerts and other updates through the internet provides many benefits to producers and agricultural professionals; Extension programs for Asian soybean rust represent an eminently successful example. Increasingly more trainings and meetings are being conducted online, promoting efficiency and maintaining Extension productivity in times of generally static-to-declining budgets, although face-to-face meetings continue to have their place. Internet-based consultation or diagnosis using digital images of symptoms – and in some cases, microscopic images – have become common. Information flow is no longer vertical, from researchers through the local Extension system to farmers. Producers now receive information from a wide range of sources, and Extension specialists are not necessarily their principal source for day-to-day information needs. More than ever, we Extension specialists must protect our credibility as unbiased, science-based experts in order to remain relevant. Nucleic acid-based techniques are becoming fundamental to Extension programs, increasing precision and/or speed of pathogen detection. Appropriate use of molecular tools requires awareness of their limitations as well as strengths.

CLASSROOM IN THE COCOA BLOCK. J. Konam, Y. Namaliu, R. Daniel and D.I. Guest. *Faculty of Agriculture, Food & Natural Resources, The University of Sydney, NSW 2006, Australia. Email: d.guest@usyd.edu.au*

More than 80% of Papua New Guinea's annual cocoa production of 42,000 t is produced by 150,000 smallholder farming families. The current average yield of 300 kg dry beans/ha is much less than the potential yield because of poor management and yield losses to *Phytophthora* pod rot and canker (*Phytophthora palmivora*) and vascular streak dieback (*Oncobasidium theobromae*). Apart from improved cocoa genotypes, technology adoption is poor and over 95% of 108 farmers surveyed had no knowledge of cocoa disease and pest management. We developed a series of low, medium, high and very high Integrated Pest and Disease Management (IPDM) options that include different levels of sanitation, harvest frequencies, canopy and shade management, fertiliser application and weed and insect control that contribute to reduced disease and increased yield. The choice of input level recognises the aspirations and resource limitations of individual smallholder farmers. On-farm demonstration plots are established in highly visible areas through participatory action research (PAR). IDPM training is scheduled with local cropping cycles and farmers are trained during establishment of the plots. Over 108 PAR trials have been established and more than 1,500 farmers trained. The uptake of options is close to 100%, and over 80% of farmers prefer the higher input options. Yield increases of more than 100% have been reported and PNG smallholders are investing in cocoa for the first time. We aim to transform the industry from the current 90% low input to 50% medium input farms.

KNOWLEDGE TRANSFER THROUGH FARMER-PARTICIPATORY TRAINING AND RESEARCH. J.G.M. Vos, S.L.J. Page and U. Krauss. *CAB International (CABI), Kastanjelaan 5, 3833 AN Leusden, The Netherlands. Email: j.vos@cabi.org*

Globalisation of trade implies that farmers in developing countries increasingly need to meet new standards of quality and reliability. With plant diseases attracting the bulk of hazardous pesticide use in some cropping systems (examples will be provided), farmers need new knowledge on crop protection methods. Implementation of sustainable crop protection methods is complex and dependent on many factors. This fact alone has implications for the style of knowledge transfer, as complex systems cannot be translated into simple messages. Farmer-participatory methods aim to mitigate situations where farmers lack access to, for them, new knowledge. Farmer Participatory Training (FPT) focuses on knowledge transfer through facilitated discovery learning. FPT in plant disease management goes through three consecutive steps: (1) Identification, (2) Understanding, (3) Informed problem solving. Farmer Participatory Research (FPR) focuses on knowledge generation through facilitated farmer experimentation. The focus in FPR is on meeting farmers' needs and demands by appropriate knowledge generation through local technology development and/or validation, and follows the four continuous problem-solving steps: (1) Identify and experience, (2) Observe and reflect, (3) Form abstract concept, (4) Test in new situation. The focus of knowledge transfer and generation is indirectly to achieve food security and/or expected product quality, but first and foremost to improve smallholder's livelihoods. Impact assessments do show the hoped-for results: more stable production with improved product quality, and increase in farmers' incomes. However, for lasting impact, more actors in the agricultural knowledge system need to become engaged.

KNOWLEDGE TRANSFER: THE EXTENSION-INDUSTRY INTERFACE. G. Munkvold. *Iowa State University, 160 Seed Science Bldg., Ames, IA 50011, USA. Email: munkvold@iastate.edu*

A multitude of information sources is available to farmers seeking to improve profit and sustainability. In the United States, university Extension services continue to be a critical resource for knowledge transfer to farmers, but their predominance as primary, direct sources of crop production information has waned. While Extension services may enjoy a high level of credibility, their direct access to farmers and their ability to be proactive have been curtailed by budget constraints. At the same time, industries that supply inputs to the farmer have assumed an ever greater role on providing advice on technology and crop production practices. These trends have converged to foster competitive, yet synergistic, relationships between public agencies, such as university Extension, and private businesses seeking to inform farmers. Although priorities may differ, public and private entities share an interest in improving farmers' efficiency, profitability, and sustainability. Public/private partnerships are increasingly important mechanisms for developing and disseminating science-based recommendations to farmers, both in relation to technology products and cultural practices. These partnerships can take many forms, and can be universally beneficial. However, attention must be paid to protecting the reputation of public agencies as unbiased information sources. In the foreseeable future, ever closer ties between public and private entities will result in timely knowledge transfer to farmers but conflicts will inevitably arise, and it may become more difficult for farmers to discern the original source of information they receive.

Plant pathogenic bacteria

FUNCTIONAL GENOMICS OF THE HRPG AND HRPX REGULON OF *XANTHOMONAS CAMPESTRIS* PV. *VESICATORIA*. U. Bonas. *Martin-Luther-University Halle-Wittenberg, Department of Genetics, Weinbergweg 10, D-06120 Halle, Germany. Email: ulla.bonas@genetik.uni-halle.de*

Pathogenicity of the Gram-negative bacterium *Xanthomonas campestris* pv. *vesicatoria* (Xcv), the causal agent of bacterial spot disease on pepper and tomato plants, depends on a type III secretion (T3S) system which translocates more than 20 bacterial effector proteins into the plant cell. The T3S system is encoded by plant-inducible *hrp* (hypersensitive reaction and pathogenicity) genes which are clustered in a 33-kb pathogenicity island on the bacterial chromosome. The master regulators of T3S are HrpG (OmpR type regulator) and HrpX (AraC-type regulator), expression of which depends on activated HrpG. HrpG is activated by so far unknown environmental signals, but a mutated derivative, termed *hrpG**, leads to constitutively active HrpG in different conditions. To identify the entire HrpG and HrpX regulons and possibly new virulence factors, we performed microarray analyses using the sequenced Xcv strain 85-10 and derivatives. Approximately 140 genes were found to be induced, whereas 50 genes were repressed under conditions simulating infection. Most upregulated genes encode T3S system components, helpers and type III effectors, and proteins of unknown function. New insights on regulation of pathogenicity of Xcv will be presented.

FUNCTIONAL GENOMICS AND HOST SPECIFICITY DETERMINANTS OF *RALSTONIA SOLANACEARUM*. S. Genin.

LIPM, INRA-CNRS, 31326 Castanet-Tolosan, France. Email: sgenin@toulouse.inra.fr

Ralstonia solanacearum, the causal agent of bacterial wilt, is considered as a species complex with an uncommonly wide host range; It can be transmitted through water, seedlings and soil, where it can survive for long periods. According to recent classification, the species complex is subdivided into four main groups called phylotypes that highly correlate with strain geographical origins. We have undertaken a microarray-based genomic diversity study aimed at the identification of host-specificity determinants within *R. solanacearum* strains. Based on the complete sequence of strain GMI1000, a genomic microarray was developed earlier. More recently, two additional 12X draft genome sequences of strain IPO1609 (potato strain) and strain Molk2 (banana strain) were obtained and 1150 new genes that are not present in strain GMI1000 were included in the microarray. We investigated the genomic repertoire of *R. solanacearum* strains showing differential behavior in their host specificity with a focus on identifying host-specificity determinants of a subset of strains that specifically attack banana. In particular, emerging strains have been identified in the West French Indies (Martinique) which share similar genomic characteristics with *R. solanacearum* strains pathogenic on banana plants (PB strains) but that are unable to cause symptoms on this host (NPB strains). Comparison using the DNA hybridization results of 5 PB strains and 6 NPB strains allowed identification of 17 candidate genes that could determine specificity on banana. The functional characterization of these candidates is currently under way.

USING FUNCTIONAL GENOMICS TO IDENTIFY *PSEUDOMONAS SYRINGAE* TYPE III EFFECTORS AND DETERMINING THEIR ACTIVITIES IN PLANTS. **J.R. Alfano.** *Center for Plant Science Innovation and the Department of Plant Pathology, University of Nebraska, 1901 Vine St., Lincoln, NE, 68588, USA. Email: jalfano2@unl.edu*

The ability of *Pseudomonas syringae* to be pathogenic on plants and to elicit the hypersensitive response (HR) is dependent upon the type III secretion system (T3SS). The genome of *P. syringae* has recently been mined for type III effector genes that encode proteins that are injected into plant cells by the T3SS. Type III effectors appear to be able to suppress both effector-triggered immunity (ETI) and pathogen-associated molecular patterns (PAMP)-triggered immunity (PTI). We developed bioassays to determine whether any of the type III effector proteins could suppress ETI and PTI. These bioassays revealed that many of these type III effectors could suppress both ETI and PTI. The high proportion of effectors that suppress plant immunity suggests that suppressing plant immunity is one of the primary roles for *P. syringae* type III effectors and a central requirement for pathogenesis. Many type III effector genes are arranged in clusters known as pathogenicity islands (Pais) within the bacterial genome. These Pais, which were likely acquired through horizontal gene transfer, were identified based on the presence of type III promoters, an atypical GC content, mobile DNA elements, and the presence of known type III effector genes. We have constructed unmarked Pai mutants and poly-Pai mutants that lack many type III effector genes allowing us to discover phenotypes that would otherwise be masked by effector redundancy. Our mutagenesis strategy and phenotypes from our initial Pai mutants will be presented.

GENOMICS OF *CLAVIBACTER MICHIGANENSIS*. **R. Eichenlaub, A. Burger, K.-H. Gartemann, M. Flügel, I. Gräfen and O. Kaup.** *Bielefeld University, Dept. of Gene Technology and Microbiology, P.O. Box 100131, 33501 Bielefeld, Germany. Email: eichenlaub@uni-bielefeld.de*

The Gram-positive actinomycete *Clavibacter michiganensis*, comprises several subspecies which are pathogens on a variety of agriculturally important plants. *C. m.* subsp. *michiganensis* (Cmm) is a pathogen of tomato and causes a tracheobacteriosis leading to wilt and canker. In earlier work we have shown that wilting of infected plants depends on two plasmid-encoded genes of Cmm, *celA*, carried by plasmid pCM1 and encoding a β-1,4-endoglucanase, and *pat*-1 mapping on plasmid pCM2, encoding a putative serine protease. Loss of the plasmids converts the pathogen to an endophyte not inducing disease symptoms. The Cmm genome sequence was recently obtained, and conclusions derived from this information will be presented. For example we identified a pathogenicity island of 130 kb flanked by 1.9 kb repeats close to the origin of replication. Deletion of this region as well as inactivation of certain serine proteases located in this region affect colonization and lead to a drastic reduction of *in planta* titre. A tomatinase which also maps on this pathogenicity island was studied and shown to deglycosylate the tomato phytoanticipine α-tomatine to the inactive compound tomatidine. However, we found that inactivation of the *tomA* gene did not significantly affect Cmm virulence. A Cmm genome chip was used to study differential gene expression of Cmm when grown in tomato xylem sap as compared to growth in minimal medium. By this approach we hope to identify further genes which may be relevant for this microbe-plant interaction.

Natural compounds and disease control

NATURAL PRODUCTS AND DISEASE RESISTANCE IN CEREALS. **A. Gierl and M. Frey.** *Lehrstuhl für Genetik, WZW, Technische Universität München, Am Hochanger 8, 85350 Freising, Germany. Email: gierl@wzw.tum.de*

Plant secondary metabolites constitute a large field of chemical biodiversity that is important for the survival strategies of plants. A secondary metabolic pathway can be defined by the branch point from primary metabolism, and the downstream reactions that lead to end product formation. Evolution has recruited specific enzymes that catalyse these reactions. The function and evolution of the biosynthesis of protective natural products will be discussed using benzoxazin biosynthesis of maize and avenacin biosynthesis in oats as examples. Gene duplication was essential for the evolution of the genes encoding these pathways. The examples show that gene duplication does not necessarily result in redundancy; rather it is a prerequisite for the generation of biodiversity.

PLANT-DERIVED NATURAL PRODUCTS – SYNTHESIS, FUNCTION AND THE BASIS OF METABOLIC DIVERSITY. **A. Osbourn.** *Department of Metabolic Biology, John Innes Centre, Norwich NR4 7UH, UK. Email: anne.osbourn@bbsrc.ac.uk*

Plants produce a huge array of natural products, many of which are specialised metabolites associated with particular species. These secondary metabolites often have important ecological roles, facilitating pollination and seed dispersal and/or providing protection against attack by pests and pathogens. Al-

though the ability of plants to perform *in vivo* combinatorial chemistry by mixing, matching and evolving the genes required for different secondary metabolite biosynthetic pathways is likely to have been critical for survival and diversification of the Plant Kingdom we know very little about the mechanisms underpinning this process. This talk will focus on the function and synthesis of plant natural products and on the origins of metabolic diversity and will draw on our research on triterpene synthesis in crop and model plants.

REGULATION AND FUNCTIONAL ANALYSIS OF BIOPROTECTIVE METABOLITE GENES FROM THE GRASS SYMBIONT *EPICHLOË FESTUCAE*. **B. Scott, K. May, D. Takemoto, C. Young, A. Tanaka, D. Fleetwood and R. Johnson.** *Molecular BioSciences, Private Bag 11 222, Massey University, New Zealand. Email: d.b.scott@massey.ac.nz*

Epichloë endophytes are biotrophic fungi that systemically colonise the intercellular spaces of leaves of grasses to form mutualistic symbiotic associations. The production of secondary metabolites by these fungi confers bioprotective benefits to the grass. However, in pastoral ecosystems some of these metabolites are toxic to grazing mammals. We have cloned and functionally analysed genes for the synthesis of three classes of these bioprotective molecules, peramine, lolitrem B and ergovaline (Tanaka *et al*., 2005; Young *et al*., 2005 & 2006; Fleetwood *et al*., 2007). A single gene, *perA*, encoding a non-ribosomal peptide synthetase appears to be required for peramine biosynthesis (Tanaka *et al*., 2005). Complex gene clusters with 10 and 11 genes, respectively, are required for lolitrem B (*ltm* genes) (Young *et al*., 2005 & 2006) and ergovaline (*eas* genes) (Fleetwood *et al*., 2007) biosynthesis. The biochemical function of some of these genes is being elucidated by a systematic deletion analysis combined with chemical analysis of intermediates that accumulate *in planta*. These experiments will allow us to propose biosynthetic schemes for the synthesis of these metabolites. Symbiota of these 'knock-out' mutants are being used to examine biological function of the metabolites. Spatial and temporal patterns of gene expression are being examined *in planta* using promoter fusions with the GUS reporter gene (May *et al*., 2008). To dissect signaling pathways required for activation of these genes *in planta* we are using *gusA* 'knock-in' constructs combined with T-DNA mutagenesis. An overview of progress to date will be presented.

EXPLOITATION OF NATURAL COMPOUNDS IN ECOFRIENDLY MANAGEMENT OF PLANT PESTS. **N.K. Dubey.** *Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi 221005, India. E-mail: nkdubey2@rediffmail.com*

In recent years, use of natural compounds for plant disease control has gained considerable attention. Pyrethrins from *Chrysanthemum cinerariaefolium* have a long record of safe use as a natural pesticide because of excellent safety profile and biodegradable nature. Allyl isothiocyanate, a component in mustard and horseradish oil is used as an effective antimicrobial in preservation of different foods. Carvone, from *Carum carvi* has been introduced as a natural pesticide to protect potato tubers from microbial rot. Azadirachtin from *Azadirachta indica* is an outstanding natural pesticide with great potential for commercial exploitation. Many natural products have been recommended for integrated pest management because of their efficacy as semiochemicals affecting the physiological behaviour of pests. The ovicidal, antifeedant and oviposition-deterrent efficacy of some natural compounds strengthen their exploitation as novel agrochemicals in pest management. Some essential oils have been recommended as substitutes for synthetic fumigants in controlling postharvest infestations of food commodities and mycotoxin secretions. These are recommended for enhancement of shelf life of commodities without showing any residual problems. Unlike the prevalent fumigants, the problem of development of resistant strains of pests may be solved by the use of essential oils because of the synergism between different components of the oils. Microencapsulation technology is being introduced for flavour stabilization of oils during their use as antimicrobials in the food industry. Naturally-occurring compounds appear to have a prominent future in the development of biodegradable and cost-effective commercial pesticides for agricultural crop productivity as well as safety of the environment and public health.

Disease management in organic farming

FUNGAL DISEASE MANAGEMENT IN ORGANIC ORCHARDS: EPIDEMIOLOGICAL ASPECTS AND MANAGEMENT APPROACHES. **I.J. Holb.** *Centre for Agricultural Sciences and Engineering, University of Debrecen, H-4015 Debrecen, P.O. Box 36, Hungary. Email: holb@agr.unideb.hu*

Environmental considerations are becoming increasingly important and, as a consequence, interest has turned from conventional to organic fruit production, where management practices differ from those in conventional production. Synthetic products are banned in organic fruit production, and in plant protection and nutrient supply, only natural products are permitted according to IFOAM standards. As a result, disease control is less effective than in conventional production with the consequence that epidemics are likely to be more serious. This lecture will provide current management options against key fungal diseases of fruit crops in the growing season. Then development of fungal disease management in pome and stone fruit species will be illustrated by focusing mainly on management practices against scab and brown rot. This will include epidemic features of apple scab and brown rot in organic apple orchards, the risk of early scab epidemics initiated by sexual and asexual forms of fungi in organic apple orchards, possible strategies for control of inoculum sources in organic production systems, efficacy and phytotoxicity of approved fungicidal products, and appropriateness of various sanitation practices in organic fruit production. The role of resistant vs. susceptible cultivars in disease epidemiology and management of organic orchards will also be discussed. Based on the above examples, a theoretical and practical decision-making approach will be provided for organic orchards based on mechanical, agro-technical, biological and chemical control options. Future trends in fungal disease management will be discussed.

MANAGEMENT OF LATE BLIGHT IN ORGANIC FARMING. **D. Shtienberg.** *Department of Plant Pathology and Weed Research, ARO, the Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel. Email: danish@volcani.agri.gov.il*

Late blight, caused by *Phytophthora infestans*, is the major foliar pathogen of tomatoes in Israel. Coping with the disease is much more difficult in organic production of tomatoes, as only copper compounds are available for use, and their efficacy is occasionally low. Moreover, it is likely that in the near future the rates of copper used will be reduced, due to consumer pressure. The objective of this study was to develop means to manage late

blight in organic production of tomatoes with reduced rates of copper. The effects of various management procedures (covering the soil with polyethylene, application of fungicides and sanitation), and their interactions, were studied in a series of experiments conducted in walk-in tunnels and commercial-model greenhouses. Under conditions of the western Negev (south west Israel) foliar infection by the pathogen could be suppressed by covering the soil with reflective polyethylene (that reduced relative humidity and increased air temperature) and by application of novel fungicides. Under conditions of high temperature (>20 ^{o}C) and low relative humidity the foliage remained dry and infections did not occur. Nevertheless, the pathogen may progress from infected leaf-blades via the petioles, to the stems, where it causes stem lesions. Stem lesions eventually lead to plant mortality. Observations made in the greenhouses suggested that the damage resulting from stem infections is more significant than that from foliar infections. It is possible to prevent stem infections by sanitation, i.e. removal of infected plant material.

CONTROL OF DISEASES ON SEED FOR ORGANIC PRODUCTION. **J.M. van der Wolf.** *Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands. Email: Jan.vander Wolf@wur.nl*

Seed-borne pathogens can seriously affect crop yield and quality in organic plant production. The most effective means of control is by exclusion and reduction of inoculum during seed production. For this, a number of measures can be used, including the use of pathogen-free basic seed, resistant cultivars, crop rotation, spacing within or between crops, the control of weeds and crop debris, and the use of appropriate fertilization, irrigation- and hygiene practices. Seed-borne inoculum can also be reduced by treating basic seed or plantlets used for seed production with natural resistance inducers. Several bacterial and fungal extracts, as well as specific organic compounds and micronutrients, have been found to induce resistance against plant pathogens after treatment of seeds or plantlets. Infection and contamination of seed, however, cannot always be avoided. To reduce seed-borne inoculum after seed production, various seed treatments can be applied. Within the EU STOVE project (2003-2006, www.stove-project.net), different physical and biological treatments suitable for use in organic vegetable seed production were developed and evaluated. Physical treatments with hot water, aerated steam and low energy electrons were found highly effective against a number of seed-borne fungal and bacterial pathogens in cabbage, carrot, parsley, bean, pea and lamb's lettuce. It also reduced disease expression in greenhouse and field experiments. From biological treatments tested, including the use of antagonists, antimicrobial compounds and resistance inducers, only thyme oil reduced fungal pathogens to levels comparable to that of physical treatments.

MANAGEMENT OF VIRUS DISEASES IN ORGANIC AGRICULTURE. **R.A.C. Jones.** *Agricultural Research Western Australia, Locked Bag No. 4, Bentley Delivery Centre, WA 6983, Australia. Email: rjones@agric.wa.gov.au*

Virus diseases often cause serious losses in yield and quality of cultivated plants grown in organic production systems. These losses, and the resulting financial damage, are limited by controlling virus epidemics using measures that minimise infection sources and suppress spread. When diverse control measures that act in different ways are combined, their effects are complementary resulting in far more effective overall control. Such experiences have led to development of Integrated Virus Disease Management (IVDM) concepts that suit organic systems. Although chemical control of invertebrate and fungal vectors with pesticides and fungicides, and of virus reservoir hosts such as weeds and volunteer crop plants, with herbicides is precluded in such systems, effective control of virus diseases can still be achieved by combining available host resistance, and phytosanitary, cultural and biological control measures. Selecting the ideal mix of measures for each pathosystem and production situation requires knowledge of the epidemiology of the causal virus and the mode of action of each individual control measure so that diverse responses can be devised to meet the special features of each of the scenarios encountered. The tactics devised must be robust and necessitate minimal extra expense, labour demands and disruption to standard practices. Examples of applying IVDM to different organic systems will be described. These examples will illustrate how to achieve effective control of virus diseases in diverse pathosystems and production situations, without compromising on the need for environmental and social responsibility.

Nematology and plant diseases

EVOLUTION AND THE TAXONOMY OF PLANT PARASITIC NEMATODES. **B. Adams.** *Microbiology and Molecular Biology Dept. 775 WIDB Brigham Young University Provo, UT, 84602-5253, USA. Email: bjadams@byu.edu*

A promising approach to nematode management is exploitation of genomics to target molecular aspects of nematode pathogen biology. These discoveries are leading to more sophisticated engineering of plant resistance via genetically modified hosts, and the ability to confound expression of specific nematode parasitism genes. However, these revolutionary approaches require a logical basis for establishing the origin and maintenance of parasitism-associated genes, and characterizing their distribution across the enormous genetic diversity of Nematoda. Apart from an evolutionary framework (phylogeny), the relevant diverse molecular genetic patterns cannot be meaningfully and efficiently explored, compared, and extended. Similarly, unless hierarchical taxonomic relationships reflect phylogeny, they will be irrelevant at best, or misleading at worst, to informing management programs. Collaborative work by several groups is gradually producing both fine-scale and deeper node resolution of nematode phylogenetic relationships. The resulting hypotheses have laid the groundwork for taxonomic revision at all hierarchical levels, and bring greater objectivity, stability and replication to the enterprise.

MOLECULAR GENETICS OF NEMATODE-HOST INTERACTIONS. **V.C. Blok.** *Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee, Scotland, UK. Email: vblok@scri.ac.uk*

During the last decade, our understanding of the molecular interactions between plant-parasitic nematodes and their hosts has greatly improved revealing that nematodes have acquired a variety of factors not found in animal-parasitic or free-living nematodes that enable them to penetrate and manipulate their hosts in order to form specialized feeding sites. A number of large-scale genomics projects involving plant parasitic nematodes are currently underway and it is expected these will reveal much more concerning the evolution of parasitism, how many genes have been acquired and how these are arranged in the genome. The interaction

between obligate endoparasitic nematodes and their hosts is complex and undoubtedly the result of extended co-evolution. Interestingly there are marked differences in these highly specialized relationships. For example the cyst nematodes *Globodera* spp. typically have very restricted host ranges within the Solanaceae whilst some root knot nematodes such as *Meloidogyne incognita* have extremely broad host ranges enabling then to develop on thousands of plant species. Elucidating the molecular bases which differentiate these interactions continues. Clearly the ability to evade host defences is central to these interactions. Comparisons of intraspecific variation within highly specialised species and those with wide host ranges has revealed contrasting levels of diversity, with implications for the genetic basis of nematode-host interactions. Topic Area: Nematology and Plant diseases.

MANAGING NEMATODES IN THE POST-METHYL BROMIDE ERA. **N. Greco and L.W. Duncan.** *CNR - Istituto per la Protezione delle Piante, Sezione di Bari, Via Amendola 122D, 70126 Bari, Italy. Email: n.greco@ba.ipp.cnr.it*

The phase-out of methyl bromide has caused problems in the management of some economically important plant parasitic nematodes. Scientists are trying to implement new strategies that are effective, convenient, safe, sustainable and profitable. Future management programmes will rely more on the integration of resistant cultivars with crop rotation and other control tactics. Increased reliance on nematode identification, spatial monitoring, and the adoption of precision agricultural methods are also likely. Greater emphasis will be placed on some existing management tactics and new tactics will be adopted. Increased reliance on quarantine measures will help to reduce the spread and establishment of exotic nematodes. Plant nurseries can play a key role by releasing only material that is free of nematodes and other pests and diseases. Managing the water and nutrient holding capacity of soil can help plants tolerate nematode infestations and some industrial and municipal wastes used as soil amendments can also suppress nematode populations. Many plant extracts are being studied for their nematicidal activity and some are available as commercial formulations. Micro-organisms that are antagonistic to nematodes or that induce resistance in plants are being commercially formulated. Physical methods of controlling nematodes are being re-evaluated. Solarization and biofumigation are increasingly adopted and new machinery is being developed to produce hot water or steam at an acceptable cost for use in protected cultivation and in the field. Although conventional nematicides (primarily organophosphate, carbamate, halogenated hydrocarbons) have decreased in availability, some new nematicides and nematicide mixtures being studied appear promising.

PERSPECTIVES IN BIOLOGICAL CONTROL OF NEMATODES. **B.R. Kerry.** *Nematode Interactions Unit, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK. Email: brian.kerry @bbsrc.ac.uk*

Screening of soil micro-organisms over >50 years has led to the development of a number of biological control products based on <10 organisms. This empirical approach has generally failed to provide growers with robust biological control strategies. The inaccessibility of nematode pests in soil and the extended period over which some crops require protection present an especial challenge for the effectiveness of biological control agents in reducing nematode damage. However, the continued identification of suppressive soils in which specific micro-organisms control nematode pest populations indicates that biological control is practical in commercial crop production in some situations. The importance of microorganisms in the regulation of nematode populations in natural ecosystems has been demonstrated in recent research in European sand dunes, and has provided new insights into pest management. If whole organisms rather than their genes or metabolites are to be used reliably in crop protection, it is essential to understand their ecology in the rhizosphere, especially the interactions with the plant and target nematode. An integrated approach that combined studies at molecular, whole organism and population scales has provided new insights into the use of the nematophagous fungus, *Pochonia chlamydosporia*, as a biological control agent. This research has resulted in a registered product based on *P. chlamydosporia* for use against root-knot nematodes by small growers in the tropics. Up-scaling from applications in small-grower plots to commercial agriculture is an additional challenge, which will demand further understanding of the spatial dynamics of host-parasite interactions in soil.

WALL-LESS, PHLOEM-LIMITED BACTERIAL PLANT PATHOGENS

DYNAMIC GENOMES AND THE EMERGENCE OF PLANT PATHOGENIC MOLLICUTES. **R.E. Davis, Y. Zhao, R. Jomantiene, W. Wei and I.M. Lee.** *Molecular Plant Pathology Laboratory, USDA-Agricultural Research Service, Beltsville, MD, USA. Email: robert.davis@ars.usda.gov*

Plants are vulnerable to attack by wall-less bacterial pathogens – phytoplasmas and plant pathogenic spiroplasmas that in nature live obligately parasitic lifestyles. Emergence of the phytoplasma clade from an *Acholeplasma*-like ancestor gave rise to an intriguing group of wall-less prokaryotes through a remarkable and continuing evolutionary process. In a ceaseless progression, phytoplasmas evolved reduced genomes, lost biochemical pathways for synthesis of nutrients supplied by hosts, and gained capabilities for transkingdom parasitism and pathogenicity in plants and insects. Complete, draft, and targeted genome sequencing has opened exceptional possibilities for understanding the evolutionary divergence of phytoplasmas and free-living acholeplasmas from a common ancestor, and has revealed dynamic structures including varied mobile elements, phase variable genes, and sequence-variable mosaics (SVMs) of clustered genes that appear to function as platforms of genome plasticity, providing loci for acquisition of new genes and for targeting of mobile genetic elements to specific regions in phytoplasma chromosomes. Similarly, available genome sequence data have provided a rich resource for understanding spiroplasma evolutionary emergence, pathogen-host interactions, cell-cell communication, motility, and potential for gene exchange through conjugation, as well as the divergence of spiroplasmas and vertebrate-pathogenic *Mycoplasma* spp. from a common ancestor. The small, AT-rich genomes of phytoplasmas and spiroplasmas have evolved unique and dynamic architectures. The future challenges us to understand how these features may enable adaptations to the continually shifting environments encountered during obligate, transkingdom parasitism.

SPIROPLASMA-HOST RELATIONSHIPS: PLASMIDS AND INSECT-TRANSMISSION OF *SPIROPLASMA CITRI*. **M. Breton, S. Duret, N. Berho and J. Renaudin.** *UMR 1090 Génomique Diversité Pouvoir Pathogène, INRA Université de Bordeaux 2, F-33883 Villenave d'Ornon, France. Email: renaudin@bordeaux.inra.fr*

Spiroplasma citri is the causative agent of lethal stunting dis-

eases in various host plants including citrus, several brassicaceaeous species and periwinkle. The spiroplasmas multiply in the phloem sieve tubes and are transmitted from plant to plant by phloem sap-feeding insects (leafhoppers) in a persistent, circulative manner. *S. citri* GII-3 contains 7 plasmids, 6 of which (pSci1-6) share highly conserved regions including the replication region, which we characterized functionally. These plasmids encode adhesion-related proteins ScARPs and the hydrophilic protein P32, which have been tentatively associated with insect transmission. When experimentally injected into insects, all *S. citri* strains multiply in the hemolymph. However, while some strains (GII-3, Alc254) are readily transmitted to host plants by the infected insects, others (R8A2, 44) are not. Interestingly, non insect-transmissible strains do not express these proteins and even do not carry pSci1-6. To further study the role of plasmid-encoded proteins in insect-transmissibility, *S. citri* strains GII-3 and 44 with modified plasmid contents were constructed and assessed for their ability to be transmitted by the leafhopper vector. Plasmids from GII-3 (as well as mutated/deleted derivatives) were introduced into the non-transmissible strain 44. Also, based on the incompatibility of their replication regions, plasmids of GII-3 were replaced by their mutated/deleted derivatives. Experimental transmission of the whole set of transformants should provide new insights into the role of plasmid-encoded determinants in the biology of *S. citri*. First data proved pSci6 to be essential for transmission.

PHYTOPLASMA-INSECT VECTOR INTERACTIONS AND SPECIFICITY. **D. Bosco and C. Marzachì.** *Di.Va.P.R.A Entomologia e Zoologia applicate all'Ambiente, Università di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: domenico.bosco@unito.it*

Phytoplasmas are transmitted by leaf-, plant-hoppers and psyllids in a persistent manner. Following acquisition from the infected source plant, there is a latent period before the vector can transmit. During this time phytoplasmas multiply in different insect tissues. The multiplication in the vector points to highly evolved interrelationships and explains the high level of specificity of transmission. An insect unable to sustain multiplication of a phytoplasma will not serve as a vector. The generally accepted model proposes the passage of ingested phytoplasmas into the midgut lumen, adhesion to midgut epithelium cells, passage between or through midgut cells, subsequent invasion of haemolymph with transport to different organs and tissues, including salivary glands, and finally excretion with saliva during feeding. Several factors are involved in acquisition and transmission specificity, among these the most important are feeding behaviour (phytoplasmas are phloem-limited and are transmitted by phloem feeders), host-plant (monophagous vs polyphagous vectors), and genetics of both the vector and the phytoplasma. Phytoplasma membrane proteins are in direct contact with insect cell membranes and are involved in mediating specific interactions with the vector. Insect proteins interact with phytoplasma membrane proteins possibly as receptors and transporters. Different phytoplasmas can also infect the same vector and compete for insect transmission. Vector specificity can be investigated, besides transmission from plant to plant, by microinjection and artificial feeding experiments. The spread of phytoplasmas results from a complex picture, involving ecological, behavioural and molecular factors that control interactions between phytoplasmas and their hosts.

PHYTOPLASMA FUNCTIONAL GENOMICS AND HOST-PARASITE INTERACTIONS. **S. Namba.** *Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan. Email: anamba@mail.ecc.u-tokyo.ac.jp*

Phytoplasmas are transmitted from plant to plant by sap-feeding insect vectors (leafhoppers), and infect more than 700 plant species worldwide. We have examined several molecular findings involved in both plant-phytoplasma interactions and insect–phytoplasma interactions. First, the complete phytoplasma genome sequence suggested that the genome encodes very few metabolic functions, implying that the consumption of metabolites by phytoplasmas in plants may cause disease symptoms (Oshima *et al*., 2004, Nature Genet.). Second, the approximately 30-kb region, including glycolytic genes, was tandemly duplicated in the genome of a severe pathogenic phytoplasmas (Oshima *et al*., 2007, Mol. Plant Pathol.). The presence of two glycolytic gene clusters suggested that a higher consumption of the carbon source may affect the growth rate of the phytoplasma and may also directly or indirectly cause more severe symptoms. Third, positive selection was recognized on Amp, a phytoplasma surface membrane protein (Kakizawa *et al*., 2006, J. Bacteriol.). This positive selection may reflect an interaction between the phytoplasma and the host cytoplasm. Fourth, an affinity-column assay showed that Amp formed a complex with insect microfilament proteins. In addition, this interaction was correlated with the phytoplasma-transmitting capability of leafhoppers (Suzuki *et al*., 2006, PNAS). Our results suggest that Amp has an important function in the insect-phytoplasma interaction. The phytoplasma genome encodes many membrane proteins whose functions are still unknown; further analysis of these will provide valuable insights into host-phytoplasma interactions.

Plenary Session PS 3
In Celebration of 100 Years of the American Phytopathological Society

STAGING A CENTENNIAL: MILESTONES IN THE DEVELOPMENT OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY. P.D. Peterson and K.B.G. Scholthof. *Department of Entomology, Soils, and Plant Sciences, Clemson University, Florence, S.C. 29506-9706, USA. Email: ppeters@clemson.edu*

As part of the growing specialization generally in the biological sciences between 1880 and 1920, the creation of the American Phytopathological Society (APS) in 1908 was a response to the developing professionalism within the agricultural sciences in the United States. Inspired and led by senior federal plant pathologist C. L. Shear, the organization and early membership reflected both the growing impact of plant disease losses and the expansion of problem-solving research by the United States Department of Agriculture, agricultural colleges and state agricultural experiment stations. At the time of the first regular meeting in 1909, at Harvard Medical School, Boston, Massachusetts, 130 plant pathologists from the U.S. and Canada had become charter members. Hosted by the American Association for the Advancement of Science (AAAS) in both 1908 and 1909, APS then met jointly with AAAS for its first 33 years before meeting alone or with other specialized scientific societies. As the profession grew in the 20th century, the Society reflected increased specialization within university departments, government, and industry, while membership increased to nearly 5000 scientists worldwide. An examination of pivotal developments and influential scientists in plant pathology during the last century provides a backdrop for understanding the past, current, and future role of the APS.

THE GROWTH OF APS AS A PUBLISHER OF PLANT PATHOLOGY LITERATURE. J.D. MacDonald. *Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616, USA. Email: jdmacdonald@ucdavis.edu*

In February, 1911, shortly after its founding, the American Phytopathological Society launched the journal *Phytopathology*, which stood as the society's primary publishing focus for approximately 50 years. During the 1960s, APS joined in a partnership with the American Association of Cereal Chemists which led the two societies to share a professional operations staff and to open a joint headquarters building in 1972. Both societies viewed publication of discipline-specific books as an important membership need enabled by their shared editorial and business staff. Over the following decade, APS published the first of its highly successful compendium series and several books that emerged largely as symposium proceedings. In 1980, APS took over *Plant Disease Reporter* from the USDA and re-launched it as *Plant Disease*. The society founded APS Press in 1984 to bring cohesion and strategic planning to its book publishing efforts and in the following 24 years, the book publishing ventures of APS grew exponentially. With the emergence of molecular plant pathology, APS collaborated in 1987 with the International Society for Molecular Plant-Microbe Interactions to launch the journal *Molecular Plant-Microbe Interactions*. Through the 1990s, APS gained experience with electronic technologies and APS Press digitized its extensive image collection to produce CDROM products. In 2000 APS launched the all-electronic journals *Plant Health Progress* and *Plant Health Instructor*, and led in founding the multi-society online resource, *Plant Management Network*. Over the past 50 years, visionary leaders, talented staff and creative members have done much to move APS into the information age.

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY: PUBLIC SERVICE AND OUTREACH. J. Fletcher. *Department of Entomology & Plant Pathology, Oklahoma State University, Stillwater, OK 74078, USA. Email: jacqueline.fletcher@okstate.edu*

From its War Emergency Boards formed during WW I and WW II to address food security issues to recent white papers and a congressional briefing on crop biosecurity, APS has sought to serve and inform the public on national issues. APS also has a history of outreach to the nation's youth, as in a 1960s series in the *American Biology Teacher*, judging at international science fairs, and an annual exhibit at the Future Farmers of America Convention. Public policy involvements initiated through coalitions include the National Academy of Sciences in the 1960s and later, the Intersociety Consortium for Plant Protection in the 1980s, and the Council for Agricultural Science and Technology and the Coalition on Funding Agricultural Research Missions since the 1990s. The APS National Plant Pathology Board (now Public Policy Board), begun in 1991, was charged to interact with federal, private, and public advocacy groups; inform APS members of national developments; and advise Society officers on policy issues. The Office of Public Affairs and Education (now Office of Public Relations and Outreach) was formed in 1995 to lead the Society's diverse public outreach programs and to interact with the media. The addition, in 2000, of Eversole Associates as a professional "Washington presence" greatly increased the visibility and leadership of APS on crop biosecurity, USDA/EPA rules and policies, the sequencing of plant pathogen genomes, sustainable agriculture, graduate education, culture collections, and many other national issues critically important to plant pathologists and the discipline of plant pathology.

APS LEADERSHIP AND PARTICIPATION IN INTERNATIONAL AGRICULTURE. C.C. Mundt. *Dept. of Botany & Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, USA. Email: mundtc@science.oregonstate.edu*

From its earliest days, the American Phytopathological Society (APS) has indicated its intention to play a significant role in international agriculture. Prominent members of APS played a key role in establishment of programs that led to the "Green Revolution". Over the years, a large number of APS members have been actively involved in international agricultural organizations and in cooperative endeavors between U.S. and foreign institutions. The International Cooperation Committee was among ten Standing Committees formed during the first 50 years of the Society and provided leadership for the establishment of the Caribbean Division in 1960. Increasing interest in international activities resulted in establishment of the Office of International Programs (OIP) in 1996. These international bodies of APS have been involved in a diversity of projects, including library assistance, programmatic activities at annual APS meetings, recommending honorary awards, administering grant and travel funds, and addressing policy issues. The most controversial policy issue centered on the relationship of human population growth to world hunger, and took nearly 20 years to resolve. International membership has been the fastest growing sector of APS for many years, and stood at 34% of total membership in 2006. Similarly, submissions to APS journals have become increasingly international. This international flavor also has brought challenges, however, and APS currently is working towards better representation of its international members in committees, governance, and editorial boards. APS is striving to build linkages with other plant pathology societies, for example, through a recent collaboration with the Chinese Society for Plant Pathology.

CONTRIBUTIONS OF PLANT PATHOLOGY TO THE LIFE SCIENCES IN THE PAST 100 YEARS. **R.J. Cook.** *Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA. Email: rjcook@wsu.edu*

Already at the turn of the 20th century, and as recently as the 1950s, the discussion was on whether plant pathology is a science in it own right or applications from other fields of science. Yet the crystallization by 1935 of TMV and the proof in 1936 that TMV is a nucleoprotein preceded the first crystallization of an animal virus by 20 years. Plant virology also produced the first evidence that RNA (not just DNA) serves in heredity. No area of the life sciences has been impacted scientifically more by plant pathology over the past 100 years than mycology, including medical mycology. Most lists of other seminal contributions from plant pathology include density gradient centrifugation and SDS-PAGE gel electrophoresis as tools of molecular biology; discovery of viroids, MLOs, spiroplasmas, and fastidious vascular-limited phytopathogenic bacteria as agents of disease; the type III and IV secretion systems in phytopathogenic bacteria; and RNA-silencing in plants. Plant pathology has also broadened its scientific leadership in plant-microbe interactions to include, in addition to host-pathogen interactions, the beneficial mycorrhiza and plant growth promoting rhizobacteria and plant-associated (food-borne) human pathogens. Interestingly, Flor's gene-for-gene model emerged from an applied program aimed at control of flax rust, but helped spark the emergence of molecular plant biology through molecular characterization of virulence, defense and innate resistance, now on the forefront in the life sciences but still to find practical application; whereas knowledge of the molecular basis for crown gall emerged from curiosity-driven research but is resulting in major practical applications.

Keynote Session
Recent developments in disease management

BIOCONTROL FUNGI ACT AS PLANT SYMBIONTS AND IMPROVE RESISTANCE TO BIOTIC AND ABIOTIC STRESSES. **M. Lorito.** *Department of Aroboriculture, Botany and Plant Pathology, University of Naples, Portici (Naples), Italy. Email: lorito@unina.it*

Our current understanding is that the activity of a fungal biocontrol agent is not limited to the direct killing or inhibition of the pathogen, but also involves extensive changes in the manner in which plants and pathogens interact. By using *Trichoderma* spp. as a model, we demonstrated that these saprophytic microbes establish a type of symbiotic relationship with the plant. They stimulate root formation, and consequently plant growth and crop yield, in order to increase the area to colonize and the quantity of exudates to use as nutrients. At the same time, effector proteins are released by the biocontrol agent within the plant tissues and "sensed" by the plant cells, resulting in defence-related reactions such as spikes in Ca^{2+} uptake and PCD. This generates a pre-activation of the systemic resistance to pathogens and a deep change in the plant "interactome" produced in response to pathogen attack. The outcome of this complex beneficial effect is that crops colonized by effective *Trichoderma* strains grow better, are more resistant to both foliar and soil-borne diseases, and are less susceptible to abiotic stresses such as lack of nutrients, drought, etc. The knowledge gathered so far allows the development of a new generation of bio-agents capable of protecting the plant from various stresses and displaying both the fertilizer and disease control effects.

POSITIONAL CLONING AND CHARACTERIZATION OF A MAJOR QTL IN MAIZE THAT CONFERS RESISTANCE TO ANTHRACNOSE SHEATH BLIGHT. **R. Broglie.** *DuPont Central Research & Development, Wilmington, DE, 9880, USA. Email: richard.m.broglie@cgr.dupont.com*

Colletotrichum is a large genus of Ascomycete fungi that infect a wide range of important crop and ornamental plants. *Colletotrichum graminicola* causes anthracnose stalk rot and anthracnose leaf blight of maize. Depending on the severity of disease, yield losses from anthracnose stalk rot can range from 2–10% or higher if lodging occurs. Although much progress has been made in understanding the signaling pathways leading to disease resistance in model plants, less is known about the biochemical and genetic basis of fungal pathogenicity in economically important crops. This has hindered the development of alternative disease control strategies for major row crops. To address this need, we set out to understand the biochemical mechanisms involved in resistance of tropical maize to *Colletotrichum graminicola*. This work was conducted in collaboration with researchers at the University of Delaware. Resistance to anthracnose stalk rot in the tropical maize line MP305 had been localized previously to a QTL region on chromosome 4 by RFLP mapping. Fine-mapping of this QTL from MP305 in near-isogenic maize lines has enabled the map-based cloning of a novel LRR-containing R gene, termed *Rcg1*. The role of *Rcg1* in conferring anthracnose stalk rot resistance was confirmed by Mu-transposon knock-out strategies. A survey of public maize germplasm showed that *Rcg1* is present rarely; when present, it is found mainly in older open-pollinated tropical material. This data indicates that *Rcg1* may have been lost in the selection of elite genetics.

THE CHALLENGE OF CHEMICAL CONTROL OF PLANT DISEASES. **A.J. Leadbeater.** *Syngenta Crop Protection AG, Schwarzwaldallee 215, CH-4058, Basel, Switzerland. Email: andy.leadbeater@syngenta.com*

Since the first fungicide, sulphur, was used to control powdery mildew on grapes, production of most crops has become dependent on the use of fungicides to avoid disease losses. In the late 1840s the Irish potato famine proved the necessity for chemical intervention to prevent human and economic disaster. Recently it has become increasingly difficult for growers to control crop diseases. Genetic resistance of crops towards diseases has been in many cases short-lived (for example cereal rusts), GMOs have only limited success for disease control and acceptability. With more intensive cropping, new diseases have arisen which are devastating if not controlled, such as Asian Rust of soybean. In addition, new more aggressive pathotypes of diseases have arisen. All these changes require the rapid development of chemical control measures to prevent economic disaster, since reliance on genetic resistance and cultural techniques have been insufficient. Intensive use of chemical control measures has in turn led to it own challenges, including fungicide resistance. The sustainable use of fungicides to prolong their effectiveness and usefulness to growers is key, and the implementation of resistance management strategies an essential part of this. Only if the long-term effectiveness of fungicides can be ensured will industry invest the money and resources required for their discovery and development, especially considering the high standards of today's registration requirements. The Fungicide Resistance Action Committee (FRAC) and its network play a vital role in the design and support of these strategies.

CONCEPTS IN CHEMICAL CONTROL

REGULATORY ASPECTS IN CHEMICAL CONTROL OF FUNGAL DISEASES: IMPACT ON EFFICIENT PLANT PROTECTION. **G.F. Backhaus.** *Federal Biological Research Centre for Agriculture and Forestry, Messeweg 11/12, Braunschweig, Germany. Email: g.f.backhaus@bba.de*

Chemical plant protection measures against plant diseases have a long history. In particular since the 19th century chemicals containing copper, sulphur, or phenolic compounds have been successfully used. From the middle of the 20th century new fungicides were invented which proved to be efficient against dangerous pathogens like powdery and downy mildews or *Phytophthora infestans*. However, very soon farmers demanded an official approval, by special authorities, for those substances to guarantee sufficient efficacy and no phytotoxicity. In Germany, this was the main reason for the first obligatory testing of plant protection products (PPPs), laid down in the plant protection act of 1968. Nowadays PPPs are intensively tested in the frame of the European evaluation of active substances and the national registration procedures for efficacy, phytotoxicity and resistance management and, in particular, for their impacts on human and animal health, the ground water and the environment. In addition, many regulations have been set up for the use of registered PPPs in the fields with special regards to application machinery, user protection, and distances to water bodies, natural habitats or human living areas. Current discussions in the European Union suggest that such regulations may become even stricter in the future, even though this might enhance harmonization between European countries. In contrast, other groups of experts wish to extend the worldwide development of strict quarantine regulations to prevent spread and establishment of dangerous pathogens including treatments with PPPs. The manifold impact of those regulations on practical plant protection against fungal diseases is discussed.

CONTRIBUTION OF GENOMICS IN REVEALING THE MODE OF ACTION OF FUNGICIDES. **R. Beffa, F. Schmitt, D. Nennstiel, J.L. Zundel, P. Perret, A.L. Mauprivez, A. Lappartient, V. Toquin, F. Villalba, T. Knobloch, V. Lempereur and M.-H. Lebrun.** *BCS Biochemistry department and UMR5240 CNRS-UCB-INSA-BCS, Functional Genomics of Plant Pathogenic Fungi, Bayer Cropscience, La Dargoire, Research center, 14-20 rue P Baizet, 69263 Lyon Cedex 09, France. Email: roland.beffa@bayercropscience.com*

The expanding field of fungal genomics fuels the development of genome wide functional tools and comparative analyses in plant pathogenic fungi. As a consequence, transcriptomics, proteomics and metabolomics studies coupled with high throughput forward and reverse genetics are now available in a significant number of fungal plant pathogens (*e.g. Ustilago maydis*, *Magnaporthe grisea*, *Fusarium graminearum*, *Botrytis cinerea*). These functional and their associated bioinformatics tools are essential in accelerating the discovery of biochemical targets required to develop novel fungicides. These tools also offer the possibility to accelerate the identification of the biochemical mode of action of novel fungicides. This knowledge is required to characterize and follow efficiently the emergence of resistance. We will review the available genomic tools in plant pathogenic fungi. Several case studies will be discussed by comparing the information obtained using classical biochemical approaches and adequate genomic and bioinformatics tools. These studies have shown that the efficient discovery of modes of action requires a multi-disciplinary approach involving cellular biology, biochemistry, molecular genetics and genomics.

GENOMICS-BASED DISCOVERY OF ANTIFUNGAL TARGETS INVOLVED IN PATHOGENICITY ON PLANTS. **M.-H. Lebrun, K. Lambou, C. Ant, J. Collemare, M. Droux, O. Frelin, C. Ribot, C. Barbisan, M.-J. Gagey, A. Lappartient, P. Perret, F. Villalba, D. Nennstiel, F. Schmitt, J.-L. Zundel, and R. Beffa.** *UMR5240 CNRS-UCB-INSA-BCS, Functional Genomics of Plant Pathogenic Fungi and BCS Biochemistry department, Bayer Cropscience, La Dargoire, Research center, 14-20 rue P Baizet, 69263 Lyon Cedex 09, France. Email: marc-henri.lebrun@bayercropscience.com*

Genomics of plant pathogenic fungi is an expanding field (12 available and 6 ongoing genome sequences). This great resource covers the main crop diseases and is boosting functional analyses. Comparative genomics is also starting to unravel the evolutionary trends shaping fungal genomes. First analyses suggest fungal plant pathogens do not significantly differ from their related saprophytes, except for the expansion of gene families involved in secondary metabolism and signaling, as well as genes families encoding secreted proteins. Some pathogenicity factors are conserved in a wide range of fungi (Pmk1 MAP kinase), while others are only involved in the infection process of few fungi (Hog MAP kinase). This information is needed to identify conserved pathogenicity factors as possible targets for novel fungicides. The tetraspanin Pls1 was first identified in the rice blast fungus *Magnaporthe* as essential for appressorium mediated penetration. Further studies showed that it is required for the penetration of unrelated fungi (*Colletotrichum*, *Botrytis*). Pls1 is a membrane protein likely involved in the traffic of appressorial membrane/secreted proteins required for the formation of the penetration peg. Identification of partners of Pls1 using yeast two hybrids allows the development of high throughput screens (HTS) for this type of target. The role of amino acid biosynthetic pathways in infection was evaluated using targeted auxotrophic mutants. Some pathways are not essential for infection, while others do, in particular the methionine biosynthesis required for penetration and colonization of the plant. Regulatory networks are also interesting possible fungicide targets. The transcription factor (TF) Bip1 is essential for the penetration of *Magnaporthe* into its host plant. Identification of the promoters controlled by Bip1 allows the setting of HTS for this type of target. Finally, the development of novel cell wall inhibitors such as echinochandins pointed out the role of the Slt2 MAP kinase pathway in the basal cellular resistance to these fungicides. In conclusion, the recent expanding field of fungal genomics is increasing our understanding of fungal pathogenicity on plants and may lead to the discovery of novel highly active and specific fungicides.

REDUCTION OF MYCOTOXINS THROUGH CHEMICAL CONTROL. **P. Nicholson and P. Jennings.** *John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK. Email: paul.nicholson@bbsrc.ac.uk*

Fusarium head blight (FHB) of wheat and barley constitutes a disease complex involving toxin-producing species (e.g. *Fusarium culmorum,* and *F. graminearum*) and non toxin-producing species (e.g. *Microdochium majus* and *M. nivale*). The chief toxins produced are trichothecenes, of which deoxynivalenol (DON) and nivalenol (NIV) are most commonly associated with FHB. Fungicides tend to be differentially active against the toxin-producing and non toxin-producing species that cause FHB. For example

strobilurin chemistry, such as azoxystrobin, tends to exhibit high activity against *Microdochium* species but only limited activity against *Fusarium* species while the converse is observed for azoles such as tebuconazole or metconazole. Effective control of FHB through the application of fungicide is challenging, not least because the proportion of toxin-producing and non-producing species differs markedly across locations and, critically, these fungi are antagonistic to one-another. Where *Fusarium* and *Microdochium* species co-exist the application of azoxystrobin may lead to increases in the toxin content of grain. Other factors affecting fungicide efficacy include application rate, with control of FHB decreasing with decreasing fungicide dose, and the time of application with fungicides being at their most effective when applied during crop anthesis and within ±2-3 days of inoculum arriving at the ear; outside of this window fungicide efficacy drops off dramatically. The new azole fungicide prothioconazole exhibits very high activity against *Fusarium* spp. while possessing moderate efficacy against *Microdochium* spp. The potency of this compound provides greater flexibility to the grower, not only due to the wider range of species it is active against, but also because it is effective over a wider application window. However, even with the introduction of this new azole chemistry, where disease pressure is high and the resistance of crop varieties is low it remains challenging to limit DON accumulation to below EU permitted levels. An integrated approach involving improved varieties and agronomic practices combined with precision application of potent antifungal compounds is required to eliminate the risk of mycotoxins entering the cereal food chain.

Induced resistance

RESISTANCE INDUCED BY RHIZOBACTERIAL VOLATILES. **C.-M. Ryu, H.-S. Yi, Y.-R. Ahn, H. Zhang, M.A. Farag, P.W. Paré, and J.W. Kloepper.** *Systems Microbiology Research Center, KRIBB, Daejeon, Republic of Korea. Email: cmryu@kribb.re.kr*

Certain plant growth-promoting rhizobacteria (PGPR), in the absence of physical contact with plants, stimulate plant growth and elicit induced systemic resistance (ISR) *via* volatile organic compound (VOC) emissions. Gas chromatographic analysis of VOCs collected from the PGPR strains *Bacillus subtilis* strain GB03 and *Bacillus amyloliquefaciens* strain IN937a reveals consistent patterns in VOC emissions in comparison with non-growth-promoting strains such as *E. coli* DH5α. The two most abundant compounds, 2,3-butanediol and 3-hydroxy-2-butanone, are consistently emitted from GB03 and IN937a while these metabolites are not released from DH5α. Of several *Arabidopsis* mutant lines tested for regulatory control of ISR against *Erwinia carotovora* subsp. *carotovora*, only the ethylene-insensitive line (*ein*2) did not exhibit an amelioration of disease symptoms when *Arabidopsis* plants were pre-treated with GB03 volatiles. To assess potential utilization of PGPR VOCs for crop plants, volatile blends from GB03 and IN937a have been applied to pepper and tobacco roots. Bacterial survival capacity of 2,3-butanediol null mutants was significantly reduced in proximity with plant tissue. These reduced bacterial survival rates suggest that in addition to bacterial VOCs triggering plant growth and ISR in plants, such chemicals provide protection for PGPR via chemical signaling within the host plant. Our results suggest that 2,3-butanediol produced by *B. subtilis* may serve dual functions: to elicit indirectly ISR in the leaves and directly the production of plant antimicrobial compounds in the roots, and to act as a protecting agent for bacterial cells against the compounds.

THE ROLE OF PRIMING IN INDUCED RESISTANCE. **B. Mauch-Mani.** *University of Neuchâtel, Laboratory of Molecular and Cellular Biology, Rue Emile-Argand 11, C.P. 158, CH-2009 Neuchâtel, Switzerland. Email: brigitte.mauch@unine.ch*

In plants, a primary infection of above-ground parts by necrotizing pathogens or the colonization of roots with certain beneficial microbes can induce a unique physiological state called "priming". This primed state can also be reached through treatment with various natural and synthetic compounds. Such primed plants display more rapid and stronger (potentiated) activation of the numerous cellular defence responses that are induced in response to an attack by pathogens or pests or exposure to abiotic stress. Although the phenomenon has been known for years, most progress in our understanding of priming has been made only recently. Priming for tolerance to biotic and abiotic stress operates via various metabolic pathways. Priming in plants shows phenotypic similarity to potentiation phenomena seen in the overall defence response of animals including man, suggesting that the mode of action of priming and the resulting potentiation of cellular defence responses, rather than the direct up-regulation of defence signalling cascades, may be of great advantage for living organisms. Consequently, plants in the primed state are efficiently protected against stress without major trade-off effects on commercially and ecologically important traits, such as growth and seed set. Current knowledge of priming in various induced-resistance phenomena in plants will be summarised.

SIGNALING DURING INDUCED SYSTEMIC RESISTANCE IN *ARABIDOPSIS*. **C.M.J. Pieterse, S. Van der Ent, M.H.A. Van Hulten, L.C. Van Loon, M.J. Pozo, J.Ton and S.C.M. Van Wees.** *Plant-Microbe Interactions, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands. Email: C.M.J.Pieterse@uu.nl*

The alarm signal molecules salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are major regulators of induced plant defence. Their signalling pathways cross-communicate, providing the plant with great potential to fine-tune its defence reaction. Plants of which the roots have been colonized by specific strains of non-pathogenic, fluorescent *Pseudomonas* spp. develop a JA/ET-dependent form of protection that is called rhizobacteria-induced systemic resistance (ISR). We identified a MYB-type transcription factor gene, *AtMYB72*, whose expression is specifically up-regulated in the roots upon colonization by ISR-inducing rhizobacteria. Mutant analysis revealed that AtMYB72 is an essential part of local signalling events in the roots, which eventually lead to systemic expression of ISR in the leaves. Transcript profiling of ISR-expressing *Arabidopsis* leaves revealed that the onset of ISR is not associated with detectable changes in gene expression. However, upon pathogen attack, a large set of predominantly JA/ET regulated genes showed potentiated expression. Evidently, ISR-expressing plants are primed for augmented expression of pathogen-inducible genes, which might allow the plant to react more effectively to a broad spectrum of pathogens. The promoters of JA-responsive, ISR-primed genes appeared to be significantly enriched in binding sites for the transcription factor MYC2, suggesting an important role for this transcription factor in priming. Priming is a cost-effective process that provides the plant with enhanced capacity for rapid and effective activation of cellular defence responses that are induced only after contact with a pathogen, resulting in ecological fitness benefits when plants are grown under pathogen pressure.

ROLE OF REDOX STATE IN SYSTEMIC ACQUIRED RESISTANCE. **P.R. Fobert, H. Shearer, K. Hahn, L. Wang, A. Rochon, P. Boyle and C. Després.** *Protein Research Group, National Research Council Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon SK, S7N 0W9, Canada. Email: Pierre.Fobert@nrc-cnrc.gc.ca*

In *Arabidopsis*, defense responses against biotrophic pathogens, including systemic acquired resistance (SAR), require the phenolic metabolite salicylic acid (SA) and the non-expression of the pathogenesis gene *NPR1*. Exogenous application of SA increases the ratio of reduced to oxidized glutathione, the major cellular redox buffer. SA treatment also triggers monomerization and nuclear localization of NPR1, presumably via reduction of conserved cysteine residues. Nuclear localization of NPR1 is required for its interaction with TGA transcription factors and the activation of *PR* genes. Our recent findings indicate that NPR1 and TGA2 form an SA-dependent enhanceosome able to activate *PR-1*. This enhanceosome requires the oxidation of NPR1 Cys-521 and Cys-529 as well as the core of the NPR1 BTB/POZ domain. With respect to redox control of TGA factors, we previously reported that NPR1 preferentially interacts with the reduced form of TGA1 clade transcription factors, which predominate following SA treatment. To ascertain the contribution of this clade of TGA factors to defense responses, we have now isolated T-DNA insertion mutants and shown that they are compromised in resistance to the bacterial pathogen *Pseudomonas syringae*. The mutants display altered levels of phytohormones and patterns of PR gene expression. Using yeast two-hybrid screens, we have also identified an interaction between TGA factors and a novel class of glutaredoxin-like proteins. These proteins co-localize with TGA2 in plants and are encoded by genes that are responsive to both SA and pathogens.

Mycotoxins

ECOPHYSIOLOGY OF MYCOTOXIGENIC FUNGI: UNDERSTANDING FUNCTION FOR BETTER CONTROL. **N. Magan and R. Geisen.** *Applied Mycology Group, Cranfield Health, Cranfield University, Bedford, MK43 0AL, UK. Email: n.magan@cranfield.ac.uk*

The production of mycotoxins such as trichothecenes, aflatoxins, ochratoxins are predominantly dependent on nutritional matrices, and interacting environmental factors such as temperature, pH and water availability. Ecophysiological studies have determined the profiles for germination, growth and mycotoxin production to enable predictive modelling approaches to be developed. Often under environmental stress, growth is unrelated to levels of mycotoxin production. Sometimes growth inhibition occurs while significant toxin production occurs, especially under fluctuating environmental conditions. The relationship between such abiotic factors, growth and the triggering of toxin gene cluster expression need to be related to phenotypic toxin production. We have thus examined the impact of interacting environmental factors on trichothecene, aflatoxin and ochratoxin biosynthetic genes and clusters using toxin gene microarrays and quantitative PCR and correlated expression with phenotypic mycotoxin expression. The efficacy of interacting abiotic stresses on gene clusters (e.g. tri genes; *F. culmorum, F. graminearum)* were elevated and paralleled high phenotypic expression of DON and vice versa. This now provides tools for targeted approaches to evaluate and test control systems directly on these toxin gene clusters under simulated environmental regimes. Recent studies with *otapks* gene expression for *P. verrucosum* demonstrated that sub-optimal preservatives, especially under water stress conditions, lead to significant stimulation of expression and phenotypic ochratoxin production. These targeted arrays will enable a better understanding of the influence of interacting abiotic and plant factors on regulation of important toxin gene clusters and be an excellent tool for examining novel chemical and non-chemical control strategies for prevention of mycotoxins entering the food chain.

GENOMICS, GENE EXPRESSION, AND THE CONTROL OF AFLATOXIN CONTAMINATION. **G.A. Payne, A.L. Dolezal, D.M. Nielsen and C.P. Woloshuk.** *Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7567, USA. Email: gary_payne@ncsu.edu*

Contamination of maize with the carcinogen aflatoxin remains a worldwide problem. Effective control strategies are lacking, leading to serious health concerns and economic losses. An available genome sequence and Affymetrix GeneChip microarrays for *Aspergillus flavus* provide new resources to better understand the regulation of aflatoxin biosynthesis and the interaction of the fungus with its host. The Affymetrix GeneChip microarray contains elements representing both the predicted genes in *A. flavus* and 8,000 genes expressed in maize seeds. Transcriptional analysis of developing seeds infected with *A. flavus* in the field revealed a suite of plant and fungal genes uniquely expressed in the host-parasite interaction. The differentially expressed fungal genes were not expressed under several cultural conditions, and the host genes were not expressed in wounded but non-inoculated kernels. Some of the differentially expressed fungal genes showed similarity to pathogenicity factors previously described for other pathogens. Among the differentially expressed genes in the host were well-characterized genes expressed in response to foliar pathogens. These data support a hypothesis that *A. flavus* and maize seeds perceive one another, and that maize seeds respond to infection by the opportunistic pathogen *A. flavus* similarly to how they respond to more aggressive plant pathogens. A better understanding of the interaction between *A. flavus* and its host will aid in the development of resistant germplasm.

CONTRIBUTION OF ERGOT ALKALOIDS TO SUCCESS OF A FUNGAL ENDOPHYTE-GRASS SYMBIOSIS. **D.G. Panaccione, C.L. Schardl and D.A. Potter.** *West Virginia University, Morgantown, WV 26506-6108, USA. Email: danpan@wvu.edu*

Fungi that produce ergot alkaloids often accumulate them in a complex profile that includes large quantities of certain pathway intermediates and spur products in addition to the pathway end product. Inefficiency in the pathway leading to accumulation of multiple ergot alkaloids may be maintained because the multiple products account for multiple benefits for the producing organism. *Neotyphodium* sp. Lp1, an endophyte of perennial ryegrass (*Lolium perenne*), accumulates high concentrations of the ergot alkaloid pathway intermediate chanoclavine and the spur product 6,7-secolysergine (both of the clavine class of ergot alkaloids), as well as ergine (from a spur in the later portion of the pathway), during its biosynthesis of the ultimate pathway product ergovaline. Perennial ryegrass infected with a strain in which the first pathway gene (dmaW) was knocked out accumulated absolutely no ergot alkaloids; strains in which a gene (*lpsA*) controlling a later step was knocked out accumulated the clavines chanoclavine and 6,7-secolysergine but not ergine or ergovaline. Comparison of grasses containing the mutant endophytes to uninfected grasses or those containing wild-type *Neotyphodium* sp. Lp1 showed that the clavines deterred feeding by rabbits (*Oryctolagus cunicu-*

lus) but were not effective against black cutworm (*Agrotis ipsilon*). Conversely, later pathway products ergine and ergovaline deterred feeding and decreased weight gain and survival of black cutworm. The data suggest that different ergot alkaloids in the complex profile produced by *Neotyphodium* sp. Lp1 contribute to different bioprotective abilities conferred by this endophyte to its host grass.

BIOLOGICAL AND CHEMICAL COMPLEXITY OF *FUSARIUM PROLIFERATUM*. **A.E. Desjardins, R.H. Proctor and M. Busman.** *National Center for Agricultural Utilization Research, United States Department of Agriculture, 1815 N. University St., Peoria, Illinois, 61604, USA. Email: anne.desjardins@ars.usda.gov*

The heterothallic ascomycete *Fusarium proliferatum* (teleomorph *Gibberella intermedia*) is a genetically diverse biological and phylogenetic species with a worldwide distribution and an unusually broad host range. *F. proliferatum* is a frequent component of the *Fusarium* ear rot complexes of maize and wheat, and also causes diseases of plants as diverse as asparagus, garlic, reed, rice, and various palm species. *F. proliferatum* produces a strikingly wide range of mycotoxins and other biologically active metabolites, including fusaric acid, moniliformin, terpenoids (fusaproliferin), cyclic peptides (beauvericin and enniatins), and polyketides (bikaverin, fumonisins, fusarins). In addition to polyketide synthase (PKS) genes for these three metabolites, *F. proliferatum* contains a PKS for perithecial pigment, and at least seven additional PKS genes of unknown function. We have begun production of PKS gene-deletion mutants of *F. proliferatum*, starting with *FUM1*, the PKS gene required for the biosynthesis of carcinogenic fumonisin mycotoxins. Our objective is to determine the functions of fumonisins and other fungal metabolites in development and plant pathogenicity of *F. proliferatum*.

Innovative disease control strategies

NEW METHODS FOR THE MANAGEMENT OF PLANT PARASITIC NEMATODES. **R.A. Sikora.** *INRES-Phytomedizin, Soil Ecosystem Phytopathology and Nematology, University of Bonn, Nussallee 9, D-53115 Bonn, Germany. Email: rsikora@uni-bonn.de*

Integrated management of plant-parasitic nematodes is undergoing major and in some cases uncontrollable changes. Rotations used for nematode management are being altered drastically by the increasing need for food, feed, fibre and more recently biofuel. Demand and high commodity prices are affecting crop selection which then dramatically changes the structure of traditional cropping systems. These alterations are often made without considering their impact on pest control. The influence of agriculture on climate will also affect how crops are managed in the future. These factors will require a new IPM mind-set with regard to combating damage caused by nematodes. For example, shorter and near-monoculture rotations will require new sources of resistance. The few genes available, coupled with near-monoculture rotations will promote pathotype selection and complicate management. However, the results from functional genomics, and development of genetically modified crops with novel genes for nematode resistance could lead to a reduction in nematode damage on some crops. New seed treatment technologies and new nematicides could be important in reducing early root infection in the pathozone with simultaneous reduction in environmental impact. Biological enhancement of planting material with biocontrol agents will also improve protection of the root system. Advances in remote sensing and site-specific treatment will be needed to reduce treatment cost. These technologies will be discussed and their advantages and disadvantages for nematode management outlined.

QUORUM-SENSING AS A TARGET FOR NOVEL BIOCONTROL STRATEGIES DIRECTED AT *PECTOBACTERIUM*. **Y. Dessaux, A. Cirou, S. Uroz, X. Latour and D. Faure.** *Institut des Sciences du Végétal, CNRS, 91198 Gif-sur-Yvette, France. Email: dessaux@isv.cnrs-gif.fr*

In bacteria, quorum-sensing (QS) permits a synchronous and cell density-dependant expression of target genes. In the plant pathogen *Pectobacterium,* production of maceration enzymes and the toxic peptide harpin are under QS control, with N-acyl-homoserine lactones (AHLs) as essential signals. However, several organisms have evolved the ability to inactivate AHLs by producing degradative enzymes (AHLases). We and others have identified numerous bacteria with AHLase activity. Amongst these, *Rhodococcus erythropolis* strain W2 showed very strong AHLase activity due to the production of an AHL lactonase, an amidohydrolase and an oxido-reductase (Uroz *et al.*, 2003; 2005). Recently, we have characterized the lactonase genetic determinant that codes for a phosphotriesterase-like enzyme, unrelated to known AHL lactonase. Strain W2 actively reduces or suppresses the virulence of *Pectobacterium carotovorum* in potato tuber assays demonstrating the value of this quorum-quenching strategy. Further experiments were undertaken to promote the bacterial community naturally degrading AHL in a plant environment (Cirou *et al.*, Env. Microbiol., in press). Based on their structural relatedness with AHL, biochemicals were assayed for their possible role in favoring the growth of this functional community. One compound was identified that indeed promoted the AHL degradative potential of the rhizobacterial community, via the selection of AHL-degrading bacteria. Preliminary experiments using a potato tuber assay showed that the community selected by this compound actively protected the plant from *Pectobacterium*-induced disease, opening a path for novel biocontrol approaches.

INNOVATIVE FUNGICIDES AND INNOVATION IN THEIR USE. **H.-W. Dehne.** *Institute of Crop Science and Resource Conservation, Nussallee 9, D-53115 Bonn, Germany. Email: hw-dehne@uni-bonn.de*

Fungicides are an important tool in plant production – especially to ensure the quality of food and feed at high yields. The development of fungicide resistance within pathogen populations and changes in the incidence of particular plant pathogens as well as changes in pathogen populations stimulate efforts to create new antifungal compounds with new modes of action. Most new compounds within a particular group of fungicides show higher biological efficacy as well as a different or broader spectrum of activity. Recently, fungicides with new modes of action have been identified. Their development has led to improved potential for plant disease control. Innovations in the use of fungicides are provided by more efficient formulations of active compounds and new additives. Furthermore the application of innovative tools for diagnosis of plant pathogens improves the decision process for use of the most efficient fungicide or fungicide combination. Methods for forecasting disease development and the influence of disease progress on yield can lead to more accurate timing of fungicide application, and this leads to more efficient

disease control. Innovative application techniques range from air-assisted spraying equipment to point-application. They lead to increased efficacy of fungicide use with simultaneously reduced environmental side effects.

MICROBIAL COMMUNITY MANAGEMENT TO CONTROL SOILBORNE PLANT DISEASES. **L. Kinkel.** *Department of Plant Pathology, 495 Borlaug Hall, University of Minnesota, Saint Paul, MN, USA. Email: kinkel@umn.edu*

Soils support a diverse microflora, including plant pathogens, symbionts, saprophytes, and pathogen antagonists. However, we have limited understanding of the ecological and evolutionary dynamics of these distinct functional groups in soil. Among these, antibiotic-producing bacterial antagonists have been used widely in the biological control of plant pathogens. In Minnesota, a naturally-occurring disease suppressive soil supported *Streptomyces* communities having significantly greater densities, inhibitory activities, and a greater diversity of antibiotic phenotypes than communities in an adjacent disease-conducive soil. We hypothesized that density- and frequency- dependent selection are important to generating and maintaining a high intensity and diversity of inhibitory activities within the suppressive soil. Extensive sampling in native prairie soils provides support for the hypotheses of frequency and density-dependent selection, and specifically that local coevolutionary interactions are important to pathogen inhibitory activity in soil. In further research, we have explored the potential for green manures to impose density-and frequency-dependent selection on the soil microbial community and enhance pathogen suppressive activity. Specifically, we have shown that green manures can enhance the frequency and intensity of antibiotic inhibitory activities within soilborne *Streptomyces* communities, and can reduce the intensity of plant diseases on multiple host crops. More broadly, our results suggest that long-term management strategies for soilborne plant pathogens should incorporate active efforts to maintain high densities of indigenous soil microbes in agricultural soils, and that regular integration of green manures into cropping systems may be beneficial to the development of effective and sustainable disease control.

Precision agriculture and plant pathology

PROSPECTS FOR PRECISION AGRICULTURE TO MANAGE AERIALLY DISPERSED PATHOGENS IN A PATCHY LANDSCAPE. **D.E. Aylor and F.J. Ferrandino.** *The Connecticut Agricultural Experiment Station, Dept. of Plant Pathology and Ecology, P.O. Box 1106, New Haven, CT 06504, USA. Email: Donald.Aylor@po.state.ct.us*

Precision application of disease control measures for an aerially dispersed pathogen depends on the spatial scale and temporal dynamics of pathogen spread and host development. These dynamics can be expressed in terms of basic biological and physical properties, *viz*., latent period, infectious period, basic infection rate, dispersal distance and survival time scales, host phenology, and the level of acceptable risk. A mean waiting time for new infections to appear on discrete patches of host plants a certain distance from a focus of disease is defined in terms of these basic parameters. We illustrate how this waiting time can be used to help establish guidelines for minimizing application of fungicides, while maintaining acceptable yield. We examine the following questions: 1) Once disease or the pathogen is detected locally, can a safety zone around a focus be protected without spraying the whole field? 2) When can small fields separated by a given distance be treated as separate management units? 3) For hosts distributed on the regional or landscape scale, can we define waiting times that allow us to forgo or delay control measures in a neighboring region? These ideas will be illustrated using apple scab, potato late blight, tobacco blue mold, and stem rust of wheat as examples.

MODELLING PLANT DISEASE EPIDEMICS FOR CROP PROTECTION. **V. Rossi.** *Institute of Entomology and Plant Pathology, "Sacro Cuore" Catholic University, Via Emilia Parmense 84, 29100 Piacenza, Italy. Email: vittorio.rossi@unicatt.it*

Initially plant disease models were developed as simple rules, graphs, or tables, and later as descriptive tools. Advances in environmental monitoring, automatic data processing, and botanical epidemiology enabled the development of a new class of mechanistic dynamic models, which are more accurate and robust. They explain mathematically the relations within a pathosystem (including both pathogens and host plants) by means of linked differential equations, and describe the way in which the system changes over time and space as an effect of external variables. Thus, the equation parameters do not have fixed values but vary according to the influencing weather conditions. These models require input data, particularly meteorological data, to be collected over time and space. Scales of time and space for inputs may differ according to the application of the model: from warning services, which use models to produce crop protection information at the collective level on a territorial scale, to precision agriculture which uses models at a within-plot scale. While the use of mechanistic dynamic models in warning services for crop protection is well established, their use in precision agriculture has yet to be developed. These models could be used to draw dynamic maps of current and future spatial distribution of both visible and latent infections within a plot, so that timing, active ingredients and rates of fungicides may be defined accordingly. The main challenge that needs to be overcome before this can be accomplished is the lack of meteorological inputs at the within-plot level.

DETECTION AND MONITORING OF AIRBORNE PATHOGENS BY OPTICAL REFLECTANCE AND AIR SAMPLING. **J.S. West, S.L. Rogers, S.D. Atkins and B.D.L. Fitt.** *Rothamsted Research, Harpenden, AL5 2JQ, UK. Email: jon.west@bbsrc.ac.uk*

To detect and monitor progress of airborne pathogens it is possible to assess disease symptoms or to detect airborne inoculum before infection occurs. Numerous optical techniques have been used to detect early disease symptoms. Chlorophyll fluorescence often changes before visible symptom expression but is difficult to measure in crops and provides only a warning of imminent disease without identifying the causal agent. Changes in the canopy spectral signature vary with host-pathosystem but generally a reduction in the ratio of green to far-red light reflected from the canopy indicates chlorophyll reduction associated with disease development. Imaging rather than spectrographic techniques are usually needed to distinguish chlorophyll reductions caused by discrete leaf lesions from overall reductions caused by nutrient deficiency. In practice, it is often necessary to apply fungicides to entire fields by the time visible foci of diseases such as stripe rust are observed. Monitoring inoculum in air can provide an earlier warning of potential disease. Immunological or molecular diagnostic techniques applied to air samples have improved accuracy and quality of information gained, compared to

identifying and counting spores by microscopy. Spore numbers can be estimated directly from the amount of pathogen DNA detected by quantitative PCR and additional genotypic information, such as presence of genes conferring fungicide resistance, can be obtained. Recent research suggests that integration of spore trapping with quantitative PCR has great potential for forecasting diseases of arable crops and for monitoring changes in populations such as development of fungicide resistance.

APPLICATION OF REMOTE SENSING TECHNOLOGIES FOR STUDY OF *WHEAT STREAK MOSAIC VIRUS*. **C.M. Rush, F. Workneh, J. Price, D. Jones and S.R. Evett.** *TAES 2301 Experiment Station Road, Bushland, TX 79012, USA. Email: cm-rush@tamu.edu*

A variety of remote sensing technologies have been developed and several have been used extensively to study plant diseases. However, no instrument or technology is suitable for all situations and care must be taken to ensure that a specific technology is capable of acquiring the type of data needed for a particular study. In preliminary research, a handheld hyperspectral radiometer was used to differentiate nitrogen deficient wheat (*Triticum aestivum*) from wheat infected by *Wheat streak mosaic virus* (WSMV). Results were often ambiguous and unreliable. Later, Landsat 5 images were acquired from January - May for one county in the Texas Panhandle. The first image was acquired in January 2006 to identify dormant wheat fields. Normalized difference vegetative index (NDVI) was calculated using ENVI software to identify wheat fields and create a mask on subsequent imagery. For ground truth data, fields with wheat streak mosaic (WSM) were identified and rated for severity. Data were used as the basis for classification within the mask of the image. Initially, 56,168 ha of wheat were detected by Landsat imagery and of those 17,893 ha were later classified as severely infected with WSMV. Furthermore, 95% of the known ground truth points exhibiting severe WSM were classified correctly. In additional studies, a handheld hyperspectral radiometer was used to quantify severity of WSM. Correlation coefficients for reflectance at 550 nm × disease rating averaged 0.83 and were highly significant. Remote sensing technologies will be useful in developing automated site-specific irrigation systems for wheat.

DISEASES OF SOILLESS CROPS

THE STATUS OF BIOLOGICAL CONTROL OF PLANT DISEASES IN SOILLESS CULTIVATION. **J.M. Whipps and J. Postma.** *Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK Email: john.whipps@warwick.ac.uk*

Avoidance of plant diseases has been a major driver for the development of soilless cultivation systems. Nevertheless, diseases still occur in these systems and the need for additional control measures exists. Traditionally, control has relied on the use of chemical fungicides but environmental pressure to reduce chemical usage in the environment, and fewer active ingredients registered for use, has stimulated the development of biological methods of disease control. One approach has been to utilise microbial inoculants as straight replacements for chemical pesticides, and some commercial products are now available. Another approach has been to utilise slow sand filters (SSFs) to remove pathogens such as *Pythium* and *Phytophthora* species, and these SSFs depend upon the development of an active microbiota. Interestingly, in some cases, the use of growing systems that recirculate nutrient solution to save on water and waste have also exhibited disease suppression and this effect appears to depend, at least in part, upon the development of an active microbiota. Recent microbial population studies examining both culturable and non-culturable bacteria and fungi from SSFs and recirculating systems have begun to reveal the microorganisms associated with the development of pathogen suppression. Although some specific antagonists such as *Lysobacter* sp. with potential for use as microbial inoculants in these systems have been identified, it seems that in some cases, the development of suppression depends upon the establishment of an active, non-specific microbiota.

CHALLENGES, OPPORTUNITIES AND OBLIGATIONS IN BIOLOGY AND MANAGEMENT OF PLANT PATHOGENS IN IRRIGATION WATER. **C.X. Hong and G.W. Moorman.** *Virginia Polytechnic Institute and State University, 1444 Diamond Springs Road, Virginia Beach, Virginia, USA. Email: chhong2@vt.edu*

Plant pathogens in irrigation water were recognized early in the last century as a significant crop health issue. This issue has increased greatly in scope and degree of impact since that time and it continues to be a challenge as horticulture and agriculture increasingly depend on recycled water for irrigation. The present paper examines this issue in light of global agricultural water security, and reviews current knowledge of plant pathogens in irrigation systems and water treatments. Major challenges, opportunities and obligations in biology and management of waterborne pathogens and resultant diseases will be highlighted. The international community of plant pathologists is called to intensify research and collaborate with hydrologists, agricultural engineers, ecologists, geneticists, economists, statisticians and farmers to attack this complex issue of growing worldwide significance.

TRANSMISSION OF VIRUSES IN SOILLESS CULTIVATION SYSTEMS. **C. Büttner, M. Bandte, H.J. Echevarria Laza, U. Paschek, C. Ulrichs, D. Schwarz and W. Pestemer.** *Humboldt-Universität zu Berlin, Department Phytomedicine, Lentzeallee 55/57, D-14195 Berlin, Germany. Email: carmen.buettner@agrar.hu-berlin.de*

It has been shown that plant pathogenic viruses are widely spread in the environment and occur in soil and bodies of water like ditches, rivers, streams, lakes, sea water and even glacier ice. Plant viruses detected in water share certain features as they are stable, except a few samples like *Cucumber mosaic virus* (CMV) and *Tomato spotted wilt virus* (TSWV), possess wide host ranges, and occur in high concentration in plant tissue. All of them can infect plants through their roots. It has been demonstrated for a number of viruses that they can be released from undisturbed roots, and obviously from micro-wounded cells into the soil and water. Infected plants growing in the vicinity of water may thus likewise be a source of plant viruses in rivers and lakes. Dump material from vegetables and ornamentals as well as composts have to be considered as sources of viruses in surface waters. Plants will be repeatedly inoculated with viruses contaminating the water, independent of whether the initial source of water harbours viruses or viruses have entered the water along its course. Hence the use of hydroponic systems with recirculating nutrient solutions may facilitate virus transmission. Experiments on the transmission of many different viruses demonstrate the infection of plants through roots in recirculating irrigation systems within 1 to 3 months. Therefore the risk of dissemination of plant virus diseases often resulting in crop losses has to be evaluated before the assignment of recirculating or reused water.

METHODS FOR DETECTION AND QUANTIFICATION OF PLANT PATHOGENS IN RECYCLED IRRIGATION WATER. **C.A. Lévesque.** *Agriculture and Agri-Food Canada, Central Experimental Farm, 960 Carling Ave., Ottawa, ON, K2A 2P8, Canada. Email: levesqueca@agr.gc.ca*

The microbial ecology of the rhizosphere and growth substrate suffers from "black box" syndrome despite being a very important factor in root and plant health. The so-called unexplained inconsistencies between treatments applied in different locations after all physical and chemical parameters are measures and considered equal are often attributed to unknown microbial differences in the growth substrate. While molecular biology is providing new tools to study microbial populations, the vast diversity in natural soils slows illumination of the box. In natural soils, the clumped distribution of many pathogens becomes a major challenge for detection and quantification because DNA is often extracted from relatively small, localized samples. Soilless crops offer a good model for comprehensive microbial studies of rhizosphere and growth substrate because the biological diversity appears to be lower than in natural soil, and representative sampling of the substrate can be easily achieved in recirculated systems. Biosensors that continuously sample and test water are the holy grail of public health authorities responsible for drinking water quality, and such devices will eventually become available for modification and application in soilless crops. Different laboratories are characterizing the microbiota in this rather unique environment to better understand the microbe-microbe relationships that lead to plant health or root disease. Such characterization is essential for developing robust molecular detection tools amenable to routine sampling and testing. Understanding the interactions among microorganisms is essential to interpret the complex molecular detection data.

HOST-PATHOGEN INTERACTIONS

STRATEGIES OF PLANT VIRUSES TO OVERCOME SILENCING BASED ANTIVIRAL RESPONSE. **J. Burgyan.** *Agricultural Biotechnology Center, Godollo, Szent-Györgyi Albert 4, Pest 2100 Hungary. Email: burgyan@abc.hu*

Viruses are inducers, as well as targets of RNA silencing-based antiviral defence. Replication intermediates or folded viral RNAs activate RNA silencing, generating small interfering RNAs (siRNAs), which are the key players in the antiviral response. Viruses are able to counteract RNA silencing by expressing silencing-suppressor proteins. Many of the identified silencing-suppressor proteins sequester siRNAs to prevent the assembly of the RNA-induced silencing complex (RISC), which targets the corresponding viral RNA. However, viruses also developed alternative strategies to inhibit the antiviral plant response by targeting different component of silencing machinery. The molecular bases of the silencing suppressor strategies and their effects on endogenous silencing pathways will be also discussed.

ACTIVATION OF DEFENCES BY OLIGOGALACTURONIDES: HOW PLANTS SENSE AND RESPOND TO A BREACH IN THE WALL. **G. De Lorenzo.** *Dipartimento di Biologia Vegetale, Università di Roma La Sapienza, 00185 Roma, Italy. Email: giulia.delorenzo@uniroma1.it*

Upon tissue injury or pathogen infection, homogalacturonan (HGA) in the cell wall is broken down into smaller fragments (oligogalacturonides: OGs). OGs activate the plant innate immune response, acting as endogenous elicitors and alerting the plant cell of a breach in tissue integrity. OGs are therefore signals derived from an altered-self (host-associated molecular patterns or HAMPs) and microarray analysis shows that they induce responses largely overlapping those activated by PAMPs. In *Arabidopsis*, OGs increase resistance to the necrotrophic fungus *Botrytis cinerea* independently of jasmonate-, salicylic acid- and ethylene-mediated signalling. OG formation may be favoured by the interaction of fungal endopolygalacturonases with plant cell wall leucine-rich-repeat proteins (PGIPs: Polygalacturonase-inhibiting proteins). PGIPs possess an OG-binding domain and may take part in the perception of these oligosaccharides. Despite their simple primary structure, OGs not only induce defences but also have a wide range of effects on plant growth and development. Their activity as regulators of growth and development is related to their auxin antagonism. Similarly, OG-induced resistance to fungal infection is antagonized by exogenous auxin. By using both biochemical and genetic methods we are dissecting the OG perception/transduction pathway to elucidate the molecular basis of the OG/auxin antagonism and its significance in defence and development.

GENOME-WIDE CATALOGS OF OOMYCETE EFFECTORS: FROM STRUCTURE TO FUNCTION. **S. Kamoun.** *The Sainsbury Laboratory, Colney Lane, Norwich, NR4 7UH, UK. Email: sophien.kamoun@tsl.ac.uk*

Eukaryotic plant pathogens, such as oomycetes and fungi, secrete an arsenal of effector proteins to modulate plant innate immunity and enable parasitic infection. Deciphering the biochemical activities of effectors to understand how pathogens successfully colonize and reproduce on their host plants became a driving paradigm in the field. This presentation will focus on effectors of oomycetes such as *Phytophthora infestans*, arguably the most destructive pathogens of dicot plants. Tremendous progress has been made recently in understanding the biology of oomycete effectors. Two classes of effectors target distinct sites in the host plant: apoplastic effectors are secreted into the plant extracellular space, while cytoplasmic effectors are translocated inside the plant cell, where they target different subcellular compartments. Of particular interest are the RXLR and Crinkler (CRN) cytoplasmic effectors that are characterized by conserved motifs following the signal peptide. The RXLR domain is functionally interchangeable with a malaria host targeting domain and appears to function in delivery into host cells. The recent completion of five oomycete genome sequences enabled genome-wide cataloguing of the effector secretome revealing hundreds of candidate effectors. Effectors are frequently organized in clusters of paralogous genes, many of which exhibit hallmarks of positive selection probably as a result of a coevolutionary arms race with host factors. We also utilized the discovered RXLR effectors in high-throughput *in planta* expression assays to screen for avirulence and virulence activities. Understanding the perturbations caused by effectors is helping to unravel mechanisms of pathogenicity and plant defense.

HIDDEN ASPECTS OF INNATE IMMUNITY OF *ARABIDOPSIS* TO *BOTRYTIS CINEREA*. **J.P. Métraux.** *Department of Biology, University of Fribourg, Ch. du Musée 10, CH-1700 Fribourg, Switzerland. Email: jean-pierre.metraux@unifr.ch*

Botrytis cinerea is a ubiquitous pre- and postharvest necrotrophic pathogen with a broad host range that causes substantial crop

losses. Germinating *B. cinerea* conidia penetrate through the cuticle and epidermal walls leading to the death of invaded cells and to tissue softening, rot or necrosis, depending on the invaded parts. The cuticle and the cell wall are considered important potential barriers for this fungus. Unexpectedly, we observed several situations where this classical and intuitive notion turns out to be invalid. Firstly, a powerful resistance was observed against *B. cinerea* in plants impaired in cuticle structure and permeability (abnormal cuticle-associated resistance; ACR). They included plants overexpressing a gene encoding a fungal cutinase as well as mutants with alterations in various genes associated with synthesis of the cuticle. Secondly, strong immunity was also obtained in plants when *B. cinerea* spores were inoculated directly at wound sites (wound-induced resistance; WIR). The absence of symptoms after *B. cinerea* inoculation in ACR and WIR was associated with a strong decrease in hyphal growth compared to wild-type plants. This implies the deployment of active defence mechanisms in the plant. This talk will present an overview of what we have learned on the molecular basis of ACR and WIR with respect to early events, defence signalling and expression of genes involved in defence.

SOILBORNE PLANT DISEASE AND THEIR CONTROL

IPM FOR SOILBORNE DISEASE MANAGEMENT FOR VEGETABLE AND STRAWBERRY CROPS IN SE USA. F.J. Louws. *Dept. Plant Pathology, North Carolina State University, P.O. Box 7616, Raleigh NC 27695, USA. Email: frank_louws@ncsu.edu*

Implementation of methyl bromide (MB) alternatives in vegetable and strawberry production systems in NC and surrounding SE-States required a tiered approach. Tier I included the research and development of new products, methods of application or farming systems research on University research stations. Tier II engaged growers and other stakeholders in on-farm-research to evaluate practical issues and solutions to ensure implementation feasibility. Research efforts generated novel information on the biology of parasitic and beneficial microbes and included the development and implementation of chemical and non-chemical dependent strategies. Sixty different taxa amongst 1300 fungi and stramenopiles isolated from strawberry roots documented *Pythium irregulare* and *Rhizoctonia fragariae* AG-A, AG-G predominated. Important and less prevalent pathogens included *Pythium* HS and Group 'F', *Phytophthora cactorum* and a newly named species *Phytophthora bisheria* Abad, Abad & Louws sp. nov. Economic analysis (Sydorovych *et al.*, 2006, HortTech. 16:118-128) based on 2-15 years of data demonstrated chloropicrin alone (under low weed pressure conditions), Telone-C35 and metam sodium generated 25- 1670 $US in higher returns/A than MB; all superior to non-fumigated controls (-$6450). Technical issues remain. Compost-based farming systems combined with cover crops generated marketable strawberry yields of 90-104% compared to MB. Non-amended plot yields were 62-80%. *Verticillium* wilt race-2, *Fusarium* wilt, and bacterial wilt (*Ralstonia solanacearum*) were documented as major tomato pathogens. Fumigants with high chloropicrin content offered superior suppression of the fungi but only grafted plants provided effective bacterial wilt management. Novel IPM-based information was translated into train-the-trainer programs for agents and other consultants and extended to growers.

SUSTAINABLE APPROACHES FOR CONTROLLING SOILBORNE PATHOGENS. C.T. Bull. *USDA/ARS, 1636 E. Alisal St., Salinas CA 93901, USA. Email: cbull@pw.ars.usda.gov*

Sustainable cropping systems for the purpose of this discussion have three major imperatives: 1) environmental; 2) economic; and 3) social. Plant pathologists have traditionally been concerned with promoting economic sustainability with little regard for the other two imperatives. Thus, almost any approach taken by plant pathologists to control disease has been touted as sustainable if an economic edge is achieved regardless of environmental and social outcomes. The limitations of our training dictate that we must work in interdisciplinary teams to develop sustainable cropping systems. In these teams, plant pathologists bring to the table powerful tools to understand microbe-microbe and plant-microbe interactions. It is the context and process in which the knowledge gained is applied to solving disease problems within the cropping system that will determine if the approach is sustainable or not.

CONTROL OF SOILBORNE DISEASES IN ORGANIC SYSTEMS. A.H.C. van Bruggen. *Biological Farming Systems, Wageningen University, Marijkeweg 22, 6709PG, Wageningen, The Netherlands. Email: ariena.vanbruggen@wur.nl*

Conceptual approaches to plant disease control may differ fundamentally between organic and conventional growers. In organic plant production, whole-system integration is the primary management approach for preventing disease build-up, especially of soilborne diseases. Curative methods are reserved as a secondary control strategy. While some organic growers substitute manure for fertilizer and plant extracts for synthetic fungicides, most of them use a wide range of soil management and cropping practices to maintain soil and plant health. Crop rotations are adjusted, and plant- or animal-based soil amendments are applied at rates needed to maintain nutrient balances. In most cases, this is sufficient to suppress soilborne diseases. Consequently, soilborne disease incidence and severity are generally less in organic than in conventional plant production. Suppression of soilborne diseases in organic soils is general rather than specific; fluorescent pseudomonads may not be involved. An exception to the rule of enhanced disease suppression in organic soils may be dampding-off by pathogens with saprophytic abilities, especially when a crop is planted within a few weeks after incorporation of a cover crop or immature compost. *Pythium* damping-off oscillates after fresh organic matter incorporation, in tandem with oscillations in bacterial populations, which can be used as a measure of soil health. Seedborne diseases can also be a problem, especially since it became mandatory to use organically produced seeds if available. Since then, a lot of research has been directed at finding plant extracts and physical methods to control seedborne diseases in organic farming systems.

INTEGRATED CONTROL OF SOILBORNE WHEAT PATHOGENS. T. Paulitz, K. Schroeder and P. Okubara. *U.S. Department of Agriculture, Agricultural Research Service, Root Disease and Biological Control Unit, Washington State University, Pullman, WA 99164-6430, USA. Email: paulitz@wsu.edu*

There are no resistant varieties or chemical controls for the major soilborne pathogens of wheat in the Pacific Northwest of the U.S. These diseases include *Rhizoctonia* root rot and bare patch (caused by *R. solani* and *R. oryzae*), *Fusarium* crown rot (caused by *F. pseudograminearum* and *F. culmorum*), *Pythium* root rot (caused by numerous *Pythium* spp.) and take-all (caused by *Gaeumannomyces graminis* var. *tritici*). Growers rely almost completely on cultural control measures, and we have evaluated many of these, espe-

cially for *Rhizoctonia*, adapted for no-till, including greenbridge (weed and crop volunteer) management, fallow (both chemical and mechanical), seed opener disturbance, precision seed row placement, crop rotation, residue and nitrogen management, and chemical seed treatment. Until recently, there was no way of accurately detecting and quantifying pathogens in soil. We have developed real-time PCR techniques to quantify 10 species of *Pythium* and 7 groups of *Rhizoctonia,* based on ITS sequences of the rDNA. In the last three years, soils were extensively sampled in eastern Washington, including grower fields and breeder variety-testing sites. By developing a pathogen profile for each testing site, breeders can focus on sites with high pathogen densities to select for tolerance. This survey has shown that pathogen species composition is affected by cropping system and rotation. With accurate detection and quantification of soilborne pathogens, growers can determine risk before planting and make management decisions to mitigate the effects of soilborne fungal pathogens.

Diseases of Mediterranean crops

CURRENT STATUS AND FUTURE PROSPECTS FOR INTEGRATED MANAGEMENT OF OLIVE DISEASES IN THE MEDITERRANEAN BASIN. **E.C. Tjamos.** *Agricultural University of Athens, Votanikos 11855, Athens, Greece. Email: ect@aua.gr*

Verticillium wilt has a significant negative impact on olive cultivation all over the world. The situation has become more complex by the appearance of a defoliating strain of *Verticillium dahliae* in the USA and recently in the Mediterranean region. Current screening of olive germplasm for selecting resistant cultivars or rootstocks appears to be promising. Soil solarization or chamber solarization have been suggested; available fungicides are unable to control the pathogen. *Spilocaea oleagina* is effectively controlled by copper compounds, while strobilurin-based fungicides could also be efficient. Trials with copper oxychlorides in Italy indicated that four treatments could control *Pseudocercospora cladosporioides*. *Clitocybe olearia* and *Armillaria mellea* are causing root rot and wood decay in old olive orchards, but *Fomitiporia mediterranea* is an emerging threat. Severity of olive knot disease is directly related to susceptibility to frost, hailstorm and harvesting injuries. Phytoplasmas are ubiquitous, but a clear correlation between a given syndrome and the presence of one or more phytoplasmas did not emerge. Olive trees host up to 13 different viruses without significant impact so far. Molecular hybridization tests on dsRNA-positive samples in Apulia, revealed the presence of three nepoviruses, *Arabis mosaic virus*, *Cherry leaf roll virus* and *Strawberry latent ring spot virus*, plus *Olive leaf yellowing-associated virus* and Olive latent virus-1. Aspects related to integrated management of the diseases and problems related to dispersal of pathogens by exporting olive plant material in southern hemisphere countries will be discussed.

CURRENT STATUS AND FUTURE PROSPECTS FOR THE INTEGRATED CONTROL OF SOILBORNE DISEASES OF LEGUMES IN THE MEDITERRANEAN BASIN. **B.B. Landa.** *Instituto de Agricultura Sostenible, CSIC, Apdo. 4084, 14080 Córdoba, Spain. Email: blanca.landa@ias.csic.es*

Management of soilborne diseases of legumes in sustainable, Mediterranean cropping systems should be based on strategies that integrate available control measures. The use of host resistance is recognized as the most practical and cost-efficient method for controlling soilborne diseases in sustainable agriculture, and should be combined with the use of pathogen-free seed, its protection from infection using biocontrol agents or chemicals, and the avoidance of highly infested soil and/or environmental conditions that favour disease development. The efficient use of resistant legume germplasm may be conditioned by the nature and diversity of the pathogen population in soil, the development of new virulent pathogen strains able to overcome the available resistance, as well as by infections by multiple pathogens which may modify the disease response of the plant to any of them. Advances in biotechnology and molecular biology may help to understand these conditioning factors and contribute to a more efficient use of legume germplasm resistant to diseases by providing answers to: Which soilborne pathogens prevail in a geographical area and what is the extent of their pathogenic diversity? What is the potential for pathogenic/virulence evolution? Can the breakdown of resistance occur as a result of co-infection by more than one pathogen? Does this phenomenon depend on the interacting pathogen genotypes? Do biocontrol agents have some specificity for legume species or cultivars? By providing these answers phytopathologists would contribute to maintain the critical role of legumes in sustainability as a major source of soil nitrogen and protein in Mediterranean farming systems.

VIRAL DISEASES THREATENING VEGETABLE PRODUCTION UNDER PROTECTED CONDITIONS IN THE MEDITERRANEAN. **F. García-Arenal.** *Departamento de Biotecnología y Centro de Biotecnología y Genómica de Plantas, E.T.S.I. Agrónomos, Universidad Politécnica de Madrid, 28040 Madrid, Spain. Email: fernando.garciaarenal@upm.es*

The largest area of Mediterranean protected crops occurs in SE Spain, where a particularly dynamic turnover of species, cultivars and innovative cultural practices have resulted in a quite unique agroecosystem. This system has been shown to be highly vulnerable to viral diseases, with the periodic occurrence of severe epidemics caused by emergent or re-emergent viruses, which often limit the production of some species. Emergence of new virus diseases is due to the evolution of virus populations resulting in increased virulence or transmissibility, and/or in host range expansion or host switches. Knowledge about the ecological and evolutionary factors leading to virus emergence is scant, and this restricts the possibility of developing sustainable control strategies. We have explored the role of some of these factors. An important one is the genetic composition of the host population: rapid turnover of cultivars, with the progressive accumulation of resistances to pathogens, may result in important selective pressures on virus populations, which could be countered or not by virulence costs. A second factor is the size of the vector population, which may determine the appearance and fixation of highly pathogenic virus genotypes, in addition to its well-known role in determining incidence. Last, high incidence of virus infection may result in a large fraction of co-infection by different viruses and strains, favouring the appearance of new genotypes and the selection of more pathogenic ones. These analyses have focussed on diseases of tomato and pepper, two of the most important crops in the region.

OCCURRENCE OF *ASPERGILLUS* SECTION *NIGRI* IN GRAPES AND ITS IMPACT ON WINES AND EUROPEAN VITICULTURE. **V. Sanchis.** *Food Technology Department. CeRTA-UTPV. Lleida University, Rovira Roure 191, 25198 Lleida, Spain. Email: vsanchis@tecal.udl.cat*

The most commonly isolated fungal genera in grapes are *Al-*

ternaria, with a decreasing percentage from setting to harvest, yeasts and *Aspergillus*, with increasing percentages from setting to harvest, and *Cladosporium*, *Rhizopus* and *Penicillium*. Black aspergilli are the main group of the genus *Aspergillu*s regarding their high capacity to colonize the grapes. This group is very important for food safety because, mainly *Aspergillus carbonarius* and members of the *Aspergillus niger* aggregate are the main source of ochratoxin A (OTA) contamination in grapes and raisins. At harvest, *Aspergillus* section *Nigri* contamination is related to latitude and longitude, being greater towards the East and the South. This indicates that meteorological conditions can contribute to the spatial distribution of black aspergilli, within the Mediterranean basin at least. Among the different wine types, red and sweet wines seem to be the most contaminated by OTA. In the last group, the wines with higher OTA levels were fortified musts followed by those made from sun-dried grapes. At the moment, no fungicides are systematically applied to control black aspergilli in this kind of product. Different treatments are able to control black rot of grapes and OTA production, although it is outstanding that, frequently, data obtained on control of black aspergilli by fungicides have been a secondary result of chemical treatments specifically designed to control other fungi more frequently found in grapes. Switch, originally designed to control *Botrytis* and *Sclerotinia* infection in different crops, is perhaps the only one directly registered against *Aspergillus* on grapes.

Plant breeding and resistance strategies

SEQUENCE-BASED BREEDING! HOW MARKERS TURN INTO SEQUENCE ALLELES. **M. Prins, M. de Both and A.P. Sørensen.** *Keygene N.V., Agro Business Park 90, P.O. Box 216, 6700 AE Wageningen, The Netherlands. Email: marcel.prins@keygene.com*

Current high-throughput sequencing technologies are revolutionizing the DNA research arena. What will the implications be for molecular plant breeding projects for researchers as well as for plant breeders? Keygene is designing and developing a range of molecular breeding applications using advanced sequencing technologies. Some will replace current molecular marker technologies and some will open up novel possibilities for genetic research. We will present a number of current and future possibilities and emphasize the power of efficient use of a combination of HT sequencing tools for trait discovery and exploitation in plant breeding. To keep up with the speed of data generation, the aggregation of data into information that can be absorbed by the researcher and plant breeder becomes an increasingly demanding task. Also in this field Keygene has developed tools that are applied for rapid identification of plant genes involved in response to insects as well as fungal and bacterial pathogens.

IDENTIFICATION OF NEW POTENTIAL SOURCES OF RESISTANCE TO SOYBEAN RUST. **S. Li.** *USDA-ARS, Crop Genetics and Production Research Unit, Stoneville, MS, 38776, USA. Email: shuxian.li@ars.usda.edu*

Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Sydow, is one of the most destructive diseases of soybean [*Glycine max* (L.) Merr.]. Before 2004, ASR was not present in the continental USA and evaluation of U.S. soybean lines for resistance was done only with foreign isolates. Now that ASR has been discovered in North America, evaluation of soybean lines using US isolates is important in order to identify new resistance sources. In this study, 10 plant introductions (PIs) previously identified as resistant lines in Paraguay were evaluated using an ASR isolate from Mississippi. Replicated experiments were conducted in a growth chamber at the USDA-APHIS PPQ approved Stoneville Research Quarantine Facility from 2006 to 2007. Results from the seedling assays indicated that most of the PIs had lower ASR severity and less sporulation than the susceptible check cv. Williams 82. Although none of the PIs were immune, PI567102B was identified as the most resistant line having the lowest severity, no sporulation, and the red-brown reaction. Further evaluation using other U.S. isolates is in progress. PIs that have resistant reactions to both U.S. and Paraguay isolates may be important sources of resistance to soybean rust.

WORLDWIDE MONITORING SYSTEMS: THE NEED OF PUBLIC AND PRIVATE COLLABORATION. **R.D. Magarey, W.E. Dolezal and T.J. Moore.** *North Carolina State University and Center for Health Plant Science and Technology, 1730 Varsity Drive, Suite 300, Raleigh, NC, 27606, USA. Email: Roger.D.Magarey@usda.gov*

In recent years, invasive plant pathogens such as *Phytophthora ramorum* and *Puccinia graminis* (TTKS) have emerged as global threats that move rapidly across international boundaries. To counter such threats, the accurate monitoring of global plant health necessitates the involvement of all agricultural sectors: government agencies, universities and the agricultural industry. Many members of industry conduct: i) scouting of research and seed production fields for pests; ii) phytosanitary field inspections; and iii) provide disease diagnostics testing for their customers. The USDA Pest Information Platform (PIPE) has proved to be an excellent vehicle for fostering collaborative efforts between the public and private sectors in monitoring for soybean rust (*Phakopsora pachyrhizi*) in North America. The American Seed Trade Association's Phytosanitary Committee has developed a draft proposal for an industry PIPE platform for a narrow list of new and emerging pests of maize, soybeans and watermelon. Combining private industry and public pest data into a central database can provide: i) useful pest distribution information for state extension specialists; ii) improved monitoring for emerging and exotic pest threats; and iii) improved pest distribution records for state and federal regulators to enhance their decision making. Expanding PIPE-like systems beyond national borders has potential to provide additional information for agencies to prepare and respond to exotic pest threats. Many members of industry with international operations have the capacity to jumpstart the development of a global PIPE. However, a global PIPE would require a comprehensive data sharing policy that nevertheless protects national, state and corporate interests.

THE DEFENSOME: A PROTEIN NETWORK REGULATING INNATE IMMUNITY IN RICE. **K. Shimamoto.** *Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology (NAIST), 8916-5 Takayama, Ikoma 630-0101, Japan. Email: simamoto@bs.naist.jp*

We have been studying the role of Rac GTPase in innate immunity of rice and found that it is a key molecular switch for the defence response. To identify components of the Rac GTPase-mediated innate immunity in rice we have taken a number of approaches including proteomics and reverse genetics and found components associated with OsRac1. They include known components such as RAR1, SGT1, and Hsp90 and novel components

such as RWD and Sti1/Hop and they interact with OsRac1. Various studies on protein-protein interactions among these components indicate that at least 10 proteins form a network in Rac GTPase-mediated innate immunity. Since most of these components are involved in both PAMP-mediated and R protein-mediated resistance in rice we propose a model in which early signaling events involved in these two types of resistance occur in essentially the same protein complex we call the "defensome" at the plasma membrane. Details of individual components in the protein complex will be discussed.

COMPARATIVE GENOMICS OF PLANT-PATHOGEN SPECIFICITY. **R. Michelmore, L. Yao, K. Caldwell, L. Williams, T. Wroblewski, J. Wong and J. Greenberg.** *University of California, Davis, USA. Email: rwmichelmore@ucdavis.edu*

We have been taking a comparative approach to dissecting the molecular basis and evolution of specificity between several dicot plant species and multiple bacterial and oomycete pathogens. We are focused on understanding the evolution of resistance genes, effector proteins from *Pseudomonas* spp. and Oomycetes and their plant targets. We screened Arabidopsis and diverse crop species, particularly tomato and lettuce, for their ability to recognize effector proteins from *P. syringae* and *Bremia lactucae*. The majority of secreted effectors from multiple strains of *P. syringae* have been assayed for their ability to elicit a macroscopic reaction using *Agrobacterium*-mediated transient assays. We have detected considerable inter- and intra-specific variation in the ability of these plant species to recognize individual pathogen effectors. Non-host resistance can be explained by the recognition of multiple effectors. We have also conducted large scale yeast two-hybrid assays and identified numerous effector-potential target interactions. In addition, we have characterized genes encoding signal transduction proteins for signatures of selection to understand the possible functional constraints on their evolution and the need for other proteins to monitor (guard) their status. Signal transduction genes exhibit very different patterns of variation from recognition (R) genes determining specificity and most signal transduction genes have significantly lower levels of polymorphism that R genes. We have investigated RIN4 in detail; this negative regulator of basal resistance seems to be targeted directly or indirectly by multiple pathogen effectors. Our data indicates there are points of vulnerability in plants that are exploited by multiple effectors from multiple pathogens.

DEPLOYMENT OF BROAD-SPECTRUM DISEASE RESISTANCE IN RICE THROUGH WHOLE-GENOME SELECTION. **H. Leung, Y. Liu, S. Madamba, M. Bernardo, R. Mauleon, C. Vera Cruz, B. Liu, X-Y. Zhu, S. Zhang, J. Leach and D. Galbraith.** *Plant Breeding, Genetics and Biotechnology Division, Manila, Philippines. Email: h.leung@cgiar.org*

Broad-spectrum disease resistance is a high-priority trait in rice breeding because it provides resistance to multiple races of a pathogen and possibly multiple pathogens. Such forms of resistance may be attributed to a combination of major resistance genes and QTL controlling quantitative resistance. Some varieties with durable disease resistance are known, based on field performance records but the challenge is to have relatively simple and robust methods to identify the right gene combinations and to reconstitute them in breeding cycles. We are using mutants and elite germplasm in parallel to identify genomic regions contributing to broad-spectrum resistance. We screen for gain and loss of disease resistance in chemical- and irradiation-induced mutants. Loss-of-blast resistance mutations were recovered from high-throughput screening in the field. Genomic deletions in these mutants can be detected through hybridization with whole-genome oligoarrays. Using "field-proven" germplasm, we identified chromosomal regions controlling quantitative resistance through a combination of QTL mapping and transcriptome analysis. To facilitate selection, we applied genotyping microarrays to monitor the introgression of candidate genes and chromosomal regions in advanced backcross populations. This approach is being practiced in breeding for blast resistance. To aid disease resistance breeding, we are producing a) breeding-ready near-isogenic lines with major genes and QTL in elite genetic backgrounds, and b) a database (Genome Browser) displaying known deletions, disease resistance QTL, and gene expression clusters anchored to the rice chromosomes. We hope these resources can facilitate the identification of new genes for resistance and accelerate the construction of genotypes useful in breeding programs.

Crop and food biosecurity

THE CROP BIOSECURITY PROJECT: AIMS AND RESULTS. **M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco, Italy. Email: marialodovica.gullino@unito.it*

The project "Crop and Food Biosecurity and Provision of the Means to Anticipate and Tackle Possible Misuse of Plant Pathogens Against Crops" has been funded by the European Union, within its VIth Framework Research Programme. This Coordination Action addressed the threat of plant pathogens as weapons against crops. Agriculture and agro-industry account for over 15% of the European Union (EU) gross annual product. A deliberate misuse in agriculture could have significant economic consequences in Europe, where consumers enjoy food of high quality and safety. The deliberate introduction of a pathogen could affect crop yield and quality, cost of disease management, trade if certain quarantined pathogens are introduced, markets, food prices and availability of certain foods. The project permitted a coalition of EU partners, plus USA and Israel, to: a) establish a strong network of interconnected laboratories able to quickly diagnose new plant pathogens/races/biotypes and to study their biology and epidemiology; b) prepare a list of pathogens considered high-risk for European crops as well as a list of the most vulnerable crops; c) propose a risk assessment methodology for epidemics resulting from incidental events; d) provide effective tools and protocols for use by inspectors for quarantine and phytosanitary controls; e) enhance surveillance capacity already in place and develop improved surveillance models and early warning surveillance systems; f) identify and prioritise fields and areas where more research is needed and suggest future investment needs; g) raise awareness of the issue of crop biosecurity, to enhance preparedness in Europe.

EVALUATION OF THE RISK POSED BY DELIBERATE USE OF PLANT PATHOGENS AS ANTI-CROP WEAPONS IN EUROPE. **F. Suffert, E. Latxague and I. Sache.** *INRA - Agro Paris Tech, UMR1290 BIOGER-CPP, F-78850 Thiverval-Grignon, France. Email: fsuffert@grignon.inra.fr*

Malevolent use of plant pathogens, i.e. agroterrorism *sensu lato* (anti-crop bioterrorism and use of bioweapons against the agricultural sector), represents a non-negligible threat for crops and

forests in Europe. In order to assess this risk objectively, a methodology based on three interdependent steps was used. (1) A list of 50 candidate pathogens (fungi, bacteria, and viruses) that may pose a threat to European agriculture and forestry was compiled. This list included exotic and quarantine pathogens, but also endemic pathogens causing recurrent epidemics because they presented particular risk profiles. (2) Theoretical scenarios of possible acts of agroterrorism were elaborated considering three types of acts: biowarfare, bioterrorism, and biocrime. Each scenario was divided into three sections: synopsis, justification and feasibility. Five types of consequences were identified. Nine key pathogens were selected to detail realistic scenarios using current geopolitical knowledge and information on the biology of the pathogen. (3) Items were added to the international standard Pest Risk Analysis (PRA) scheme to adapt the methodology of risk assessment to the context of agroterrorism. Our risk assessment scheme (RES) included the following five sections: importance of the target crop (acreage and uses), availability and ease of use of the pathogen, epidemic potential of the pathogen, obstacles to swift and effective response, and potential global or regional consequences. No fundamental distinction was made between regulated and non-regulated pests. The RES was applied to the nine selected pathogens. The five sections were documented using ten selected scientific publications. The different risk profiles were analysed and discussed.

CROPS UNDER RISK IN EUROPE. **H.W. Dehne, E. Leiritz and U. Steiner.** *Institute of Crop Science and Resource Conservation, University of Bonn, Germany. Email: hw-dehne@uni-bonn.de*

The agro-food sector is of major importance for the European economy, because the EU is a key producer of food in the world market. For several commodities it shares about a quarter of global food production. EU agriculture is therefore vulnerable to agro-terroristic attacks. Most of the crops grown are susceptible to a broad spectrum of pathogens and in general the genetic diversity is low for cultivars grown. For any given crop produced in Europe, there are several pathogens that do not yet occur, but cause major losses elsewhere in the world. The impacts of these pathogens and their detrimental effects in biological warfare have to be differentiated depending on economic relevance and psychological effects. Losses from agro-terrorism would include the value of lost crop production, the cost of destroying contaminated products and the costs of containment (diagnostics, pesticides). They may include major economic crises in the agricultural and food industries, loss of confidence in government and possibly human casualties. People could be at risk in terms of food safety. Export markets would be lost as importing countries placed restrictions on EU products to limit disease spread. Useful criteria for risk assessment are the area of production and yield as indicators of economic importance, climatic conditions in the growing area, the availability of pathogen control measurements in the culture, the potential as broad-spectrum host plant and complementation by global plant production.

DIAGNOSTIC TOOLS FOR MAINTAINING CROP BIOSECURITY – AVAILABILITY AND LIMITATIONS. **C. Henry, D.M. Kenyon, J. Morris and J.E. Thomas.** *Central Science Laboratory, Sand Hutton, York, Y04 1LZ, UK. Email: c.henry@csl.gov.uk*

The role of diagnostics for detection of organisms which threaten biosecurity is well established, with increasing use of sophisticated high-throughput and potentially mobile systems. However, techniques used to detect the accidental introduction of quarantine organisms at ports of entry and elsewhere may not always be suitable for the early detection of organisms which might be introduced deliberately, in a variety of ways, as potential agents of bioterrorism. A range of pathogens on key EU crop and amenity species has been identified as having the means to cause disruption either to supplies or to normal trading procedures, or to affect social and cultural conditions in member states. The availability of diagnostic systems for these pathogens has been reviewed, taking into account applicability to different substrates, rapidity, specificity, cost, availability of field test kits, validation status and the capability of testing laboratories. For several pathogens, there are no satisfactory diagnostic systems available. For a few, simple diagnostic tests are adequate and require little or no development. However, gaps in technology exist for a significant number of the pathogens. Additionally, there is only very limited potential for differentiating new or altered pathotypes without lengthy host tests, and this would hinder both detection and the ability to gather forensic information which would be needed to investigate a possible deliberate introduction. Diagnostic methods exist for the majority of the high risk pathogens which are also listed as quarantine diseases but in selected cases these still require further development, especially of field tests. Diagnostics for indigenous diseases in the context of biosecurity have been largely ignored to date and will require further development.

CROP BIOSECURITY: CONTAINMENT AND ERADICTION OF INVASIVE PATHOGENS. **A. Gamliel.** *Agricultural Engineering Institute, ARO, Volcani Center, Bet Dagan 50250, Israel. Email: agamliel@agri.gov.il*

Plant disease management aims to retard disease progress and keep its development below an acceptable level. The focus, however, with invasive species deliberately introduced to a new area is to contain the pathogen within the invaded site and eliminate it. These objectives are stated in absolute terms (e.g., "eliminate", or "eradicate") that imply a goal of zero inoculum and obviously, disease. For achieving this goal we have to consider the dynamics of plant disease, that is, changes in the incidence and severity of disease in time and space. Furthermore, we need to assess the relative effectiveness of the various measures for the control of a particular disease and the different interactions in their effects on disease dynamics. We have to measure the effects of various eradication strategies, alone and in combination, and their impact on inoculum reduction and disease progress. Finally, we need to fit each measure into an adequate integrated strategy. Thus, an eradication strategy requires a series of concerted actions in order to achieve the goal of eliminating the invasive pathogen. The main eradication steps include prevention of the invasion (also refers to as exclusion), and exclusion of the pest after its invasion. No individual action will advance the progress of eradication toward the final objective unless it has a coherent relationship with all of the other necessary steps.

BIODEFENCE: VISION OF A BROADER COOPERATION. **M.L. Gullino, R. Lausevic and J. Stack.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco, Italy. Email: marialodovica.gullino@unito.it*

Agriculture, as well as food production and distribution systems, are global in nature. They are relatively soft targets for crime and terrorism, offering many points at which commodities

could be deliberately contaminated. Since 2002, there has been worldwide recognition of the genuine threat by terrorists or criminals to the agricultural sector and global food supply. An attack on agriculture and food would have devastating consequences for human health and safety, resulting in significant social and economic impacts. The EU and the USA have well-developed regulations for crisis and consequence management following the introduction of high-consequence pathogens. In addition, preparedness programs have been implemented that include biosecurity research, education, and training programs aimed at early detection, accurate diagnostics, secure communications, and contingency plans of action. The ability to coordinate food defense across borders has been tested many times in both the U.S. and EU with real-life events and is an important food security feature. Among the differences between a terrorist attack and an accidental event would be the dimensions of the initial phase and the number of primary outbreaks. Whatever the cause of an outbreak, a comprehensive biosecurity system is essential in order to protect agriculture, food, and citizens. Because of the global nature and complexity of food production and distribution systems, international cooperation for food defense is essential. Significant challenges exist including the development of: vehicles for sharing data on the geographic distribution of pathogens, secure communications platforms for collaborative diagnostics, transnational security strategies, and international outbreak response protocols and preparedness exercises. Agricultural biosecurity and food defense are international issues requiring a global strategy to ensure food security for all nations.

Teaching Plant Pathology

THE VIRTUAL PLANT PATHOLOGY FACULTY: DEVELOPING AN AUSTRALASIAN PLANT PATHOLOGY *CURRICULUM*. **D. Guest and G. Ash.** *Faculty of Agriculture, Food and Natural Resources, University of Sydney, NSW 2006, Australia. Email: d.guest@usyd.edu.au*

Plant pathology has traditionally been taught at an introductory level in generalist degrees followed by small-group, senior undergraduate specialisations. In parallel with declining student numbers, Plant Pathology departments have been merged or dissolved, fragmenting the specific academic expertise offered to students by research-active plant pathologists. Consequently, the nexus between teaching and research is threatened and practical laboratory and field experience with plant diseases is often very limited during undergraduate training. The development of an Australasian Plant Pathology *Curriculum* has as its central aim the promotion of education opportunities and employment prospects for students of plant pathology, meeting industry's needs for plant pathology expertise into the future. A series of stakeholder forums organised by the Cooperative Research Centre for Tropical Plant Protection resulted in the development of a decentralised, consortium-based model of coursework postgraduate training. The modular structure also provides an opportunity for skills upgrades for plant health professionals, non-specialists and para-professionals. The principles underpinning the Australasian Plant Pathology *Curriculum* recognise that Australia needs to maintain and enhance a comprehensive education and training capacity in plant pathology, and that the most effective way to achieve this is to develop an agreed set of stand-alone courses that build to a coursework postgraduate qualification. The Australasian Plant Pathology *Curriculum* will develop a coursework Masters program (incorporating a Graduate Certificate, Graduate Diploma and stand-alone courses) in plant pathology, and will be delivered using a flexible model through a consortium of Australasian universities.

TRAINING IN PLANT PATHOLOGY FROM AN INDUSTRY PERSPECTIVE. **U. Gisi.** *Syngenta Crop Protection, Research Biology, CH-4332 Stein, Switzerland. Email: ulrich.gisi@syngenta.com*

Plant pathology is a complex area of science because it includes knowledge on the biology of host plants, plant pathogens and their interactions as well as the interaction with their environment. For elucidating processes and mechanisms of interaction, university teachers tend to rely on easy to handle 'model' systems such as downy mildew on *Arabidopsis*, rust on bean, powdery mildew on barley, or single fungal species like yeast (*Saccharomyces*), *Aspergillus* and *Ashbya*, in which complete genomic information is available. However, industry researchers and extension pathologists teaching at universities, and training non-pathologists within and outside their organisations are often asked to understand and explain the full range of agronomically important plant pathogens for which much less basic information is available. Therefore, basic knowledge on molecular biology, genetics, biochemistry, physiology, symptomatology and epidemiology has to be combined with applied aspects derived from agronomic problems. For illustration, a simplified scheme of interaction between the most important crop plants and plant pathogens will be presented which can be used both as a learning tool for plant pathogen nomenclature and for visualising the disease spectrum covered by the major classes of fungicide. Fungicide resistance will also be discussed as an ideal marker for studying and explaining mechanisms of inheritance in plant pathogens.

SCHOLARSHIP OF TEACHING AND LEARNING (SOTL) PROJECTS IN PLANT PATHOLOGY. **D. Eastburn and C. D'Arcy.** *Department of Crop Sciences, University of Illinois, Urbana, Illinois, USA. Email: eastburn@uiuc.edu*

The scholarship of teaching and learning (SoTL) requires systematic investigation of an instructional problem with the findings made public through presentations or publications. We will present two examples of SoTL studies in an introductory, general education plant pathology course. The first study was undertaken to evaluate effects of a supplemental course web site (www.ppp.uiuc.edu) on student learning. When students read factual material and completed virtual laboratory activities on the site their comprehension of course material increased somewhat, but improvements were not as consistent or large as we had hoped. However, when students completed a series of virtual experiments across the semester and were provided with prompt instructor feedback for each experiment, significantly improvements were found in students' abilities to describe the general concepts of the scientific method and to apply that knowledge to specific situations. The second study was designed to determine how students use what goes on in the classroom in their everyday life. Students recorded periodic reflections of how they used, or did not use, course information in non-class settings. Preliminary findings indicate that students found stories particularly memorable and were apt to retell them to friends and family while sharing their new knowledge about plant pathology. Students also connected class material to past and present life experiences, particularly with food or with nature. Our SoTL studies support the use of a variety of teaching methodologies, with attention given to providing opportunities for students to learn through repetition, and to presenting material that is connected to students' lives.

PLANT CLINICS AND PHYTOPATHOLOGY TRAINING. **H. Maraite, C. Bragard and A. Legève.** *Université Catholique de*

Louvain, Unité de Phytopathologie, Croix du Sud2/3, B-1348 Louvain-la-Neuve, Belgium. Email: henri.maraite@uclouvain.be

In several western European countries, there is a decline in the number of students registered for agricultural degrees at MS level and in state-organized extension services. Nevertheless, phytopathology as a research topic and job opportunity has expanded to cover more intricate approaches of plant health management as well as to various areas of biotechnology, environment management and new types of extension and support to farmers or other customers. There are still job opportunities for graduates able to combine knowledge of various upstream biological disciplines with applied ones, in particular for the complex area of plant health management. In this context the capacity to train students in plant disease diagnosis, and formulation of crop protection advice adapted to a particular situation or problem, is of prime importance. Having seen the constraints of integrating the learning of students into plant clinic service activity, we have developed at UCL an interactive Plant Clinic course stretching over 30 weeks at half a day/week for up to 15 participants. These are final-year students of the Plant Health Management MS program and of the international postgraduate Master in Protection of Tropical and Subtropical Crops, organized with FUSA Gembloux and CIRAD-ENSAM, France. Training ranges over *in situ* disease detection and evaluation, application of various diagnostic tools, literature search, and discussion of crop-protection strategies. The cases analysed are actual problems or examples provided by the Plant Clinic service or by the students. Our experience will be compared to that of other approaches.

Keynote Session

Knowledge and technology transfer for plant pathology

DECISION MAKING IN AGRICULTURE. S. Parker, J. Turner and S. Elcock. *Central Science Laboratory, Sand Hutton, York, North Yorkshire, YO41 1LZ, UK. Email: s.parker@csl.gov.uk*

Timely and reliable evidence is necessary to inform sound decision-making. This is true at all decision-making scales; from policy formulation to in-field crop management. However, despite substantial investment to develop decision support tools to underpin these activities, especially at the field scale, there is little evidence that decision makers use them routinely. Indeed, it remains a moot point whether the benefits from their use are truly great enough to justify adoption. As a consequence, close scrutiny of decision processes in agriculture show that they often lack a quantitative basis and instead depend on 'rules of thumb', subjective judgements and sometimes arbitrary choices. The presentation will consider why recent systems have failed to gain trust and uptake of the intended beneficiaries. In examining these failings and addressing how they can be corrected, we will discuss the UK CropMonitor initiative. This aims to deliver an infrastructure for providing measurements important for decision-making at policy, industry and farm scales. CropMonitor has a growing user base and is used by many of the key influencers in UK agriculture. The system design recognises that the needs of the various user groups and the focus of their decision-making differs. At a generic level, UK CropMonitor supports decisions by providing rapid access to accurate relevant data and, where appropriate, its interpretation. This information is carefully collated, so that the interests of the various user groups are catered for and delivered in an appropriate context and via efficient routes.

PLANT HEALTHCARE FOR POOR FARMERS AROUND THE WORLD: GATHERING DEMAND AND INNOVATIVE RESPONSES. E. Boa. *Global Plant Clinic, CABI E-UK, Bakeham Lane, Egham, Surrey, TW20 9TY, UK. Email: e.boa@cabi.org*

Since 2003 the Global Plant Clinic (GPC) has helped establish over 60 plant health clinics in Africa, Asia and Latin America. These independent clinics are held in public spaces once a week for a few hours and are staffed by agronomists, extension staff and others, including research scientists. The 'plant doctors' are employed by NGOs, farmer cooperatives, research institutes and other organisations with close ties to farmers. The plant doctors are trained to give advice on all types of plant health problems. The GPC delivers original courses to identify possible causes and recommend suitable ways to manage them. In Nicaragua, the most advanced scheme, there are 18 clinics operating (November 2007) and 28 plant doctors have completed the three courses that comprise a curriculum on 'how to become a plant doctor'. The demand gathered by the clinics has stimulated the creation of a diagnostic and plant health management network and rejuvenated attempts to broaden access to technical support and validated control technologies. Similar changes are taking place in Bangladesh, Bolivia and Uganda and I will present an overview of innovative responses by local partners. I will discuss the wider implications for extension and crop protection and suggest how researchers can play a stronger role in helping improve the impact of development projects.

LINKING DIAGNOSTIC LABORATORIES TO ENSURE RAPID DETECTION AND ACCURATE DIAGNOSIS OF PLANT DISEASE. J.P. Stack. *Biosecurity Research Institute, Kansas State University, 1041 Pat Roberts Hall, Manhattan, Kansas, 66506-7600, USA. Email: jstack@ksu.edu*

Early detection and accurate diagnosis can minimize the impact of a disease outbreak. With hundreds of host species and thousands of pathogen species, a plant diagnostician cannot be an expert with all plant systems. The National Plant Diagnostic Network (NPDN) was created to link plant diagnostic labs throughout the United States to enhance diagnostic capability and increase sample capacity. The application of advanced computer, communications, and molecular technologies has enhanced NPDN's ability to rapidly detect outbreaks, to accurately identify newly introduced pathogens and insect pests, and to securely communicate that information to those with the authority and capability of responding effectively. Through NPDN's communications system, diagnosticians have access to expertise wherever it exists. Among the deployed technologies underlying NPDN's capabilities are web-enabled microscopy and video conferencing to facilitate collaborative diagnostics. These same technologies are also being used to conduct diagnostic training workshops over the internet and to distribute the latest diagnostic protocols to all labs. The impact of plant diseases on natural and cultivated plant systems ranges from minor aesthetic effects to major declines in productivity that result in landscape perturbations or food shortages leading to famine. Linking local, regional, and national plant diagnostic resources and expertise into global networks should become a priority for ISPP. This may be of greater significance to resource-poor nations that lack modern diagnostic infrastructure and experience. Providing plant diagnosticians in resource-poor nations the access to global diagnostic expertise may greatly reduce the impacts of local disease outbreaks. Networking is essential for global plant biosecurity.

CONCEPTS IN CHEMICAL CONTROL

CARBENDAZIM-RESISTANCE AND ITS MOLECULAR MECHANISM IN *GIBBERELLA ZEAE*. **C.J. Chen, C.W. Bi, J.J. Yu, J.X. Wang, H.X. Li, Q.Q. Luo and M.G. Zhou.** *College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, P.R. China. Email: mgzhou@njau.edu.cn*

In China, *Gibberella zeae* (anamorph *Fusarium graminearum*) is the dominant pathogen causing wheat head blight. The first case of resistance of *G. zeae* to MBC i.e. benzimidazoles (MBC-fungicides) was detected in 1992 in the Zhejiang province of China after these fungicides had been widely used for about 20 years. From then on, MBC resistance was found to be common in pathogen populations on the eastern coast and along areas of the Yangtze River. Our research showed both field-resistant and MBC-sensitive strains shared similar temperature sensitivity, fitness and virulence on ears. But no mutation in beta-tubulin was found in *G. zea*e. To identify the MBC resistance mechanism of *G. zeae,* a microtubule-associated protein gene (*map*) and other members of tubulin gene family, were also cloned and analyzed. Alterations at amino-acid codons 167, 198 or 200 in a *novel* tubulin gene, corresponded to the different phenotype sensitivities. We have called this novel tubulin, a β_2-tubulin. At codon 167 in this gene, TTT encoding phenylalanine in MBC-sensitive isolates was converted to TAT (tyrosine) in moderately resistant field strains; at codon 198, GAG was altered into CTG resulting in substitution of glutamic acid for leucine in a highly MBC resistant field strain. Deletion and complementation of the β_2-tubulin gene validated this gene conferring resistance of *G. zeae* against benzimidazoles. DMIs and JS399-19(2-cyano-3-amino-3-phenylancryic acetate), are recommended to control FHB.

QOI FUNGICIDE RESISTANCE: CURRENT STATUS AND THE PROBLEMS ASSOCIATED WITH DNA-BASED MONITORING. **H. Ishii.** *National Institute for Agro-Environmental Sciences (NIAES), Kannondai 3-1-3, Tsukuba, Ibaraki 305-8604, Japan. Email: hideo@niaes.affrc.go.jp*

QoI fungicides, which inhibit mitochondrial respiration at the Qo site of the cytochrome *bc1* enzyme complex, are the second most important class of fungicides. QoI fungicides generally carry a high risk of pathogen resistance development with resistance occurring in over twenty pathogens, such as powdery mildew, downy mildew, anthracnose, *Alternaria* spp., scab, and grey mould. Molecular mechanisms of QoI resistance have been intensively studied; a single point mutation which causes an amino acid change in cytochrome *b* is thought to govern the expression of high resistance. A range of molecular methods including PCR-RFLP and quantitative real-time PCR have all been developed, enabling the rapid detection of resistance. However, the status of heteroplasmy in the mitochondrial genome which encodes the cytochrome *b* gene can cause instability over time, making it difficult to precisely monitor QoI resistance in some pathogens. The role of the alternative oxidase pathway in QoI resistance is not clear as yet, although this enzyme is very likely implicated in resistance development of grey mould in particular. Novel QoI fungicides have been developed and some of them interestingly show differential patterns of cross-resistance to pre-existing QoI fungicides. This paper briefly summarizes QoI resistance development over the last decade as well as future research prospects.

ROLE OF DRUG TRANSPORTERS IN FUNGICIDE RESISTANCE AND PLANT DISEASE CONTROL. **M.A. de Waard.** *Wageningen University, Phytopathology, P.O. Box 8025, 6700 EE, Wageningen, The Netherlands. Email: maarten.dewaaard@wur.nl*

ATP-binding Cassette (ABC) transporters are membrane transporters that constitute a large protein family within all kingdoms of life. Particular ABC-transporters transport natural toxic compounds and xenobiotics such as fungicides and can reduce the intracellular accumulation of fungicides within fungal cells. This property can protect fungi against the toxic activity of plant defense compounds, fungicides and drugs. The transporters are described as drug transporters. Similar properties have been described for Major Facilitator Superfamily (MFS) transporters. Several drug transporters of the filamentous fungi *Aspergillus nidulans, Botrytis cinerea*, and *Mycosphaerella graminicola* function in protection against azoles, as knockout mutants are relatively sensitive to these fungicides and overexpression mutants relatively insensitive. These results suggest that overexpression of drug transporters is a potential mechanism of resistance to azoles in practice. Overexpression of drug transporters can also result in resistance to chemically unrelated fungicides and natural toxic compounds. This phenomenon, known as multidrug resistance (MDR), is a serious problem in chemical control of human pathogens, and also exists for plant pathogens. Specific drug transporters function as virulence factors in pathogenesis since they provide protection against plant defense compounds. Inhibitors of drug transporter activity, known in literature as modulators, may reduce the protective activity of transporters against plant defense compounds and delay or prevent disease development on host plants. A number of medical modulators display disease control activity against *M. graminicola* on wheat seedlings but possess hardly any *in vitro* toxicity. Such compounds may be leads in the discovery of new disease control agents.

DEMETHYLATION INHIBITOR RESISTANCE IN CEREAL PATHOGENS AND CONSEQUENCES OF RESISTANCE MANAGEMENT. **B.A. Fraaije, H.J. Cools, J. Motteram and J.A. Lucas.** *Plant Pathology and Microbiology Department, Rothamsted Research, AL5 2JQ, Hertfordshire, UK. Email: bart.fraaije@bbsrc.ac.uk*

The azole class of DeMethylation Inhibitors (DMIs) have been the leading agents for control of fungal pathogens of both plants and humans since their introduction in the early 1970s. Despite early reports of DMI resistance development in the cereal powdery mildew pathogen *Blumeria graminis*, resistance in this and other plant pathogens has developed relatively slowly without affecting practical disease control. Resistance mechanisms identified so far are enhanced active efflux, over-expression of the target-encoding sterol-14a-demethylase gene (*CYP51*) and *CYP51* mutations. Recent work on control of *Septoria* leaf blotch (*Mycosphaerella graminicola*) with 12 currently marketed azoles in the UK showed clear differences in efficacy. Many of the older azoles gave poor control of *Septoria* leaf blotch when applied in two-spray programmes at the maximum recommended dose whereas the newer azoles epoxiconazole and prothioconazole performed well. Interestingly, additional testing of isolates and DNA samples of infected leaves from these experiments revealed that particular CYP51 alterations are differentially selected by different azoles in the field. These correlations were confirmed for a selection of strains with both *in vitro* and *in planta* fungicide sensitivity testing. Based on this knowledge we tested if alternations or combinations of different azole fungicides can be used as an effective resistance management strategy.

TAXONOMY OF PLANT PATHOGENS

TAXONOMY OF PLANT PATHOGENIC FUNGI: SPORE DIMENSIONS OF THE PAST ARE REFUTED BY GENOMES OF THE FUTURE. **P.W. Crous.** *Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands. Email: p.crous@cbs.knaw.nl*

Phytomycologists form an essential bridge between mycology as science, and phytopathologists as its user community. Most of the names currently used for phytopathogenic fungi are based on former opinions, and clash with current data. Many genera are shown to be poly- or paraphyletic, and most morphological species to be assemblages of different phylogenetic taxa, many of which frequently turn out to be geographically separated. Furthermore, dual nomenclature for pleomorphic fungi should be eliminated as soon as possible, as it is redundant in the DNA era. Although the Linnaean system was based on the phenotype, I argue that inconspicuous differences may in some cases be more relevant for trade and quarantine, and hence a more accurate naming system based on DNA barcodes is called for. This is an achievable goal if scientists embrace the virtual laboratory of the future, and deposit related data in interactive, linked databases such as GenBank and TreeBase, with metadata in MycoBank. This process should be strongly enforced as part of the editorial policy of all reputable journals. Techniques to detect minute quantities of DNA, and compare partial or whole genomes are continually evolving. Although we need to improve our understanding of population dynamics and gene flow, we should also acknowledge the importance of new emerging diseases caused by novel organisms, or formerly less relevant pathogens that have gained new importance due to climate change. Molecular techniques and data will play a central role in the future taxonomic system adapted for plant pathogenic fungi.

TAXONOMY OF PLANT PATHOGENIC NEMATODES - USE OF SSU AND LSU rDNA SEQUENCES FOR PHYLOGENETIC RECONSTRUCTION AND DNA BARCODING. **H. Helder, M. Holterman, G. Karssen, R. Landeweert, P. Veenhuizen, S. van den Elsen, H. van Megen and J. Bakker.** *Laboratory of Nematology, Department of Plant Sciences, Wageningen University, Binnenhaven 5, 6709 PD Wageningen, The Netherlands. Email: hans.helder@wur.nl*

Nematodes are thought to constitute the most numerous group of multicellular animals, and are present in virtually all terrestrial, freshwater and marine habitats. Several different feeding types are represented within the phylum Nematoda, and plant parasites constitute a minority. Here we will focus on three major groups of plant parasites – Tylenchida, Dorylaimidae and Trichodoridae – causing worldwide losses which are estimated at about $80 billion annually. Most notorious are cyst, lesion and root-knot nematodes. The morphology of nematodes is highly conserved, and microscope-based analysis of nematode communities is notoriously time-consuming, and can only be done by trained experts. DNA barcoding is a powerful alternative for the identification and quantification of this kind of organism. Among animals the small subunit ribosomal DNA (SSU rDNA) gene (≈ 1,700 bp) is often used to reconstruct deep phylogenetic relationships. Over the last few years we have sequenced a substantial part of the NW European terrestrial and freshwater nematode fauna (in total about 1,500 genotypes, up to Autumn 2007), and we observed that plant (and animal) parasitism has resulted in accelerated evolution of the SSU rDNA gene. The availability of sequence information for non-parasitic nematodes allowed us to define species-specific DNA signature sequences for the identification of plant pathogenic nematodes (including quarantine species such as *Meloidogyne chitwoodi, M. fallax*, *Globodera rostochiensis* and *G. pallida*) in highly complex backgrounds such as soil samples. Currently this rDNA signature-sequence-based identification and quantification system is used for high throughput routine screening of soil and plant samples.

STAR-BURST EVOLUTION OF THE PICORNAVIRUS SUPERFAMILY: TAXONOMIC IMPLICATIONS. **V.V. Dolja, Y.I. Wolf and E.V. Koonin.** *Department of Botany & Plant Pathology, Oregon State University, Corvallis, OR 97331, USA. Email: doljav@science.oregonstate.edu*

Phylogenetic analysis revealed that the picornavirus superfamily includes 14 families, 4 floating genera and 14 unclassified viruses that infect plants, fungi, oomycetes, vertebrates, arthropods, and a variety of unicellular eukaryotes. This vast assemblage of viruses contains seven evolutionary lineages that combine viruses with distinct host ranges. For instance, lineage 2 includes plant viruses of the family *Luteoviridae* and genus *Sobemovirus* along with fungal *Barnaviridae* and unclassified viruses that infect a dinoflagellate and an oomycete. The branching pattern of the lineages indicates a star phylogeny in which the principal diversification events appear to have occurred in a burst in a relatively short period of time. Because this pattern is similar to that described for supergroups of eukaryotes, it is proposed that the picornavirus-like viruses emerged and diverged into major lineages near the time of eukaryogenesis. The subsequent processes likely involved sampling the space of potential hosts by viruses and a slower virus-host coevolution that proceeded in a conventional, tree-like mode. Evolutionary affinities of the proteins that are most broadly conserved in the picornavirus superfamily suggest their origins from bacteriophages or, in the case of the trypsin-like proteinases, from bacteria themselves. Generally, our results support the continuing use of biological features such as viral host range, in addition to phylogenetic analysis, for virus classification. More specific implications for plant virus taxonomy involve proposals to elevate the genus *Sobemovirus* to a family status and to establish a new family including genera *Sadwavirus* and *Cheravirus*.

TAXONOMY OF PLANT PATHOGENIC BACTERIA. **C.T. Bull and B.A. Vinatzer.** *USDA/ARS 1636 E. Alisal St., Salinas CA 93905 USA. Email: Carolee.Bull@ars.usda.gov*

Taxonomy is the process of the classification, the application of names (nomenclature), and the practice of identification. Classification is an iterative process that follows the scientific method. Knowledge of relationships from previous findings is refined using the most powerful methods available. The validity of the results and conclusions are scrutinized through the peer review process. The scientific community begins to use the new classification system if, in their scientific opinion, it is an improvement over previous classifications. Over the past 20 years DNA:DNA hybridization and sequence analyses of individual genes informed classifications. Now new inexpensive technology allows researchers to compare sequences of multiple genes and even of entire genomes to study the genetic diversity of bacterial populations and classify organisms. If these studies include appropriate type strains, the information generated may be developed into proposals that could result in changes in nomenclature. The application of names to circumscribed taxa is conducted according

to rules and standards presented by internationally recognized codes of nomenclature. The International Code of Nomenclature of Prokaryotes regulates the nomenclature for species, subspecies and higher taxa. The International Standards for Naming Pathovars of Plant Pathogenic Bacteria, regulates the nomenclature of pathovars and provides recommendations for changes in nomenclature that involve the proposal of pathovars as species or subspecies. Results from current genomic studies may lead to the identification of genetic signatures for *Pseudomonas syringae* pathovars that could be used as justification for proposal of some pathovars as distinct species.

Biosecurity and quarantine

THE REVISED INTERNATIONAL PLANT PROTECTION CONVENTION – A NEW CONTEXT FOR PLANT QUARANTINE. **W.P. Roberts.** *Department of Agriculture, Fisheries and Forestry, GPO Box 858, Canberra, ACT 2601, Australia. Email: bill.roberts@daff.gov.au*

The purpose of the International Plant Protection Convention (IPPC) is to secure common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control. Under the IPPC, pests include: "any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products". Although the first version of the IPPC came into force in 1952 it was the recognition of the IPPC by the Sanitary and Phytosanitary Agreement of the World Trade Organization that provided the impetus to revise the IPPC and substantially increase standard setting activities. Today there are over 160 contracting parties to the IPPC. There are a significant number of approved standards covering a wide range of plant protection issues particularly relating to preventing or controlling pests moving in international trade. Every year a series of working groups produce new standards and revise old standards for approval by the meeting of the parties to the convention.

PEST RISK ANALYSIS AS APPLIED TO PLANT PATHOGENS. **F. Petter, S. Brunel and M. Suffert.** *European and Mediterranean Plant Protection Organization, 1 rue Le Nôtre, 75016 Paris, France. Email: hq@eppo.fr*

The European and Mediterranean Plant Protection Organization (EPPO) is a regional standard-setting organization created in 1951. One of EPPO's main priorities is to prevent the introduction of dangerous pests from other parts of the world, and to limit their spread within the region should they be introduced. In recent years, trade networks have expanded and diversified, increasing the risks of introducing pests to new geographical areas. Measures adopted by countries to protect their territories from these introductions should be technically justified and an International Standard for Phytosanitary Measures (ISPM) on Pest Risk Analysis (PRA) has been developed in the International Plant Protection Convention framework (ISPM no. 11). Since the 1990s, EPPO has also been involved in developing schemes for PRA. EPPO member countries favoured maintaining an EPPO scheme as it presents a logical sequence of questions which address all the elements mentioned in ISPM no. 11. The scheme provides detailed instructions for the different stages of PRA for quarantine pests: initiation, pest categorization, probability of introduction, assessment of potential economic consequences and pest risk management. Its application to pathogens is presented. Guidance for the use of additional tools, e.g. climatic predictions, and impact assessment tools are also being developed to assist pest risk analysts in mapping endangered areas for establishment and impact. Since 2006, a system has also been established to perform PRA at the EPPO level and Expert Working Groups (EWGs) are convened to conduct PRAs on specific pests. This system is presented. The PRA scheme and PRAs prepared within the EPPO framework are available on the EPPO website (www.eppo.org).

BIOTERRORISM AND BIOSECURITY. **M.L. Gullino, F. Suffert and J. Stack.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: marialodovica.gullino@unito.it*

Agriculture and related sectors are essential to social, economic and political stability worldwide. Food production and distribution systems are global in nature and pose relatively soft targets, offering many points at which commodities could be deliberately contaminated. Since 2002, there has been strong recognition worldwide of the existence of a genuine threat from terrorists or criminals to the agricultural sector and global food supply. Crop biosecurity is a new field of research, aimed, in its broadest sense, at protecting agriculture (crops), food supply and natural resources from the natural or intentional introduction, establishment and spread of plant pests, pathogens and noxious weeds. Securing agro-food systems requires the ability to identify threats and then to prevent, detect, respond to and recover from actual events. Most work in this field has been done in the United States, Australia and New Zealand. Existing programmes mostly focus on high-consequence, existing, or near-term threatening agents and events and their rapid detection, leading to numerous lists of plant and animal pathogens, compiled independently by several Agencies. European research defined a first list of high-threat candidate pathogens for Europe as well as a list of crops at risk. Results achieved to date are discussed as well as the importance of international cooperation in order to develop long-range strategic planning, to enhance the current surveillance infrastructure, to develop biosecurity capabilities in the agro-food sector, and to ensure worldwide optimal capacity to anticipate, prevent, respond to, and recover from acts of agroterrorism.

CONSEQUENCES OF MODERN DIAGNOSTICS FOR PLANT QUARANTINE. **M. Maes.** *Institute for Agricultural and Fisheries Research, Plant-Crop Protection, Burg, Van Gansberghelaan 96, 9820 Merelbeke, Belgium. Email: martine.maes@ilvo.vlaanderen.be*

Isolation, pathogenicity testing and host indexing are reliable diagnostic methods for bacterial, fungal and viral plant pathogens. Visual symptom detection combined with these methods have been used for phytosanitary control of quarantine pathogens. But obviously, several of these pathogens were able to spread despite extensive control of the traded plant material, probably through symptomless latent infections. Real-time PCR provides the sensitivity needed for detection of latent pathogens present in low concentrations or fastidious in growth. This method can be designed for high through-put and even on-site analyses. PCR, real-time PCR and sequence analysis are also interesting tools for symptom diagnosis and identification of new isolates. However, the reliability of molecular tests must be of great concern, since decision on pathogen contamination or identity is reduced to a blind DNA test and may be performed by analysts with a limited knowledge of microbiology, symptoms and

epidemiology. False results in quarantine matters can have an important impact. Therefore, taxonomists, curators of collections and phytopathologists have to cooperate to support the biotechnologists with data on the correct identity of the quarantine organism in terms of relatedness and variability within the taxon, host range and symptoms. The molecular method has to be thoroughly validated with strains from certified collections, working collections and with biological samples. In DNA/RNA detection technology only small samples can be processed. Hence, sampling methodology and knowledge on the *in planta* distribution and extraction of the pathogen are crucial. Preliminary selective enrichment of the pathogen or its DNA/RNA from larger sample volumes could be an interesting strategy for future developments.

Plant Pathology in industrialized and developing countries

PLANT PATHOLOGY: CURRENT AND PROPOSED DIRECTIONS IN THE UNITED STATES. J. Leach. *Bioagricultural Scineces, Colorado State University, Fort Collins, CO, 80523-1177 USA. Email: jan.leach@colostate.edu*

Plant pathology in the U.S. has been and will continue to be profoundly influenced by societal, institutional, environmental, and technological changes. The American Phytopathological Society established a series of Ad hoc committees to study plant pathology as a discipline and a profession. These committees were charged with assessing our current position and providing a vision of how best to best position plant pathology to meet future societal needs and scientific opportunities. The foundation for each study was the overarching vision statement: "Plant pathology will be a global and integrated discipline that will develop and apply the fundamentals of plant-microbe interactions to advance plant health and productivity". A summary of the key findings, predictions, and directions from these studies, particularly as they relate to plant pathology in the U.S., will be presented.

MOLECULAR PLANT PATHOLOGY IN SOUTHERN HEMISPHERE INDUSTRIALISED COUNTRIES. J. Manners. *CSIRO Plant Industry, Brisbane, QLD 4067, Australia. Email: john.manners@csiro.au*

The following are some of the key plant pathology challenges facing plant production systems in Australasia. 1. Maintaining biosecurity, including quarantine, incursion management and pre-emptive breeding, 2. Resisting and managing diseases that are re-emerging as a result of altered agricultural practices, 3. Resisting rapidly evolving pathogens, and 4. Nurturing a science capability that will lead to durable solutions to major diseases. Molecular plant pathology will contribute to all of these challenges. The recent incursion of smut disease into the tropical sugarcane industry has required an overhaul of cultivars and breeding germplasm, and DNA markers are assisting in this process. Necrotrophic pathogens, for example Fusaria causing crown rot, are now major issues for the wheat industry because of practices such as stubble retention and minimum tillage, and molecular approaches are used to increase our knowledge and to both breed and engineer resistance to these pathogens. Rust fungi have been a long-standing threat to the Australian wheat industry and CSIRO in Canberra have led the world in understanding host-rust fungus interactions at the molecular level. Based on structure-function studies of resistance and avirulence genes, this work is moving towards engineering durable resistance. Finally, in the São Paulo region of Brazil a new force in molecular pathology has emerged based on a substantial genomics capability developed to underpin the large agricultural industry of this region. This has led to sequencing the genomes of important bacterial and viral pathogens and suggests a global shift of capability in molecular pathology.

PLANT PATHOLOGY IN EUROPE: PRESENT STATUS, IMPACT AND FUTURE CHALLENGES. J.A. Lucas. *Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK. Email: john.lucas@bbsrc.ac.uk*

Plant pathology has traditionally had a strong base in many European countries, with research networks of Institutes, specialised extension services and academic groups in universities. This has changed in recent years with reduced emphasis on and support for agricultural sciences across much of Western Europe, with consequences including the closure of some Institutes, contraction of government-funded consultancy services, and consolidation of the agrochemical sector. Concerns have also been voiced about a potential loss of skills in specialist disciplines such as mycology, plant nematology and virology. At the same time the biological sciences have been invigorated by a revolution in molecular genetics and genomics, with European researchers making key contributions in high profile and fast moving areas such as host-pathogen interactions, plant innate immunity and defence. Genome projects are delivered by research consortia and this has accelerated integration and globalisation of the research community. A challenge for plant pathology is to ensure that advances in the fundamental science translate into practical applications not only in Europe but beyond. This will be crucial given current concerns about sustainability of the food chain, and the need for a new green revolution, to meet not only demand for food but also industrial crops, such as biofuels, and to adapt to the likely impacts of climate change.

THE MANY CHALLENGES FOR PLANT PATHOLOGY IN AFRICA. J. Roux, A. Muimba-Kankolongo, M. Apetorgbor, D.A. Begoude, G. Nakabonge and M.J. Wingfield. *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa. Email: jolanda.roux@fabi.up.ac.za*

Plants, including trees for fuel wood, construction timber and fruit, present a hugely important resource in Africa. In the majority of African countries, wood provides the most important source of fuel and lighting, while fruit and agricultural crops provide staple diets for millions. Despite seemingly good soil fertility and opportunities for enhanced agricultural production, most African farmers rely on subsistence agriculture. The impact of pathogens and pests on agricultural crops and trees in Africa is huge. Studies in several countries have shown that losses to agricultural crops caused by virus diseases reached up to 90%, while in most of Africa, mycotoxin-producing fungi present a substantial threat to human, animal and plant health. Likewise, fungal diseases of forest trees have caused the planting of preferred tree species to be discontinued. Yet, despite the often difficult conditions, plant health specialists in the region have contributed substantially to plant health management. One of the greatest constraints to plant health management in Africa is a severe shortage of trained plant health experts. In many cases, where these experts are available, they are seriously limited in what they can achieve due to low levels of funding, poor infrastructure and in-

adequate support structures. For example, many African countries do not have reliable or stable power supplies, making the application of modern diagnostic and other techniques impossible or very costly. These poor conditions result in many African plant pathologists finding employment elsewhere in the world. This is an unfortunate situation that deserves the attention and support of plant pathologists who operate under more fortunate conditions.

Transgenic plants

GENERAL OVERVIEW OF GM CROPS 1996-2007; ADOPTION, IMPACT AND FUTURE PROSPECTS. J. Clive. *International Service for the Acquisition of Agri-biotech Applications (ISAAA), P.O. Box 427 SAV, Grand Cayman, Cayman Islands, UK. Email: cjames@candw.ky*

In the early 1990s, some were skeptical that genetically modified (GM) or transgenic crops, now more often referred to as 'biotech crops', could deliver improved products and make an impact at the farm production level. There was even more skepticism that the developing countries of Asia, Latin America and Africa could access and adopt biotech crops developed by industry. The first decade of commercialization of biotech crops, 1996-2005, witnessed unprecedented rapid global adoption in both industrial and developing countries. 2007 was the second year of the second decade of commercialization of biotech crops (1996-2015). The adoption of biotech crops, in 2007 and the period 1996 to 2007 is reviewed, within a broad framework, that addresses their adoption and impact (economic, environmental and humanitarian) including their contribution to food, feed, fiber and fuel security on a global basis. The future prospects of biotech crops are summarized including their potential contribution to a more sustainable agriculture.

MECHANISMS OF RESISTANCE TO VIRUS DISEASES. R.S. Nelson. *Plant Biology Division, 2510 Sam Noble Parkway, Samuel Roberts Noble Foundation, Inc., Ardmore, Oklahoma, 73401, USA. Email: rsnelson@noble.org*

Two procedures, traditional crossing and stable transformation, are the most common methods used to produce plants resistant to virus-induced disease. The mechanisms employed by these plants to resist virus disease, however, are astonishingly variable. They vary from expression of a nucleic acid or polypeptide sequence that directly inhibits virus accumulation to the loss of expression of a sequence necessary for virus accumulation. The former relies on a sequence from the plant or virus while the latter relies on a sequence from the plant. Specific examples include expression of plant-derived or virus-derived genes for resistance to virus accumulation [e.g. respectively, resistance (R) gene-mediated and RNA silencing] and loss of expression of plant-derived genes necessary for virus accumulation (e.g. eIF4E down-regulation). Resistance phenotypes are manifest as a block in virus replication, cell-to-cell movement, or vascular-mediated accumulation. A summary of these and other novel resistance mechanisms to virus infection will be presented. It is clear that researchers now have identified resistance mechanisms which, either singly or combined, can be used to produce plants resistant to most, if not all, viruses. The reasons that this ultimate goal has not been achieved will be discussed.

RISK ASSESSMENT OF GM PLANTS AND PRODUCTS: FACTS, THE PUBLIC AND POLITICS. T. Twardowski. *Instytut Chemii Bioorganicznej i Politechnika ¸ódzka, Noskowskiego 12/14, 61-704 Poznaf, Poland. Email: twardows@ibch.poznan.pl*

So far, only four kinds of plant and two traits have occupied up to 99% of the total area of GM plantings. However, up to 70% of the products available in supermarkets are affected by genetic engineering. Statistically, every single person on the globe has contact with GMO or GM products. Risk evaluation of GMO and GM products is well developed and scientifically advanced, though it consumes time, money and labor. Probably biotechnology is the only innovation technology having such an advanced self-security program developed by biotechnologists themselves. During the first ten years of commercialization, no severe incident has been reported. However, science fiction stimulates the imagination and history of opposition. The sociotechnique of GMO opponents is extremely effective and so is that of politicians, through general public influence. This political environment has borne fruit in legislation, which is directly connected with the economy and development of many states. An example is the formation of 'GMO-free zones', the most important aim for those who oppose modern biotechnology. According to lawyers and biotechnologists, this concept is: 1) contrary to present legislation, 2) without scientific background, and 3) against the development of the national economy.

Global seed health: concerns and solutions

DIAGNOSTIC TARGETS, MOLECULAR TAXONOMY AND THE BARCODING OF LIFE. G. Vannacci and G. Firrao. *Dip. CDSL - Sez. Patologia Vegetale, University of Pisa, Via del Borghetto, 80, 56124 Pisa, Italy. Email: g.vannacci@agr.unipi.it*

Technical advances in molecular biology provided us with an array of finely tuned seed health testing tools that entailed major progress in sensitivity and specificity. However, methods are still based on defined molecular features that do not account for the complexity of living organisms. High specificity reduces false positives but we should know exactly what we need, or we want, to diagnose and how the molecular feature that we plan to diagnose fits our target. Specificity derives from "species" which is the preferred reference in fungal detection and diagnosis in a seed lot. Thus, changes in the species concept have marked consequences in diagnostics. Since, at least in fungi, the operational definition of species is more and more frequently based on the concordance of different genes genealogies, the strict relationship between a specific target and a given species may get lost. Many morphospecies are under revision and some of them have been, and others will be, split in different molecular species. These molecular differences do not correlate with pathogenicity or other relevant traits (such as mycotoxin production), leading to the paradox that a too high specificity would not be advisable for such pathogens. Conversely, we will need diagnostic tools targeting groups of harmful organisms with similar traits. In these cases, diagnostic should move from a taxonomic to a physiological framework. On the other hand, species revisions based on molecular analyses in obvious contrast with morphological evidences contributed substantially to clarify several enigmatic case studies. Examples from seed borne fungi will be given to illustrate these points.

TRANSBOUNDARY MOVEMENT OF SEEDS AS INFLUENCED BY THE WTO: A DEVELOPING COUNTRY'S PERSPECTIVE. **R.K. Khetarpal, V.C. Chalam and K. Gupta.** *Division of Plant Quarantine, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110012, India. Email: ravi@nbpgr.ernet.in*

Seeds are potential carriers of a number of destructive pests or virulent strains thereof during transboundary movement. Many developing countries are unable to comply with the Agreement on Sanitary and Phytosanitary (SPS) Measures of the WTO to which they are signatory members. India has recently taken some initiative for developing its National Standards of Phytosanitary Measures in line with International Standards on Phytosanitary Measures, initiating Pest Risk Analysis, strengthening its survey and surveillance programme, upgrading its quarantine facilities and expertise including establishment of containment facilities and laboratories for detection of transgenes. A number of supportive research programmes viz., molecular detection of pests, development of digitized keys for identification of pests, development of disinfestation protocols based on thermal and irradiation treatments and detection of transgenicity in undeclared samples during exchange or trade have also been recently initiated. The biosafety issues related to safe trade in Genetically Modified Organisms also need to be addressed. Besides, the seed certification programme of a country impinges upon its export market and quarantine. In India, seed health testing standards are prescribed for only a few cases with none in respect to seed-transmitted viruses. For these a multi-institution project was recently undertaken to develop diagnostic kits and standards for quality control of important legume viruses. Information on distribution of pests and their epidemiology needs to be constantly generated in identifying both recently introduced pests, and the pest-free areas for facilitating export. Compliance with the SPS Agreement is a multifaceted task where comprehensive policy and operational reforms are needed simultaneously.

INFORMATION RESOURCES AND THEIR APPLICATION IN RATIONALIZING SEED HEALTH REGULATIONS. **P. Scott, L. Charles, J. Cortes and R. Day.** *CABI, Wallingford, UK. Email: l.charles@cabi.org*

National phytosanitary regulation, including seed heath considerations, must be based on the available information. Where information is lacking, regulations are still necessary, but they can be modified when new information becomes available, promoting dissemination and trade of improved seed, without increasing risk. Case studies are described in which countries have been ready to simplify their seed health regulations when an independent, science-based information source is invoked to show, for example, that pest organisms intended to be excluded from entering a country by its regulations are in fact already present. Such a source might also show that seed-transmission of the organism is improbable, or that impact of the organism is low. Or it might indicate opportunities for the regional harmonization of phytosanitary measures. To be accepted as a basis for risk analysis in contexts such as these, an information source needs to be readily identified as science-based, independent of sectoral interests, comprehensive, and preferably widely used. Examples of information resources that complement and enhance the official exchange of information facilitated by the International Plant Protection Convention (IPPC) are discussed.

FACTORS AFFECTING RISKS RELATED TO SEED-TRANSMITTED *FUSARIUM* SPP. IN MAIZE. **G.P. Munkvold.** *Iowa State University, 160 Seed Science, Ames, IA 50011, USA. Email: munkvold@iastate.edu*

Several species of *Fusarium* can be seed-transmitted in maize, and these fungi are both pathogenic to the plant and toxigenic toward maize-consuming livestock and humans. *Fusarium proliferatum*, *F. subglutinans*, and *F. verticillioides* are commonly seed-transmitted, but the impact of this phenomenon depends on environmental (biotic and abiotic) and management factors in the seed production field, the seed conditioning environment, and the planted field. In this presentation, the influence of selected factors in each of these environments will be discussed, including insect infestation in seed production, gravity separation of seeds, fungicidal seed treatment, and temperature conditions in the planted field. Insect management can play a significant role in reducing seed infection, which can be further reduced in a predictable way through gravity separation of seeds. Fungicidal seed treatment may contribute to minimizing the risk of seed transmission. The consequences of *Fusarium* seed transmission include reduced emergence, poor seedling health, and systemic infection of maize plants. The importance of systemic infection is not completely understood, but it occurs more extensively under warm temperature conditions; therefore the risks associated with systemic *Fusarium* infection are likely enhanced under these conditions. The implications of these environmental and management factors taken together will be discussed.

What future for plant pathology

THE FUTURE OF PLANT PATHOLOGY. CHALLENGES/OPPORTUNITIES: A U.S. PERSPECTIVE. **M.B. Dickman.** *Institute for Plant Genomics and Biotechnology, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, 77843-2123, USA. Email: mbdickman@tamu.edu*

Plant pathology is a discipline borne of necessity. Since this science of approximately 150 years has changed, evolved, and advanced, the perception of the importance and relevance of plant disease research fluctuates from the classic Irish potato famine to the sobering fact that numerous U.S. university plant pathology departments are closing or being consolidated with other disciplines. Historically, in the U.S., the overall report card for plant pathology has been quite good; food is generally abundant and the comparative cost quite reasonable. It is evident that previous and ongoing research has been quite successful in the U.S., although this statement cannot be said for other areas of the world. With the world shrinking, usable land decreasing, and the population increasing, coupled with global warming, and the threat of bioterrorism, an important consideration is whether we are adequately preparing the next generation of students/researchers (the numbers of which are also decreasing in the U.S.) to meet the inevitable demands in the future? When food safety and food security issues are added, the task ahead becomes even more daunting. We are now in the era of post-genomics, transgenic plants, precision breeding, and overall remarkable technological advances. Can these rapidly advancing technologies be implemented with a new core of dedicated trained professionals to provide an adequate supply for the world?

EFFECTS OF PLANT DISEASES ON CROP PRODUCTION: A EUROPEAN PERSPECTIVE. **E.-C. Oerke.** *Institute of Crop Sci-*

ence and Resource Conservation – Phytomedicine, University of Bonn, Germany. Email: ec-oerke@uni-bonn.de

When growing plants for food, feed and energy, man has to compete with pests for this limited resource. Incidence and severity of diseases often increase with production intensity, and crop productivity is endangered especially in high-input systems predominating in Europe. Yield losses to pathogens may reduce both crop quantity and quality, and in developed countries the high consumer standards for food quality are still increasing. Detection of mycotoxins in plant produce has increased the interest in effective control of pathogens in cereals, grapevines, and other crops. Fungicides are used to sustain productivity at high levels but it is also necessary to preserve natural resources. High productivity agriculture will become more important in future, as bio-energy production promotes changes in land use and results in competition for areas at present devoted to food and feed production. Climate change is likely to affect the spatial distribution of crops and pathogens as well as their impact on productivity. Innovative technologies like remote sensing, GPS-based monitoring of pathogens, GIS-based disease mapping and technologies for exact applications may contribute to reduce pesticide use in spite of emerging disease problems. Consumers want to have food not treated with pesticides or free from pesticide residues, but producers do not have the technologies to control disease without chemicals. Public awareness of the origins of food which seems to superabound, and of the ongoing thread to crops is often low in industrialized countries and has resulted in uninformed prejudices as well as in closure of university departments of plant pathology.

ASIAN PERSPECTIVE ON THE FUTURE OF PLANT PATHOLOGY. **E.W. Park.** *Department of Agricultural Biotechnology, Seoul National University, Seoul, 151-921, Republic of Korea. Email: ewpark@snu.ac.kr*

Asia is the world's largest and most populous continent covering 29.4% of Earth's land area and containing almost 4 billion people, more than 60% of the world population. The developing economies in Asia including East Asia, Pacific region and South Asia have grown rapidly, increasing their share of global output from 19% in 1995 to 27% in 2005. Considering its land area, human population, and economic growth, Asia will become increasingly influential to global food security and economy in the future. For example, rice production in Asia, which covers more than 90% of the world production, clearly affects food supply not only in Asia but also in other continents. In the 21st century, globalization is a major issue in all aspects of human life, and convergence of technologies offers immense opportunities for the improvement of human abilities, social outcomes, productivity and quality of life. The rapidly expanding volume of agricultural products in global trade has greatly accelerated the introduction and establishment of invasive plant pathogens and pests. Consequently, international and interdisciplinary collaboration becomes more and more important to investigate fundamentals of plant-microbe interactions and to develop and apply innovative tools for plant health and productivity. This presentation will discuss the future of plant pathology in Asia in the light of societal, environmental, and technological changes with special emphasis on East and South East Asian countries.

GLOBAL PERSPECTIVE OF ENVIRONMENTAL AND AGRICULTURAL SUSTAINABILITY. **J. Gilliland.** *Rural Generation Ltd, Brook Hall Estate, 67 Culmore Road, Londonderry, BT48 8JE, Northern Ireland. Email: jgilliland@twisel.com*

With an annual increase of 70 million people on this planet to feed, one might say that the future for both environmental & agricultural sustainability is a real challenge. But it is not until one adds in key issues such as food, fuel & water poverty; climate change; dietary change with in the Pacific Rim; and the need for energy and water security; do you realise the truely enormous challenges facing us in the future. The last 18 months have been a truly definitive tipping point, for global sustainability. The first real inflation in food prices; food riots on the streets of Mexico; exotic livestock diseases rampant across Europe; and an International Agreement to find a replacement for Kyoto, by 2009; clearly proves that we have turned a definitive corner and we now need, more than ever, smarter tools if global sustainability is ever to be achieved. Investment in R&D, in this area, is probably now at its lowest level for a Generation. The ultimate goal, a truly multifunctional Agriculture can only be achieved from using the products of increased investment in R&D, to allow farmers and land managers to deliver on multiple societal benefits, off the same piece of land, with in the same year. Without this, global environmental & agricultural sustainability will never be achieved!

PLANT PATHOGENS AND MICROBIAL INTERACTIONS IN SOIL

TRIPARTITE INTERACTIONS AMONG PLANTS, ARBUSCULAR MYCORRHIZAL FUNGI AND ENDOCELLULAR BACTERIA. **P. Bonfante.** *Department of Plant Biology and IPP-CNR, Viale Mattioli 25, 10125 Torino, Italy. Email: p.bonfante@ipp.cnr.it*

For efficient uptake of nutrients most land plants need to be associated with arbuscular mycorrhizal (AM) fungi which are vital components of rhizospheres. AM fungi supply plants with nutrients, thus increasing their productivity and conferring resistance to stress. Exploitation of these symbioses in agro-environments is of high environmental and economic value. However, mostly due to the obligate biotrophic status of AM fungi, there is still a lack of knowledge on their biology. The aim of this presentation is to illustrate a peculiar feature of these symbionts, i.e. the presence of bacteria in their cytoplasm as a rather common event. Intracellular bacteria were characterized in the isolate BEG 34 of *Gigaspora margarita* as a new bacterial taxon, '*Candidatus* Glomeribacter gigasporarum', which has a reduced (1.4 Mb) genome. A sequencing project is currently in progress, and will allow direct investigation of the bacterium's impact on the fungal host. The bacteria are vertically transmitted through fungal spore generations and thanks to a protocol based on repeated passages through single-spore inocula, cured spores were obtained, giving a line that has a distinct phenotype regarding cytoplasm and cell wall organization. The absence of bacteria severely affects presymbiotic fungal growth such as elongation and branching after root exudate treatment, suggesting that *Ca*. Glomeribacter is important for optimal development of its fungal host. Under laboratory conditions, the cured fungus could be propagated, representing a stable variant of the wild type. Taken as a whole the data reveal arbuscular mycorrhizas as true tripartite interactions.

FATE, PHYSIOLOGY AND IMPACT OF FIELD-RELEASED *PSEUDOMONAS FLUORESCENS* SBW25. **J.K. Jansson.** *Department of Microbiology, Swedish University of Agricultural Sciences, Box 7025, 750 07 Uppsala, Sweden. Email: Janet.Jansson@mikrob.slu.se*

The plant growth promoting bacterium, *Pseudomonas fluorescens* SBW25 was marked with genes encoding GFP, luciferase and tellurite resistance and released in the first Swedish field trial of a genetially modified microorganism (GMM). The GMM strain survived well on all wheat plant surfaces over the entire 8 month experimental period. No treatment resulted in reduction of disease symptoms caused by *Microdochium nivale* and no GMM cells were ever found in bulk soil or uninoculated plants. The indigenous soil and plant-associated microbiota was impacted to a minor extent by the GMM inoculant as assessed by T-RFLP using primers directed towards bacteria and fungi. By contrast, time and plant part sampled had significant effects on the community composition of the indigenous microbiota. In addition, the physiological status and biocontrol effects of SBW25 were studied in more detail using proteomics and a combination of marker genes and the results support the use of SBW25 as a plant growth promoting inoculant.

TYPE III PROTEIN SECRETION SYSTEM IN FLUORESCENT PSEUDOMONADS CAPABLE OF BIOCONTROL. F. Rezzonico, D. Gobbin, G. Défago and Y. Moënne-Loccoz. *UMR CNRS 5557, Ecologie Microbienne, Université Lyon 1, F-69622 Villeurbanne, France. Email: moenne@biomserv.univ-lyon1.fr*

Many root-colonizing fluorescent pseudomonads can protect plants from soil-borne fungal pathogens. They act on pathogens via competition or antagonism, and/or by inducing systemic resistance in the plant host. In recent years, new properties such as the presence of genes encoding a type III protein secretion system (T3SS) have been identified in biocontrol pseudomonads. In pseudomonads, this feature was considered a virulence trait, but inactivation of the T3SS gene *hrcV* in biocontrol strain *Pseudomonas fluorescens* KD reduced protection of cucumber from *Pythium ultimum*. In biocontrol pseudomonads, the occurrence of T3SS gene *hrcN* was more frequent in strains that can produce the antifungal metabolite 2,4-diacetylphloroglucinol (Phl). In Phl-producing pseudomonads, *hrcN* was found in five of the six main MLST groups, corresponding to the *P. fluorescens* complex. Statistical analyses showed that the percentage of biocontrol pseudomonads with gene *hrcN* was higher among tomato isolates than in strains from cucumber, tobacco or wheat. The occurrence of *hrcN* in biocontrol strains was associated with superior disease suppression ability in the *Fusarium oxysporum* f. sp. *radicis-lycopersici*-tomato pathosystem and especially the *Pythium ultimum*-cucumber pathosystem, but this correlated largely with Phl production ability and other plant-beneficial traits. Phylogenetic analysis suggested that the occurrence of *hrcN* is ancient in biocontrol pseudomonads, which mostly displayed specific *hrcN* alleles in comparison with phytopathogenic pseudomonads. Overall, the occurrence of T3SS genes is likely to be a basic ecological trait in most biocontrol pseudomonads.

ADAPTATION AND DEFENCE OF PLANT PATHOGENS TO MICROBIAL ANTAGONISM. J.M. Raaijmakers and A. Schouten. *Laboratory of Phytopathology, Wageningen University, P.O. Box 8025, 6700 EE Wageningen, The Netherlands. Email: jos.raaijmakers @wur.nl*

Microbial interactions in soil and plant-associated environments are mostly viewed from the perspective of how beneficial microorganisms inhibit the growth or activity of pathogens. However, pathogens themselves have a diverse array of mechanisms to counteract antagonism, including active efflux and degradation of antimicrobial compounds, and interference with the regulation and biosynthesis of enzymes and antimicrobial metabolites produced by antagonistic microorganisms. Development of resistance in pathogen populations to chemical control agents is common and well-studied. In contrast, resistance in pathogens to antimicrobial compounds produced by antagonistic microorganisms is presumed not to develop or at least to do so relatively slowly, because antagonistic microorganisms operate in microsites where only a small fraction of the pathogen population is exposed to the antimicrobial compounds during a short period of its life cycle. Furthermore, in contrast to the inundative application of chemical pesticides, only minute amounts of the antimicrobial compounds are presumed to be produced by the antagonistic microorganisms in situ. Studies focusing on the antimicrobial metabolites 2,4-DAPG and cyclic lipopeptide surfactants show that substantial variation in sensitivity exists within pathogen populations and that pathogens can deploy a wide range of defence mechanisms against microbial antagonism, including detoxification and active efflux.

PLANT PATHOLOGY IN INDUSTRIALIZED AND DEVELOPING COUNTRIES

PLANT PATHOLOGY IN CHINA, PRESENT STATUS AND FUTURE PRIORITIES. Y.-L. Peng. *Department of Plant Pathology, China Agricultural University, Beijing 100094, P.R. China. Email: pengyl@cau.edu.cn*

China has 1.3 billion people but 0.127 billion hectare arable land. A major challenge for the Chinese government is to maintain food self-supplying during a time that crop-land is declining due to rapid urbanization and industrialization. Since plant diseases are an important threat to food production, and the central and provincial governments have made great efforts to reduce crop loss due to the diseases that include setting up more research facilities, increasing researching funds and enlarging community of extension experts. At present, more than 40 universities established department of plant pathology, in each of which there are 10 to 30 faculties. In the Chinese academy of agricultural sciences (CAAS), there are 14 institutes with plant protection research groups, each of which involves 5-10 researchers. Plant Protection Institute is a CAAS-affiliated institution with more than 200 researchers who are aiming at developing technology for controlling pests and diseases that occur in the whole country. In addition, each provincial academy of agricultural sciences has its own plant protection institute to deal with local crop diseases. There is also a huge plant pathology extension service with about 4000 specialists working in the county or district extension stations to provide farmers with plant disease control guidance. As economy is improved in China, more research funding from diverse resources was recently set up. For example, in 2007, the central government provided a special fund of one billion RMB for the start-up of ten programs aimed at solving technology restraints to increase the production of ten major agricultural crops, including rice, corn and wheat. The Ministry of Science and Technology and the National Science Foundation of China also provide funding for basic research on plant pathology. Special funds from the China Scholarship Council and from the National Science Foundation of China have also been set up to support the student and visiting scientist exchange program and the international collaboration, respectively.

PLANT PATHOLOGY IN INDIA: HISTORICAL PERSPECTIVES, PRESENT STATUS AND FUTURE CHALLENGES. **U.S. Singh and N.W. Zaidi.** *Department of Plant Pathology, College of Agriculture, G.B. Pant, University of Agriculture & Technology, Pantnagar 263145, India. Email: umesh146@yahoo.com*

Indian agriculture witnessed growth and development that have a few parallels in the world. The country emerged from an era of food deficiency to that of food sufficiency. Plant pathology developed as a science in India about hundred years ago. Prior to this, British officers having Botany as a hobby made several valuable observations and notings. Plant pathology has been and will persist to be philosophically influenced by major changes in the novel technologies, organizations, communities and the ecology. Presently, plant pathology in India has a fairly strong research base with linkages of research Institutes, specialised extension departments and academic groups in universities. This has gained momentum in recent years, as a consequence of which several big research organizations, institutes, government-funded extension services and NGO's have come forward to work together. A couple of good Phytopathological Societies have been established in the country to give an insight of plant pathology as a discipline and a profession. However, establishment of a number of small societies is proving to be counter productive. Plant pathological research in India is striving not only to keep pace with modern and applied aspects of the subject, but to also deal with its traditional branches like mycology, fungal pathology, virology and nematology. However, bacteriology and phytoplasmology are yet to find due recognition. Use of molecular genomics and genetic tools is the latest attraction of Indian plant pathologists by which, this discipline as a whole has taken a giant leap in the perception of complex research areas such as host-pathogen interactions, signal transduction, population biology etc. However, in spite of all these advancements primarily in research, we are still far from performing our duties as a practitioners (i.e. plant doctors). Limited success of IPM programme in the country, in spite of strong research base and huge investment by both Federal and States governments, speaks of failure of plant pathologists to perform their duties as plant doctors. One of the major lacunae in this programme was identified to be poor access of disease diagnostics and advisory services to the farmers particularly to small and marginal farmers. Because of economic liberisation, entry of big corporate houses in agriculture and popularisation of contract farming, there is pressure on plant pathologists to come out of lab and concentrate more on practice, otherwise they will loose their relevance. There is need to create a cadre of plant doctors who can take up private practice on the pattern of human and animal doctors. Indian agriculture would need a new vision to make rapid progress in the ensuing millennium to ensure food security without sacrificing food safety. One of the important challenges for the planners, decision-makers and plant pathologists is to make sure that the technologies developed should take a shape of practical convenience and reach up to benefit the end user i.e., the farmer. There is also need to concentrate on overall plant health management rather than plant diseases alone. Probably time is ripe to group different agricultural sciences in two major divisions i.e. Plant improvement and plant health management. Plant pathology, entomology, agronomy, soil science and weed science should be a part of division of plant health management.

APPLICABILITY OF PLANT PATHOLOGY RESEARCH AT IRRI AND OTHER CGIAR INSTITUTES TO INDUSTRIALIZED AND DEVELOPING COUNTRIES. **R.S. Zeigler.** *International Rice Research Institute (IRRI), Los Baños, Laguna, Philippines. Email: r.zeigler@cgiar.org*

The international agricultural research centers (IARCs) have conducted research on plant diseases of the world's principal food crops since the founding of the International Rice Research Institute in 1960, followed by the creation of other IARCs through the mid 1970s. Prior to this, little research was done on tropical food crops of importance to developing countries. Research first focused on the etiology of poorly understood diseases, clarification of pathogen-vector relations, and the development of disease management using host-plant resistance. Major contributions include the development of effective field – and controlled-environment screening procedures, elucidation of the genetics of resistance, and the development of pathogen-resistant cultivars. Disease management approaches have also been developed based on epidemiological modeling and understanding of pathogen population dynamics. A significant contribution of the IARCs has been the compilation of compendia and other means of assembling readily available information about heretofore poorly studied diseases. Perhaps most important has been the building of plant pathology research capacity in developing countries. Thousands of developing country scientists have been exposed to plant pathology as an integral part of any crop-improvement program through the efforts of the IARCs. Finally, the products of IARC research have directly benefited industrialized countries, especially by providing sources of durable resistance to key pathogens in crops such as wheat, maize, potato, dry beans and rice. Specific cases from Asia, Africa and Latin America will be presented to illustrate the above points.

GLOBAL TRANSFER OF PLANT PATHOLOGY TECHNIQUES. **R. Strange.** *School of Biological ad Chemical Sciences, Malet Street, London, UK. Email: r.strange@sbc.bbk.ac.uk*

Country boarders are not recognised as barriers by plant pathogens and, in this era of mass air transport, even oceans provide only temporary respite from the onslaught of serious pathogens of our most important crops. Moreover, indigenous pathogens evolve more virulent forms *in situ*. It is therefore imperative that Plant Pathologists recognise the global dimensions of their discipline and unite in developing techniques that mitigate the worst effects of such pathogens and facilitate their deployment where required. Knowing your enemy is a sound policy in Plant Pathology and, in this context, means accuracy both in the identification of the pathogen and in estimates of the amount of damage it causes. The former is mandatory *inter alia* for quarantine procedures and the latter essential for establishing priorities for developing control measures. Knowledge of the epidemiology of a given pathogen may be crucial in developing techniques to limit its spread and knowledge of its specificity for the discovery of potential breeding material for resistance. The elucidation of mechanisms of resistance and susceptibility hold the prospect of producing cultivars of crop plants either by conventional breeding or genetic transformation with durable resistance to their worst pathogens. The talk will be illustrated with examples which show how the application of such techniques have the potential to do much to alleviate the hunger experienced by at least one-eighth of the world's population and will also accentuate the difficulties in applying such techniques in poor Developing Countries where the spectre of hunger is ever present.

OFFERED PAPERS

Abstracts marked with asterisk were selected for short oral presentations.

AIRBORNE PLANT DISEASES

15.1 *BOTRYTIS CINEREA* ON TOMATO IN HEATED GREENHOUSES. **R. Aerts, L. Vogels, S. Gielen, B. Seels and K. Heyens.** *Research Group Sustainable Crop Protection, Department Industrial and Bio Sciences, Katholieke Hogeschool Kempen, Kleinhoefstraat 4, BE-2440 Geel, Belgium. Email: rudi.aerts@khk.be*

In heated greenhouses *Botrytis cinerea* is the most important pathogen of tomato. By studying infections in greenhouses it became clear that *B. cinerea* can not infect all parts of the plant. Spores can only germinate in the presence of water or high humidity. This means that healthy, unwounded tissue cannot be infected. Dying tissue still retains some moisture and can be easily infected. Thus two kinds of infection appear: those on wounds and those on necrotic tissues. Healthy leaves cannot be infected but sometimes leaves may develop some necrosis. On this necrotic tissue *B. cinerea* can germinate and grow towards the healthy parts of the leaves. Prevention of necrosis should be part of *Botrytis* control measures. Tomato plants are wounded by cutting the leaves or by harvesting the tomatoes. These wounds can be infected by *B. cinerea*. Stem infections are much more important then leaf infections. The number of infections depends above all on the concentration of spores in the greenhouse and the shape of the wounds. The risk of an infection can be reduced by making on the stem a very smooth wound that will dry more rapidly and have less necrotic parts that can be infected. In the study presented here, many infections were measured. More than 90% of the infections on stems appeared at irregular, rough wounds, so without necrotic tissue it is very difficult for *B. cinerea* to infect tomato plants. A control method based on this knowledge was very successful. The number of infections decreased by more than 90%.

15.2 GUIDELINES FOR MANAGEMENT OF POWDERY MILDEW ON CAPSICUMS. **C. Akem, Z. Baron, G. MacManus and P. Boccalatte.** *Horticulture and Forestry Science, Department of Primary Industries and Fisheries, P.O. Box 15, Ayr, QLD 4807, Australia. Email: Chrys.Akem@dpi.qld.gov.au*

Powdery mildew caused by the fungus *Leveillula taurica* (Lev.), is a common disease of capsicums in tropical and subtropical environments of Australia where most of the capsicums are produced. It is typically a late-season disease, difficult to control with protectant fungicides such as sulphur, once fully established in the field. The need for a more consistent and reliable approach to manage the disease necessitated a study to establish a set of guidelines for growers to sustainably manage the disease. A number of environmentally friendly procedures including screening and identification of resistant varieties, evaluation of foliar fertilizers and other soft chemical alternatives for disease control were assessed. The results led to a set of guidelines, distributed to growers for use in managing the disease in a more sustainable way. These guidelines included the need to start disease management early in the season, using an appropriate foliar fertilizer application such as silicon; following up with the first systemic fungicide spray only when initial infection has been detected; and alternating between fungicide groups to minimise the development of resistance. Also important is the need for good spray coverage, ensuring that the disease is controlled on both leaf surfaces. The ultimate aim of the recommended management program is to encourage growers to apply as few or minimal inputs as possible, but as much as needed to manage the disease for sustainable yields.

15.3 UNRAVELLING THE EVOLUTIONARY DYNAMICS OF A NOVEL CERCOSPOROID FUNGUS. **O.A. Akinsanmi, A.K. Miles and A. Drenth.** *Tree Pathology Centre, The University of Queensland and Department of Primary Industries and Fisheries, 80 Meiers Road, Indooroopilly, QLD 4068, Australia. Email: uqoakins@uq.edu.au*

The fungus *Pseudocercospora macadamiae* has no known sexual state. It is novel, unique and spreading to new macadamia-growing regions in Australia. As with other cercosporoid fungi, initial findings showed that the asexual spores mostly spread by rain-splash; however, the prevalence and virulence on different macadamia varieties and the distribution pattern of the fungus indicates the involvement of sexual spores. We tested the hypothesis that the sexual structure is produced only under specific conditions and is involved in the evolutionary dynamics of the fungus. The putative geographical isolation observed in the pathogen populations may account for the low level of recombination between strains from different regions. Primers designed for *MAT 1-1* and *MAT 1-2* genes differentiated the isolates into different mating types. Using both indirect and direct bio-assays, we identified significant ($P<0.05$) association between the disease caused by the pathogen and some host characters. Discriminant analysis notably differentiated the host varieties based on their propensity to heighten the dispersal and possible recombination in the pathogen populations. These preliminary results imply a role for ascospores in the biology and epidemiology of the pathogen, but further studies to confirm sexual recombination and establish the distribution of the putative mating types are currently underway.

15.4 VERTICAL DISTRIBUTION OF LEAF BLAST LESIONS IN MIXTURES OF RICE CULTIVAR SASANISHIKI AND ITS RESISTANT NEAR-ISOGENIC LINE. **T. Ashizawa, K.S. Zenbayashi and S. Koizumi.** *National Agricultural Research Center, 1-2-1 Inada, Joetsu, Niigata, Japan. Email: toketa@affrc.go.jp*

The vertical distribution of leaf blast lesions caused by the fungus *Pyricularia grisea* was studied to estimate the degree of leaf blast suppression in rice multilines in experimental paddy fields for 4 years. Leaf blast in 1:1 and 1:3 mixtures of susceptible rice cultivar Sasanishiki and its resistant near-isogenic line, Sasanishiki BL7, developed slower than in pure stands of Sasanishiki. The average distance of lesions on leaves from the ground in the 1:3 mixtures was significantly lower than that in the pure stands at the end of leaf blast epidemics (at booting stage). This result shows that the distribution of leaf blast lesions in the upper layer differs between the susceptible pure stands and the 1:3 mixtures at the end of leaf blast epidemics.

15.5 POPULATION GENETIC STRUCTURE OF THE POPLAR RUST FUNGUS *MELAMPSORA LARICI-POPULINA* AT DIFFERENT SPATIAL SCALES. **B. Barrès, C. Xhaard, F. Halkett, J. Pinon and P. Frey.** *INRA, Nancy-University, UMR1136, Tree – Microbe Interactions, F-54280 Champenoux, France. Email: frey@nancy.inra.fr*

Poplar cultivation in Europe is highly intensive and there have been many efforts by breeders during the past decades to develop cultivars with a good level of disease resistance. However, the Eurasian poplar rust fungus, *Melampsora larici-populina* has successively broken down all the complete resistances deployed so far in poplar cultivation, causing severe economic losses. Alternative strategies based on spatial management of available resistanc-

es and on breeding for partial resistance are on-going, but they need a good knowledge of the adaptation potential of the pathogen. Biological characteristics, such as the dispersal distances or the relative importance of sexual vs. asexual reproduction, are often difficult to measure for such phytopathogenic fungi. A way to infer these characteristics is the use of molecular markers. Fifteen microsatellite markers were developed in order to study the population genetics of *M. larici-populina* at different spatial scales. At a regional scale, the study of *M. larici-populina* populations in the Durance River valley corridor revealed the co-occurrence of two epidemics, originating from two primary inoculum sources in the wild and in the cultivated trees. Furthermore, gene flow from cultivated stands to wild stands was shown. The long-distance migration capacity of the rust was assessed by studying eleven European populations and two populations recently found in Iceland and Canada. European populations exhibited an isolation-by-distance pattern, whereas non-European populations appeared to result from a strong founder effect with long-distance dispersal of a limited number of individuals.

15.6* COMPARISON OF METHODS FOR DETECTION OF ASCOSPORES OF *SCLEROTINIA SCLEROTIORUM*. **G.J. Boland, M.R. McDonald, S.M. Westerveld, M.S. Melzer, C. Saude and M. Tesfaendrias.** *University of Guelph, Guelph, Ontario, N1G 2W1, Canada. Email: gboland@uoguelph.ca*

Ascospores of *Sclerotinia sclerotiorum* are the primary source of inoculum for epidemics caused by this pathogen in numerous crops. In carrot (*Daucus carota* subsp. *sativus*), the concentration of ascospores sampled within the crop canopy is related to the severity of disease that subsequently develops in the field. Several methods of sampling and detecting ascospores of *S. sclerotiorum* are being compared to identify the most effective method(s) to incorporate this variable into a disease forecasting model to determine the need and timing of applications of fungicide for management of sclerotinia rot of carrot. The methods include: 1) growth on semi-selective medium directly exposed within and above the crop canopy; 2) aerosol sampling within and above the crop canopy using a N6 (Anderson) single stage viable impactor with detection by growth on semi-selective medium, 3) aerosol sampling using a Zefon Bio-Pump with Air-O-Cell (assessment using microscopy) or 4) Via-Cell cassettes (assessment by serial dilution and growth on semi-selective medium), and 5) a Burkard 7-day recording volumetric spore sampler with detection using a polymerase chain reaction (PCR) assay. All of the methods detected ascospores on at least some sampling dates during 2006-07, except the Zefon Bio-Pump when using the Via-Cell cassettes. On most sampling dates, direct exposure of Petri dishes containing semi-selective medium within and above the crop canopy were equally effective in detecting ascospores. Selection of the most effective method for a specific application is dependent on the cost and availability of technical expertise, and laboratory equipment and supplies.

15.7 MOLECULAR DETECTION OF AIRBORNE *SCLEROTINIA SCLEROTIORUM* SPORES ON MEXICAN CROPS. **C. Calderón-Ezquerro, H.A. Guerrero-Parra, R. Reyes-Montes, C. Toriello-Nájera, J. West and J. Atkins.** *Centro de Ciencias de la Atmósfera, Circuito exterior, Ciudad Universitaria, Universidad Nacional Autónoma de México, UNAM. C.P. 04510 México D.F., Mexico. Email: mclce@atmosfera.unam.mx*

Sclerotinia sclerotiorum is a plant-pathogenic fungus affecting a wide variety of hosts and having a vast geographical distribution; it has been ranked among one of the most destructive plant pathogens in the world. *S. sclerotiorum* causes considerable losses in ornamentals and vegetables such as lettuce, broccoli, cabbage, carrot, bean and potato. Early detection and diagnosis of *S. sclerotiorum* inoculum improves our understanding of the pathogen's epidemiology, allows more accurate prediction of disease and improves control measures. Therefore, it is important to optimise detection of the fungus in the environment, with a main focus on the airborne spores. Consequently, rapid and precise molecular techniques are appropriate to achieve this goal. In this particular case, modern methodology based on DNA detection (PCR) showing great potential in fungal identification, has been chosen. DNA detection of ascospores of *S. sclerotiorum* collected in the air of fields growing lettuce, broccoli, cabbage, carrot, and bean, enable us to estimate the presence and airborne spore concentrations through a crop cycle; thus presence of the fungus may be evaluated prior to crop infestation and /or before symptoms of the disease appear. Knowing the ascospore concentration in the air during the different seasons can give more accurate disease prediction, leading to optimization of fungicide application and/or other control measures.

15.8* DYNAMICS OF AIRBORNE CONIDIA OF *ERYSIPHE NECATOR* IN RELATION TO WEATHER AND HOST FACTORS. **O. Carisse and R. Bacon.** *Agriculture and Agri-Food Canada, 430 Gouin, St-Jean-sur-Richelieu, QC J3B 3E6, Canada. Email: carisseo@agr.gc.ca*

Production of airborne conidia is a major component of grape powdery mildew epidemics. Conidia of *E. necator* are produced on lesions induced by ascospores from overwintered cleistothecia or conidia from previous infections. The objectives of the study were to investigate the dynamic of aerial conidium concentration (ACC) in relation to weather and host factors and to develop a prediction model for categories of ACC. ACCs were monitored in a grape canopy in 2000, 2001, and 2002 with a 7-day recording Burkard spore sampler. Weather (temperature, relative humidity, rainfall, wind speed, leaf wetness, and light intensity) data were collected with a data logger located within few meters from the spore sampler. A new set of weather data that contained 150 new weather variables was constructed from original weather data. Regression tree analysis was used to represent and explain the relationship between weather variables and log ACC data and to develop decision rules to predict risk of powdery mildew. The regression tree model was validated against 12 independent data sets collected in unsprayed vineyards from 2003 to 2006. In 78 % of the cases, the regression tree model adequately predicted the risk of powdery mildew from weather data. Decision rules derived from the regression tree could be used by advisors to estimate risk of powdery mildew and hence need for fungicide application.

15.9 COMPARISON OF PREDICTORS OF *BOTRYTIS* LEAF BLIGHT OF ONION AND DETERMINATION OF BEST OPERATIONAL THRESHOLDS. **O. Carisse, N. McRoberts and L. Brodeur.** *Agriculture and Agri-Food Canada, 430 Gouin Boulevard, St-Jean-sur-Richelieu, QC J3B 3E6, Canada. Email: carisseo@agr.gc.ca*

Botrytis leaf blight (*Botrytis squamosa*) predictors were developed to identify the best time to initiate fungicide spray programs or to time spray intervals. These predictors are either based on le-

sion or spore monitoring or on weather conditions that favour sporulation or infection. The cost associated with using each of the predictors varies and the choice of a predictor should be based on a balance between the efforts required to obtain the information, and the reliability of the predictor. Receiver operating characteristic curve (ROC) analysis was used to evaluate the reliability of seven predictors: 1) number of lesions on the oldest leaves; 2) number of lesions on the youngest leaves; 3) concentration of airborne conidia; 4) sporulation index (SI); 5) inoculum production index (IPI); 6) infection probability (IP); and 7) disease severity index (DSI). Predictors based on biological monitoring were better predictors than those based on weather. This is important considering that, in general, biological monitoring is more expensive than weather monitoring. The operational threshold for ACC was around 10 conidia per m^3 of air with a range of 8 to 14 conidia per m^3 depending on the damage threshold. The operational threshold for number of lesions on the oldest and youngest leaves was 3 and 5 lesions and of 0.15 and 0.50 lesions per oldest and youngest leaves, respectively. Among the weather-based predictors, the most reliable were the sporulation index at a threshold of 80 and the DSI at a threshold of 0.50 and 2.5.

15.10 EVOLUTION AND DYNAMICS OF *PUCCINIA STRIIFORMIS* f.sp. *TRITICI* RACES IN THE USA FROM 2000 TO 2006. **X.M. Chen.** *USDA-ARS, 361 Johnson Hall, Washington State University, Pullman, WA 99164-6430, USA. Email: xianming@wsu.edu*

Virulences of the stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*) to resistance genes *Yr8* and *Yr9* were first detected in the south-central states and California in 2000. This new group of races were represented by race PST-78 with virulence to varieties Lemhi (*Yr21*), Heines VII (*Yr2* and *YrHVII*), Lee (*Yr7*, *Yr22* and *Yr23*), Fielder (*Yr6* and *Yr20*), Express (*YrExp1* and *YrExp2*), AVS/6*Yr8 (*Yr8*), AVS/6*Yr9 (*Yr9*), Clement (*Yr9* and *YrCle*) and Compair (*Yr8* and *Yr19*), and race PST-80 with the virulences of PST-78 plus virulence to Produra (*YrPr1* and *YrPr2*). Since then these races have evolved into more virulent races by combining their virulences with previously existing virulences. The virulences present in new races with combination of the virulences of PST-78 and PST-80 include those to Tres (*YrTr1* and *YrTr2*), Stephens (*Yr3a*, *YrSte* and *YrSte2*), Yamhill (*Yr2*, *Yr4a* and *YrYam*), Chinese 166 (*Yr1*), Paha (*YrPa1*, *YrPa2* and *YrPa3*), Moro (*Yr10* and *YrMor*) and Duchamp (*Yr3a*, *YrDru* and *YrDru2*). These new races have become more and more predominant. Race PST-100 with virulences of PST-80 plus virulences to Stephens and Yamhill has replaced PST-78 and PST-80 as the most predominant race throughout the United States since 2003. Races with the *Yr1* virulence and those of PST-100 have become predominant in California. Races with virulences to Moro and/or Paha in combination of the PST-100 virulences have become predominant in the US Pacific Northwest, and are associated to genes for race-specific resistance deployed in these regions. These race dynamics warrant the use of non-race-specific resistance.

15.11 RESISTANCE TO *HELMINTHOSPORIUM* LEAF BLIGHT AND BIOCHEMICAL RESPONSES OF WHEAT GENOTYPES OF DIVERSE ORIGINS. **A.K. Chowdhury.** *Department of Plant Pathology, Uttar Banga Krishi Viswa Vidyalaya, Pundibari, Coochbehar, West Bengal, 736165, India. Email: apurba1996@yahoo.co.in*

Foliar blight is a major constraint to wheat production and productivity particularly in the north eastern plains zone of India which has more than 9.0 million hectares of area under wheat. It is a complex disease as a number of pathogens, viz. *Bipolaris sorokiniana, Alternaria triticina, Drechslera gigantea,* and *Pyrenophora tritici-repentis,* were found pathogenic and can cause significant yield losses. We have studied the pathogenicity of *B. sorokiniana,* the major pathogen involved and confirmed by immuno-electrophoresis assay. The variability among different isolates of *B. sorokiniana* was also observed both morphologically and biochemically. The estimated yield losses were recorded to the tune of 15% on average in eastern India during the crop season 2004-05. More than 3000 germplasm lines belonging to the Indian and CIMMYT wheat programme were evaluated for their tolerance to foliar blight disease, and 52 genotypes were found highly resistant. When biochemical parameters were analyzed in two resistant wheat genotypes (Bhrikuti and Rayon F89) and one susceptible cultivar (Sonalika), results indicated that the resistant genotypes always had higher levels of phenol and OD-phenol, and enhanced PPO and PO enzyme activities than the susceptible genotype, and the activity was further increased following infection. When the protein profile was examined, it was observed that a new protein band of 29.4 kDa was present in Bhrikuti germplasm both in healthy and diseased conditions. The same band appeared in Sonalika only in the diseased condition. The possible role of PR protein is discussed.

15.12* DEVELOPMENT AND IMPLEMENTATION OF SAMPLING-DRIVEN PROGRAMS FOR MANAGEMENT OF GRAPEVINE POWDERY MILDEW IN EASTERN WASHINGTON. **L. Costadone and G.G. Grove.** *WSU Irrigated Agriculture Research and Extension Center 24106 N Bunn Rd. Prosser, WA 99350, USA. Email: lcostadone@wsu.edu*

Powdery mildew of viniferous grape (*Vitis vinifera*), caused by the ascomycete *Erysiphe necator*, is one of the most severe diseases of grapevine worldwide. Disease management can be expensive because in many instances control requires the intensive use of QoI, DMI, sulfur, and oil fungicides. Widespread resistance of *E. necator* to QoI and DMI fungicides has been documented. Therefore, lowering selection pressure is a key component of fungicide resistance management in grapevine IPM programs. A PCR assay using species-specific primers was developed to identify propagules of *E. necator* in vineyard air samples collected by Rotorod sampling devices. Molecular techniques provide a rapid and accurate way to identify *E. necator* in air samples, compared to the more conventional tools. Our research indicates that microscopic signs of powdery mildew occur about 1 week days after PCR detection, and 7 days after *E. necator* conidia were detected by the Rotorod sampler/PCR assay. Volumetric spore traps confirmed the presence of *E. necator* ascospores in the vineyard during the period when PCR detected the fungus in air samples. Fungicide programs initiated upon first detection of *E. necator* in the vineyard reduced fungicide usage without compromising control. Four years of field research indicate that PCR used in combination with Rotorod samplers can be used to signal the initial presence of *E. necator* in the vineyard air, and represents the first step in incorporating an inoculum component in the powdery mildew risk assessment model in widespread use in Eastern Washington.

15.13 GENETIC STRUCTURE OF *BOTRYTIS CINEREA* POPULATIONS FROM TOMATO GREENHOUSES IN FRANCE. **V. Decognet, M. Bardin, A.S. Walker, M. Fermaud and P.C. Nicot.**

INRA, Centre de Recherches d'Avignon, Unité de Pathologie Végétale, B.P. 94, F-84143 Montfavet Cedex, France. Email: veronique.decognet@avignon.inra.fr

The effect of three factors (geographic scale, cropping system and host plant) on the structure of *B. cinerea* populations was investigated using 8 microsatellite markers. Strong genetic differentiation was observed between populations in tomato greenhouses and those sampled outside in the close vicinity, on grapevine, litter, or blackberries. Great differences were also repeatedly observed within a greenhouse between tomato and lettuce populations consecutively produced in yearly rotations. Among populations sampled on tomato stem lesions, geographic differentiation was observed at a national scale (comparison of the Bordeaux, Champagne and Provence areas) and at a regional scale, for glasshouses sampled in Provence from 2002 to 2004. In Provence, populations from tomato were characterized by the presence of one or several dominant genotypes in each greenhouse, combined with an extreme diversity of the remaining isolates. The sampling sites shared few common genotypes and none of the genotypes dominant at one site were dominant at another site. Unexpectedly, isolates collected in three greenhouses in Provence in 2005 and 2006 shared the same dominant genotype (more than 80% of all collected isolates). Our results suggest frequent exchange of inoculum among greenhouses and a possible host specialization of *B. cinerea*. The systematic occurrence of dominant genotypes in all greenhouses suggests that the cropping system influences the genetic structure and that endogenous secondary inoculum (produced on diseased plants) plays an essential role in the development of grey mould epidemics in tomato greenhouses. All these findings have a direct impact for the management of grey mould in vegetable greenhouses.

15.14 *BLUMERIA GRAMINIS* f.sp. *HORDEI* POPULATION AS A TEXTBOOK OF EVOLUTIONARY BIOLOGY. A. Dreiseitl. *Agricultural Research Institute Kromeriz Ltd., Havlickova 2787, CZ-76701 Kromeriz, Czech Republic. Email: dreiseitl.antonin@vukrom.cz*

Powdery mildew, caused by the ascomycete fungus *Blumeria graminis* f.sp. *hordei*, is the most widespread disease of barley in the Czech Republic as well as in other parts of central and north west Europe. To limit the damage to barley, genetic resistance is an effective, economically sound and safe alternative to fungicide application. However, the pathogen can adapt to specific resistance genes, reflect the corresponding host genetic structure and overcome its resistance in the field. Adaptation of the pathogen is based on operation of all known evolutionary forces. During our study of the national pathogen population we have obtained evidence for processes like mutation, recombination (as a result of pseudosexual reproduction), gene flow (immigration as well as emigration), genetic drift (after a drastic reduction of the population, i.e. a bottleneck, followed by a founder effect), hitch-hiking and permanent directional selection. Our study of the host-pathogen relationship *Hordeum – B. graminis* f.sp. *hordei* was aimed at directly increasing grain yield and quality, and reducing indirect harmfulness (application of environmentally unacceptable and costly fungicides), in particular using genetic host resistance. We report results on the frequency of virulence on corresponding resistance genes, and the diversity and complexity of individuals as well as the entire population. We also document the effects of a broad spectrum of evolutionary forces on the population. This is important not only in its practical aspects but also for its value in theory and teaching.

15.15* ADAPTATION OF THE CLONAL PATHOGEN *PUCCINIA STRIIFORMIS* f.sp. *TRITICI* TO RESISTANT CULTIVARS AND TEMPERATURE. J. Enjalbert, M. Mboup, B. Bahri, M. Leconte and C. de Vallavieille-Pope. *UMR BIOGER CPP, INRA Agro-Paris-Tech, Pathologie Végétale et Epidémiologie, B.P. 01, 78850 Thiverval-Grignon, France. Email: pope@grignon.inra.fr*

The genetic diversity of the biotrophic wheat pathogen *Puccinia striiformis* f.sp. *tritici* is strongly structured by host resistance genes with the successive failure of five specific resistance genes during a 20-yr period. Based on both molecular markers and pathotypes, a strong and steady spatial structure between northern and southern populations in France was shown. The strong genomic divergence may result from a long-term accumulation of mutations in two major clonal lineages following their divergence from a common ancestor. The northern isolates belong to north-west European populations and the southern isolates belong to a Maghreb and a south-west European population. A specific resistance gene found in Maghreb wheat cultivars but not in north–west European cultivars can explain part of this structure. Furthermore, this structure corresponds not only with cultivar selective pressure. The southern isolates were found to be more adapted to high temperature than the northern ones, both in controlled and field conditions. Both selective forces, resistance genes and temperature, are driving the evolution of yellow rust populations in Europe. Wheat yellow rust populations in Europe thus provide one of the few examples of how selective forces (host and environment) influence the distribution of clonal pathogens.

15.16 ENVIRONMENTALLY SAFE COMPOUNDS FOR CONTROLLING SUGAR BEET POWDERY MILDEW. A.M.H. Esh and M.S. Shalaby. *Agricultural Research Center, Sugar Crops Research Institute, 9 Cairo University Street, Giza, Egypt. Email: aymanesh@gmail.com*

Sugar beet powdery mildew disease caused by *Erysiphe betae* is a serious disease in Egypt, occurring during the ripening stage in March, April and May. Six calcium salts, Ca sulphate, Ca hydroxide, Ca disodium-ethylenediaminetetraacetate, Ca chloride, Ca polysulfate and Ca carbonate as well as salicylic acid and 3-aminobutyric acid were evaluated for protective and curative effects in controlling the disease in the greenhouse, and their effect on the fungus conidiospores in the laboratory. All the calcium salts tested significantly decreased the severity of the powdery mildew and the percentage of mature leaf area diseased (MLAD) when used as a protective. The percentage of MLAD of the first three treatments above was 6.88%, 10.45% and 11.96%, respectively, while that of the control treatment was 78.5%. Ca chloride, Ca disodium-ethylenediaminetetraacetate and Ca polysulfate showed a significant reduction of the disease when used as protective and curative. All the compounds tested significantly decreased the germination percentage of *Erysiphe betae* conidiospores. Compared to the untreated control, the reduction in spore germination was moderate. Calcium- EDTA, $CaCl_2$ and Ca sulphate were the most effective in reducing spore germination.

15.17 THE BIOLOGICAL CHARACTERISTICS AND VIRULENCE OF *MAGNAPORTHE GRISEA* SPORES. L. Fan, Z. Jiannong, L. Yongfeng, C. Zhiyi, L. Youzhou and N. Yafeng. *No.50 Zhongling street, Nanjing, P.R. China. Email: lufan@jaas.ac.cn*

Rice blast is an important disease of rice. We discuss the biological characteristics of *Magnaporthe grisea* spore germination,

and virulence of *M. grisea* in this paper. The results showed that the best temperature for germination of *M. grisea* spores was 25°C-30°C, optimum 28°C. Best germination pH value of the spores was pH6.5-7.0 at 28°C. The germination rate of spores was more than 80% in 8 h in the presence of water, and in these conditions germinating spores produced appendiculate cells in 6 h. The results implied that spores of *M. grisea* finished preparing for infection within 8 h in the presence of water. When germinating spores of isolate 2005-50 were inoculated on 'Lijiangxintuanheigu' (a cultivar sensitive to rice blast), the disease index was 26.06%; when non-germinating spores were inoculated, disease index was 11.13%. When liquid substrate of germinating spores was inoculated, the disease index was 8.03%, while germinating spores mixed with liquid from germinating spores gave a disease index of 49.01%. The results showed that there was a substance in liquid of germinating spores which plays an important role during spore infection of rice. The liquid was analysed by SDS-PAGE and many protein brands was detected. The results imply that *M. grisea* spores maybe produce certain proteases which help the *M. grisea* spore to infect rice.

15.18 RELATIONSHIPS BETWEEN WATER AVAILABILITY AT THE GRAPE BERRY SURFACE AND INFECTION BY *BOTRYTIS CINEREA*. **M. Fermaud, C. Deytieux-Belleau, J. Roudet, V. Veyssière, B. Donèche and L. Geny.** *INRA, UMRSV, B.P. 81, ISVV, 33883 Villenave d'Ornon, France. Email: fermaud@bordeaux.inra.fr*

In grapevine, grey mould (*B. cinerea*) is a major airborne disease that can drastically reduce yield and wine quality. To improve disease control, it was necessary to better understand some determinants involved in the duration and dynamics of fruit susceptibility to the pathogen. Because exosmosis increases during fruit ripening and modifies the availability of water at the surface of grape skin, external free water was assessed by measuring water activity (a_w) at different berry stages. Possible relationships were investigated between a_w and the time course of berry susceptibility. First, a laboratory bioassay allowed us to assess resistance at different stages in undamaged berries of Sauvignon blanc. By analyzing some major elementary infectious steps (incidence of infected berries, severity of rot expansion, sporulation intensity), significant differences in disease intensity were shown between the berry stages tested from bunch closure onwards. The increasing susceptibility of grape berries during ripening was confirmed. The time course of water availability showed a decrease in a_w during berry development which was more marked after veraison. This may be due to the increasing amount of solutes at the berry surface, following exosmosis, which bind with free water. A significant linear regression was established between a_w at the skin surface and berry susceptibility level. Thus, the measurement of water activity could constitute a good indicator to characterize grape susceptibility. Lastly, the effect of water availability on *B. cinerea* growth under *in vitro* conditions was investigated, showing the highest radial growth for a_w of *ca*. 0.99, whereas growth was significantly reduced and very low for a_w of *ca*. 0.93.

15.19 THE USE OF MICROSATELLITE DNA FOR MONITORING STRAINS OF *PHRAGMIDIUM VIOLACEUM* (UREDINALES) RELEASED FOR THE BIOLOGICAL CONTROL OF EUROPEAN BLACKBERRY. **D.R. Gomez, L. Morin, K. Garsia and K.J. Evans.** *CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601 and TIAR, University of Tasmania, 13 St Johns Avenue, New Town, TAS 7008, Australia. Email: don.gomez@csiro.au*

European blackberry (*Rubus fruticosus* aggregate) is a genetically diverse plant comprising more than 15 taxa that have invaded regions of southern temperate Australia. *Phragmidium violaceum* is a macrocyclic and autoecious rust pathogen that has coevolved with blackberry in Europe. The pathogen was first reported in Australia *circa* 1984, following an unauthorised introduction to the state of Victoria. Since then, nine strains of the pathogen, collected in France, were released with the aim of enhancing biological control of European blackberry in Australia: strain F15 was released in 1991 and 1992 and eight additional strains were released in 2004. While anonymous-DNA fingerprinting has resolved considerable genetic diversity in *P. violaceum,* the use of this for monitoring pathogen strains in sexually recombining populations is limited due to issues with dominant loci. The development of polymorphic, co-dominant loci from simple sequence repeats (SSRs), was conducted with the aim of identifying strain-specific alleles for efficient post-release monitoring of released pathogen strains. Nine SSR loci were characterised from a DNA library of *P. violaceum* enriched for the $(GAA)_n$, $(GA)_n$ and $(GACA)_n$ motifs. Wildtype strains, defined as isolates collected prior to authorised releases in 2004, were homozygous or heterozygous for the same alleles at each of the nine loci. However, at some loci, strains released for biological control in 2004 were heterozygous with each allele unique only to this group. These loci are being used in current studies investigating the successful establishment, persistence and spread of unique biocontrol strains, and their genes, post release.

15.20 *FUSARIUM* INTERNAL FRUIT ROT OF SWEET PEPPERS IN FLANDERS. **T. Goossens, C. Sauviller, L. van Herck, K. Heungens, K. Heyens and R. Aerts.** *Department Industrial and Bio Sciences, Katholieke Hogeschool Kempen, Kleinhoefstraat 4, BE-2440 Geel, Belgium. Email: rudi.aerts@khk.be*

During the last four years a new problem has emerged in the cultivation of sweet peppers in Flanders. *Fusarium* spp. cause internal fruit rot, which can develop external necrotic lesions at the end of the ripening period or on mature fruit. Several species of *Fusarium* can cause internal fruit rot. Two isolates were identified as *F. proliferatum*, 13 isolates are related to *F. nygamai* and *F. lactis*, and 4 isolates belong to the *F. oxysporum* complex. Infection with these *Fusarium* spp. Begins at flowering, in the pistil. Monitoring two greenhouses, we detected a higher incidence of internal fruit rot in the house with a higher energy input. Studies on condensation on flowers during the night showed that all flowers became wet at night, most of them for several hours. A survey in 2007 of flowers did not show any correlation between flower size and fruit rot incidence. Petal fall after flowering is probably more important. Some petals remain on the young fruit and can cover the pistil. Moreover many petals are infected by *Fusarium* spp. Thus it seems that the petals are the main source of inoculum, and this can influence the search for a control method. However a high incidence of *Fusarium* spp. on the petals did not necessarily result in a high incidence of internal fruit rot, so a lot of parameters remain unknown. Removing the petals from the plant and from the greenhouse could be an important control measure.

15.21 USE OF FLOW CYTOMETRY FOR THE EARLY DETECTION LATE BLIGHT CAUSED BY *PHYTOPHTHORA INFESTANS*. **G.W. Griffith, J.P. Day and H.M. Davey.** *Institute of Biological Sciences, Cledwyn Building, Aberystwyth University, Penglais, Aberystwyth, SY23 3DD, Ceredigion, Wales, UK. Email: gwg@aber.ac.uk*

Late blight is the most damaging disease affecting potato crops in many parts of the world, with current control measures often involving >10 fungicide sprays at 1-2 wk intervals. Environmental concerns, recent legislation and evolution of fungicide resistance have provided an impetus for reduced usage and more efficient targetting of pesticides. Decision support systems (DSS) for late blight based on meteorological data are well established but it is recognised that the amount of pathogen inoculum present in the air around potato crops is the key parameter not yet integrated into DSS. We have developed a novel use of flow cytometry combined with high volume air sampling for the sensitive and specific detection of *Phytophthora infestans* sporangia. Using a Partec PAS-III flow cytometer, light scatter and intrinsic fluorescence parameters were used to differentiate sporangia from fungal conidia (*Alternaria*, *Botrytis*), rust urediniospores and pollen. Use of the fluorescent brightener Calcofluor white M2R allowed differentiation between *P. infestans* sporangia and *Erysiphe* conidia. Data analysis rules ('gating') to specifically identify *P. infestans* sporangia were developed using the methods of Genetic Programming, leading to very low false positive/negative counts. Initial field data are presented and the potential application of these techniques to predicting late-blight epiphytotics in the field is discussed.

15.22 DISPERSAL OF AIRBORNE SPORES FROM PLANT PATHOGENIC FUNGI IN CEREALS. **N. Havis, B. Fraaije and S. Oxley.** *SAC, West Mains Road, Edinburgh EH9 3JG, Scotland, UK. Email: Neil.Havis@sac.ac.uk*

Spore release events were monitored in Scotland as part of Scottish Government-funded research to investigate the epidemiology of major cereal pathogens including *Rhynchosporium secalis* and *Ramularia collo-cygni* in barley and *Mycosphaerella graminicola* in wheat. Three Burkard cyclone spore samplers and one seven-day volumetric spore trap sampler were set up at four locations in cereal-growing regions in autumn 2004, and molecular techniques were used to detect and quantify the spore levels at each site over the next three years. Automated weather stations were also placed next to the samplers to determine the influence of local meteorological conditions on spore release events. Analysis of the DNA results from the sites demonstrated different spore release patterns for each pathogen. *R. collo-cygni* spore levels increased towards the end of the growing season and peak levels correlated with extended periods of surface wetness in the crop. *Rh. secalis* and *M. graminicola* spore release events appeared to be less influenced by weather patterns. *M. graminicola* spore levels reached a peak in late autumn while *Rh. secalis* produced a number of peaks throughout the growing season. Accurate detection of spore levels offers the potential for targeted crop protection measures, which could lead in turn to economic and environmental benefits.

15.23 FUNGICIDE RESISTANCE AND HOST SPECIFICITY OF *PSEUDOPERONOSPORA CUBENSIS* IN THE EASTERN U.S. BASED ON FIELD STUDIES. **G.J. Holmes, S.J. Colucci and L. Kanetis.** *Dept. Plant Pathology, NC State University, P.O. Box 7616, Raleigh, NC 27695, USA. Email: gerald_holmes@ncsu.edu*

In 2004 a dramatic shift in virulence of *Pseudoperonospora cubensis* occurred on cucumber. From the 1960s until 2004 cultivar resistance in commercially grown cucumbers (fresh market and processing) was sufficient to control downy mildew without fungicide use. In 2004, downy mildew was extremely severe on cucumbers throughout the southeastern U.S. Previously resistant cultivars seemed to provide no protection and several fungicides were ineffective against the disease. This resulted in an estimated 40% crop loss region-wide. We are documenting fungicide resistance to mefenoxam and azoxystrobin and characterizing the host specificity (i.e., pathotypes) in isolates collected in the eastern half of the U.S. To complement laboratory-based assays, we conducted field experiments in 7 states (Florida, Georgia, South Carolina, North Carolina, Delaware, New York and Michigan). Mefenoxam (Ridomil Gold) and pyraclostrobin (Cabrio) were applied at weekly intervals beginning at the seedling stage, prior to symptom development. In most locations fungicides were applied at least four times. Based on disease development on plants treated with mefenoxam or pyraclostrobin, practical resistance to these fungicides is widespread. Nevertheless, cymoxanil, cyazofamid, fluopicolide, propamocarb and zoxamide have shown good to excellent activity under field conditions. Host specificity of *P. cubensis* is being determined based on the ability of isolates to cause disease on 12 cucurbit hosts described by Lebeda and Widrlechner. These hosts were planted in the field in 9 locations in the same 7 states during the 2007 season. The disease was present at each location and reactions on the 12 hosts will be reported.

15.24 TARGETING THE SYMPTOMLESS PHASE OF PATHOGEN GROWTH TO UNDERSTAND QUANTITATIVE RESISTANCE TO *LEPTOSPHAERIA MACULANS*. **Y.J. Huang, C. Jestin, R. Delourme, G.J. King and B.D.L Fitt.** *Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK. Email: yong-ju.huang@bbsrc.ac.uk*

Many fungal plant diseases have a symptomless phase between infection and appearance of symptoms or production of new inoculum. To improve control of such diseases, whether by breeding disease-resistant cultivars or by use of fungicides, there is a need to understand the symptomless phase of epidemics. Phoma stem canker, caused by *Leptosphaeria maculans*, is an important disease on *Brassica napus*. Epidemics of phoma stem canker are initiated by air-borne ascospores. After initial infection of leaves, *L. maculans* has a long period of symptomless growth down leaf petioles to reach stems where, in spring/summer, it causes stem cankers and resulting yield loss. Quantitative trait locus (QTL) mediated resistance to *L. maculans* is considered to be race non-specific and durable. It is hypothesised that QTL-mediated resistance to *L. maculans* operates by decreasing pathogen growth in petiole and stem tissues. However, it has been difficult to test this hypothesis due to the lack of symptoms during pathogen growth. To visualise this symptomless growth, *L. maculans* was transformed with the *GFP* reporter gene, providing a method to investigate the growth of *L. maculans* before stem cankers can be seen. Quantitative PCR techniques have been developed to quantify the growth of *L. maculans* in *B. napus* tissues. This poster will report work using the new molecular tools to investigate QTL-mediated resistance to symptomless growth of *L. maculans* along leaf petioles and in stem tissues before stem cankers can be seen, which will improve our understanding of quantitative resistance to *L. maculans*.

15.25 DEVELOPMENT OF STRATEGIES FOR THE MANAGEMENT OF FROGEYE LEAF SPOT OF BIDI TOBACCO IN INDIA. **S. Jahagirdar.** *Department of Plant Pathology, Agricultural Research Station, Nipani, 591 237, India. Email: shamaraoj@rediffmail.com*

Frogeye leaf spot caused by *Cercospora nicotianae* Ell. and

Eve. has become a major threat in recent years accounting for economic losses of 5-50% in India. Research on management of frogeye leaf spot with new molecules was taken up in 2005 and 2006 at ARS, Nipani on tobacco cultivar A-119. Spraying with 0.1% hexaconazole, 0.1% propiconazole or 0.05% carbendazim gave a Percent Disease Index (PDI) of 27.5, 26.7 and 24.3 % respectively, all very similar. The maximum incidence of 55.1% was recorded in untreated controls. Growth parameters such as plant height, leaf length and leaf breadth were significantly superior in these treatments. The mean maximum cured leaf yield (1190 kg/ha) was recorded with propiconazole followed by 1153 kg/ha with carbendazim. The minimum yield (996 kg/ha) was recorded in the untreated control. The economics of disease management showed highest net return of US$ 626 with propiconazole followed by carbendazim (US$ 618) and US$ 568 for hexaconazole. The maximum benefit/cost ratio (1.52) was obtained with carbendazim followed by propiconazole (1.45) and hexaconazole (1.36). The nicotine percentage ranged from 2.64 to 3.56. Samples treated with hexaconazole recorded the maximum nicotine percentage followed by propiconazole (3.46). Reducing sugars were in the range of 7.17 to 7.88%, with the highest percentage obtained with carbendazim followed by 7.51% for a treatment combining carbendazim + mancozeb. The chloride percent was well within the range of < 1.0. Thus, spraying twice with 0.1% hexaconazole or 0.1% propiconazole or 0.05% carbendazim at the 65th and 80th days after transplanting helps to manage frogeye leaf spot with production in India of excellent quality bidi tobacco.

15.26 EXPLOITATION OF NEWER MOLECULES KX 007 42SC AND JE 874 10EC IN THE MANAGEMENT OF DOWNY MILDEW OF GRAPE INCITED BY *PLASMOPARA VITICOLA* (BERK. & CURT.) BERL. & DET. IN INDIA. **S. Jahagirdar, S. Kulkarni, V. Devappa and H.M. Venkatesh.** *University of Agricultural Sciences, Dharwad. 2. Du Pont India Ltd., Bangalore, India. Email: shamaraoj@gmail.com*

Grape is an important fruit crop in Indian horticulture. In Karnataka, increasing area under grape cultivation has led to multifold dynamism in horticultural systems. Diseases like downy mildew, powdery mildew and anthracnose have become major management targets in this area. Downy mildew incidence is often influenced by mean temperature as well as dry periods in Northern Karnataka (Shamarao Jahagirdar *et al.*, 2001). Hence management options always include searching for new antifungal molecules. During 2003 and 2004, a bioefficacy and phytotoxicity trial on KX 007 42SC and JE 874 10EC was conducted at the Regional Research Station, Bijapur. The first spray was applied after appearance of the disease followed by two sprays at ten day intervals. Following the third spray, the final observations were recorded. The results showed that the minimum incidence 12.0 PDI was recorded with Curzate 50WP at 1.4g/l, which was on par with JE 874 at 1.2g/l, giving incidence of 14.0 PDI and KX007 at 2.8 g/l (14.5 PDI). The other treatments Mancozeb 72WP at 2.0 g/l and Metalaxyl MZ72WP at 1.4 g/l gave incidences of 26.9 and 5.7 PDI respectively. The maximum incidence (51.5 PDI) was recorded in the untreated control. The phytotoxicity trial showed that there were no phytotoxic symptoms at tested concentration levels. Symptoms of leaf necrosis, epinasty, fruit injury etc. were not noticed. Hence, KX007 and JE 874 can be used as effective components in development of spray schedules against downy mildew of grape in Northern Karnataka.

15.27 *OIDIUM NEOLYCOPERSICI*: INTRA-SPECIFIC VARIABILITY INFERRED FROM AFLP ANALYSIS AND RELATIONSHIP WITH CLOSELY RELATED POWDERY MILDEW FUNGI INFECTING VARIOUS PLANT SPECIES. **T. Jankovics, Y. Bai, G.M. Kovacs, M. Bardin, P.C. Nicot, H. Toyoda, Y. Matsuda, R.E. Niks and L. Kiss.** *Plant Protection Institute of the Hungarian Academy of Sciences, H-1525 Budapest, P.O. Box 102, Hungary. Email: tjan@nki.hu*

There is considerable variation in the pathogenicity, virulence and host range of *Oidium neolycopersici* isolates causing tomato powdery mildew epidemics in many parts of the world. In this study, rDNA ITS sequences and AFLP patterns were analyzed in 17 *O. neolycopersici* samples collected in Europe, North America and Japan, including those which overcame some of the tomato major resistance genes. The ITS sequences were identical in all the 10 samples tested and were also identical to ITS sequences of eight previously studied *O. neolycopersici* specimens. The AFLP analysis revealed high genetic diversity in *O. neolycopersici* and indicated that each of the 17 samples represented different genotypes. No clear correlation was found between virulence and AFLP pattern of the isolates studied. Cross-inoculation tests, as well as analyses of ITS sequences and AFLP patterns of powdery mildew anamorphs collected from tomato, *Aquilegia vulgaris*, *Chelidonium majus*, *Passiflora caerulea* and *Sedum alboroseum* indicated that these morphologically indistinguishable pathogens represent distinct, but phylogenetically closely related species. All could only infect their original host plant species, except *O. neolycopersici* which infected *S. alboroseum*, tobacco and *Arabidopsis thaliana* in addition to tomato in cross-inoculation tests. This appears to be the first genome-wide study investigating the relationships among powdery mildews that are closely related, based on ITS sequences and morphology. The results indicate that morphologically indistinguishable powdery mildews that differed in only 1 to 5 single nucleotide positions in their ITS region are to be considered as different taxa with distinct host ranges.

15.28 LEAF SPOTS AND BLIGHTS OF KIWIFRUIT (*ACTINIDIA DELICIOSA*) AND THEIR CONTROL IN KOREA. **I.H. Jeong, M.T. Lim, K.S. Lee, S.J. Cho, G.H. Kim, T.W. Han, H.C. Kim, S.H. Shin, H.S. Park, M.J. Kim, J.S. Shin and Y.J. Koh.** *Department of Plant Medicine, Sunchon National University, Suncheon 540-742, Korea. Email: pretyjeong@nate.com*

Leaf spots and blights have been severe in orchards of kiwifruit (*Actinidia deliciosa*) in Korea in recent years. Angular leaf spots appeared at early infection stages in June and several other symptoms were recognized as the disease developed. Brown leaf blights were the most frequent, followed by greyish brown ringspots in open-field orchards and silvering grey leaf blights in rain-proof orchards, plus dark brown ringspots in both orchards. *Phomopsis* sp. was the dominant fungus associated with the leaf spot and blight symptoms on kiwifruit, followed by *Glomerella cingulata*, *Alternaria alternata*, etc. *Phomopsis* sp. was commonly isolated from plants with angular leaf spot, silvering grey leaf blight, and zonate brown leaf blight, and *G. cingulata, A. alternata,* and *Pestalotiopsis* sp. were isolated from greyish brown ringspots, dark brown ringspots and zonate dark brown leaf blights, respectively. Sometimes *Phomopsis* sp. mixed with *A. alternata* or *G. cingulata* was isolated from severely blighted leaves. Diseased leaves were recorded as about 70% in open-field orchards late in the season but use of vinyl rain-proof shelters limited the disease incidences to under 20%. Four consecutive sprays of fludioxonil SC from late June at 10-day-intervals successfully controlled the diseases even in open-field orchards.

15.29 MORPHOLOGICAL MARKERS, MYCELIAL COMPATABILITY AND AGGRESSIVENESS USED TO STUDY VARIABILITY IN FIELD POPULATIONS OF *SCLEROTINIA SCLEROTIORUM*. **S. Kaur, P. Grover and P.S. Sandhu.** *Assistant Pl. Pathologist, Oilseeds Section, Department of Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana, Punjab,India. Email: jsarb@rediffmail.com*

Stem rot and head rot caused by *Sclerotinia sclerotiorum* are economically devastating diseases of sunflower. These infections can cause partial or total crop destruction. In the absence of documentation of pathotype variation in the causal organism, past attempts at germplasm screening showed poor repeatability, due to yearly variation in prevalent pathogenic profile. The objectives of this study were to understand genetic and pathogenic variation of *S. sclerotiorum* isolates prevalent in field populations. Forty-eight sclerotial isolates of *S. sclerotiorum* were collected. All of them were inoculated onto potato dextrose agar (PDA) and their mycelial tips were cut and re-cultured three times to obtain genetically identical cultures. The isolates were characterized on morphological traits i.e. colony colour, shape and sclerotial number, size and pattern when grown on PDA. Variation was recorded with a range of 10-84 mm for colony diameter. Average size and number of sclerotia were 3.6 mm and 7.8 respectively and they were arranged in three patterns; ring at center, ring at periphery and scattered over colony. Colonies were white with smooth or wavy margins and thick velvety to sparse growth. Based on similarities, 11 groups (G_1 to G_{11}) of isolates were established, representing a wide range of diversity. All the isolates were evaluated for the existence of mycelial compatible groups (MCGs). Most isolates (69.68%) showed incompatible reaction leading to identification of 31 MCGs. We inferred that field populations of *S. sclerotiorum* were genetically heterogeneous. Data on aggressiveness of these isolates will be presented.

15.30 DETECTION OF *PYRENOPEZIZA BRASSICAE* ASCOSPORES IN AIR SAMPLES AS A CRITERION FOR FUNGICIDE APPLICATION IN BRUSSELS SPROUT CROPS. **R. Kennedy and A. Wakeham.** *University of Warwick, Warwick HRI, Wellesbourne, CV35 9EF, UK. Email: roy.kennedy@warwick.ac.uk*

The light leaf spot pathogen (*Pyrenopeziza brassicae*) occurs commonly on vegetable brassica crops in Scotland. Wind-dispersed ascospores of *P. brassicae* are responsible for transmitting light leaf spot to uninfected Brussels sprout crops. Using air-sampling techniques, *P. brassicae* ascospores were detected in crops of Brussels sprouts in the absence of visible infection. The occurrence of ascospore inoculum was successfully used as a criterion for fungicide applications to control light leaf spot in Brussels sprout crops. The increasing occurrence of fungicide-resistant *P. brassicae* isolates resulted in reduced light leaf spot control. Disease control was observed where fungicides with different modes of activity were applied at times when *P. brassicae* ascopores were detected in the air. Greater use of fungicides with tebuconazole as the active ingredient resulted in the occurrence of insensitive *P. brassicae* isolates. There was variation in *P. brassicae* ascospore concentration in air samples under field conditions, depending on the cultivar present.

15.31 PHOTORESPONSES OF THE RICE BROWN SPOT FUNGUS *BIPOLARIS ORYZAE*. **J. Kihara, A. Moriwaki, N. Tanaka, M. Ueno and S. Arase.** *Faculty of Life and Environmental Science, Shimane University, Matsue 690-8504, Japan. Email: j-kihara@life.shimane-u.ac.jp*

Light serves as an environmental signal to regulate fungal development and behaviour. *Bipolaris oryzae* is the causal agent of brown leaf spot disease in rice. First, we isolated and characterized three melanin biosynthesis genes, the polyketide synthase gene, the scytalone dehydratase gene, and the 1,3,8-THN reductase gene. Northern blot analysis showed that expression of these genes was significantly enhanced in mycelia exposed to near-ultraviolet (NUV) radiation, but not blue or red radiation. We next isolated and characterized *BLR1* and *BLR2*, genes that encode a putative blue-light regulator similar to white-collar 1 (WC-1) and white-collar 2 (WC-2) in *Neurospora crassa*. The deduced amino acid sequence of *BLR1* and *BLR2* showed high degrees of similarity to other fungal blue-light regulator proteins. Disruption of the *BLR1* gene or RNA-silencing of the *BLR2* gene showed that both genes are essential for conidial development after conidiophore formation and for photolyase gene expression enhanced by NUV radiation. Finally, suppression subtractive hybridization (SSH) was performed in order to identify the novel NUV radiation-regulated genes of *B. oryzae*. As a result, we found 18 genes up-regulated after NUV irradiation. Furthermore, 14 of the 18 genes did not increase after NUV irradiation in a blue-light regulator (BLR1)-deficient mutant of *B. oryzae*, suggesting that the 14 genes could be regulated by the blue-light regulator (BLR1). Additional molecular characterization of the roles of light in fungal development may provide us with valuable insights on control methods for fungal pathogens.

15.32 IMMUNOLOGICAL ANALYSIS OF *USTILAGO MAYDIS* PHENYLALANINE AMMONIA-LYASE. **S.H. Kim and M.W. Hyun.** *Department of Microbiology and Institute of Basic Sciences, Dankook University, Cheonan, Chungnam 330-714, Korea. Email: piceae@naver.com*

Phenylalanine ammonia-lyase (PAL) from the maize pathogen *Ustilago maydis* was analysed immunologically to obtain insights into the structural relationships between plant PAL and fungal PAL and between PAL and histidine ammonia-lyase (HAL). Cross-reactivity was found among all the PAL proteins from different species tested, using antibodies raised against both plant and fungal PALs. Both anti-alfalfa and anti-popular PAL antibodies strongly recognized plant PALs but only weakly recognized fungal PALs. Antibodies raised against *U. maydis* PAL only weakly recognized the *Rhodotorula glutinis* yeast PAL. The anti-*U. maydis* PAL antibodies showed low affinity for the plant PALs but they bound strongly to *Pseudomonas* bacterial HAL. Significant cross-reactivity between the two plant PAL antibodies and the bacterial HAL was also observed. Both the anti-*Ustilago* PAL and the anti-poplar PAL antibodies displayed similar enzyme inhibition patterns, including moderate inhibition of bacterial HAL activity. However, the bacterial HAL antibody inhibited only *Ustilago* PAL. The PAL and HAL antibodies tested showed no inhibition against yeast PAL. *Ustilago* PAL contains two putative antigenic determinant sites with high hydrophilicity but the sequences of poplar PAL, alfalfa PAL, *Rhodotorula* PAL, *Pseudomonas* HAL and rat HAL, contain only one site. An overall pattern of similarity is present only between the two plant PALs and the yeast PAL. By contrast, the most hydrophilic sites of *U. maydis* PAL are located closer to the amino- and carboxyl-terminal ends.

15.33 POWDERY MILDEW FUNGI AS INVASIVE PLANT PATHOGENS: RECENT EPIDEMICS CAUSED BY NORTH AMERICAN AND ASIAN SPECIES IN EUROPE. **L. Kiss.** *Plant*

Protection Institute of the Hungarian Academy of Sciences, H-1525 Budapest, P.O. Box 102, Hungary. Email: lkiss@nki.hu

Some species of the Erysiphales, such as the grapevine powdery mildew pathogen, *Erysiphe necator*, have long been known to cause biological invasions. The histories of two recent severe European powdery mildew epidemics seem to be similar to that of the grape powdery mildew disease in the 19th century. In both cases, species known from North America only, namely *E. flexuosa* infecting horse chestnut (*Aesculus* spp.) and *E. elevata* infecting Indian beam (*Catalpa bignonioides*) trees, appeared in Europe and spread rapidly from one country to another causing serious epidemics. These disease outbreaks were, most probably, the results of new biological invasions because there are no previous records of these powdery mildew species on horse chestnut and Indian beam trees in Europe. Some other species of the Erysiphales thought to be native to North America have also started to spread in Europe quite recently. Examples are *E. symphoricarpi* infecting snowberry (*Symphoricarpos albus*) and *E. azaleae* infecting *Rhododendron* spp., as well as a number of taxa thought to be native to Asia, such as *E. arcuata* infecting hornbeam (*Carpinus betulus*) and an *Erysiphe* sp. infecting lilacs (*Syringa* spp.). Powdery mildews are ubiquitous plant pathogens and the symptoms they cause are obvious on leaves and other aerial parts of their host plants. Thus their occurrence and spread can easily be monitored. This should make the invasive species of the Erysiphaceae ideal targets for future studies of biological invasions caused by plant pathogenic fungi.

15.34 PATHOGENIC AND GENETIC DIVERSITY AMONG *MYCOSPHAERELLA PINODES* ISOLATES DURING THE WINTER PEA GROWING SEASON. **C. Le May, M. Guibert, A. Leclerc, L. Lebreton and B. Tivoli.** *INRA-Agrocampus, UMR 1099 BiO3P, Domaine de la Motte, 35653 Le Rheu, France. Email: lemay@agrocampus-rennes.fr*

Plant diseases are caused by pathogen populations made up of individuals, continuously subjected to evolutionary forces. Ascochyta blight, caused by *Mycosphaerella pinodes*, is one of the most damaging necrotrophic pathogens of field pea worldwide. Many studies on variation through time and according to location have shown genetic and pathogenic diversities among isolates. However, understanding of the pathogenic and molecular variations, population structures and abilities to overcome resistance is currently required at the field level. Winter pea is particularly subject to the disease because of factors such as length of the growing season, conducive climatic conditions, and the high level of infection. Isolates of the pathogen were characterised by morphological, biological and molecular methods, and the evolution of the pathogen population was studied over the growing season in Rennes (western France). Isolates (200) were collected in the field every two weeks during the winter growing season (December to June). All isolates were identified using morphological descriptors. Four pea genotypes were used to test the pathogenic reaction of each isolate in a controlled environment (Onfroy *et al.*, 2007). Finally, three AFLP markers (Zhang *et al.*, 2003) were used to assess the variation in the population. Variability was detected at the field scale and variations in the population structure during the growing season were observed. Possible explanations of these variations will be discussed.

15.35 REACTIONS OF AUSTRALIAN *BRASSICA NAPUS* GENOTYPES TO ISOLATES OF THE DOWNY MILDEW PATHOGEN (*HYALOPERONOSPORA PARASITICA*) FROM WESTERN AUSTRALIA. **H. Li.** *School of Earth and Geographical Sciences, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, WA 6009 Australia. Email: hli@cyllene.uwa.edu.au*

Downy mildew, caused by the oomycete *Hyaloperonospora parasitica*, is an important disease of brassicas worldwide. Downy mildew is particularly damaging when it attacks plants at the seedling stage. In Australia, downy mildew frequently occurs on oilseed rape (*Brassica napus*) and, especially since 1998, severe outbreaks of downy mildew have been widespread on oilseed rape in Western Australia on seedlings, in some areas severely retarding seedling growth and vigour. As no previous studies had been undertaken to determine if resistance to *H. parasitica* existed within Australian oilseed rape germplasm, an investigation was carried out to screen 65 Australian spring type oilseed rape genotypes against 5 isolates of *H. parasitica* inoculated onto seedlings at the cotyledon stage. There were significant effects of genotypes, isolates and a significant genotype × isolate interaction. Two genotypes were classified as highly resistant, and an overall majority of the oilseed rape genotypes tested showed at least partial resistance. This is the first study in Australia to investigate the range of resistance of different Australian oilseed rape genotypes to *H. parasitica*. The resistance to *H. parasitica* identified in this study will not only enable Australian oilseed rape breeders to incorporate resistance to *H. parasitica* into new cultivars for enhanced disease resistance, but also allow direct deployment of the highly resistant genotypes in to situations and regions most conducive to the development of severe downy mildew.

15.36 STRATEGIES FOR INTEGRATED CONTROL OF BROWN SPOT OF PEAR (*STEMPHYLIUM VESICARIUM*, TELEOMORPH *PLEOSPORA ALLII*). **I. Llorente, A. Vilardell, P. Vilardell, C. Moragrega, L. Ruz, G. Santamaria and E. Montesinos.** *Institute of Food and Agricultural Technology- CeRTA-CIDSAV, University of Girona, Av. Lluís Santaló s/n, 17071 Girona, Spain. Email: isidre.llorente@udg.edu*

Brown spot of pear (BSP) is caused by the fungus *Stemphylium vesicarium* (Wallr.) Simmons, and causes high economic losses in several pear-growing areas in Europe including Spain, Italy, France, The Netherlands, Belgium and Portugal. The use of protective fungicides applied to a fixed schedule or according to the BSPcast model is the most common management tactic. However, the level of control is limited, especially when disease pressure is high. This limitation may be overcome by incorporating additional methods to reduce the disease pressure. *S. vesicarium* overwinters on pear in fallen infected leaves or fruits as pseudothecia of its teleomorph *Pleospora allii*. Control of the primary inoculum may be critical for management because a reduction of levels or a delay in production of inoculum may considerably decrease disease intensity at the end of the epidemic. New strategies to decrease primary inoculum and limit ascospore discharge have been tested, and different trials were performed in experimental orchards in Spain. Biological (*Trichoderma* sp.) and mechanical methods (leaf shredding and removal) were evaluated in the field for decreasing ascospore discharge. Mechanical methods were the most effective and biological control was partially effective. Integrated control of BSP requires a sustained strategy based on factors affecting disease mostly derived from forecasting systems (BSPcast, PAMcast), as well as on host susceptibility, inoculum levels, pathogen biology and fungicide characteristics.

15.37 CHARACTERIZATION OF *PHYTOPHTHORA INFESTANS* ISOLATES FROM THE CZECH REPUBLIC DURING 2006 AND 2007. **J. Mazáková, M. Zouhar, V. Táborský and P. Ryšánek.** *Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague, Kamýcká 129, 165 21 Prague 6 - Suchdol, Czech Republic. Email: mazakova@af.czu.cz*

Phytophthora infestans (Mont.) de Bary is an economically important plant pathogen, worldwide. Global changes in pathogen populations in the past two decades have significantly influenced *P. infestans* genetic variability, which it is necessary to monitor every year. *P. infestans* isolates were obtained from late blight-infected potato plants from different commercial fields, research station fields, variety-testing fields and gardens of the Czech Republic during 2006 and 2007. A total of 137 isolates were used to study the pathogen's population structure using basic markers such as mating types, resistance to systemic fungicides and mitochondrial DNA haplotypes. Mating type determination by pairing test and cleaved amplified polymorphic sequence (CAPS) analysis showed that 85 and 50 isolates were of A1 and the A2 mating type, respectively. Sensitivity to the systemic fungicides metalaxyl, dimethomorph and propamocarb-hydrochloride was tested in vitro on agar. All the isolates appeared to be sensitive to each of the fungicides. Analysis of mitochondrial DNA using PCR and restriction digestion showed that the majority of isolates (134) were haplotype Ia and the rest (3 from 2007) were the IIa haplotype, referring to the distribution of a new migrant population of *P. infestans*. This work was supported by the Grant Agency of the Agriculture Research (NAZV) of the Ministry of Agriculture of the Czech Republic, grant QG 50055 and Ministry of Education of the Czech Republic, grant No. MSM 6046070901.

15.38 FUNGICIDE SENSITIVITY IN *PODOSPHAERA XANTHII* AND EFFICACY FOR CUCURBIT POWDERY MILDEW IN NY, USA, IN 2003-2006. **M.T. McGrath.** *Department of Plant Pathology, Cornell University, Long Island Horticultural Research and Extension Center, 3059 Sound Avenue, Riverhead, NY, 11901-1098, USA. Email: mtm3@cornell.edu*

Sensitivity of the cucurbit powdery mildew fungus, *Podosphaera xanthii*, to fungicides at risk for resistance development was determined using a seedling bioassay conducted in crops and a laboratory leaf-disk assay conducted with individual isolates collected from production fields and a research field where fungicide efficacy was assessed through replicated field experiments with pumpkin. Qualitative resistance to QoI fungicides was detected every year; trifloxystrobin and pyraclostrobin were ineffective when tested (2003 and 2006). Qualitative resistance was also detected to MBC fungicides, which are no longer used for powdery mildew. Resistance to DMI fungicides is quantitative. Sensitivity has been declining. The leaf-disk assay revealed 3% of isolates tolerated 50 ppm myclobutanil in 2004 and 10% in 2005; while in 2006, 80% tolerated 40 ppm and 7% tolerated 100 ppm. The DMIs myclobutanil and triflumizole were highly effective in 2005 (82-93% control), but moderately effective (34%) to ineffective in 2006. Quinoxyfen, a quinoline fungicide, has continued to be effective (88% control in 2006). Few isolates have been found tolerating greater than 5 ppm. Boscalid, a carboxamide fungicide, provided 89% control in 2006 when 20% of isolates tolerated 50 ppm. Only 56% control was obtained in 2005. Resistance to QoI and MBC fungicides was also detected with the bioassay every year examined. Strains were detected able to tolerate 80 ppm myclobutanil in 14 of 15 commercial fields in 2006. Near the season end, strains were found tolerant of 10 ppm quinoxyfen in one of six fields and 150 ppm boscalid in three fields.

15.39 SENSITIVITY OF *PODOSPHAERA XANTHII* TO REGISTERED FUNGICIDES AND EXPERIMENTALS IN GA AND NY, USA, IN 2007. **M. Miazzi and M.T. McGrath.** *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via Amendola 165/A, 70127 Bari, Italy. Email: m.miazzi@agr.uniba.it*

Fungicide resistance is a major constraint to effectively managing cucurbit powdery mildew caused by *Podosphaera xanthii*. A seedling bioassay was used to monitor sensitivity to currently-registered, at-risk fungicides on Long Island, NY, in commercial and research cucurbit fields during the 2007 growing season. Resistance to QoI fungicides was common based on results with trifloxystrobin (Flint). Strains tolerating high concentrations of boscalid (175 ppm, using unregistered Endura rather than Pristine which also contains pyraclostrobin) and myclobutanil (120 ppm formulated as Nova) were present at powdery mildew onset in spring-planted zucchini. Similar strains were found at a higher frequency later in the year on the main season crop pumpkin. Strains tolerating quinoxyfen (Quintec) at low concentration (5 ppm) were not detected in zucchini but were at very low frequencies in pumpkin. Quintec received its first registration in the USA, which was for melon, in 2007. Tolerance of the DMI fungicides myclobutanil and triflumizole (Procure) was similar. An *in vitro* leaf-disk assay was conducted with isolates collected in Georgia from cucurbit fields where control was inadequate despite fungicide applications (mostly Procure and/or Pristine). Twenty percent of isolates tested were able to grow on 100 ppm myclobutanil, 78% on 10 ppm quinoxyfen, and 28% on 100 ppm boscalid. For the isolates tested from Long Island cucurbits fields, 24% and 19% were able to grow respectively on 100 and 120 ppm myclobutanil, 70% and 38% respectively on 10 and 15 ppm quinoxyfen, and 21% and 9% respectively on 100 and 125 ppm boscalid.

15.40 BASELINE SENSITIVITY TO NEW FUNGICIDES OF *PODOSPHAERA XANTHII* IN NEW YORK AND GEORGIA, USA. **M. Miazzi and M.T. McGrath.** *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via Amendola 165/a, 70127 Bari, Italy. Email: m.miazzi@agr.uniba.it*

An important step in managing fungicide resistance is to obtain information on the initial sensitivity of pathogen populations to a new fungicide before it is widely used. These data are essential to be able to detect when a shift occurs in pathogen sensitivity to a fungicide as resistance begins to develop. In 2007, 73 isolates of *P. xanthii*, causal agent of cucurbit powdery mildew, were tested for their sensitivity to three fungicides not yet registered for this use in the USA: difenoconazole, belonging to the triazole group of fungicides (FRAC Group 3), and two undisclosed compounds. Isolates were collected from greenhouse-grown verbena and cucurbit fields in Long Island (NY) and Georgia and tested using fungicide-treated leaf discs on water agar in Petri dishes. Based on results to date, compound V10118 appears to be very active on most of the isolates, with growth reductions between 22 and 100% at 0.1 ppm, the lowest rate tested. At 0.5 ppm of V10118, only 7% of the isolates grew. For the other undisclosed compound, growth was reduced 3% to 70% on discs treated with 0.1 ppm compared to nontreated disks. Isolates from Georgia resulted more sensitive (no growth at a dose of 0.5 ppm) than iso-

lates from Long Island (38% of isolates grew at the same concentration). For difenoconazole the growth inhibition of the isolates was between 22 to 100% when they were exposed to 80 ppm, and 35 to 100% at 120 ppm. Interestingly, the range of sensitivity to difenoconazole was dissimilar to that to myclobutanil, a triazole registered for cucurbit powdery mildew since 2000 in the USA.

15.41 FACTORS AFFECTING SUSCEPTIBILITY OF SAUVIGNON BLANC GRAPE BERRIES TO *BOTRYTIS CINEREA* INFECTION. **D.C. Mundy, G.N. Hill and R.M. Beresford.** *The Horticulture and Food Research Institute of New Zealand Limited (HortResearch), Marlborough Wine Research Centre, P.O. Box 845, Blenheim 7240, New Zealand. Email: dmundy@hortresearch.co.nz*

Anecdotal evidence suggests that New Zealand Sauvignon blanc vineyards with low berry yeast-assimilable nitrogen (YAN) suffer less botrytis bunch rot. The direct influences of YAN, sugar accumulation and wounding on susceptibility of Sauvignon blanc grape berries to *Botrytis cinerea* infection was investigated in laboratory studies in 2005 and 2007. Berries with a range of YAN concentrations were obtained from field plots with added nitrogen (urea) in a vineyard with naturally low fertility. A range of sugar concentrations was obtained by either sampling berries 0-4 weeks before harvest (2005) or using natural variability in berry sugar content at harvest (2007). Susceptibility was measured by inoculating wounded and unwounded berries with *B. cinerea* spores and assessing the percentage of berries showing infection after 6 days of moist incubation. In 2005, YAN concentrations ranged from 134-442 mg/l and accumulated sugar concentrations from 14.0-23.0 °Brix. In 2007, YAN concentrations ranged from 199-654 mg/l and soluble sugars ranged from 17.1-23.3 °Brix. A significant ($P<0.05$) relationship between susceptibility of unwounded berries and sugar concentration was observed in both years. Wounding caused a relatively greater increase in susceptibility of unripe berries (<17.5 °Brix) than ripe berries (>21 °Brix). No clear relationship was found between berry susceptibility and YAN in either experiment. It was therefore concluded that berry susceptibility is strongly correlated with sugar concentration, that wounding increases susceptibility of unripe berries and that YAN does not have an important direct influence on berry susceptibility.

15.42 PATHOGENIC AND MOLECULAR VARIABILITY OF *ALTERNARIA* SPP., INFECTING BT-COTTON. **M.K. Naik, G. Ramegowda, K.V. Bhat, Gururaj Sunkad and M.B. Patil.** *Department of Plant Pathology, University of Agricultural Sciences, College of Agriculture, Raichur, Karnataka, 584101, India. Email: manjunaik2000@yahoo.co.in*

Nine isolates of *Alternaria macrospora* and five of *A. alternata* were obtained from infected Bt cotton leaves from different regions of Karnataka and Maharashtra states of India. The isolates were analysed for pathogenic and molecular variability. The isolates, when inoculated on Bt cotton differentials using the detached leaf technique, significantly differed in their ability to infect them. Isolate A_1 showed susceptible reaction on moderately susceptible genotype and isolates A_5, A_7, A_8 and A_{11} showed moderately resistant reaction even on the resistant genotype indicating hyper-virulence. However, the isolates A_6, A_{10}, A_{12}, A_{13} and A_{14} showed moderately susceptible reaction even on the susceptible genotype indicating hypo-virulence. The isolates were thus grouped into two variants, hyper-virulent (A_1, A_2, A_3, A_4, A_5, A_7, A_8, A_9, A_{11}) and hypo-virulent (A_6, A_{10}, A_{12}, A_{13} and A_{14}). The variants with higher virulence in a pathogenic population are of interest because they were more virulent on Bt cotton genotypes resistant to predominant pathotypes. Such variation in pathotype within the population will have implications for stability of cultivar resistance. When the *Alternaria* isolates were analyzed for genetic variability using RAPD-PCR, with fourteen primers, as many as seven primers generated polymorphism and based on Jaccard's similarity coefficient and dendrogram, the isolates were grouped into three clusters. Cluster one (A_1, A_4 and A_{11}) shared 65-52% similarity, cluster two (A_3, A_5, A_7, A_8, A_{10}, and A_{14}) showed a similarity value of 63-52%. Isolate A_2 from cluster three was genetically distinct and divergent, sharing only 42% similarity.

15.43 EFFECT OF MIXED PLANTINGS OF RICE CULTIVARS FOR WHOLE-CROP SILAGE ON OCCURRENCE OF RICE BLAST DISEASE. **T. Nakajima, H. Sekiya, M. Tachibana, K. Zenbayashi, H. Kito, Y. Yaji and A. Oshibe.** *National Agricultural Research Center for the Tohoku Region, Daisen, Akita 014-0102, Japan. Email: tonko@affrc.go.jp*

The effect of mixed plantings consisting of different rice cultivars for livestock feeding on occurrence of rice blast disease was investigated under natural conditions. For mixed planting, three Japanese rice cultivars for whole-crop silage, Bekoaoba (with *Pita-2* true resistant gene, and resistant to the major race of blast fungus under field conditions), Yumeaoba (a resistant variety with *Pita-2, Pib* resistant genes), and Kusayutaka (a susceptible/resistant variety with *Pia, Pik* resistant genes), and two Japanese economic rice cultivars, Hitomebore (susceptible, with *Pii* resistant gene) and Manamusume (susceptible, with *Pii* resistant gene), were used. They were mixed in several ratios by seed numbers. Blast disease severities and percentages of diseased plants in the single plantings and mixtures of the forage and economic rice cultivars were examined. No disease occurred in the two whole-crop silage cultivars Bekoaoba and Yumeaoba, whether in the single plantings or mixtures. The disease severity index and the diseased plant percentage of the compatible varieties in the mixtures were significantly lowered, compared with the single compatible cultivar plantings, even in the 1:1 mixture (susceptible variety: resistant variety). In the mixture of 1:3 (susceptible: resistant), rice blast disease suppression was more effective than that of the single plantings treated with chemicals. The yields of the mixed and single plantings were analyzed.

15.44 EFFECT OF MIXED PLANTINGS OF A RICE CULTIVAR, KOSHIHIKARI, AND ITS ISOGENIC LINES ON SUPPRESSING RICE BLAST. **T. Nakajima, N. Yasuda, M. Tsujimoto, J. Moriwaki, K. Zenbayashi, Y. Sasaki and T. Hoshi.** *National Agricultural Research Center for the Tohoku Region, Japan. Email: tonko@affrc.go.jp*

The effect of mixed planting of a rice cultivar, Koshihikari, compatible line, and its isogenic lines, some Koshihikari Niigata BL lines, compatible and incompatible lines, on the occurrence of rice blast disease caused by *Pyricularia grisea* (Cooke) Saccardo, telomorph *Magnaporthe grisea* (Hebert) Barr, was investigated under natural conditions. The seeds tested came from the Niigata Crop Research Center, Niigata Prefecture Agricultural Research Institute. Blast disease severities and the percentages of diseased plants in 1:1, 1:3 and 1:7 mixtures of compatible to incompatible lines, and their component lines single plantings were examined. No disease in incompatible lines was seen in the single

plantings and mixtures. The disease severity index and the diseased plant percentage of compatible lines in the mixtures were significantly reduced, compared with those of the single compatible line, even in the 1:1 mixture. However, in the mixtures of compatible and incompatible lines, blast disease suppression in the compatible line was not more effective than that of the single line treated with chemicals. In the case of the rice cultivar Sasanishiki multilines, disease suppression was more effective for leaf blast than for panicle blast. On the contrary, in Koshihikari and its isogenic multilines, panicle blast disease suppression was more effective than leaf blast suppression.

15.45 INTEGRATION OF TEMPERATURE, HUMIDITY AND LOCALITIES ON YELLOW RUST INCIDENCE IN ISFAHAN, IRAN. **M. Nasr Esfahani.** *Islamic Azad University - Ardestan Branch and Agriculture and Natural Resources Research Center, Iran. Email: m_nasresfahani@yahoo.com*

One of the important diseases of wheat (*Triticum aestivum*) is yellow rust and/or stripe rust*, P. striiformis* (*P. glumarum*) in Iran. Field surveys in 2005-06 in Isfahan wheat growing areas indicated that yellow rust was already present, with patches in some fields where infection was 100% with 39.29% severity and scoring scale of 25.30. There was a very low correlation between infection % and severity % (r=-0.19) whereas there was a very high correlation between the severity and scoring scale (r=0.99). The conditions favouring yellow rust incidence were cool temperatures, high humidity and cloudy weather along with irregular rains. The susceptibilities of 35 bread wheat lines to yellow rust in glasshouse conditions were assessed. Plants were inoculated with lyophilized rust urediospores from seedlings up to middle age, four times at 15-day intervals. The results indicated that the infections varied from 63.33 to 100%, which can not be a scale for determination. Disease severity, graded in seven classes showed great variation among the wheat lines, ranging from 34.70 to 93.46%. There was also no high correlation between severity and infection % (r=-0.45). The scoring scale within 0–32 was highly significant (p=0.01).There was also a very high correlation between severity and scoring scale (r=0.99). These results placed the wheat lines in 5 distinct groups. Cluster analysis also placed the lines in 5 distinct groups similar to the above at distances below 5 of rescaled distance cluster combine, as was in DMRT.

15.46 DEPLOYMENT OF HETEROGENEITY IN CEREALS FOR DISEASE CONTROL AND YIELD ENHANCEMENT. **A.C. Newton, D.C. Guy and J.S. Swanston.** *Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland, UK. Email: adrian.newton@scri.ac.uk*

Cereal varieties deployed in multi-component mixtures show enhanced resilience to abiotic and biotic stress. However, the expression of such characteristics is dependent on the interactions between trait expressions such as resistance and canopy morphology. Such interactions depend on spatial and temporal factors such as the patchiness of component distribution and their scale. Coarse patchy mixtures control the spread of some pathogens better than homogeneous mixtures. Yield is enhanced in most interactions whether diseased or not and there is a positive relationship between yield and number of components in a mixture. Developmental traits tend to converge in mixtures and within a moderate range, quality is not reduced and can even be enhanced. In Scotland wheat is used for grain distilling, but varieties differ greatly in alcohol yield. Distillers and growers are now recognising mixtures as a means of deploying varieties that are high in alcohol yield, but show agronomic weaknesses, such as lodging. Maltsters remain resistant to the use of barley mixtures, citing the risk of uneven processing, but this view underestimates the variation due to effects of environment and genotype × environment interaction. Research has shown some well-matched mixtures to give more stable malting performance across sites than their component cultivars. Disease control tends to be greater under high nutrient conditions. Mixtures are affected far less by changes in climate or soil cultivation methods than single varieties.

15.47 *BOTRYTIS*-SCARRING IN PERSIMMON FRUIT: INFECTION AND DISEASE CYCLE. **P.A. Rheinländer, R.A. Fullerton and P.W. Sutherland.** *HortResearch, Mt Albert Research Centre, Private Bag 92169, Auckland, New Zealand. Email: PRheinlander@hortresearch.co.nz*

Dark brown, corky spots on the skin of persimmon fruit (*Diospyros kaki* L.) are a cosmetic disorder known as scarring. In New Zealand the disorder causes substantial revenue losses to the persimmon industry. Histological examinations showed that the scarring is associated with *Botrytis cinerea* Pers. In spring the fungus first colonises senescent petals adhering to the fruitlet and subsequently attempts to infect the underlying epicarp tissue. Scanning electron microscopy and fluorescence immunolabelling demonstrated that the fungus produces mycelial cushions composed of finger-like clusters of hyphae on the fruit surface. These function as infection points and trigger a defence response in the fruit which prevents the fungus from establishment, and causes death and discolouration of the surrounding epidermal cells. Affected areas appear as dark specks to the naked eye and develop into scars as the fruit develops. Monitoring in persimmon orchards showed that conidia produced from infected senescent petals during humid weather facilitates colonisation of new emerging flowers leading to repeated cycles of infection during the flowering period. Conidia from over-wintering sclerotia in calyxes, pedicels and fruit mummies represent the primary inoculum sources for petal infection in spring. In humid districts of New Zealand, the first-formed leaves in spring often become infected by *B. cinerea* resulting in necrotic lesions at the leaf tips, expanding to produce large quantities of conidia under humid conditions. These leaves represent a secondary source of inoculum for the persimmon flowers. A proposed disease cycle for *B. cinerea* in persimmon orchards in New Zealand is presented.

15.48 DETECTION AND QUANTIFICATION OF AIRBORNE ASCOSPORES OF *SCLEROTINIA SCLEROTIORUM* BY QPCR. **S.L. Rogers, S.D. Atkins, J.S. West and B.D.L. Fitt.** *Rothamsted Research, Harpenden, AL5 2JQ, UK. Email: sarah.rogers@bbsrc.ac.uk*

A technique to detect ascospores of *Sclerotinia sclerotiorum* in air samples by quantitative PCR is more sensitive than traditional microscopy as it can detect the presence of only a few spores on a Hirst or rotating-arm trap surface, in a background of thousands of other spores and pollens. The technique was tested on samples produced by inoculating different numbers of *S. sclerotiorum* ascospores onto wax-coated plastic tape of the type used in Burkard spore traps. Spores deposited on duplicate sub-samples, produced by cutting inoculated tape in half, were counted by microscopy to validate the qPCR quantification result. DNA was first extracted from spores on pieces of tape before qPCR. The

primers produced PCR products from DNA of both *S. sclerotiorum* and *Botrytis cinerea* but differences in the melting points of the two products enabled quantification of only *S. sclerotiorum* DNA. This was confirmed in tests with DNA from both species mixed in different ratios. When tested on outdoor air samples from spore traps operated at Rothamsted over recent years, the method demonstrated that airborne spores of *S. sclerotiorum* were present in unusually large numbers in April 2007 at Rothamsted, just before a severe epidemic of *Sclerotinia* stem rot (SSR) on oilseed rape at Rothamsted and throughout northwestern Europe in 2007. Low rainfall throughout April 2007 produced a low predicted risk of SSR from weather-based forecasts. Air sampling combined with qPCR showed that high concentrations of spores were present in air, although local rainfall was minimal.

15.49 F1 PROGENY ISOLATES OF *PHYTOPHTHORA INFESTANS* MAY EXHIBIT ENHANCED AGGRESSIVENESS TO POTATO AND TOMATO. A.E. Rubin, S. Klarfeld and Y. Cohen. *Bar Ilan University, Ramat Gan, Israel. Email: Ycohen@mail.biu.ac.il*

Oospores were produced in tomato leaves infected with A1 and A2 isolates of *P. infestans*. The oospores were extracted, exposed to two cycles of drying-wetting to kill the pathogen's vegetative structures, and mixed with perlite and water. Tomato leaves floating on this mixture served as bait for oospore infection. F1 lesions (isolates) were recovered from 4 crosses. Single-sporangium isolates were examined for resistance to metalaxyl, mating type and compatibility with potato or tomato differential lines each carrying a major gene for late blight resistance. While parental isolates were either sensitive or resistant to metalaxyl, most progeny isolates (n=296) exhibited various levels of intermediate resistance to metalaxyl. Most isolates belonged to the A1 or A2 mating type, depending on the cross and lesion. Some were sterile or had the unusual mating type A1A2. Fifty different races were identified on potato differentials. A few isolates gained new virulence factors while others lost parental virulence factors. On tomato, many progeny isolates possessed greater sporulation capacity, compared to their parents, on cultivars carrying the resistance gene *Ph0, Ph1* or *Ph2*. In one cross, 28 progeny isolates out of 130 were able to sporulate on *Lycopersicon pimpinellifolium* L3707 carrying *Ph3*, on which all parents failed to grow, whereas 16 isolates lost the ability to grow on *Ph1*, *Ph2,* or both. The data suggest that sexual reproduction may produce isolates aggressive to otherwise late blight-resistant potato and tomato cultivars.

15.50 PHYSIOLOGICAL VARIABILITY OF *CERCOSPORA COFFEICOLA*. G.C. Souza, L.A. Maffia and E.S.G. Mizubuti. *Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Brasil. Email: agcsouza@yahoo.com.br*

Cercospora leaf spot, caused by *Cercospora coffeicola*, is an important coffee disease in Brazil. However, little is known about its biology. Thus, under laboratory and greenhouse conditions, we evaluated the variability of 60 pathogen isolates, sampled in three regions under both conventional and organic crop systems in Minas Gerais State. In the laboratory, areas under the mycelial growth curve (AUGC), cercosporin production at 18, 22, or 26ºC, and sporulation at 25ºC were evaluated. Six isolates were selected and inoculated on seedlings of Catuaí and Catucaí coffee varieties, either fertilized or not with potassium chloride, and kept in a growth chamber at 18, 22, or 26°C for 48 h after inoculation. Incubation period (IP), latent period (LP), disease severity (SEV), and leaf fall were evaluated under greenhouse conditions. High variability among *C. coffeicola* isolates was detected regarding all the variables assessed. The isolates were grouped according to sporulation, AUGC, and cercosporin production. Of the 60 isolates, 27 did not sporulate. Cercosporin production and mycelial growth tended to increase with temperature. Positive correlations were detected between: severity and leaf fall for both varieties; severity and cercosporin production for 'Catucaí'; and potassium chloride and SEV for both varieties. Variability of *C. coffeicola* was found to be affected by temperature, potassium fertilization, and plant genotype.

15.51 *COLLETOTRICHUM ACUTATUM* FROM NORWAY FREQUENTLY DEVELOPS PERITHECIA IN CULTURE. A. Stensvand, H.U. Aamot, G.M. Strømeng, V. Talgø, A. Elameen, J. Børve and S.S. Klemsdal. *Norwegian Institute for Agricultural and Environmental Research, Høgskoleveien 7, 1432 Ås, Norway. Email: arne.stensvand@bioforsk.no*

The ascigerous stage (formation of perithecia with viable ascospores) of *Colletotrichum acutatum* was recently reported to occur on fruits of highbush blueberry (*Vaccinium corymbosum*) in Norway. When 113 isolates of *C. acutatum* from various plant species were cultured on strawberry leaf agar, nine developed perithecia with viable ascospores. Four isolates originated from apple (*Malus domestica*) and one each from sweet cherry (*Prunus avium*), raspberry (*Rubus idaeus*), highbush blueberry (*V. corymbosum*), hollyberry cotoneaster (*Cotoneaster bullatus*), and northern dock (*Rumex longifolius*). Except from blueberry, we never detected the ascigerous stage on decaying fruits or any other parts of the above mentioned plant species. On potato dextrose agar, the colour of the underside of the cultures forming perithecia varied from light grey-green to dark grey-green or dark brown-green. The colour of the upperside varied greatly, being dark grey-green, grey-brown, grey, and beige-pink; only the raspberry and blueberry isolates were beige-pink. Amplified fragment length polymorphism (AFLP) analysis of the isolates using six primer combinations resulted in 103 clear polymorphic bands. A dendrogram was constructed, and based on cluster analysis using genetic similarity, the isolates could be divided into several clusters. Eight of nine perithecia-forming isolates grouped together in the dendrogram, indicating genetic difference from other isolates. This was also supported by principal coordinate (PCO) analysis.

15.52 IDENTIFICATION OF A PUTATIVE NEW BARLEY LEAF RUST RESISTANCE GENE. Y. Sun and S.M. Neate. *Department of Plant Pathology, North Dakota State University, Fargo USA. Email: stephen.neate@ndsu.edu*

Resistance is the preferred means of controlling leaf rust of barley caused by *Puccinia hordei* G. Otth. However, changing virulence in *P. hordei* has rendered ineffective many of the known resistance genes. In this study, isolates 'Race 8', '90-3', '90-15', '89-3', and 'Neth 202' of *P. hordei* were used to differentiate resistance genes in 82 selected barley lines. Putative new resistance was identified in barley line 'C2-02-134-2-2' and separated from *Rph15* also known to be in that line. Based on disease reaction when challenged with isolate 'Race 8', the F_2 population of a cross between 'C2-02-134-2-2' and a susceptible line 'ZA47', segregated into a 15:1 resistant to susceptible ratio (χ^2=0.853) indicating the presence of more than one gene. In the F_2 generation, the *Rph15* phenotype (00;) separated from a second resistance

gene phenotype (0;12-). To isolate the gene which gave the (0;12-) phenotype from *Rph15*, the 10 F_2 plants bearing the (0;12-) phenotype were transplanted and selfed, and the $F_{2:3}$ families were screened for homogeneity of disease reaction. To determine inheritance and to undertake gene mapping using diversity array technology (DArT) a new cross was made between 'Bowman' and a plant from a homogeneous family that showed the (0;12-) phenotype. The putative new gene was mapped to barley chromosome 7H and when inoculated with a selected set of *P. hordei* races showed different phenotypic reactions from *Rph3* and *Rph19* known to be on the same chromosome. Allelism studies with *Rph3* and *Rph19* are in progress.

15.53 BIO-EFFICACY OF NEW COMBI-FUNGICIDE FORMULATIONS AGAINST MAJOR DISEASES OF RICE IN TUNGABHADRA PROJECT AREA. **G. Sunkad and M.V. Chandrasekhar.** *Division of Plant Pathology, Regional Agricultural Research Station, Raichur-584 10, Karnataka, India. Email: gsunkad@rediffmail.com*

Rice is one of the popular food crops grown in Tunga Bhadra Project command area of northern Karnataka and suffers from major diseases such as leaf and neck blast (*Pyricularia oryzae*), sheath blight (*Rhizoctonia solani*) and brown spot (*Helminthosporium oryzae*). A field experiment on bio-efficacy of different new combi-fungicide formulations and new fungicides against these major diseases was conducted in the area during Rabi/Summer, 2005-06 and Kharif, 2006. Among them, the combi product Zineb+Hexaconazole at 2.0-2.5 g/l was found very effective in reducing the disease incidence of sheath blight and brown spot, besides also reducing the incidence of leaf and neck blast. The combi product also recorded higher grain yields and cost/benefit ratios compared to other fungicides combination treatments tested. Further, there were no phytotoxic symptoms (scorching, necrosis, hyponasty, leaf tip injury, leaf surface injury, wilting and vein clearing) on plants due to spraying of Zineb+Hexaconazole at all the dosage levels tested up to the 10th day of treatment during both seasons. Hence, spraying of Zineb+Hexaconazole at 2.0-2.5 g/l at tillering and panicle emergence can be recommended for effective and economical management of major diseases of rice in the command area.

15.54 STRUCTURAL AND BIOCHEMICAL MECHANISM OF RESISTANCE IN GROUNDNUT TO *PUCCINIA ARACHIDIS*. **G. Sunkad and S. Kulkarni.** *Division of Plant Pathology, Regional Agricultural Research Station, Raichur-584 10, Karnataka, India. Email: gsunkad@rediffmail.com*

Groundnut rust (*Puccinia arachidis* Speg.) is an important disease causing yield losses in all major groundnut-growing areas of India. Six groundnut genotypes, GPBD-4 and DH-22 (resistant), K-134 and R-8808 (moderately resistant) and KRG-1 and TMV-2 (susceptible) were selected to study the mechanism of resistance on the basis of structural and biochemical changes. The healthy and diseased leaves of the different genotypes were subjected to structural and biochemical changes due to infection of the pathogen. Among histological parameters, cuticular thickness, epidermal cell thickness and wax contents were greater in the resistant and moderately resistant genotypes. Number and size of epidermal cells and stomata were greater in susceptible genotypes. Diseased leaves of resistant and moderately resistant genotypes showed more total reducing and non-reducing sugars compared to susceptible genotypes. Further, the sugars were more in resistant and moderately genotypes than susceptible genotypes. Phenol, orthodihydroxy phenol and protein content were greater in healthy leaves compared to disease leaves, unlike sugars. Within the genotypes, the resistant and moderately resistant genotypes showed higher concentrations of these biochemicals than susceptible genotypes.

15.55 *KABATINA ABIETIS* AS A POSSIBLE CAUSE OF CURRENT-SEASON NEEDLE NECROSIS (CSNN) ON FIR. **V. Talgø, T. Cech, G. Chastagner, I.M. Thomsen, K. Riley, K. Lange and A. Stensvand.** *Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway. Email: venche.talgo@bioforsk.no*

Current-season needle necrosis (CSNN) is a poorly understood disease with significant impact on the marketability of fir (*Abies* spp.) Christmas trees and boughs in North America and Europe. Initial symptoms appear on needles soon after bud break as tan to yellow-coloured spots, which turn reddish brown during the summer. The symptoms are observed on noble fir (*A. procera*), nordmann fir (*A. nordmanniana*) and grand fir (*A. grandis*) on both continents. The etiology of CSNN is unknown. In the USA, Ireland and Denmark, research has indicated that CSNN is a physiological disorder. Isolation and examination of host tissue at the onset of symptoms failed to identify a pathogen. In the USA, foliar applications of very high rates of calcium, or shading noble and grand fir shoots during shoot elongation, significantly reduced CSNN damage. Research in the USA and Denmark also showed that CSNN susceptibility in noble fir is under strong genetic control. In Germany, the fungus *Kabatina abietis* Butin & Pehl. was isolated for the first time in 1992 from grand fir needles with CSNN symptoms, and later from nordmann and noble fir. *K. abietis*. It was also recently isolated from symptomatic nordmann fir needles on samples from Austria, Norway and Denmark. Given the uncertain etiology of CSNN, inoculation tests will be carried out in Norway, and fungicide trials will be performed in Austria, Denmark, Germany, Norway and the USA in 2008, in an effort to determine the potential role of *K. abietis* in the development of this disease.

15.56 ASSESSMENTS OF WILLOW LEAF RUST (*MELAMPSORA* SP.) IN WILLOW PLANTATIONS. **M. Toome, K. Heinsoo and A. Luik.** *Estonian University of Life Sciences, Kreutzwaldi 1a, Tartu 51014, Estonia. Email: merje.toome@emu.ee*

Willow leaf rust (*Melampsora* sp.) is a major disease problem in plantations of willow grown for renewable energy. It is important to make accurate assessments on field experiments to estimate the susceptibility of different clones and the effect of treatments on rust resistance. Although visual assessment is the most commonly used method, it is very subjective. Therefore the aim of this study was to test a more objective, precise and fast method for rust assessment. Four willow clones in three experimental Estonian short-rotation willow plantations were used. Three different methods were collated to compare their accuracy and suitability for field experiments. The new method was to scan the leaves using the Winfolia 5.0a program, and measure the area of different colours, where orange or yellow was indicated as rust and green as healthy. The results indicated a very weak correlation between the exact and measured area covered with rust pustules. Therefore the suitability of this computer-based method is not recommended since in plantations there are other factors that can cause colour changes on leaves. In addition, visual assessments were made in plantations and the number of uredinia per unit leaf area was counted on the same leaves using a stereomi-

croscope. Visual assessment was correlated to the real rust levels only in case of heavy infections. Low rust levels were not differentiated and therefore this method is reliable only in cases of heavy rust damage.

15.57 *CERCOSPORA* LEAF SPOT OF MUNGBEAN AND ITS CONTROL IN THAILAND. **B. Udomsak, S. Seemadua and P. Thammakijjawat.** *Plant Protection Research and Development Office, Department of Agriculture, Ministry of Agriculture & Cooperatives, Chatuchak, Bangkok, 10900, Thailand. Email: boossaracum@yahoo.com*

Cercospora leaf spot is caused by *Cercospora canescens* Ellis & Martin. In Thailand this disease is widely distributed and most serious wherever mungbean is grown, especially during the rainy season. On susceptible varieties of mungbean (U-Thong 1), the disease can reduce yield up to 68%. The symptoms first appear as water-soaked spots on leaves, and as the spots become older, they turn reddish or brown around the circumference with grey or white centres. Heavy infection can cause defoliation. *C. canescens* can sporulate on mungbean leaf agar. For control, systemic fungicides such as benomyl 50% WP and thiophanate methyl 75% WP are effective in controlling *Cercospora* leaf spot; planting mungbean to avoid the disease should be done during May to June and use of resistant varieties such as Kamphaeng Saen 1 and Chai Nat 36 can reduce the disease.

15.58 SIMPLIFYING SURVEILLANCE FOR AIR-BORNE FUNGAL PATHOGENS. **B.K. Vogelzang, E.S. Scott, K. Ophel-Keller and J.A. Davidson.** *South Australian Research and Development Institute, The University of Adelaide and The Cooperative Research Centre for National Plant Biosecurity, Australia. Email: Vogelzang.bonny@saugov.sa.gov.au*

Monitoring for plant pathogens currently relies on detection of symptoms by suitably skilled personnel. The difficulties of distinguishing diseases based on symptoms, and of timing surveillance to coincide with symptom expression, can result in new pathogens not being detected until they are already widespread. In epidemiological studies, monitoring has relied on symptom expression in crops or trap plants. However, there may be logistical challenges in assuring a timely supply of trap plants, and the amount of inoculum may be underestimated if conditions are suboptimal for disease development. The combination of air sampling and molecular diagnostics allows fast, reliable, accurate, sensitive and specific detection of air-borne fungal pathogens. Research is underway to develop methodology for nucleic acid-based detection and quantification of fungal plant pathogens from spore traps, using pathogens of pulse and oilseed crops as models. Sensitivity, specificity and factors affecting detection thresholds, such as temperature, relative humidity and presence of non-target particles will be determined. The robustness of the methodology in answering epidemiological questions of potential importance in plant biosecurity, such as the distance, direction, rate and timing of spread of pathogens, will be tested. As it is difficult to predict which exotic pathogens will enter, become established and cause significant losses in a new area, research is also proposed into the detection of unanticipated plant pathogens in spore traps, using nucleic acid-based community profiling methods. This approach may lead to early detection of incursions, early warning of epidemics and identification of weather conditions favouring aerial dispersal of spores.

15.59 REACTION OF CELERY TO INFECTION BY *CERCOSPORA APII*. **W. Wakuliński, Zamorski, Nowicki and Przybył.** *Department of Plant Pathology, Warsaw University of Life Science, Poland. Email: wojciech_wakulinski@sggw.pl*

Early blight is one of the most destructive fungal diseases of *Apium graveolens* worldwide. However its etiology is complex, the key causal agent being *Cercospora apii*. Occurrence of the fungus has been noted in Poland since the early XX century but its increased incidence in recent years was the reason to examine the response of celeriac to *C. apii* infection. Disease severity, yield loss and phenolic compounds content was assayed. In field trials all 17 cultivars tested were found susceptible to infection, and symptoms appeared on all above-ground parts of plants. Cultivar reaction to early blight ranged from 1.3 to 3.5 on a 6-degree scale. Disease significantly reduced both the mass of leaves and roots, the fresh weight of which were respectively over seven and six times less than healthy ones. No clear relationship existed between foliar disease severity and crop reduction. This suggests that the pathogen strongly influences the production and distribution of carbohydrates. Infection also resulted in changes in the balance of phenolic compounds. Leaves and roots of infected plants accumulated significantly more apigenin and rosmarinic acid, compared to the control. Increased levels of ferulic and chlorogenic acids were found in leaves and roots respectively.

15.60 DISEASE STRESS IN MAIZE PRODUCTION IN CHINA. **X.M. Wang.** *Institute of Crop Science, Chinese Academy of Agricultural Sciences, No.12 Zhongguancun South Street, Beijing 100081, P. R. China. Email: wangxm57@sina.com*

China is the second largest country in maize production with 27.2 million ha. in sown area and 1.42 billion t in production in 2006. With the change of global climate, growing of new varieties and use of new cultural techniques, field diseases have also changed and are a threat for maize production in China. In spring maize areas, 15 races of *Exserohilum turcicum* were detected and caused the epidemic of turcicum leaf blight after 2002. Continuous accumulation of *Sporisorium holci-sorghi* teliospores causes head smut as one of the important diseases. Grey leaf spot, an epidemic in the wet-hot zone, is spreading to cool areas with lat. 46 N. In summer maize areas using no-tillage and straw-back-to-soil methods, *Pythium* and *Fusarium* stalk rot is severe again and brown spot becomes a new problem. Since 2005 under the warmer winter conditions, over-winter populations of *Laodelphax striatellus* have enlarged and make more severe the maize rough dwarf disease caused by *Rice black streaked dwarf virus* in the Yellow-Huai river Valley. In recent years *Polysora* rust has become a new problem in southern parts of summer maize areas because only a few varieties are resistant. For controlling these diseases some new strategies should be used, such as screening the disease resistance of newly released varieties and breeding lines, using breeding lines with moderately resistance as well as high level resistance, and extending techniques of seed-dressing and biocontrol in soil-borne diseases. Cultural methods and biodiversity techniques will be useful in maize disease control.

15.61 GENETICS OF THE RESISTANCE OF MAZHA TO WHEAT POWDERY MILDEW, AND SSR MARKERS LINKED TO THE RESISTANCE GENE. **W. Zhai, X. Duan and Y. Zhou.** *State Key Laboratory of Biology of Plant Diseases and Insect Pests,*

Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, P.R. China. Email: xyduan@ippcaas.cn

Powdery mildew, caused by *Blumeria graminis* f.sp. *tritici*, (Bgt), is one of the most important diseases of wheat in China. A gene that confers the resistance to Bgt in a Chinese wheat landrace Mazha has been genetically analyzed and SSR markers linked to the gene have been screened. The results show that a single recessive gene, which fitted the 1R:3S segregation ratio in reciprocal F2 populations, determined the resistance of Mazha to a mildew isolate E30 in the greenhouse. Analysis of the TC1 population, which fitted the 1R:1S ratio, confirmed the result. Based on the genetic analysis, 178 pairs of SSR primers, published in the public domain, from wheat 5D and 7B were used to analyze the DNA polymorphisms between the parental DNAs and the resistant and susceptible DNA pools. Three SSR markers, wmc396, wmc218 and gwm400, were identified among the population of 145 wheat plants, and their genetic distance from the gene was determined as 14.5 cM, 22.2 cM and 36.7 cM, respectively. The molecular marker analysis indicates that the resistance factor in Mazha locates on chromosome 7BL of wheat. This gene is different from the known gene *Pm5e*, which also locates on long arm of 7B, as indicated by analyses of the location of the two genes on the arm and their virulence. We suggest that the gene from Mazha is most likely a novel resistance gene. This research is funded by the National Basic Research Programme (2006CB100203), the High-Tech Research and Development Programme (2006AA10Z1C1) and the National Key Technology R&D Programme (2006BAD13B02).

Bioremediation

18.1 DIVERSITY AND TOLERANCE OF FUNGI IN PERI-URBAN AGRICULTURAL SOIL WITH THE POSSIBILITY OF HEAVY METAL CONTAMINATION.

I. Ahmad and S. Iram. *Member Crop Sciences/Natural Resources,Pakistan Agricultural Research Council, Plot No. 29, Sector G-5/1, P.Box 1031, Islamabad, Pakistan. Email: iftahmad@gmail.com*

Diversity and tolerance of fungi to heavy metals was studied in soil samples from peri-urban agricultural fields of Faisalabad and Rawalpindi areas, Pakistan, irrigated by sewage and industrial /municipal wastewater. Zn, Pb, Cd, Ni, Co were analysed in soil samples by atomic absorption spectrophotometry. In the Faisalabad area Pb, Cd and Zn concentrations were high while in Rawalpindi concentrations of Pb, Cd, Zn and Ni were high. Diversity of fungi was investigated by the soil dilution plate method. Overall 41 different fungi were isolated. The percentage frequency of fungi was higher in the Faisalabad area than Rawalpindi. *Aspergillus niger*, *A.* sp, *Penicillium* sp, *Fusarium* sp *and Rhizopus* sp were the most common. Only the first four fungi were selected for heavy metal) tolerance at different concentrations ($NiSO_4$, $ZnSO_4$, $CdSO_4$, $PbNO_3$ at 1, 5, 10, 15, 20, 25, 30, 35, and 40 ppm) on potato dextrose agar medium. *A. niger* was the most tolerant fungus against all the heavy metals tested and showed strong radial growth (8-9cm) from 0-40ppm. It was followed by *Aspergillus* sp, *Fusarium* sp and *Penicillium* sp. Minimum inhibitory concentrations for all the metal salts were determined. *A. niger* showed highest inhibition minimum (= highest tolerance) at 30ppm ($ZnSO_4$) in the Faisalabad area while in the Rawalpindi area it showed an inhibition minimum of 25pp ($ZnSO_4$) as compared to other fungal species.

18.2* HEAVY METAL ADSORPTION BY A POLYSACCHARIDE OBTAINED FROM THE FILAMENTOUS FUNGUS *PHOMOPSIS* SP.

A. Chimento, E. Raspanti, F. Saiano and S.O. Cacciola. *Dipartimento S.En.Fi.Mi.Zo., Università degli Studi di Palermo, Viale delle Scienze, 90128, Palermo, Italy. Email: cacciola@unipa.it*

A polysaccharide has been obtained by thermal alkali treatment of the mycelium of an isolate of *Phomopsis sp.* from sunflower grown in vitro. The FT-IR spectrum and nitrogen content suggest that chitosan and glucans are the main components, and the ability to complex various metal species is mainly due to chitosan. Information on Lewis base sites has been used as a guideline in evaluating the complexing ability against a number of metal ions in aqueous media at pH in the range 4-6. After 24 h contact time, up to 870 mmol/g of lead, 390 mmol/g of copper, 230 mmol/g of cadmium, 150 mmol/g of zinc and 110 mmol/g of nickel ions were adsorbed by the material. About 70% of the overall adsorption process was already completed after approximately 10 min. Adsorbed metal ions could be recovered by washing with dilute acid. The material has been successfully used as a depolluting agent of wastewaters. The results show that this method of removing heavy metal ions is promising compared to other conventional and generally more expensive processes because of the low growing-cost for a large amount of fungal biomass, a cheap procedure to obtain the treated biomaterial and a process with low environmental impact for recovery of surface-bound metals.

18.3 GLUTATHIONE TRANSFERASE ACTIVITY AND IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN *TRICHODERMA HARZIANUM* GROWN WITH HEAVY METALS.

R. Faedda, I. Puglisi, A.R. Lo Piero, G. Petrone and S.O. Cacciola. *Dipartimento di Scienze Agronomiche, Agrochimiche e delle Produzioni Animali, Università degli Studi di Catania, Via S. Sofia 98, Catania, Italy. Email: rfaedda@unict.it*

Glutathione S-transferases are a family of multi-functional enzymes involved in cellular detoxification processes that catalyze the conjugation of reduced glutathione to various xenobiotics. They have also been implicated in heavy metal tolerance by different species of fungi. In this study the effect of four different heavy metal ions (Cd, Hg, Pb and Zn) on glutathione transferase (GST) activity of *Trichoderma harzianum* (IMI 393899) has been evaluated. Furthermore, differentially expressed genes from heavy metal-exposed *T. harzianum* have been identified. GST activity of *T. harzianum*, grown in Czapek Dox liquid medium supplemented with the different heavy metals at 1 and 10 ppm respectively, was significantly higher with respect to the control. The highest GST activity was observed with 10 ppm of Cd (22.5 nmol/min/mg) and 10 ppm of Hg (16.2 nmol/min/mg), which is respectively 2.5 and 1.8 times higher than the control enzyme activity (9 nmol/min/mg). These data would indicate that GST activity of the fungus is correlated with its ability to tolerate heavy metal contamination. To identify differentially expressed genes from *T. harzianum* exposed to the heavy metals ions, suppressive subtraction hybridization (SSH) tests were prepared using PCR-select™ cDNA Subtraction kit (Clontech Laboratories, Inc.). In conclusion, these results shown that *T. harzianum* can be considered as a universal model for studying the regulation of gene expression in response to heavy metal stress in fungi.

18.4 ANALYSIS OF HEAVY METALS AND FUNGI OF CONTAMINATED SITES IN GERMANY AND PAKISTAN.

S. Iram, L. Ahmad and D. Stuben. *Department of Environmental Sciences,*

Fatima Jinnah Women's University, Rawalpindi, Pakistan. Email: iram.shazia@gmail.com

We collected soil samples from peri-urban soils irrigated by industrial and sewage waste in Lahore, Pakistan and mine waste in Wiesloch, Germany, for analysis of fungal diversity and tolerance to heavy metals. Heavy metal analysis was done by X-ray florescence and ICP-MS. X-ray florescence showed that Zn, As and Pb concentrations were higher in the mine waste sample from Wiesloch. ICP-MS appeared to be more sensitive and showed that Fe, As, Zn and Pb were present in higher concentrations in the mine waste. Soils from peri-urban areas of Pakistan had lower concentrations of heavy metals than in the German mine waste. The diversity and frequency of fungi was analyzed using the soil dilution method. Overall frequency percentage and diversity was higher in Pakistan soil than in Germany. The main fungi included *Aspergillus nidulans, Aspergillus niger,* and species of *Acremonium*, *Alternaria, Aspergillus, Aureobasidium*, *Chaetomium*, *Coniothyrium*, *Curvularia*, *Fusarium, Humicola*, *Monilia*, *Monocillium* and *Mortierella. Aspergillus niger, Aspergillus flavus* and *Aspergillus nidulans* were checked for tolerance to toxic metals ($CdCl_2$, $CuSO_4$, $NiCl_2$ and $ZnCl_2$) at different concentrations (1, 5, 10, 15, 20, 25, 30, 35, 40 ppm) by measuring radial growth. All the fungi tested showed tolerance to $ZnCl_2$ (25 ppm) and $NiCl_2$ (12 ppm) but no tolerance against $CdCl_2$ and $CuSO_4$.

18.5 ENHANCING WOOD DECOMPOSITION IN LAND CONVERTED FROM FOREST TO PASTURE USING *TRICHODERMA* SPP. FORMULATION IN NEW ZEALAND. **D.R.W. Kandula, L.M. Condron, A. Stewart, A.J. Marshall and G.R. Edwards.** *National Centre for Advanced Bio-Protection Technologies, P.O. Box 84, Lincoln University, New Zealand. Email: kandulaw@lincoln.ac.nz*

Wood debris is one of the major soil components in forest to pasture conversions and its decomposition is an important factor determining the success of this large scale operation in New Zealand. Wood decomposition and crop growth were measured for a dryland mixed pasture in Darfield (Canterbury) treated with a prill formulation made of humic acid and five isolates of *Trichoderma* spp. and compared to an untreated control and nitrogen (150 kg/ha/yr) treatment. There was a huge variation in wood debris recovered (ranging between 2.5 t/ha and 32.6 t/ha) from the root zone of different plots, though the mean values were not significantly different. Significantly higher levels of *Trichoderma* spp. were recovered from the root zone and from soil adhering to the wood debris in plots treated with the prill. There were also significantly higher levels of decomposed soft wood in the *Trichoderma*-treated plots compared to the control or N fertiliser treatment. Incubation of the decomposed wood under high humidity revealed *Trichoderma* spp. to be the dominant coloniser. A significant increase in pasture dry matter was evident with added nitrogen, and no yield differences were observed between treatments when nitrogen application ceased. There was evidence of higher numbers of *Trichoderma* spp. in the root zone, heavy colonisation of wood debris by *Trichoderma* spp. and greater percentage of soft wood in *Trichoderma*-treated plots; however this did not translate into a pasture yield increase over the course of the experimental period.

18.6* SCREENING FUNGI FOR HYDROLYTIC AND OXIDATIVE ENZYME ACTIVITIES POTENTIALLY USEFUL IN BIOREMEDIATION. **H. Puhakka-Tarvainen, M. Martinez Subirá, R. Linnakoski and A. Pappinen.** *Faculty of Forest Sciences, University of Joensuu, P.O. Box 111, 80101 Joensuu, Finland. Email: helena.puhakka-tarvainen@joensuu.fi*

Fungi are interesting organisms in terms of bioremediation because of their robustness and tolerance to high concentrations of polluting chemicals. The supposed mechanism for fungal bioremediation is the non-specific degradation by lignocellulose degrading enzymes, a.k.a. hydrolases (e.g. cellulases) and oxidases (laccases, peroxidases). Potential target molecules for bioremediation are, among others, polycyclic aromatic hydrocarbons (PAHs), which are ubiquitous and have carcinogenic and genotoxic properties. In this experiment, almost 200 fungal species isolated from eastern Finland (North Karelia) and eastern China (Wuyi Mountains) were screened for enzymatic activities. Most species were polypores or plant endophytes, and screening was based on agar plate tests with decolourization. For hydrolases, colour reactions were obtained using Remazol Brilliant Blue-coupled fibres, or by staining fibre-containing agar plates with Congo red. For oxidases, the polymeric dye Poly-R478 was used for staining. Screening revealed several fungal strains expressing enzymatic activity. It has been recognized for a long time that polypores are effective producers of hydrolytic and oxidative enzymes, but in this experiment some endophytic fungi also showed activity. One of the most effective endophytes found was birch-associated *Melanconium bicolor*. It was notable that several species expressed both hydrolytic and oxidative activities in parallel. That is probably caused by the fungal need for a complete enzymatic armoury prior to being able to degrade lignocellulose for nourishment. Our study has shown that this poorly explored group of fungal endophytes has great potential for bioremediation and merits further study.

18.7 SUPPRESSION OF SEEDLING DISEASES OF SILK COTTON (*CEIBA PENTANDRA* L.) AND EUCALYPTUS (*EUCALYPTUS TERETICORNIS* SMITH.) BY *PLEUROTUS SAJARCAJU* (FR.) SING. BY DECOMPOSED COIRPITH AND PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR)-BASED POTTING MEDIA. **V. Sendhilvel, T. Marimuthu and C. Arokiaraj.** *Department of Plant Pathology, TNAU, Coimbatore 641 003, India. Email: veltnau@rediffmail.com*

Studies on decomposition of coir waste by yeast slurry and *Pleurotus sajarcaju* showed that *P. sajarcaju* was found to effectively decompose the coir waste. Nutrient analysis of the decomposed coirpith showed that the *Pleurotus*-decomposed coirpith (PDCP) possessed greater nutrient status than yeast slurry-decomposed coirpith (YDCP). The effect of decomposed coirpith, biocontrol agents and PGPR-based potting media on forest tree seedling disease and their growth was studied. The various combinations of normal nursery mix and *P. sajarcaju*-decomposed coirpith (PDCP) used were 0:100, 25:75, 50:50 and 100:0. The media with equal proportion of normal media mix (NMM) and PDCP gave maximum germination of the forest tree seeds (*Eucalyptus tereticornis*) and Silk cotton (*Bombax ceiba*). Seeds treated with *Trichoderma viride* sown in PDCP 50:50 ratio along with amendment of *Pseudomonas fluorescens* + *Azospirillium* + *Phosphobacteria* at 10 per cent (v/v) showed the maximum shoot and root growth in both silk cotton and eucalyptus. Minimum disease incidence was observed of 8.6% wilt in eucalyptus caused by *Fusarium oxysporum* and 6.3% root rot in silk cotton caused by *Verticillium albo-atrum*.

18.8* BIOREMEDIATION OF MUNICIPAL SEWAGE EFFLUENT USING ENERGY CROPS. A. Werner and A.R. McCracken. *Applied Plant Science Division, Agri-Food & Biosciences Institute, Newforge Lane, Belfast BT9 5PX, N. Ireland, UK. Email: alistair.mccracken@afbini.gov.uk*

Willow is fast growing, with a high water demand. When grown in short rotation coppice (SRC) as a source of biomass for renewable energy it offers an excellent opportunity for use as a biofilter for polishing high-level nutrient, particularly sewage effluent rich in nitrogen (N) and phosphorus (P). In a replicated trial at Culmore, Co. Londonderry, Northern Ireland, SRC willow, and single-stem poplar were planted in 2005. A grass plot was also established. Plots of the three crops were irrigated during 2006 and 2007 with effluent from the city Wastewater Treatment Works (WTW) at levels which deliver approximately 150 t ha^{-1} yr^{-1} nitrogen and 15t ha^{-1} yr^{-1} phosphorus. Soil water samples, taken every two weeks at a depth of approximately 60cm, ground water, sampled monthly from boreholes to a depth of at least 5 m and soil and tissue samples taken twice annually were analysed for N and P and a range of other nutrients. All three plant-systems removed nitrogen and phosphorus from the effluent and no leakage into the groundwater has been detected. Even with exceptionally high rainfall in summer 2007 no nutrients were washed out from the system, although there were problems with ponding on the soil surface. The pH of soil water from irrigated plots did not change compared to the non-irrigated control plots. In contrast, electric conductivity showed elevated levels in the grass and willow plots compared to their non-irrigated controls, although this did not affect the nutrient removal capacity of willow and grass.

Biosecurity and quarantine

45.1 PLANT BIOSECURITY EDUCATION AND TRAINING IN AUSTRALIA. K.L. Bayliss and S. McKirdy. *Biological Sciences, Murdoch University, South St Murdoch 6150 WA, Australia. Email: k.bayliss@crcplantbiosecurity.com.au*

Plant Biosecurity is a set of measures designed to protect a crop, crops or a sub-group of crops from emergency plant pests at national, regional and individual farm levels. Australia is relatively free from many of the plant pests and pathogens that seriously impact agricultural and horticultural industries in other countries. This gives Australia a valuable competitive advantage in terms of securing market access and maintaining lower production costs through the absence of many plant pests commonly found overseas. To sustain that advantage into the future, Australian plant industries need the support of world-class plant biosecurity science and education. The Cooperative Research Centre for National Plant Biosecurity (CRCNPB) plays a vital role in enhancing the scientific effort to enable Australian plant industries to pre-empt and, therefore, diminish the economic, social and environmental impact of emerging plant pathogens. The CRCNPB has a strong commitment to the training of high quality PhD students and postdoctoral scientists, providing the nucleus of Australia's future plant biosecurity capacity. CRCNPB provides regular training courses and workshops for our students, staff and scientists already working in the plant biosecurity field. The CRCNPB is involved with the development of a national postgraduate curriculum in plant biosecurity, which aims to graduate students with a Graduate Certificate, Diploma or Masters in Plant Biosecurity. We also have a very popular primary and secondary school education program. Essentially we are training new and existing scientists and raising awareness of plant biosecurity issues at all levels from industry, through to the general public.

45.2* USING UNMANNED AERIAL VEHICLES TO DEMONSTRATE FREEDOM FROM EXOTIC PLANT PATHOGENS. K.L Bayliss, T. Jensen, L. Zeller, F. Wagner, R. Walker, B. MacLeod, L. Vawdrey and G. Kong. *Biological Sciences, Murdoch University, South St Murdoch 6150 WA, Australia. Email: K.Bayliss@crcplantbiosecurity.com.au*

Conventional suction-sampling spore traps have limited value for surveillance of exotic pathogens because their 'catch' capability is limited by the amount of air that passes through them and the spores are collected inside a single chamber making the detection of hourly and daily spore numbers impossible. Typically, they are passive, stationary devices that sample from fixed locations. Some of these limitations have been addressed by development of a slit-type volumetric spore trap. The spore trap contains a rotating drum on which the spores are collected, with spore numbers being determined over 24 h or 7 day periods. The tape that collects the spores can be easily analysed to provide data not only on the number of spores collected within the set time period, but can also be narrowed down to calculate the number per hour of operation, allowing correlation with weather data. In addition, a method has been developed to extract DNA from the spores collected for molecular identification of species present. The objectives of this project are to design and develop a lightweight, compact and on-the-go spore sampling device and to install it on an unmanned aerial vehicle that can traverse a predetermined path with the aim of enhancing the remote sensing detection methods for exotic plant pathogen surveillance.

45.3* FUNGI, CHROMISTS, BACTERIA, AND NEMATODES INTERCEPTED IN NEW ZEALAND DURING 2003-2007. M. Braithwaite, C.F. Hill, S. Ganev, J. Pay, W.H. Ho, R. Thangavel and B.J.R. Alexander. *Plant Health and Environment Laboratory, MAF Biosecurity, New Zealand. Email: mark.braithwaite@maf.govt.nz*

A list of unwanted fungi, chromists, bacteria and nematodes intercepted at the New Zealand border for the period 2003-2007 is presented. The type of associated consignment, the country of origin, and the interception frequency of these unwanted organisms are analysed. All non-compliant consignments were fumigated or treated by other methods, reshipped to their original countries, or destroyed.

45.4 THREATS FROM INVASIVE *PHYTOPHTHORA* SPECIES: FLAWS IN INTERNATIONAL BIOSECURITY. C. Brasier. *Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK. Email: clive.brasier@forestry.gsi.gov.uk*

Until the 1990s, considerable emphasis was placed on the role of Phytophthoras in agriculture, but recently the focus is increasingly on natural ecosystems. Many unidentified *Phytophthora* species probably occur around the world, causing limited damage in their natural environment. However, recent events show that unknown, invasive *Phytophthora* pathogens are being spread via international trade in nursery stock. Examples include *P. ramorum*, responsible for the death of millions of trees in California, *P. alni*, currently causing serious damage to alders in Europe; and *P.*

ilicis, blighting *Ilex* in the UK. Another example is the widespread establishment of the tropical tree *P. palmivora* in nurseries in Italy; and dangerous incursions, such as findings of *P. lateralis* in France and the Netherlands in the last decade. *P. lateralis* currently threatens forests of native *Chamaecyparis lawsoniana* in the Pacific Northwest. The origins of most of these species remain unknown but probably they are native to as yet unexplored ecosystems. If only 7-10% of fungi are known to science, it suggests 200-600 extant *Phytophthora* species, with some 150-450 still unknown. Based on current evidence, roughly 10% could be seriously damaging to forests, natural ecosystems and horticulture outside their natural ranges. Intermixing of related but previously geographically isolated species through international plant trade also promotes novel and dangerous evolutionary risks, and the nursery environment may be particularly favourable to development of *Phytophthora* hybrids. Climate change will also influence the behaviour of invasives, with Phytophthoras likely to show enhanced activity under the conditions of greater climatic perturbance.

45.5 THE THREAT FROM INVASIVE RED BAND NEEDLE BLIGHT DISEASE IN BRITAIN. A. Brown. *Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK. Email: anna.brown@forestry.gsi.gov.uk*

Red band needle blight caused by the introduced pathogen *Dothistroma septosporum* has increased markedly in severity in Europe since the 1990's. Black pine (*Pinus nigra*) is most frequently affected in Europe, whereas in Canada lodgepole pine (*Pinus contorta* ssp. *latifolia*) is the major host. In Britain the disease was first recorded in 1954 in a nursery in Dorset. Outbreaks reoccurred sporadically at the same location until 1966, with two occurrences in Wales on plantation trees in 1958 and 1989. After these records, there were no reports of the disease until the late 1990s. Since then, disease levels in Britain have increased dramatically, particularly on Corsican pine (*Pinus nigra* spp. *laricio*). As a result, in 2006 all Corsican pine stands under the age of 30 years in state owned forests were assessed for disease. This revealed that the disease was present in all Forest Districts surveyed in England and Wales and half of those assessed in Scotland. Overall, disease was found in 71% of the stands assessed covering a total of 7,051 hectares. The disease was also reported on a further 13 pine species in Great Britain during this survey, with lodgepole pine the second most frequently infected species. The increased incidence of the disease has resulted in a five-year moratorium on the planting of Corsican pine, one of the most important softwood timber species grown in southern Britain. The threat this disease poses to lodgepole pine, a major forestry species in Scotland, is also of increasing concern.

45.6 POPULATION GENETIC EVIDENCE FOR HOST SPECIALIZATION BETWEEN RICE- AND WHEAT-INFECTING *MAGNAPORTHE GRISEA*. P.C. Ceresini, M. Levy, M. Rossinelli, M.A. Basseto and B.A. McDonald. *ETH Zurich, Institute of Integrative Biology (IBZ), Plant Pathology, Universitaetstrasse 2, LFW B28, 8092, Zurich, Switzerland. Email: paulo.ceresini@agrl.ethz.ch*

The rice blast pathogen *Magnaporthe grisea* (anamorphase *Pyricularia grisea*) is a complex species composed of groups with pathogenic specializations for poaceous species such as rice, wheat, oats, barley and foxtail millet, besides other grasses. Blast is a major rice disease worldwide, but particularly in Brazil it has caused considerable losses on wheat as well. To our knowledge, the wheat-infecting *M. grisea* is still restricted to Brazil, but by could pose a threat to wheat production areas throughout the world. There has been no attempt, up to know, to resolve the controversy about host specialization of *M. grisea* and to explain its adaptation to wheat. The main objective of our study was to determine whether speciation driven by host-specialization is under way or has already occurred between rice- and wheat-infecting populations of *M. grisea*. To answer this question, two populations were analyzed: a world-wide collection of 190 rice-infecting isolates from Brazil, Argentina, Uruguay, Colombia, USA, Europe, China, Indonesia, India, Philippines and Thailand, and 26 wheat-infecting isolates from São Paulo, Brazil. The isolates were genotyped using sequence-haplotyping for ITS-rDNA, MPG1, NUT1, and CH7-BAC7. Multilocus genotyping was done using 12 distinct microsatellite loci, mating-type locus and avirulence genes (Avr-Pita, and Avr-Co39). Specialized pathogen populations existed for each host population, and rice- and wheat-infecting populations were reproductively isolated. We discuss the hypothesis that the wheat-infecting population originated *de novo* in Brazil, perhaps from local wild grass species.

45.7* INTERCEPTION OF EXOTIC SEED-TRANSMITTED VIRUSES IN LEGUME GEMPLASM IMPORTED INTO INDIA. V.C. Chalam, R.K. Khetarpal, D.B. Parakh, Anju Jain and A.K. Maurya. *Division of Plant Quarantine, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110012, India. Email: mailcelia@gmail.com, celia@nbpgr.ernet.in*

More than 100 viruses are known to be seed-borne and seed-transmitted in plants and may also be spread by vectors, causing severe crop losses. This calls for stringent quarantine processing of imported seeds. Adopting a strategy of post-entry quarantine and laboratory testing, a number of exotic viruses have been intercepted in germplasm imported into India over the last two decades. These include destructive viruses not yet reported from India viz., *Barley stripe mosaic virus* on *Hordeum vulgare* and *Triticum* spp.; *Broad bean stain virus* on *Vicia faba*, *Cherry leaf roll virus* on *Glycine max* and *Phaseolus vulgaris*; *Cowpea mottle virus* on *Vigna unguiculata, Raspberry ringspot virus* and *Tomato ringspot virus* on *Glycine max*. Besides, nine viruses viz., *Alfalfa mosaic virus*, *Bean common mosaic virus*, *Cowpea aphid borne mosaic virus*, *Cowpea mosaic virus*, *Pea seed-borne mosaic virus*, *Southern bean mosaic virus*, *Soybean mosaic virus*, *Tobacco streak virus* and *Tomato black ring virus* are not known to occur on the hosts in India on which they were intercepted. Even though many of the intercepted viruses are not known to occur in India, their potential vectors exist and so also the congenial conditions for them to multiply, disseminate and spread the destructive exotic viruses/strains and even native strains more efficiently. The present scenario of global exchange of seed and liberalization in trade under the WTO regime has potential to enhance the long-distance dissemination of destructive viruses or their virulent strains, and an effective quarantine strategy is thus indispensable.

45.8 DEVELOPMENT OF MONOCLONAL ANTIBODIES TO *PHYTOPHTHORA RAMORUM* AND *P. KERNOVIAE*. K. Chung, F. Avila, B. Schoedel and A. Frick. *Agdia, Inc., 30380 County Road 6, Elkhart, IN 46514, USA. Email: favila@agdia.com*

Phytophthora ramorum has become an important economical pathogen in the USA and Europe. *P. kernoviae*, which causes similar symptoms to *P. ramorum*, is also a serious problem in Europe but has not been found in the USA. The objective of this project

was to develop serological tests for these two pathogens. Mycelium suspensions of *P. ramorum* (isolates from USA and Europe) and *P. kernoviae* (European isolate) were used as antigens to produce monoclonal and polyclonal antibodies. The antibodies were selected for specificity to *P. ramorum* and *P. kernoviae* and for non-cross-reactivity to other species of *Phytophthora* or *Pythium* in ELISA and immunoblot. The monoclonal antibodies were grouped in four categories: 1) specific to *P. ramorum*; 2) specific to *P. kernoviae*; 3) recognition of both *P. ramorum* and *P. kernoviae*; and 4) recognition of any *Phytophthora* spp. but not *Pythium* spp. The polyclonal antibodies developed reacted equally with *Phytophthora* and *Pythium* species. The monoclonal antibodies specific to *P. ramorum* did not cross-react with *P. kernoviae* in an immunoblot assay but cross-reacted in DAS ELISA. Our results indicated that *P. ramorum* and *P. kernoviae* are serological related, and the monoclonal antibodies developed in this research could be used to optimize a test to recognize *P. ramorum* and *P. kernoviae* indistinctly, a species-specific test for *P. ramorum*, and a test specific to the genus *Phytophthora* that does not cross-react with *Pythium* spp.

45.9 THE SUCCESSFUL ERADICATION OF GRAPEVINE LEAF RUST FROM DARWIN, AUSTRALIA. A.M. Daly, S.J. West, G.C. Schultz and E.S.C. Smith. *Diagnostic Services Division of the Department of Primary Industry, Fisheries and Mines; G.P.O. Box 3000 Darwin, Northern Territory 0801 NW, Australia. Email: andrew.daly@nt.gov.au*

The fungus *Phakopsora euvitis* Y. Ono (2000) is the cause of grapevine leaf rust. The rust was detected for the first time in Australia in the urban area of Darwin, Northern Territory (NT) in 2001. Grapevines grow continuously throughout the year in Darwin and are used as ornamental plants or for culinary purposes. The nearest commercial fruit production is 1300 km south of Darwin. In response to the detection, the National Grapevine Leaf Rust Eradication Program (NGLREP) was implemented and a Grapevine Leaf Rust Quarantine Area (GLRQA) declared, placing movement control on all grapevine material within that area. Grapevines were surveyed in the regions of Katherine, Ti Tree, and Alice Springs. All urban properties in Darwin and the neighbouring city of Palmerston were surveyed. *P. euvitis* was only detected in these two localities. Grapevines infected with *P. euvitis* were removed and destroyed and ongoing surveillance was conducted on the remaining grapevines. During the program 746 grapevines were located in the Darwin/Palmerston localities. 83% were removed, 39% of which were infected with *P. euvitis*. Surveillance and sampling continued until June 2007. *P. euvitis* has not been detected in Darwin since May 2006, a period encompassing 8 surveillance rounds and the potential for at least 26 *P. euvitis* lifecycles. The survey results and modelling of the likelihood of the presence of *P. euvitis* indicated with a high degree of confidence that the NGLREP was successful. *P. euvitis* was declared eradicated from the NT and from Australia in November 2007.

45.10 GRAPEVINE LEAF RUST RESEARCH: ENHANCING PREPAREDNESS FOR INCURSION AND ERADICATION. A.M. Daly and C.R. Hennessy. *Diagnostic Services Division of the Department of Primary Industry, Fisheries and Mines, G.P.O. Box 3000 Darwin, Northern Territory 0801, Australia. Email: andrew.daly@nt.gov.au*

Following the incursion of *Phakopsora euvitis,* the causal agent of grapevine leaf rust, in Darwin (Northern Territory, Australia) a research program to identify resistant *Vitis* genotypes to replace susceptible vines was initiated. This was considered an important quarantine measure because, under existing jurisdictional plant health legislation, only diseased vines could be removed. 411 registered genotypes, almost 100 unidentified *Vitis* spp. from Darwin homes and native Vitaceae species were inoculated with *P. euvitis in vitro.* Only four, namely '41 B', 'Aurora', 'Siebel 128' and '554-5 seedlings', were moderately resistant or better. The majority, including *Ampelocissus acetosa* and *A. frutescens* (native) were susceptible or highly susceptible. In addition, fungicide efficacy was investigated to identify products for use in commercial vineyards if required. 11 products prevented *P. euvitis* infection and six reduced disease as post-infection treatments. Disease behaviour was elucidated by examining environmental influences on *P. euvitis*. 21-22°C was optimal for spore germination. Latent period was shortest (six days) at 24°C. Pustule formation and sporulation was greatest at 20-21°C. Six hours of leaf wetness significantly increased disease. Lastly, a protocol for PCR-RFLP identification of *P. euvitis* was adapted and implemented. Outcomes of the research program contributed to the successful eradication of *P. euvitis* from Darwin and will allow a more efficient response to future incursions. Future eradication attempts could be further enhanced by development of a PCR test to detect the presence of *P. euvitis* in symptomless leaf tissue and identification of the possible pathways of entry into Australia and spread following establishment.

45.11* ALIEN INVASIVE FUNGI IN EUROPE: INVENTORY AND SPATIO-TEMPORAL PATTERNS WITH A PARTICULAR FOCUS ON FRANCE. M.L. Desprez-Loustau, C. Robin, D. Blancard, R. Courtecuisse, C Husson, B. Lung, P.A. Moreau, M.A. Selosse and I. Sache. *INRA, UMR1202 BIOGECO, Equipe de Pathologie forestière, 71 avenue Edouard Bourleaux, B.P. 81 F33883 Villenave d'Ornon Cedex, France. Email: loustau@bordeaux.inra.fr*

Biological invasions are a crucial component of global change, with potentially dramatic adverse effects on biodiversity. The European project DAISIE aims at inventoring alien invasive species of all taxonomic groups in Europe. We present here the data compiled for fungi. With the exception of a few well known examples of invasive plant or animal pathogens, fungi are usually poorly, if at all, represented in alien species databases. A comprehensive list of alien fungi was established for France, including pathogenic as well as non-pathogenic species. The date and place of first observation, as well as region of origin and pathway of introduction were documented as far as possible, by extensive literature search. From this list and a few other national checklists, a list of 84 alien invasive fungi, *sensu stricto* (i.e. threatening biodiversity) was established for Europe, by taking into account fungal pathogenic species occurring in wild environments. The date and country of first observation of these species, as well as occurrence in all European countries were compiled from EPPO and CABI databases, principally. Spatio-temporal patterns of invasions were analysed from these two datasets. An exponential trend of alien species records since 1820 was demonstrated. Countries and regions with the highest numbers of alien species exhibit the highest level of commercial imports. Results suggest a varying degree of invasion success, estimated by the number of invaded countries adjusted for the time of invasion, between taxonomic and biological groups of fungal pathogens. Implications for quarantine regulations are outlined.

45.12 INVESTIGATING THE POTENTIAL DISTRIBUTION IN AUSTRALIA OF *NEONECTRIA GALLIGENA*, THE CAUSE

OF EUROPEAN APPLE CANKER. J. Edwards, O. Villalta and R. Powney. *Department of Primary Industries Victoria, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia. Email: Jacky.edwards@dpi.vic.gov.au*

European canker is one of the most important diseases of pome fruit and many species of hardwood forest trees worldwide. Cankers develop on the woody tissues, girdling and killing branches and, occasionally, the whole tree. Predictive modelling with the CLIMATE and CLIMEX software packages was used to identify regions in Australia with environmental conditions favourable for establishment and spread of the exotic fungal pathogen, *Neonectria galligena*, which causes European canker. *N. galligena* has been recorded on more than 60 plant species from 20 genera, and from climates ranging from sub-arctic (Iceland, Sweden, Canada), temperate (Europe, USA, Chile), arid (Syria, Saudia Arabia, Afghanistan) to tropical (Java, Florida), indicating that it is highly adaptable. European canker occurred on apple in Tasmania in 1958 and an extensive eradication program was carried out, resulting in area freedom declared in 1991. At the time, no estimate was made of the cost of this incursion to Australia. The predictive models indicated that it has the potential to establish in many parts of Australia if reintroduced, particularly the southern and eastern coastal regions. Industries such as apple, pear, walnut, loquat, ornamentals and the nurseries that supply these industries could all be adversely affected if that occurs. Of particular concern is the absence of data on the susceptibility or otherwise of Australian native flora. The pathogen has been recorded on three species of New Zealand native flora and it is unknown what the source of the infection was.

45.13 *FUSARIUM* WILT OF BANANAS IN INDONESIA AND PAPUA NEW GUINEA (PNG). L.M. Gullino, W. O'Neill, C. Hermanto, A. Molina and A.B. Pattison. *Department of Primary Industries and Fisheries, 80 Meiers Rd, Indooroopilly, 4068 QLD, Australia. Email: lisa-maree.gullino@dpi.qld.gov.au*

Panama disease, caused by *Fusarium oxysporum* f.sp. *cubense* (Foc), is one of the most devastating diseases of many banana producing countries (1). Of particular concern is 'Tropical' Race 4 (TR4), belonging to VCG 01213/16. In Australia TR4 has been detected and is restricted to the Northern Territory, but remains a biosecurity threat to commercial banana plantations. In the Asia-Pacific region, TR4 has caused substantial production losses for commercial and subsistence farmers. Since many of these countries have not been extensively surveyed for Foc since the mid 1990's, the exact distribution of TR4 is unknown. Consequently, to protect the Australian banana industry and develop management plans for the Asia-Pacific region, a comprehensive survey and characterisation of Foc isolates is required. Isolates of Foc from Indonesia and PNG, have been characterised using traditional (e.g. vegetative compatibility testing/volatile analysis) and molecular techniques (PCR, AFLP). Results from Indonesia show that most isolates belong to VCG 01213/16 (TR4). The isolates were confirmed as being TR4 using TR4-specific PCR (2). However, inconsistencies have been found between VCG tests/PCR results for several isolates. Some isolates grouping as VCG 01213/16 gave inconsistent band heights and artefacts when subjected to the TR4 PCR. Current research is focused on sequencing the isolates to determine if they are a distinct clade within the VCG 01213/16–TR4 group. Subsequently, we are refining/redeveloping the current TR4 diagnostic, with the intention of converting the diagnostic test to a Real-Time PCR assay to improve sensitivity, specificity and turn-around time.

45.14 *TUBER BRUMALE*, A NEW TRUFFLE IN NEW ZEALAND. W.H. Ho, S. Anderson, A. Guerin-Laguette, N. Hesom-Williams, Y. Wang, M. Braithwaite, C.F. Hill and B.J.R. Alexander. *Plant Health and Environment Laboratory, MAF Biosecurity New Zealand, P.O. Box 2095, Auckland 1140, New Zealand. Email: wellcome.ho@maf.govt.nz*

Tuber melanosporum (black Perigord truffle), a high-value crop, has been imported into New Zealand to inoculate trees for truffle production since 1985. *T. brumale* (winter truffle), commonly regarded as a weed truffle, was detected on tree stocks inoculated with *T. melanosporum* in a quarantine facility in New Zealand in 2006. The inoculants of these tree stocks, which were prepared from a consignment of *T. melanosporum* imported from Bologna (Italy), were also found to be contaminated with *T. brumale*. The identification of *T. brumale* was confirmed using a PCR-based technique with species-specific primers and the sequences obtained were compared to the sequences in GenBank. An eradication programme was initiated to remove and destroy tree stocks in the quarantine facility and affected trees in truffières (truffle orchards). However sequencing results on root samples collected from trees planted in earlier years in other truffières confirmed that *T. brumale* had already been present in the country for some time. Subsequently, the eradication programme was terminated and *T. brumale* is considered present in New Zealand.

45.15 SUSCEPTIBILITY OF AUSTRALIAN PLANT SPECIES TO *PHYTOPHTHORA RAMORUM*. K.B. Ireland, D. Hüberli, B. Dell, I.W. Smith, E.J. Fichtner, D.M. Rizzo and G.E.StJ. Hardy. *CRC National Plant Biosecurity and Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Murdoch, 6150 WA, Australia. Email: k.b.ireland@crcplantbiosecurity.com.au*

Phytophthora ramorum is an invasive plant pathogen causing considerable and widespread damage in nurseries, gardens and natural woodland ecosystems of the USA and Europe and is classified as a Category 1 pest in Australia. It is of particular interest to Australian plant biosecurity as, like *Phytophthora cinnamomi,* it has the potential to become a major economic and ecological threat in areas with susceptible hosts and conducive climate. At least three Australian host species have been discovered so far, *Eucalyptus haemastoma*, *Eucalyptus gunnii* and *Pittosporum undulatum*. Asymptomatic sporulation has been confirmed recently for a number of New Zealand species, including a species of *Leptospermum*, a genus that is widespread throughout Australia within areas of climatic suitability for *P. ramorum*. Results will be presented of research undertaken in the USA to assess pathogenicity of *P. ramorum* on Australian native plants and to determine sporulation and survival potential on both symptomatic and asymptomatic tissues. Test species have been selected and sourced from established gardens and nurseries based upon their provenance in regard to areas of climatic suitability for *P. ramorum*, their relation to known hosts of *P. ramorum* and their ecological and economical importance to Australian plant industries. Prior screening for the presence of *Phytophthora* species in the soil profile, and on foliage of selected test species is being conducted. Susceptibility is to be tested using unwounded, detached plant materials, and then scaled up to natural infection studies. Presence and abundance of reproductive structures, sporangia and chlamydospores, will be assessed for all hosts.

45.16 GENETIC DIVERSITY OF *FUSARIUM OXYSPORUM* f.sp. *CICERIS* POPULATIONS IN KERMANSHAH PROVINCE,

IRAN, BASED ON VEGETATIVE COMPATIBILITY GROUPS. **F. Karamian, S. Rezaee, H. Zamanizadeh and M. Javan-Nikkhah.** *Dept. of Plant Pathology, Faculty of Agriculture & Natural Resources, Science & Research Branch, Islamic Azad University, Tehran, Iran. Email: srezaee@sr.iau.ac.ir*

Chickpea (*Cicer arietinum* L.) is a major crop in Kermanshah province in the west of Iran, the fourth producer in the world. *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc) is one of the most common diseases in the region. During 2006 and 2007, 26 fields in different regions of the province were surveyed. From about 200 infected samples, we obtained 52 *Fusarium* isolates. Based on the Nelson *et al.* key, and on testing host range, 46 isolates were identified as Foc and 6 as *F. solani*. On minimal media containing 15 g/l potassium chlorate, 296 chlorate-resistant mutant sectors of 34 representative isolates from all regions were produced. Of these mutants, 42.5%, 21.3% and 34.4% were identified as nit1, nit3 and nitM respectively. After checking the self-compatibility of all the mutants, complementary tests were conducted between different nit mutants of all isolates. The mutants that could form heterokaryons and produce wild-type mycelial growth were placed in the same vegetative compatible groups (VCGs). The results show that the Foc isolates in Kermanshah province belonged to 8 different VCGs. As there were no significant correlations between VCG grouping and geographical source, the importing of the seeds could be the source of variation of the isolates. Changing cultivars may be another source of variation because of its selection pressure on fungus populations. In future we will compare isolates by using DNA molecular markers.

45.17 FIRST REPORT OF *CUCUMBER GREEN MOTTLE MOSAIC VIRUS* CAUSING WATERMELON MOSAIC DISEASE IN LIAONING PROVINCE, CHINA. **M. Li, H. Chen, Y. Zhang, G.L.S. Zhu, H. Chen, Y. Wang and F. Wang.** *Chinese Academy of Inspection and Quarantine, Beijing, P.R. China. Email: limf9@pvchina.org*

In April 2006, Watermelon mosaic disease was observed on watermelon seedlings grafted on gourd in a commercial propagation field in Liaoning Province, China. It was said the disease had been found last year. The mosaic symptom first appeared on seedling leaves; with the growing of the plants, different symptoms could be found such as mottle, leaf distortion, dwarfing and so on. The most severe problem is internal discoloration and decomposition in the fruit while the surface still looks fine. It caused huge economic losses in this area last year. Due to the difficulty of performing tests in the field, the samples were taken and planted in the lab. DAS-ELISA tests were carried out for *Cucumber green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV) and *Zucchini yellow mosaic virus* (ZYMV). The positive control was CGMMV. In order to confirm the result, the samples were also tested by RT-PCR. The specific fragments of CGMMV were amplified and detected. The results showed that the disease was caused by CGMMV. The coat protein gene of this isolate was sequenced, comprised 486 nucleotides and encoded a putative protein of 161 amino acids. This CP amino acid sequence was identical to the CGMMV-W strain in Japan. This is the first report of CGMMV occurring in a production field in China. It is suggested that the virus was introduced from outside of China with the movement of germplasm.

45.18 OPHIOSTOMATOID FUNGI ASSOCIATED WITH AN INVASIVE PEST OF PINE IN CHINA, *DENDROCTONUS VALENS*. **M. Lu, X.D. Zhou, M.J. Wingfield, N. Gillette and J.H. Sun.** *Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, 0002, Pretoria, South Africa. Email: xu.zhou@fabi.up.ac.za*

Bark beetles are common vectors of ophiostomatoid fungi, especially those species in genera such as *Leptographium* and *Ophiostoma.* These include several serious tree pathogens and many important sapstain agents. *Dendroctonus valens* LeConte (Scolytinae), which is native to North America, has caused unprecedented mortality of *Pinus tabuliformis* in China. Little research has been conducted on fungi associated with this pest in that country. Adult *D. valens* and its galleries were, therefore, collected in order to characterize the *Leptographium* and *Ophiostoma* species associated with this insect in China. Nine ophiostomatoid fungi were collected and were characterized on morphology and by DNA sequence comparisons. The fungi isolated included *L. terebrantis*, *L. koreanum*, *L. pini-densiflorae*, *O. ips*, *O. flocossum*, *O. minus*, *O. piceae*, *O. rectangulosporium*, and *O. nigrocarpum*. Of these fungi, seven species are recorded for the first time from China. The fungus most commonly collected was *L. terebrantis* which importantly is also a fungus commonly associated with *D. valens* in its area of origin. It is most likely that *L. terebrantis* was introduced into China from North America, and this might also be true for some of the other fungi found associated with *D. valens* in this study. Our results provide a foundation for population genetic comparisons of fungi associate with *D. valens* in its native range and as an introduced forest pest. Pathogenicity tests using some of the fungi are underway and these should provide deeper insights into the importance of their introduction into a new environment.

45.19 PATHOGENS OF IMPORTANCE AND THEIR ECONOMIC IMPACT ON THE AUSTRALIAN VEGETABLE INDUSTRY. **I.J. Porter, E.C. Donald and E.J. Minchinton.** *Department of Primary Industries Victoria, Private Bag 15, Ferntree Gully Delivery Centre, Victoria, 3156 WA Australia. Email: ian.j.porter@dpi.vic.gov.au*

The Australian vegetable industry has significant annual investment in pathology-based research and development. A national program to determine the major pathogens affecting vegetable crops (excluding tomatoes and potatoes) and develop new sustainable IPM programs for their control commenced in 2006. A series of eight industry workshops was held nationally in key vegetable-producing regions during 2007 to objectively identify pathogens of importance and estimate their economic impact. Workshop participants included over 120 key growers, consultants, chemical resellers, researchers and private sector providers. Participants were presented with a list of all pathogens known to affect Australian vegetable crops. Each nominated six from this list that they felt were 'priority pathogens' either because they currently could not be controlled, they were expensive or difficult to control or because they were at risk of future control failure due to chemical resistance, product withdrawal or regulatory changes etc. Individual priority rankings were combined to determine the top six pathogens for the group. Nationally, diseases caused by *Sclerotinia minor* and *S. sclerotiorum* and viral pathogens ranked the highest. *Sclerotinia* ranked as the most important pathogen in Victoria, Tasmania and Western Australia. Viral pathogens ranked highest in Queensland and New South Wales. *Tomato spotted wilt virus* was the most predominant viral pathogen. Downy mildew, *Fusarium* and *Pythium*, together with powdery mildew and *Rhizoctonia* were the next most important pathogens in the vegetable industry. Annually, pathogens caused crop losses of up to Aus 150000/ha in greenhouse crops and Aus 54000/ha in field crops.

45.20 A CLIMATE-DRIVEN PEST RISK ASSESSMENT OF FIRE BLIGHT IN NORWAY. T. Rafoss, A. Sletten and L. Sundheim. *Norwegian Institute for Agricultural and Environmental Research, Norway. Email: leif.sundheim@bioforsk.no*

Fire blight caused by *Erwinia amylovora* is under eradication in Norway. The pathogen is present in *Cotoneaster bullatus*, *C. salicifolius* and some other *Cotoneaster* spp. Occasional cases have been detected in *Sorbus aria*, *Pyracantha* spp. and apples and pears in home gardens. The disease has not reached commercial fruit orchards and nurseries. The geographical distribution is along the coast from Vest Agder County to Sogn og Fjordane County. The Norwegian Scientific Committee for Food Safety has made a Pest Risk Assessment (PRA) of the disease. Phenology of apple and pear varieties was studied during 2006, including secondary blooming throughout the season. Also, historical variation in the phenological full bloom stage for two apple varieties was studied for the last 17 years and compared with climate data. The PRA identified long and short distance movement of plants, and short distance movement of beehives and farm machinery as high risk pathways. Also, movement of equipment for mechanical vegetation control in gardens, parks and roadsides from infected areas to fire blight-free areas are highly likely to spread the disease. When climatic and phenological data were examined, it was concluded that the climate of the PRA area in most years will prevent fruit tree blossom infection, and that if *E. amylovora* is introduced into main fruit districts damage will be limited under the current phytosanitary regime in Norway. Relaxation of the regulations will increase the expected damage and losses to commercial fruit production and nurseries to a moderate level.

45.21 THE FRENCH CITRUS CERTIFICATION SCHEME. J.P. Thermoz. *INRA-GEQA, 20 230 San Giuliano, France. Email: thermoz@corse.inra.fr*

Many major threats around the world can cause important citrus yield losses, due to viruses, phytoplasmas, bacteria and fungi. When accessing new varieties or propagating trees we have to take care not to spread the pathogens. Within the French certification scheme for fruit trees, partners from research, development, official services and nurseries cooperate to propagate citrus trees free of diseases. French citrus production is located in Corsica and in overseas departments. Corsica is free of airborne and vector-transmitted diseases. For 50 years INRA-GEQA has managed a large germplasm collection of about 5000 trees grown outdoors and is able to provide seeds of rootstocks, and budwood of most citrus varieties. INRA-GEQA is moving the scheme of sanitary control to a quality insurance system in order to secure budwood quality.

45.22 MICROFLUIDIC CHIP COMBINED WITH DOT-ELISA FOR DETECTION OF *POTATO VIRUS A*. L. Wang, S.H. Tian, M.Q. Zou, P. Zhou, J.F. Li, Y. Ch. Chen, Q. Xue, X.H. Qi and N. Wang. *Institute of Inspection Technology and Equipment, Chinese Academy of Inspection and Quarantine, Beijing 100025, P.R. China. Email: shimintian@yahoo.com.cn*

A microfluidic chip device combined with dot-ELISA for detection of *Potato virus A*, the causal agent of some potato diseases, was tested. PVA is one of the important quarantine plant viruses in China. All reagents used for ELISA, like antibodies and washing buffer were prepared and added in the corresponding chip reagent pools. Each chip had six channels. Nitrocellulose membrane test strips containing eight round pieces of the NCM with a diameter of 2 mm were inserted into the polyplastic chip channels. Running the special PC program, the ELISA procedures like coating, washing, and adding of antibodies and substrate were automated in the chip. After about 200 min, the strips could be taken out from the channels, and the results visually observed, red-brown colour showing a positive detection. Different dilutions of PVA antibodies, incubation time and washing times were optimized by performing the dot-ELISA in the chip device. Results confirmed that this alternative microfluidic chip device performed well and steadily. Compared with the normal microplate ELISA or dot-ELISA performed on NCM by hand, the microfluidic chip dot-ELISA for potato virus A was faster, automatic, of high quality and with high-throughout. A further study will be conducted. Supported by the state funds for detection of micro-organisms on food and plants based on microfluidic chip methods (No.2005DIA2J128, No.2005IK0073).

45.23 THE PROBLEM WITH GRUBBY FOOTWEAR AT INTERNATIONAL BORDERS: A NEW ZEALAND CASE STUDY OF GOLFERS. T.D. White, T.A. Payne and M.R. McNeill. *AgResearch Ltd, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand. Email: tracy.payne@agresearch.co.nz*

Due to New Zealand's geographical isolation, the country is free from many of the pests and diseases that are problematic in agricultural, horticultural, silvicultural and natural environments elsewhere in the world. To help protect the country from incursions by new pest and diseases, biosecurity officers check and, if necessary, clean travellers' footwear as they enter the country. Footwear worn outdoors in unpaved areas can collect and carry contaminants such as soil and leaf matter. If contaminated footwear is not detected at the border, it can pose a significant biosecurity risk by providing an entry pathway for unwanted pests and diseases associated with soil and leaf matter. Research elsewhere has identified a diversity of hazards on a range of footwear types including spiked or sprigged footwear, such as that used by golfers. This research examined the experiences of golfers returning to New Zealand after playing golf overseas both in terms of their risk awareness and of their biosecurity experiences at the New Zealand border. Preliminary findings suggest that golfers' awareness of the biosecurity risks from dirty footwear is relatively low and this may influence their response to biosecurity issues when travelling. This work was funded by New Zealand's Foundation for Research, Science & Technology through contract CO2X0501, the Better Border Biosecurity (B3) programme (www.b3nz.org).

CLIMATE CHANGE AND PLANT DISEASES

5.1 THE RELATIONSHIP BETWEEN CLIMATE AND THE INCIDENCE OF RED BAND NEEDLE BLIGHT IN THE EAST ANGLIA FOREST DISTRICT, BRITAIN. S. Archibald and A. Brown. *Forest Research Agency, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK. Email: sarah.archibald@forestry.gsi.gov.uk*

Since the late 1990's there has been a dramatic increase in the extent and severity of red band needle blight (RBNB) (*Dothistroma septosporum*) in Britain, particularly in the East Anglia Forest District. In Britain and the main infection period for *Dothistroma septosporum* is thought to be between May and September. Meteorological data from East Anglia suggests that since the late 1990's the climatic conditions appear to have been favourable to

the disease, with mean annual maximum temperature and rainfall having increased since 1998 by 0.9°C and 0.3 mm respectively. In addition, as was found by Woods (2005) in British Columbia, the frequency of prolonged periods of precipitation and temperatures of 18-20°C during summer months has increased during this period. These factors are likely to have influenced the rate of colonisation of *D. septosporum*. The increase in temperature over the past eight years supports the prediction of Broadmeadow and Ray (2005) that mean annual temperature will increase by between 3-6°C by 2080. The increase in temperature observed in this study, if it continues as experts predict, is likely to benefit the spread and severity of RBNB.

5.2 PHYSIO-PATHOLOGICAL EFFECTS OF CLIMATE CHANGE ON POPLAR. E. Casulli, G. Mosso, A. Giorcelli, P. Chiarabaglio, G. Nicolotti, M. Gennaro, P. Gonthier, A. Garibaldi and M.L. Gullino. *Centre of competence in the agro-environmental sector (AGROINNOVA), University of Torino, Via L. da Vinci 44 - 10095 Grugliasco (TO), Italy. Email: enzo.casulli@unito.it*

Poplar has been chosen by the scientific community as a model organism for studying the development and function of trees. Trials in phytotrons and in the field were carried out. In phytotrons we assessed the effect of increases of carbon dioxide and temperature. The first phytotron parameters were set at a reference level for carbon dioxide concentration (400 ppm), humidity (40-70%) and temperature (18-26°C). The second phytotron was set with higher carbon dioxide concentration (800 ppm) and the third with both higher carbon dioxide concentration (800 ppm) and temperature (22-30°C) levels. In each phytotron three different *Populus nigra* L. genotypes from Belgium, northern Italy and southern Italy were used. Results confirmed that high carbon dioxide concentrations increased diameter, height, chlorophyll content, net photosynthesis (An) and Performance Index (PI) of all the genotypes. High carbon dioxide concentrations plus high temperature levels gave different results depending on the provenance and time period. High temperature reduced the positive effect of carbon dioxide except with the genotype from southern Italy. In the field trial, different water rates were applied on the same poplar genotypes. Pathological surveys were carried out at the end of different water regime periods to assess how plants (bark extract) reacted when inoculated in the laboratory with conidia of the weakness pathogen *Discosporium populeum* Sacc. Germ tube development was measured after 24 h at 22°C using light microscopy. No significant differences were observed among *P. nigra* genotypes although differences were observed between *P. nigra* genotypes and other hybrid poplar clones.

5.3 DIVERSITY AND DENSITY OF MICROBIAL POPULATIONS ON THE RICE PHYLLOSPHERE UNDER ELEVATED ATMOSPHERIC CARBON DIOXIDE. D.M. De Costa and N. Kishimoto. *Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka. Email: devikacos@yahoo.com*

Diversity and density of epiphytic microorganisms on the phyllosphere (i.e. leaf surface) of rice under elevated atmospheric CO_2 was determined over two growing seasons in Sri Lanka using three rice varieties. The three varieties were grown under three treatments, namely elevated atmospheric CO_2 (570 ppm) in open-top chambers (OTCs), ambient CO_2 (370 ppm) in OTCs and in open field conditions under ambient CO_2. Epiphytic microorganisms were isolated from rice leaves at four different growth stages. Each treatment had two replicates and five plants from each replicate were used for isolations. In both seasons, the lowest microbial densities and diversities were observed in rice grown in open field conditions. However, in both seasons, no significant difference was shown in the microbial density and diversity between plants grown under elevated and ambient CO_2 concentrations within OTCs. The three rice varieties did not show significant variation in the response of their phyllosphere epiphytic microbial populations to elevated CO_2. However, density and diversity of microbial populations showed significant variations between the two growing seasons and between different growth stages. Among the epiphytic microorganisms, several fungi and bacteria which showed antagonism against *Magnaporthe grisea*, the rice blast pathogen were found. However, a clear relationship was not shown between the occurrence of antagonists and the CO_2 treatment from which they were isolated.

5.4 BIOCHEMICAL AND MOLECULAR RESPONSES OF *MENTHA* × *PIPERITA* TO UV-B TREATMENT. Y. Dolzhenko, C.M. Bertea and M. Maffei. *University of Turin, Plant Biology Department and Centre of Excellence CEBIOVEM, Viale Mattioli, 25 Turin, Italy. Email: yulia.dolzhenko@unito.it*

Decrease in ozone concentration in the stratosphere causes increase in the amount of UV-B radiation (260-320 nm) that reaches the earth's surface, causing many physiological and biochemical changes in photosynthetic organisms. Photosynthesis is often reduced and the production of plant secondary metabolites increased. One of the most important species producing secondary metabolites is peppermint, *Mentha* × *piperita* L. (Lamiaceae), which is grown intensively for its essential oil employed by the flavouring and pharmaceutical industries. The composition of this oil is particularly affected by environmental factors, including light. Since changes in the oil's chemical composition often lead to drastic changes in its commercial value, we aimed to analyze the effects of UV-B on several aspects of peppermint primary and secondary metabolism. Field plants were irradiated with additional UV-B (310 nm; 2W m-2) for 1 h in the middle of the day for two days. Leaves were collected 4 h, 20 h, 24 h, 28 h, 44 h and 48 h after the first irradiation and used for essential oil extraction; scavenger enzyme activities (catalase, peroxidase, superoxide dismutase) and terpene-related gene expression were also analysed. We report the preliminary results of an effect of UV-B radiation on essential oil composition, activity and expression of some scavenger enzymes and on the expression of genes related to monoterpenoid biosynthesis. qRT-PCR experiments on scavenger enzymes and terpenoid biosynthetic gene are underway.

5.5 RANGE AND SEVERITY OF *PHOMA* STEM CANKER (*LEPTOSPHAERIA MACULANS*) OF WINTER OILSEED RAPE (*BRASSICA NAPUS*) INCREASED BY GLOBAL WARMING. N. Evans, A. Baierl, M.A. Semenov, P. Gladders and B.D.L. Fitt. *Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK. Email: neal.evans@bbsrc.ac.uk*

Climate change affects plants in natural and agricultural ecosystems throughout the world but little work has been done to predict the effects of climate change on plant disease epidemics. To illustrate such effects, a weather-based disease forecasting model was combined with a climate change model predicting UK temperature and rainfall under high and low carbon emissions for the 2020s and 2050s. Multi-site data collected over a 15-year period were used to develop and validate the weather-

based model forecasting severity of *Phoma* stem canker (*Leptosphaeria maculans*) epidemics on oilseed rape (*Brassica napus*) across the UK. This was combined with climate change scenarios to predict that epidemics will not only increase in severity but also spread northwards from England to Scotland by the 2020s. The results provide a stimulus to develop models to predict effects of climate change on other plant diseases, especially in delicately balanced agricultural or natural ecosystems. Such predictions can be used to guide policy and practice in adapting to effects of climate change on food security and wildlife.

5.6 EFFECT OF ELEVATED ATMOSPHERIC CARBON DIOXIDE CONCENTRATION ON RICE BLAST IN OPEN-TOP CHAMBERS IN BRAZIL. **R. Ghini and W. Bettiol.** *Embrapa Environmental, CP69, 13820000, Jaguariúna-SP, Brazil. Email: raquel@cnpma.embrapa.br*

Atmospheric carbon dioxide concentration is projected to increase rapidly and is expected to affect agroecosystems. Rice production is severely limited by blast, caused by *Pyricularia grisea*, worldwide. The effect of elevated atmospheric CO_2 concentration on rice blast incidence was studied in open-top chambers (OTC) in Jaguariúna, São Paulo state (latitude 22º 41' S, longitude 47º W), Brazil. The OTCs were roofless cylinders measuring 1.9 m in diameter by 2 m in height, and constructed with aluminum frame covered with transparent plastic. The trial included three treatments: OTC with elevated CO_2 concentration (approximately, 550 ppm), OTC with ambient atmosphere, and control without OTC. The treatments were randomized in blocks with three replications. Air sampling, gas measurement and gas injection were automatically performed at 10-min. intervals, 24-hours a day. The pathogen was inoculated using a conidial suspension. The experiment was repeated twice with two rice cultivars (IAC 202 and Agulha Precoce). For both cultivars, the occurrence and severity of the disease were higher for plants grown under elevated CO_2 concentration as indicated by the increased number of diseased plants, lesioned leaves per plant, symptom severity and number of sporulating lesions per plant.

5.7 RISK ANALYSIS OF CLIMATE CHANGE ON BLACK SIGATOKA IN BANANA IN BRAZIL. **R. Ghini, E. Hamada, R.R.V. Gonçalves, L. Gasparotto and J.C.R. Pereira.** *Embrapa Environmental, CP69, 13820000, Jaguariúna-SP, Brazil. Email: raquel@cnpma.embrapa.br*

Knowing the probable impacts of global climate change on the occurrence of plant diseases is of great importance for the agricultural sector, since it allows elaborating control strategies. This study aimed to evaluate the potential impacts of climate changes on black Sigatoka disease of banana in Brazil, elaborating distribution maps of the disease based on climatological normal data from 1961-1990 and on future climate. Future scenarios focused on the decades of the 2020's, 2050's, and 2080's (scenarios A2 and B2) were obtained from five General Circulation Models available from the Data Distribution Centre of the Intergovernmental Panel on Climate Change. It was assumed that development of the disease was favoured by mean temperatures between 20°C and 30°C, and relative humidity above of 70%. The maps showed a reduction of the area favourable to the disease in the country, except in the south-east and south, which would become more favourable due to higher humid period. Such reduction would be gradual for the decades of 2020, 2050 and 2080 and higher for scenario A2 than for B2, even though extensive areas would still continue being favourable to occurrence of the disease, especially in the period of November to April.

5.8 RISKS TO SITKA SPRUCE AND OTHER FOREST TREE SPECIES IN SCOTLAND DUE TO DROUGHT AND FUNGAL DISEASE. **S. Green, D. Ray and G.A. MacAskill.** *Forest Research, Northern Research Station, Roslin, Midlothian, Scotland, UK. Email: sarah.green@forestry.gsi.gov.uk*

Drought in 2003 caused extensive damage to pole stage and older Sitka spruce along the Dee valley in north-east Scotland. Three affected sites were assessed between 2004 and 2006 to monitor the development of drought damage. Symptoms on surviving trees included varying degrees of top dieback, resinosis, and the formation of elongated lesions on stems and branches. An unidentified species of *Phomopsis* was isolated consistently from the margins of these lesions. An inoculation study was undertaken in 2007 to determine whether an interaction exists between drought stress and disease caused by this *Phomopsis* sp. on Sitka spruce. A number of other fungal pathogens are known to be more damaging when their tree hosts are stressed by drought. Since eastern Scotland is predicted to suffer an increase in the frequency and severity of drought periods due to climate change, drought risk maps were also developed based on climatic and soils data to identify other drought prone forest sites in Scotland. Based on these maps, an assessment has been undertaken of the potential future risks to forest tree species planted on these drought prone sites due to a combination of drought stress and fungal disease.

5.9* EFFECTS OF OZONE AND OZONE-PROTECTANT CHEMICALS ON PHYSIOLOGY AND GROWTH OF EGYPTIAN CLOVER GROWN IN OPEN-TOP CHAMBERS AT A RURAL SITE IN EGYPT. **IA. Hassan, J.N.B. Bell and F.M. Marshall.** *Department of Botany, Faculty of Science, Alexandria University, 21526 El Shatby, Alexandria, Egypt. Email: i_hassan82@hotmail.com*

Plants of an Egyptian variety of clover (*Trifolium alexandrinum* L. cv. Messkawy) were either untreated (control) or treated with a soil drench of ethylenediurea (EDU) at 50, 100, 150, or 200 ppm or benomyl at 250, 350, 450 or 550 ppm for the whole growing season and sown in the soil either under field conditions at a rural site in northern Egypt or in open-top chambers receiving either charcoal-filtered or unfiltered air. Both EDU and benomyl caused increased growth and provided some protection against visible O_3 injury symptoms, with EDU giving better protection than benomyl. Moreover, photosynthetic rates were increased in treated plants while stomatal conductance did not show any significant difference from control plants under OTC conditions. Our results show that benomyl and EDU would be useful tools to assess the effects of ambient O_3 on plants under Egyptian field conditions. The implications for Egyptian agriculture are discussed.

5.10* INVOLVEMENT OF *PHYTOPHTHORA* ROOT AND BARK INFECTIONS IN THE WIDESPREAD DECLINE OF EUROPEAN BEECH IN BAVARIA. **T. Jung.** *Phytophthora Research and Consultancy Thomastrasse 75, D-83098 Brannenburg, Germany. Email: competence@tree-diseases.com*

During the past decade, and in particular after the wet summer of 2002, an increasing number of trees and stands of Euro-

pean beech (*Fagus sylvatica* L.) in Bavaria were showing symptoms typical for *Phytophthora* diseases: thinning and dieback of the crown, small and often yellowish leaves, root and collar rot and aerial bleeding cankers up to stem heights of >20 m. Between 2003 and 2007, 100 mature beech stands on a broad range of soils were examined, and typical *Phytophthora* symptoms were found in 94 stands. In most stands the majority of beech trees were declining, and scattered or clustered mortality occurred. Eight different *Phytophthora* species were recovered from symptomatic tissues or rhizosphere soil of 243 of 300 trees investigated. The most frequent species were *P. citricola* and *P. cambivora* followed by *P. cactorum*, *P. gonapodyides*, *P. pseudosyringae* and *P. syringae*. Primary *Phytophthora* lesions were soon infected by a series of secondary bark pathogens and wood-rotting fungi; the predisposed trees were usually attacked by several bark- and wood-boring insects leading to rapid mortality. A small-scale survey in nine Bavarian nurseries demonstrated regular infestations of all 12 beech fields with the same range of *Phytophthora* species. The results indicate that (1) *Phytophthora* species are regularly associated with beech decline, and (2) widespread *Phytophthora* infestations of nursery stock might endanger current and future silvicultural projects aiming to replace pure conifer stands by beech-dominated mixed stands.

5.11* EFFECTS OF ELEVATED CO_2 AND TEMPERATURE ON INFECTION OF ZUCCHINI BY POWDERY MILDEW. **J.Z. Liu, P. Titone, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: junzhi_liu@hotmail.com*

Plant responses to elevated CO_2 and temperature, such as changes in morphology or photosynthesis, have been much studied in recent years, especially since climate change has become a by-word, but effects of climate change on pathological responses are largely unknown. The zucchini-powdery mildew pathosystem was chosen as a model to assess the potential impact of increased CO_2 and temperature on disease incidence and severity. Zucchini plants (*Cucurbita pepo*) were grown under three different environmental conditions, which were 450 ppm of CO_2 with normal temperatures (18 to 24 °C or 18 to 26 °C), high CO_2 (800 ppm) with the same temperatures, and high CO_2 with higher temperatures (4 °C more than the above). They were artificially inoculated with *Podosphaera xanthii*. Disease effects were evaluated by comparing the size of powdery mildew colonies, number of tips, conidiophores and spores per colony, and the disease index of the whole plant. The results showed that increase of CO_2 alone generally caused no significant difference in either pathogen development or disease index, whereas increase of both CO_2 and temperature always stimulated pathogen development and disease severity.

5.12* EXAMINING THE EFFECTS OF ELEVATED CO_2 ON KEY PATHOGENS OF WHEAT. **J. Luck, G. Hollaway, A. Freeman, R. Norton and S. Chakraborty.** *Department of Primary Industries, Private Mail Bag 15, Ferntree Gully Delivery Centre, VIC 3156, Australia. Email: jo.luck@dpi.vic.gov.au*

Climate change is increasingly recognised as a major threat to natural and agricultural systems, yet the potential effects of pathogens on these changing systems are not clearly understood. The Department of Primary Industries Victoria and The University of Melbourne have established a Free-Air CO_2 Enrichment (FACE) research facility at Horsham, Victoria, to study the effects of elevated CO_2 on wheat production in Australia. This facility provides a unique opportunity to study possible changes in host pathogen-interactions and the effectiveness of partial resistance genes in the presence of elevated CO_2 in the field. Worldwide, studies on host-pathogen interaction in the presence of elevated CO_2 have been limited. In this work, we studied the influence of CO_2 on the interaction between wheat and *Puccinia striiformis* (wheat stripe rust), *Fusarium pseudograminearum* (crown rot) and *Barley yellow dwarf virus*. The major aim of this project was to understand the effects of projected CO_2 concentrations (550 ppm) under field conditions on key disease threats to wheat. Preliminary analysis of the first season's data suggested limited effects on fecundity and disease progress of stripe rust and severity of crown rot, however further analysis is required. A background infection of BYDV made it difficult to interpret any CO_2 effect on the virus but visual observations showed little difference between the CO_2 treatments. Results from the statistical analysis of the experiments will be presented.

5.13 EFFECT OF TEMPERATURE ON *FUSARIUM* WILT IN TOMATO PLANTS. **N. Pshybytko, L. Zenevich, N. Zhavoronkova and L. Kabashnikova.** *Institute of Biophysics and Cell Engineering, National Academy of Sciences of Belarus, Akademicheskaya 27, 220072 Minsk, Belarus. Email: pshybytko@rambler.ru*

The main physiological processes in tomato plants infected by *Fusarium oxysporum* were studied. The pathogen was inoculated in 4-month-old tomato plants grown in a trough root system. The response of the plants to inoculation depended on temperature, humidity and light intensity, with temperature the most important. When *F. oxysporum* was inoculated at over 35°C the plants dried up after 7-10 days. In more usual conditions the disease continued for about 30-40 days, terminated by yellowing and drying. The different visible symptoms were caused by various mechanisms of withering. Slow pathogenesis was caused by *F. oxysporum* toxins, which suppressed the plant's physiological processes, with inhibition of biosynthesis of main cell components. Under high temperature the fungus proliferated actively in root tissues and mycelium grew, blocking the phloem, with decreased xylem flow and dehydration of the plant. Activation of destructive processes (lipid peroxidation and generation of reactive oxygen) occurred under both types of pathogenesis. Photosynthesis suppression under *Fusarium* wilt was caused by decrease in photosystem II photochemistry and limitation in rate and affectivity of linear electron flow in chloroplasts. The reduction of chlorophyll content and increase in anthocyanin content indicated destructive processes in the pigment system. The intensity of destructive processes was greater under high temperature. The mechanisms of combined impact of biotic (fungi) and abiotic (high temperature) stress factors are discussed.

5.14 VIRULENCE FREQUENCIES OF WHEAT LEAF RUST (*PUCCINIA TRITICINA*) IN IRAN. **F. Rafiei, F. Afshari and A. Arzani.** *Agronomy and plant breeding division, Agriculture department, Isfahan University of Technology, Isfahan, Iran. Email: raf_fariba@yahoo.com*

In many regions of Iran, leaf rust caused by *Puccinia triticina* Eriks is an important disease for wheat production but there is little information about virulence frequencies of *P. triticina* in Iran. In the present work, data collected from a leaf rust survey in 2004, 2005 and 2006 have been used to characterize virulence and diversity of prevalent isolates of *P. triticina* in North and South provinces of Iran. Analysis of variance using transformed percent-

age data showed that the different frequencies of virulent isolates is a main source of variation. The frequency of virulent isolates also varied significantly in each region but no significance variation could be detected between regions and virulence × region interactions. This may be attributed to low variability for the frequencies of virulence to *Lr* genes in the northern and southern regions. The results also indicated that some of the near isogenic lines (*Lr2b*, *Lr2c*, and *Lr20*) are attacked by a high number of isolates whereas others are only susceptible to either a few or no isolates. Linear regression analysis showed that virulence frequency was significantly reduced for genes *Lr2b*, *Lr10*, *Lr17* and *Lr26* in the northern region. Similarly in the southern region, reduced virulence was observed for two *Lr* genes: *Lr2a* and *Lr2b*. In both regions there were no virulent isolates for *Lr9*, *Lr19* and *Lr25* genes, indicating the effectiveness of these resistance genes as well as low variation of virulence frequency for the leaf rust pathogen in Iran.

5.15 LONG-TERM DYNAMICS IN WHEAT OF *PHAEOSPHAERIA NODORUM* AND *MYCOSPHAERELLA GRAMINICOLA*. **M.W. Shaw, S.J. Bearchell, B.D.L. Fitt and B.A. Fraaije.** *Rothamsted Research, Harpenden AL5 2JQ, Hertfordshire, UK. Email: bruce.fitt@bbsrc.ac.uk*

We used the polymerase chain reaction (PCR) to study the presence of pathogens in archived wheat grain and leaves from a long-term experiment started in 1843. The data were used to construct a unique time-series of the abundance of two important pathogens, *Phaeosphaeria nodorum* and *Mycosphaerella graminicola*. During the last 30 years the relative abundance of these pathogens reflected their importance in UK wheat disease surveys. Relationships between amounts of pathogens and environmental and agronomic factors were examined by multiple regression. Fluctuations in amounts of *P. nodorum* in grain were related to changes in spring rainfall, summer temperature and national SO_2 emissions. Differences in amount of *P. nodorum* between grain and leaf were related to summer temperature and spring rainfall. In leaves, annual variation in spring rainfall affected both pathogens similarly, but SO_2 had opposite effects. SO_2 was the dominant effect on *M. graminicola*; the effect on *P. nodorum* was similar to spring rainfall. Previous summer temperature had a highly significant effect on *M. graminicola.* Annual variability was dominated by weather factors occurring over a period longer than the growing season. Long-term variation in *P. nodorum* and *M. graminicola* DNA in leaf and grain over the period 1844-2003 was dominated by factors related to atmospheric pollution, as measured by national SO_2 emissions. This suggests that long-term, economically important, changes in pathogen populations can be influenced by anthropogenically induced environmental changes.

5.16 THE EPIDEMIC OF ALDER DECLINE IN THE CZECH REPUBLIC AND THE DISASTROUS FLOODS IN 2002. **V. Strnadova, K. Cerny, B. Gregorova, V. Holub and S. Gabrielova.** *Department of Plant Protection, Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Kvetnove nam. 391, Pruhonice 25243, Czech Republic. Email: strnadova@vukoz.cz*

Phytophthora alder decline is serious in many European countries. The first marks of this decline in the Czech Republic were found in the 90s and the causal agent, *Phytophthora alni*, was isolated here in 2001. In August 2002, the western part of the country was affected by disastrous floods. Many hundreds kilometres of riparian alder stands in several catchments especially in western, middle and southern Bohemia were stressed by one-hundred or by one-thousand-year floods. Since 2003, the number of riparian alder stands damaged by *P. alni* has greatly increased in the affected catchments. The largest losses of alder trees are localised there as well, especially in the Vltava river catchment. The spread of the pathogen and/or its ability to infect the host during flooding is documented by several authors and by our own investigation. In 2005 we obtained preliminary evidence that alder trees stressed by flooding are damaged by the pathogen much more than non-stressed ones. Although our field studies are still in progress, we are convinced that the epidemic of *Phytophthora* alder decline in riparian stands in the Czech Republic (leaving aside the rest of Europe) was catalysed by the floods of 2002.

5.17 EFFECT OF GLOBAL CHANGES ON THE POWDERY MILDEW-GRAPEVINE PATHOSYSTEM: STUDY UNDER CONTROLLED ENVIRONMENT. **P. Titone, M.L. Gullino and A. Garibaldi.** *AGROINNOVA, University of Torino, Via L. da Vinci, 44, 10095 Grugliasco (TO), Italy. Email: patrizia.titone@unito.it*

Climate change involves rising CO_2 levels and rising temperature. To evaluate the influence of global changes on plant disease, we chose the powdery mildew-grapevine pathosystem. Trials were carried out with 2 grape varieties (Moscato and Barbera) grown in phytotrons, using the following environmental parameters: (1) temperature between 18°C and 26°C, CO_2 concentration 450 ppm, (2) temperature between 18°C and 26°C, CO_2 concentration 800 ppm, (3) temperature between 22°C and 30°C, CO_2 concentration 800 ppm. Disease index and physiological parameters (chlorophyll content, fluorescence, assimilation rate) were assessed. In the first 2 trials the results obtained showed an increase of powdery mildew development with temperature between 18°C and 26°C and CO_2 concentration of 800 ppm. Plants grown at 800 ppm of CO_2 generally had higher chlorophyll content, fluorescence and assimilation rate compared to plants developed at 450 ppm of CO_2.

5.18 MORPHOLOGICAL AND HISTOCHEMICAL CHANGES IN *SEIRIDIUM* SPP. UNDER WATER STRESS. **E. Turco, B. Mori, P. Raddi and A. Panconesi.** *CNR, Institute for Plant Protection, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy. Email: e.turco@ipp.cnr.it*

Reduced annual rainfall and increased mean temperature are two of the many consequences of the greenhouse effect. Organisms have to adapt to climate change, and plant pathogens and pests must similarly co-evolve to avoid any break in their life cycle. In the Mediterranean region, these climate changes are marked: long, dry summers and rainfall mostly occurring in winter favour the spread of desertification with consequent death of plants or increased susceptibility to disease. The high mortality of cypress (*Cupressus* spp.) observed in all the Mediterranean area is mostly due to *Seiridium cardinale*. This pathogen, and the other two related species (*S. cupressi* and *S. unicorne*) cause cypress canker with heavy damage in forests, nurseries and ornamental plantations. The ability of the fungus to survive for years on dead plant material as resting mycelium suggested a study on the morphological and histochemical changes induced by increasing water stress. Isolates of the three *Seiridium* species and of *Sphaeropsis sapinea* f.sp. *cupressi* (causing canker in north Africa but rarely observed in Italy) were cultured on media with osmotic potential ranging from 0 to -15 MPa. Histochemical changes, observed by light and fluorescence microscopy, revealed lipid accumulation and cell wall thickening under the

highest osmotic stress. Disorganization of hyphae, granular cytoplasm and shorter distance between septa were also observed. ESEM analysis gave further information on the chemical composition of intracellular vacuoles.

CONCEPTS IN BIOLOGICAL CONTROL OF PLANT PATHOGENS

1.1 DEVELOPMENT OF RESISTANCE IN *BOTRYTIS CINEREA* TO PYRROLNITRIN, AN ANTIBIOTIC PRODUCED BY BIOLOGICAL CONTROL AGENTS.

S. Ajouz, M. Bardin and P.C. Nicot. *INRA, Centre de Recherches d'Avignon, Unité de Pathologie Végétale, B.P. 94, F-84143 Montfavet Cedex, France. Email: philippe.nicot@avignon.inra.fr*

In order to control *Botrytis cinerea*, many biological control agents (BCAs) have been described, but information regarding their durability is lacking. The objective of the present study was to estimate the risk of losing biocontrol efficacy due to selection pressure exerted by BCAs on *B. cinerea*. Efforts have been focused first on the antibiotic pyrrolnitrin which was identified in various BCAs known to have significant efficacy against *B. cinerea*. To evaluate a possible decrease in sensitivity to pyrronitrin under selection pressure, ten successive generations of 5 isolates of *B. cinerea* were produced in vitro in the presence of a sub-lethal dose of the antibiotic. For each isolate, three independent repetitions were carried out. As a control, ten successive generations were also produced for each isolate in absence of pyrrolnitrin. We observed a significant reduction in sensitivity to pyrrolnitrin in the 10^{th} generation produced in the presence of the antibiotic and different evolution patterns were observed among the 5 isolates. The production of 10 additional generations with increasing doses of pyrrolnitrin resulted in the development of variants of *B. cinerea* with higher levels of resistance. These results suggest that continuous exposure to pyrrolnitrin may lead to adaptation of this fungus. Work in progress includes analysis of the fitness of pyrrolnitrin-resistant variants, and bioassays on plants using pyrrolnitrin-producing bacteria to evaluate a possible loss of efficacy against the resistant variants. We will also test the possibility of "reverse adaptation" of resistant variants in absence of selection pressure.

1.2 EVALUATION OF *TRICHODERMA* ISOLATES FOR CONTROL OF *FUSARIUM* WILT OF TOMATO.

J. Amini. *Department of Plant Protection, Agriculture Faculty, P.O. Box 416, University of Kurdistan, Sanandaj, Iran. Email: aminij2002@yahoo.com*

Fusarium oxysporum f.sp. *lycopersici* is a fungal pathogen that causes wilt of tomato. Antagonistic effects of 28 isolates of *Trichoderma* spp. were evaluated for biological control against the *Fusarium* wilt of tomato in vitro and in the greenhouse. The dual culture, cellophane overlay technique and volatile metabolites were used in the in vitro assay. Greenhouse experiments were carried out to test *Trichoderma* isolates against *F. oxysporum* f.sp. *lycopersici* by seed and soil treatment. Results of in vitro assays indicated that 6 tested isolates of *Trichoderma* inhibited growth of the pathogen. Mycelium inhibition varied between isolates of *Trichoderma* and ranged from 13 to 66 % in dual culture and from 13 to 100% using the cellophane overlay method. The pathogen's mycelial growth was reduced 9-46% by volatile metabolites of *Trichoderma* isolates throughout 120 hours inoculation. Also in the greenhouse, results obtained indicated that 6 *Trichoderma* isolates could reduce the disease incidence by 2.3 to 9.7 times and stimulated plant growth up to 3 times in comparison with infected controls of Antagonists and pathogen populations were estimated (cfu/g) in potting mix 15 and 40 days after planting. The pathogen and antagonist populations (cfu/g) were fixed during experiments.

1.3 A DOUBLE-STRANDED RNA MYCOVIRUS ASSOCIATED WITH IMPAIRED GROWTH IN *ALTERNARIA ALTERNATA*.

N. Aoki, H. Moriyama, M. Kodama, T. Arie, T. Teraoka and T. Fukuhara. *Department of Applied Biological Sciences, Tokyo University of Agriculture and Technology, 3-5-8 Saiwaicho, Fuchu, Tokyo 183-8509, Japan. Email: 50006951012@st.tuat.ac.jp*

Four double-stranded RNAs (dsRNAs) referred as L- (3.6kb), M1- (2.7kbp), M2- (2.6kbp) and S-dsRNA (1.5kbp), respectively, were detected in the EGS35-193 strain of *Alternaria alternata* with high concentration (~3 mg/g dried mycelium). These dsRNAs were transmitted to 100% of the conidia. The buoyant density of the isometric virus particles (33 nm in diameter) in CsCl was 1.35-1.40 g/cm^3 depending on the size of dsRNAs packaged. The sequence of 3,567 nucleotides (nt) of L-dsRNA had a single open reading frame (3,447 nt) that contained the conserved motifs of viral RNA-dependent RNA polymerase (RdRp) characteristic of RdRps of *Totiviridae* and *Chrysoviridae*. It is noteworthy that the coding strand of L-dsRNA has a 3-poly (A) tail ranging from 40 to 50 nt in length. We named this novel dsRNA mycovirus in the EGS35-193 strain of *A. alternata* as Alternaria alternata virus (AaV). The EGS35-193 strain showed impaired growth. By combination of cycloheximide treatment and hyphal tip isolation, we obtained cured strains, in which the amounts of the dsRNAs were reduced (~0.3 mg/g dried mycelium). They restored normal mycelial growth and pigmentation. These results indicated that AaV might be involved in modulating traits of the fungal host *A. alternata*.

1.4* DEVELOPMENT OF AN ATTENUATED TOMATO SPOTTED WILT VIRUS (TSWV) STRAIN, TSWV-19B TO CONTROL TSWV DISEASES IN CHRYSANTHEMUM.

H. Atarashi, H. Sayama, T. Murai, T. Natsuaki, D. Peters and R. Goldbach. *R & D, Nippon Del Monte Corp. 3748 Shimizu-cho, Numata, Gunma 378-0016, Japan. E-mail: hsayama@delmonte.co.jp*

TSWV isolates obtained from chrysanthemum, tomato, pepper and *Eustoma russellianum* were sequentially inoculated 8 to 10 times to *Nicotiana rustica*, *N. benthamiana* and/or *Datura stramonium*, and raised at 16°C. The virus symptoms appearing on these plants were alleviated gradually with passages from necrosis or necrotic ringspots to necrotic local lesions, severe mosaic or rugose, and then to mosaic or mild mosaic. Seven TSWV isolates with selected mild symptoms were inoculated to chrysanthemum to assess their adverse effects, and their protective effects against a virulent TSWV, TSWV-JM1 were evaluated by inoculating those isolates to chrysanthemum and later challenge-inoculating with TSWV-JM1. A promising TSWV isolate, TSWV-19B, with less adverse and more protective effects than others was selected among those isolates tested. Protective effects of TSWV-19B against the virulent TSWV were also confirmed in *D. stramonium*. TSWV-19B and TSWV-JM1 were tested for their transmissibility by two thrips species, *Frankliniella occidentalis* and *F. intonsa*. TSWV-JM1 was transmitted with an efficiency of 37.3% by *Frankliniella occidentalis* and 24.4% in *F. intonsa*, while TSWV-19B showed zero transmission by both species. Northern hybridization and RT-PCR using primers specific to the 5' and 3' termini of L RNA revealed that TSWV-19B contained a

defective interfering (DI) L RNA of about 2.5 kb conserving the 5' and 3' terminal regions. These results suggest that DI RNA-containing TSWV-19B is a non-transmissible, safe and practically useful attenuated virus strain for control of TSWV disease in chrysanthemum. Further experiments are in progress in TSWV-infested chrysanthemum fields.

1.5 EPIPHYTIC FITNESS AND SPREAD OF A BIOLOGICAL CONTROL AGENT OF FIRE BLIGHT USING CULTURE-BASED AND REAL TIME PCR METHODS. **E. Badosa, M. Pujol, J. Cabrefiga and E. Montesinos.** *Institute of Food and Agricultural Technology-CIDSAV-CeRTA, University of Girona, 17071 Girona, Spain. Email: esther.badosa@.udg.edu*

Pseudomonas fluorescens EPS62e was isolated from a healthy pear fruit and is an efficient biological control agent of fire blight, controlling infections in immature pear fruit, flowers and whole plants. Knowledge of the environmental fate of this strain is of great importance for field studies. A real-time PCR monitoring method was developed for EPS 62e on the basis of a SCAR-specific marker. The ability of the strain to colonize pear and apple trees under Atlantic and Mediterranean climatic conditions was evaluated with the simultaneous use of real-time PCR and culture-based methods. EPS62e was an efficient colonizer of flowers, reaching very high population levels, between 10^7-10^8 CFU per blossom, after a single treatment during bloom, according to both methods of analysis. The plant host species (pear or ap[ple) did not influence colonization rate, and the biocontrol agent completely dominated the microbiota of flowers. In contrast, in inoculated leaves, population levels decreased over time according to both methods, but the real-time PCR values were higher than in the culture-based method. The combined use of molecular and culture-based methods of analysis identified three physiological states in the field, which consisted of active colonization, survival and entry into a viable, but nonculturable state, and cell death. The strain spread moderately in the orchard, being detected in untreated flowers of trees 15-35 m from the inoculation site.

1.6 BIOCONTROL ACTIVITY OF *PSEUDOMONAS FLUORESCENS* AGAINST *RHIZOCTONIA SOLANI*. **G. Bagyanarayana and K.V.B.R. Tilak.** *Department of Botany, Osmania University, Hyderabad-500 007, A.P., India. Email: gbagyan@yahoo.com*

Several strains of *Pseudomonas* were isolated from the rhizosphere of pigeonpea (*Cajanus cajan*) and were identified following morphological and biochemical methods. The strains included both fluorescent and non-fluorescent types. One strain each of *P. fluorescens* (Pf 1) and *P. putida* (Pp) were found to have broad-spectrum antifungal activity against many soil plant pathogenic fungi (*Rhizoctonia solani*, *Sclerotium oryzae*, *Macrophomina phaseolina*, *Fusarium oxysporum* etc.) under *in vitro* conditions. Though strain PF 1 produced siderophores, i.e. iron-chelating ligands, production of an antibiotic or toxin was found to be responsible for the fungal growth inhibition in vitro. Cell-free extracts of the strain also inhibited the growth of *R. solani*. We concluded that the antifungal toxin was produced inside the cell and transported to the extra-cellular environment. Chemical mutagenesis of Strain Pf 1 using nitrosoguanidine helped to isolate an antifungal toxin-deficient phenotype which did not produce any antifungal metabolite in vitro. This mutant was isogenic to the wild type (parent) strain in other phenotypic characters like colony morphology, nutritional requirements, generation time and siderophore production. Since the mutant was changed only in antifungal metabolite production, it could be used for genetic analysis of antifungal toxin production.

1.7 CHARACTERIZATION OF *BEAUVERIA BASSIANA* AND ITS ANTAGONISMS TO *PHOMOPSIS VEXANS*. **A. Bala and J.S. Bedi.** *Department of Plant Pathology, Punjab Agricultural University, Ludhiana 141004, India. Email: anjuchani@yahoo.co.in*

Phomopsis blight and fruit rot is a serious disease of brinjal in India. Preliminary investigations yielded three isolates of *Beauveria bassiana* showing marked antagonism against *Phomopsis vexans* in vitro. A clear zone of inhibition against *P. vexans* colony growth was shown by all three isolates of *B. bassiana*. Isolate Bb-III formed the largest inhibition zone (8.3 mm). Maximum reduction in *P. vexans* mycelial weight (48.84%) and sporulation (56.34%) was observed at 50% concentration of culture filtrates of this isolate. Isolates Bb-I and Bb-III had cottony smooth growth while Bb-V had wrinkled growth. All three isolates had hyaline, septate, branched mycelium. Conidiophores had clustered flask-shaped phialides to which single-celled, smooth, hyaline, globose to broadly ellipsoidal conidia were attached. Isolates Bb-I and Bb-V produced moderate numbers of conidia (1-2.5×10^6conidia/5mm culture disc) whereas Bb-III had higher sporulation (2.5-5.0×10^6 conidia /5mm culture disc). All three isolates had similar breadth of conidia (3.8-3.9µm) whereas Bb-III conidia were shorter than those of Bb-I and Bb-V. Length of phialides was similar in Bb-I and Bb-III whereas Bb-V had smaller phialides. The isolates, when studied for their field efficacy, were found to reduce *Phomopsis* leaf blight of brinjal considerably. The studies indicated that *B. bassiana* isolates have potential to control *P. vexans* and can be exploited further by developing suitable biofungicide formulations to control the disease under practical conditions.

1.8 STRAIN-SPECIFIC SCAR MARKERS BASED ON UP-PCR FOR *TRICHODERMA HARZIANUM* T-023 FROM ARGENTINA. **V.A. Barrera, M.C. Martínez and A.L. Gasoni.** *IMYZA, INTA, CC 25 (1712) Castelar, Buenos Aires, Argentina. Email: vbarrera@cnia.inta.gov.ar*

In northwestern Argentina, citrus, sugarcane, potato and tobacco are major crops. Tobacco is heavily affected by *Fusarium oxysporum* var. *nicotianae*, which may also reduce potato production, and chemical fungicides have become ineffective. Many studies are concentrated on the application of *Trichoderma* spp. isolates as biological control agents (BCAs), but several useful *Trichoderma* strains are difficult to distinguish from others found in the field, so there is a need to find ways to monitor these strains when applied to natural pathosystems. Among different antagonistic agents, isolated from tobacco rhizosphere in Villa Alberdi (Tucumán), the strain *T. harzianum* T-023 showed efficient biocontrol activity against the pathogenic strain of *F. oxysporum* var. *nicotianae* isolated from damaged tobacco tissues in the same plots. The study was conducted using universally primed PCR (UP-PCR) markers to estimate genetic variation among 40 strains of *T. harzianum*, *T. longibrachiatum* and *T. atroviride*, previously selected as BCAs, and to obtain fingerprint patterns. Fifty eight polymorphic bands were obtained among the samples. Some of the products generated were used to design specific primers, showing that DNA markers may be successfully applied for identification purposes. Further experiments are in progress to perfect a diagnostic PCR using these primers to specifically identify the strain *T. harzianum T-023*. The SCAR markers generated will

be useful for identification of this strain in the soil rizosphere after applying microbial inoculant and may have significant implications in understanding its biological role.

1.9 BIOLOGICAL CONTROL OF AN ISOLATE OF *TRICHODERMA BREVICOMPACTUM* ON COCOA WITCHES' BROOM DISEASE. **C. Bastos.** *CEPLAC/ESJOH, C.P. 46, CEP 67105-970, Marituba, Par., Brasil. Email: cleber@ufpa.br*

Witches' broom caused by the basidiomycete *Crinipellis perniciosa* is the most important factor constraining cocoa production in the main cocoa growing regions of Brazil. Pod losses of up 90% are experienced in affected areas in Bahia and Amazon regions. Biological control by means of micro-organisms is a powerful tool that could contribute substantially as an alternative or a part of an overall integrated management strategy of the witches' broom disease. The aim of this work was to study *in vitro* and *in vivo* the effect of an isolate of *Trichoderma brevicompactum* as a possible biocontrol agent for witches' broom disease. The antagonist was isolated from inner trunk tissues of cocoa, after surface desinfection with sodium hypochlorite solution, rinsing once in 70% ethanol and twice in sterile distilled water. The isolate of *Trichoderma* produced, in liquid medium, metabolites able to inhibit 100% the germination of basidospores and mycelial growth of *C. perniciosa* when the culture filtrate was used at concentrations of 2% and 10%, respectively. Production of basidiocarps on dead brooms was significantly reduced by one application of spore suspension (2×10^7 spores/ml) obtained from *T. brevicompactum* grown on autoclaved rice grains. Experiments carried out in greenhouse showed that spore suspension (2×10^7 spores/ml) and culture filtrate at a concentration of 5% could reduce by over 70.8% the incidence of witches' broom disease in cocoa seedlings, when applied six days before inoculation with the pathogen.

1.10 TALC FORMULATION OF *STREPTOMYCES* ANTAGONIST AGAINST *MYCOSPHAERELLA* FOOT ROT AND BLIGHT. **S. Benali and M. Bencheikh.** *B.P. 920, Chlef 02000, Algeria. Email: benseti@yahoo.fr*

Peas are highly susceptible to pre-emergence damping off caused by *Mycosphaerella pinodes* in western Algeria. Rhizosphere Actinomycetes antagonistic to the growth of this pathogen were isolated from chellif soils. An isolate of *Streptomyces* (St7c5) provided superior seed protection. Increase in both germination and plant growth were recorded following treatment of seeds with *Streptomyces* formulated with inert or organic carrier when compared to controls. Application of the antagonist agent resulted in a significant reduction of *Mycosphaerella* foot rot to 5% compared to untreated seeds (30.5%). It also resulted in a significant reduction of blight symptoms when compared to the control or when treated with talc alone. The talc formulation of the *Streptomyces* agent can therefore be recommended as one of the crop strategies for management of foot rot and blight caused by *Mycosphaerella pinodes*.

1.11 FINDING AND UTILIZING BACTERIAL AGENTS OF SOILBORNE DISEASE SUPPRESSION. **M.S. Benitez, F. Baysal, S.A. Miller, M. Kleinhenz and B. McSpadden Gardener.** *The Ohio State University – OARDC, Wooster, OH, USA. Email: bbmg+@osu.edu*

Diverse microbes inhabit agricultural environments, and some fraction of those populations can suppress the development of soilborne plant diseases. Identifying and recovering microbes which consistently act to suppress plant diseases is a necessary first step in the development and application of biocontrol seed treatments. Historically, the approach has been to recover bacteria then screen them for activity. While this approach to "recover then identify" biocontrol agents has been successful, most of the recovered isolates belong to a limited set of genera. To overcome this limitation and expand our view of the diversity of bacterial biocontrol agents present in nature, we have developed and applied culture-independent molecular tools to first "identify then recover" important biocontrol bacteria. We have applied terminal restriction fragment length polymorphism (TRFLP) analyses to compare the bacterial community structure in soils differing in their disease suppressive capacity. The TRFLP analyses indicated that multiple bacterial populations were involved, but among them three signals appeared repeatedly, indicating that the bacteria giving rise to these signals were correlated with the development of this form of induced suppression. These TRF were found to be derived from novel species not previously associated with biological control. We then used the TRF-derived molecular markers to design a recovery strategy that allowed us to culture isolates of this novel species from the soils in which they were first associated with disease suppression. Multiple isolates harbouring the markers for M139 have been recovered and shown to inhibit pathogen growth *in vitro* and *in situ*.

1.12 INTERACTION OF CELL WALL RECEPTORS OF *ARABIDOPSIS THALIANA* WITH LEAF EXTRACTS OF *AZADIRACHTA INDICA*. **A. Biswas, D. Ghosh, S. Chakraborti, S. Chandra and P.K. Paul.** *Amity Institute of Biotechnology; Amity University, Uttar Pradesh, Sector 125, Express Highway, Noida 201301, India. Email: prabir_kp@rediffmail.com*

Increased application of agrochemicals to boost agricultural yield has led to massive ecological, physiological and environmental hazards. This led to search for environment friendly alternatives. Aqueous extracts of several plant species particularly from *A. indica* have been shown to be important biocontrol agents; for example, *A. indica* extracts induce SAR in host plants, leading to protection against pathogens. The results of our present study indicate that in the process of SAR induction, the *A. indica* leaf extract interacts with a number of cell wall-bound proteins. The extract induces molecular changes in these proteins which then activate a signalling pathway for the transduction of molecular signals from receptors to the nucleus leading to the expression of SAR-linked genes. The present study demonstrates that *A. indica* leaf extract establishes a signalling pathway through interaction with wall receptors leading to establishment of SAR in the host plant.

1.13 TOOLS FOR STUDYING VIRUS-FUNGUS INTERACTIONS. **B. Boine, M.N. Pearson, R. Beever, A. Bailey and G. Foster.** *School of Biological Sciences, University of Auckland, P.O. Box 92019, Auckland, New Zealand. Email: bboi001@ec.auckland.ac.nz*

Understanding the nature of the relationship between viruses and their fungal hosts is critical in determining the ecological significance of mycoviruses and their potential use as biological control agents. Two potex-like flexous viruses, *Botrytis virus F* (BVF) and *X* (BVX), from *Botrytis cinerea* (Howitt *et al.*, 2001; Howitt *et al.*, 2005), have been completely sequenced, providing the op-

portunity to examine their interaction with *B. cinerea* at molecular level. In order to study the virus-fungus interaction at cellular level three tools were developed: (i) an efficient fungal transformation protocol to study the molecular interaction between the virus and the fungus, (ii) a sensitive and reliable real time-PCR detection method for BVF and BVX for studying the impacts of transformation on virus replication, and (iii) an antibody against BVX to visualize its distribution and movement within the mycelia and also between compatible fungal strains. Results will also be presented on the transformation of *B. cinerea* with a PAC1-construct that expresses a dsRNase in *Botrytis* designed to eliminate the virus from the fungal mycelia and spores, and fitness studies on virus infected and virus free mycelia.

1.14 INCREASING SURVIVAL AND EFFICACY OF BIOLOGICAL CONTROL AGENTS BY MEANS OF HYPEROSMOTIC ADAPTATION. **A. Bonaterra, J. Francés, J. Camps, J. Cabrefiga and E. Montesinos.** *Institute of Food and Agricultural Technology-CIDSAV-CeRTA, University of Girona, 17071 Girona, Spain. Email: anna.bonaterra@udg.es*

The efficacy of biological control agents depends on the colonization of plant surfaces after application. A procedure to increase survival under water stress limitation has been developed consisting of saline stress and osmolyte amendment to the growth medium during inoculum preparation. The procedure was tested in the biocontrol agent of postharvest fungal diseases *Pantoea agglomerans* EPS125 and in the biocontrol agent of fire blight *Pseudomonas fluorescens* EPS62e. Hyperosmotic stress induced the synthesis and intracellular accumulation of compatible osmolytes but decreased growth significantly. Amendment of the saline medium with glycine betaine restored growth and promoted its intracellular accumulation. Osmoadaptation of *P. agglomerans* EPS125 increased considerably its survival on apple fruit surfaces under low relative humidity (RH) conditions and significantly improved blue mould control under conditions where the standard biological control treatments were ineffective. Osmoadaptation of *P. fluorescens* EPS62e increased cell survival on plant surfaces by 10 to 100-fold under low RH controlled conditions. In the field, cell survival increased 100 to 1000 times in immature fruit upon osmoadaptation but was not significantly affected in flowers. The efficacy of control of fire blight infections upon osmoadaptation was increased 30 to 50 % on immature fruits but was not affected in blossoms. The implications of the method for increasing epiphytic fitness and the efficacy in other biological control agents of plant pathogens are discussed.

1.15 SCREENING OF HIGH-YIELDING MUTANTS OF THE BIO-CONTROL BACTERIUM *BACILLUS SUBTILIS* BS-916 OBTAINED BY ION IMPLANTATION, AND THE MOLECULAR MECHANISM OF ANTAGONISTIC ABILITY. **Z.-Y. Chen, D.-Q. Li, Y.-F. Liu, Y.-Z. Liu and C. Lou.** *Institute of Plant Protection, Jiangsu Academy of Agricultural Science, Nanjing 210014, P.R. China. Email: chzy@jaas.ac.cn*

The mutation and screening of *Bacillus subtilis* Bs-916 by ion implantation is an important factor in bio-control of rice disease. To improve the growth rate and antagonistic ability of the strain, and to obtain high-efficiency strains, N^+ ions at different doses were implanted into Bs-916. the results showed that the mutated doses from $150\text{-}250\times2.6\times10^{13}$ N^+/cm^2 were better than 10 doses from $30\text{-}80\times2.6\times10^{13}$ N^+/cm^2. Repeated tests of mutation of the strain by ion implantation showed that it is a method of improving mutation efficiency. In 1300 mutation-treated strains, 11 strains showed antagonistic ability increased by more than 10% over that of Bs-916, by the first and the second screening, and fix quantify screening. One of the 11 strains had antagonistic ability increased by 30.7% over that of Bs-916. Analysis of the antagonistic substance in Bs-916 and high-yielding strains mutated by ion implantation, using TLC and HPLC indicated that the lipopeptides produced by Bs-916 and the mutant strains belonged to the surfactin family. High-yielding mutant strains produced more surfactin than the parental strain. The regulatory region of the surfactin operon was examined and we found that in the promoter of surfactin synthesis of Bs-H74, which is located upstream of the srfA operon in *B. subtilis*, some genes were different from the original strain Bs-916. The results indicated that the changes in the promoter gene induced by N^+ implantation may have enhanced the surfactin product.

1.16* EFFECT OF PROTECTIVE AGENTS ON THE VIABILITY OF THE FREEZE-DRIED BIOCONTROL AGENT *METSCHNIKOWIA PULCHERRIMA*. **A. Ciavorella, D. Spadaro, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: annalisa.ciavorella@unito.it*

Biological control of postharvest diseases of fruit has improved recently as an alternative to fungicide treatment, to protect human safety and limit environmental impact. Among the most studied microorganisms used as biocontrol agents, we have focused on *Metschnikowia pulcherrima*, a yeast that has antagonistic effects on *Penicillium expansum*, *Botrytis cinerea* and *Monilia* spp., agents of blue, grey and brown mould on apple and kiwi fruit. To be commercially available, microbial agents should be formulated to optimize the efficacy and stability of the final product. This work aimed to evaluate the best agents to be added to *M. pulcherrima* to protect it against the effects of freeze-drying. Several kinds of sugar in solutions of different concentration were added to a centrifuged yeast cell suspension, to give a final concentration of 10^9 cfu/ml. We then determined which sugar solution gave the best protection against freeze-drying. Viability was evaluated periodically by calculating the cfu concentration on NYDA after rehydration of the freeze-dried cells with Ringer solution. We obtained the highest percentage viability using a 25% v/v maltose solution as protective agent. No significant loss of viability of the formulated freeze-dried cells was recorded after storage of 3 months at 4°C.

1.17 PRODUCTION OF *METSCHNIKOWIA PULCHERRIMA* BY FERMENTATION: EFFECT OF NITROGEN AND CARBON SOURCES ON BIOMASS. **A. Ciavorella, D. Spadaro, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy. Email: annalisa.ciavorella@unito.it*

Microbial antagonists have been developed as alternatives to chemicals, to reduce the amounts of fungicides and their residues on fruits. One strain of the yeast *Metschnikowia pulcherrima* showed effective biocontrol properties against the main postharvest pathogens on apple and kiwi fruits. Mass production of biocontrol agents is a focus of research and industrial development to obtain a product of high quality and low cost on a large scale. The aim of this work was to find the nitrogen and carbon sources producing, through fermentation technology, maximum biomass of *M. pulcherrima* with optimal antagonistic activity. Different ni-

trogen and carbon substrates were tested under different conditions of temperature and pH. A microbial concentration of 10^9 cfu/ml after 48 h of fermentation was obtained in a substrate containing one organic nitrogen source (yeast extract) and two organic carbon sources (sugars). The pH values during the fermentation gave the trend of the process: a neutral pH was typical of the initial inoculation step, whereas a basic pH suggested the end of the growth process, with an available growth range between pH 4 and 8. The best biocontrol on apples against *Penicillium expansum* and *Botrytis cinerea* was obtained when the yeast was grown in a sugar-enriched substrate.

1.18 BIOLOGICAL CONTROL OF STRAWBERRY GREY MOULD BY *CLONOSTACHYS ROSEA* UNDER FIELD CONDITIONS. L.V. Cota, L.A. Maffia, E.S.G. Mizubuti, P.E.F. Macedo and R.F. Antunes. *Departamento de Fitopatologia, Universidade Federal de Viçosa, 36570-000, Viçosa, MG, Brazil. Email: lamaffia@ufv.br*

Grey mould, caused by *Botrytis cinerea*, is an important strawberry disease in Brazil. As a component of our grey mould management program, we have been evaluating pathogen biocontrol with *Clonostachys rosea*, and selected four isolates as potential antagonists to *B. cinerea*. In 2006 and 2007, under field conditions, we compared the efficiency of a mixture of the four *C. rosea* isolates (applied once or twice a week) to a weekly spray of procymidone alternated with captan. Following the applications and up to harvest, we evaluated weekly: leaf colonization by *C. rosea* (LCCr), average number of *B. cinerea* conidiophores on leaves (CBc), incidence of grey mould on flowers (IFlower) and fruits (IFruit), incidence of latent infections on fruits (LI), and fruit production. With applications of *C. rosea* twice a week, we got higher LCCr (16.97%), smaller CBc (10.28; 78.22 in the control treatment, sprayed with water), smaller IFlower (10.02%; 50.55% in the control), and smaller IFruit (5.95%; 25.10% in the control). Production ranged between 3.49 and 3.75 kg with applications of *C. rosea* twice a week and between 1.74 and 1.91 kg in the control. LI was 20% in the control and less than 10% in the other treatments. From our results over the two years, we recommend at least two weekly applications of *C. rosea* to successfully manage grey mould in strawberry.

1.19* DIFFERENTIAL TRANSCRIPTION OF PATHOGENESIS RELATED PROTEINS (PRS) IN TOMATO PLANTS TREATED WITH THE BIOLOGICAL CONTROL AGENT *PENICILLIUM OXALICUM*. J. Cubero, M.P. Sabuquillo, R. García-Jiménez, A. De Cal and P. Melgarejo. *Dept. Plant Protection, INIA, Crtra de La Coruña km 7, 28040 Madrid, Spain. Email: melgar@inia.es*

Penicillium oxalicum is a filamentous fungus that induces resistance against tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. Treatment of tomato plants with conidia of *P. oxalicum* induces a reduction in disease severity but the biocontrol mechanism is still not clear. In this study, we have evaluated the possible role of two pathogenesis-related proteins (PRs). Transcription of PRs was studied by Retrotranscription Real Time PCR at different time periods on healthy tomato plants grown in controlled conditions and treated with three formulations of fungal conidia or untreated. After seven days post-treatment, significant differences were found between the groups of tomato plants. Especially significant was the difference shown when a formulation was used in combination with fungal conidia. If total RNA was extracted and amplified by RT-PCR after two, three and fifteen days pots-treatment, no significant difference in PR transcription was shown between groups of treated and non-treated plants. However, at periods of less than seven days after the treatment, some individual plants showed PR induction. These results show that systemic acquire resistance may play a role in the biocontrol process with *P. oxalicum* although some other mechanisms may also be involved. Moreover, we will show that some additives in the conidial formulates may render more uniform the plant response to the biocontrol agent.

1.20 SYNERGISTIC INTERACTION OF PHENAZINES AND BIOSURFACTANTS IN THE BIOCONTROL OF *PYTHIUM* WITH FLUORESCENT *PSEUDOMONAS* SPP. J. D'aes, K. De Maeyer, M. Perneel and M. Höfte. *Laboratory for Phytopathology, Faculty of bioscience engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium. Email: jolien.daes@ugent.be*

Fluorescent *Pseudomonas* spp. are well known for their potential as biocontrol agents of soil-borne pathogens. Many fluorescent pseudomonads, including strains of *P. aeruginosa*, *P. fluorescens* and *P. chlororaphis*, produce phenazine antibiotics, shown to be toxic to a broad range of soil-borne pathogens. In many cases, these strains also produce biosurfactants, such as rhamnolipids or cyclic lipopeptides, which can also be involved in their biocontrol capacity. Research in our lab showed that *P. aeruginosa* PNA1, which produces both phenazines and biosurfactants, is effective in controlling *Pythium myriotylum* root rot on the tropical tuber crop cocoyam (*Xanthosoma sagittifolium*) and damping-off caused by *Pythium splendens* on bean (*Phaseolus vulgaris*). From experiments with phenazine and biosurfactant mutants of PNA1, it appeared that production of both metabolites is required for the biocontrol effect to occur. Moreover, phenazines and biosurfactants were proven to act synergistically in both plant-pathogen systems. We are currently investigating the mechanism for this synergism and its importance for phenazine-producing fluorescent pseudomonads. It is known that biosurfactants can affect cell wall and cell membrane integrity of several fungi. Consequently, we hypothesize that phenazines require this action of biosurfactants to be able to penetrate the *Pythium* hyphae, where they can exert their toxic effect. So far, it seems that this synergistic interaction can be generalized, as only those strains that produce phenazines as well as biosurfactants, show an optimal biocontrol effect on *Pythium*.

1.21 ENVIRONMENTAL FACTORS AFFECTING THE BIOCONTROL OF OCHRATOXIGENIC *ASPERGILLUS CARBONARIUS* ON WINE GRAPES. D.V. de Felice, F. De Curtis, V. De Cicco and R. Castoria. *Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università del Molise, Via De Sanctis, Campobasso, Italy. Email: dariodefelice@unimol.it*

Wine contamination with ochratoxin A (OTA) is due to the attack of ochratoxigenic *Aspergillus carbonarius* on grapes, which can begin in the early ripening stages. We investigated the influence of temperature and relative humidity (R.H.) on the activity of three biocontrol agents (BCAs): one yeast, *Metschnikowia pulcherrima* LS16, and two yeast-like fungi, *Aureobasidium pullulans* LS30 and AU34-2) against *A. carbonarius* and OTA accumulation on intact and artificially wounded grapes. Berries were pretreated with the BCAs, challenged with strain A1102 of *A. carbonarius* and incubated for 6 days at two R.H. levels (60% and 100%) and three different temperatures (20, 25 and 30°C). Unwounded berries, both treated with the BCAs and untreated, showed no

symptoms of infection by *A. carbonarius* in all conditions tested, suggesting that wounds on grape skin are crucial for colonization by the pathogen. In wounded berries incubated at 60% R.H. and 20°C, LS16 showed the highest biocontrol activity, lowering infections up to 69%. At 100% R.H., LS16, LS30 and AU34-2 showed effective protection of grapes only at 20 °C, reducing *A. carbonarius* infections by 86%, 68% and 82%, respectively. HPLC analyses showed that at 100% R.H. and at all temperatures, the BCAs lowered contamination with OTA, up to 93% at 20°C, as compared to untreated controls. These results highlight the importance of environmental factors in the effectiveness of BCAs in the control of *A. carbonarius* and of wine contamination with ochratoxin A.

1.22 INFLUENCE OF ECOLOGICAL CONDITIONS ON THE EPIPHYTIC MICROFLORA COMPOSITION OF WHEAT SEEDS. E. Demchenko and V. Roginskaya. *Department of Ecology and Plant Biotechnology, Krasnoyarsk State Agrarian University, Prospect Mira 90, Krasnoyarsk, Russia. Email: roginskaya48@yahoo.com*

Epiphytes are a natural element protecting plants from pathogens. The composition of seed microflora varied among the different cultivars 'Alenkaya-23', 'Skala', 'Irtyshanka-10' and 'Lustescens-25' grown in the same area under optimal conditions. During an investigation into resistance of wheat to common root rot, the positive influence of epiphytes on the shoot mass and decrease of disease intensity was confirmed. Differences in epiphyte composition have been found on the seeds of the same cultivar (Omskaya-32) germinated in eco-geographical areas of Central Siberia with different climatic and soil conditions. Epiphytes of healthy seeds matured in forest-steppe areas were represented only by bacteria, dominated by the genera *Bacillus* (57.1%) and *Pseudomonas* (25.4%). On seeds matured in a south-steppe area, *Micrococcus* (38,2%), *Pseudomonas* (18,4%) and *Mycobacterium* (9,0%), plus fungi and *Actinomycetes* were also found. The epiphyte composition of black germ seeds was more diverse with greatly increased proportion of seed mould-causing fungi. On diseased seed from the south-steppe, *Trichoderma* and *Streptomyces* were discovered, and these are antagonists to the mould pathogens. The presence of these antagonists can be explained by the soil acidity and lower humidity in the area, favourable for those micro-organisms. Higher humidity during maturation and harvesting in the forest-steppe resulted in decrease of grain quality, allowing pathogenic fungi to suppress the development of epiphytic bacteria. This resulted in 14% lower germination compared with south-steppe seeds. Therefore, ecological conditions are key in the composition of epiphytic microflora and its protective effects.

1.23 EFFECT OF INOCULATION METHOD, INOCULUM DENSITY, AND FUSACLEAN G (FO47) ON DEVELOPMENT OF CYCLAMEN WILT. W. Dercks, A. Keuck, M.L. Kreller and F. Hennig. *Fachhochschule Erfurt - University of Applied Sciences, Fachbereich Landschaftsarchitektur, Gartenbau und Forst, Leipziger Straße 77, D-99085 Erfurt, Germany. Email: dercks@fh-erfurt.de*

Cyclamen wilt caused by *Fusarium oxysporum* f. sp. *cyclaminis* (FOC) is difficult to control and can lead to severe crop loss. Fusaclean G is a product containing mycelium and spores of the antagonistic non-pathogenic *Fusarium* strain FO47 and was developed to prevent or suppress disease when added to the substrate. This greenhouse study investigated whether this effect might be dependent on FOC inoculation method and inoculum density. Two methods (substrate inoculation, drench with spore suspensions) and four inoculum densities (0, 10,000, 20,000 and 30,000 spores per pot) of FOC were tested. Fusaclean G was used as supplied by the producer and applied according to the company's recommendations. Substrate inoculation by FOC preceding potting caused faster, less variable, and stronger disease development than spore suspensions poured into the pot following potting. Thus, the former method is more suitable for trials than the latter. Initially, disease incidence increased with FOC inoculum density. However, at the end of the trial, disease levels were the same for all densities. Fusaclean G delayed onset and development of disease compared with untreated cyclamens but was not able to prevent infection or reduce final disease levels. It remains to be determined whether Fusaclean G might be more effective at lower FOC inoculum densities. However, the efficacy of any control measure is difficult to evaluate with low inoculum because wilt develops more slowly. In such cases, cyclamens are often latently infected by FOC and sold at bloom. The disease then strikes later on the buyer's windowsill.

1.24 MECHANISM OF ACTION OF *METSCHNIKOWIA PULCHERRIMA* STRAIN MACH1 AGAINST *BOTRYTIS CINEREA* ON APPLE. D. Saravanakumar, A. Ciavorella, D. Spadaro, A. Garibaldi and M.L. Gullino. *AGROINNOVA, University of Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy. Email: agrisara@rediffmail.com*

We studied the mechanism of action of the biocontrol yeast *Metschnikowia pulcherrima* strain MACH1 against grey mould (*Botrtyis cinerea*) on apple. We tested the ability of MACH1 to compete with the postharvest pathogen for acquisition of nutrients, especially for iron compounds. The yeast produced a pigment called pulcherrimin in presence of iron and showed antagonistic activity against *B. cinerea in vitro* and *in vivo*. Quantitative assay of extracellular enzymes from MACH1 culture filtrates showed that it was able to produce chitinase and beta-1,3-glucanase, and the study indicated the probable influence of these lytic enzymes in the biological control of grey mould. In addition, the state of host tissues in response to MACH1 treatment and challenge inoculation with *B. cinerea* was studied. The results revealed the greater accumulation of defense enzymes, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in apples treated with MACH1 and challenged with *B. cinerea* compared to untreated controls. The prolonged activity of defense enzymes in apples treated with MACH1 was able to limit the further growth of the pathogenic fungus in host tissues. In conclusion, the study revealed that competition for nutrients, production of extracellular enzymes and enhancement of host resistance by MACH1 could act together in the biological control of postharvest pathogens in apples.

1.25 BIOLOGICAL SOIL TREATMENT WITH *TRICHODERMA HARZIANUM* TO CONTROL ROOT ROT DISEASE OF GRAPEVINE (*VITIS VINIFERA* L.) IN NEWLY RECLAIMED LANDS IN NOBARIA PROVINCE. R. El-Mohamedy, E. Zedan and A. Adalla. *Plant Pathology Department, National Research Center, Dokki, Cairo, Egypt. Email: riadelmohamedy@yahoo.com*

Augmentation of soil with biological treatments i.e., *Trichoderma harzianum* cultured on sugar cane bagasse, *T. harzianum* (spore suspension 5×10^6 cfu/ml) and Plant Guard (biocide), successfully controlled *Fusarium solani, F. oxysporum* and *Macrophomina phaseolina*, the main causes of root rot disease on grapevines in Nobaria

province**.** Complete reduction of linear growth for these pathogens was recorded at 4 ml/l of plant Guard. Meanwhile, *T. harzianum* cause a reduction of 80.0, 84.4 and 88.9% of the same pathogens respectively. Treatment of artificially infested soil by bio-enhancing bagasse at a rate of 10% (w/w) of soil, 4 ml/l Plant Guard and 100 ml/l *T. harzianum* gave highly effective control of root rot on grapevine seedlings. In field experiments, two soil applications with 10 % bio-enhancing bagasse and 4 ml/l Plant Guard significantly reduced numbers of infected vines as well as the percentages of disease severity. Moreover, these treatments increased population density of *Trichoderma* spp. in rhizosphere soil of treated vines, whereas, it decreased in rhizosphere soil of untreated vines (control). A large increase in yield/vine was recorded on vines treated with 10% bio-enhancing bagasse and 4 ml/l Plant Guard. We suggest that manipulating soil by biocontrol agents and agricultural waste formulations can be safely used commercially as a substitute for fungicides for controlling soilborne plant pathogens.

1.26 CULTURAL, PHYSIOLOGICAL AND BIOCHEMICAL FEATURES OF *TRICHODERMA HARAZIANUM* ISOLATES IN RELATION TO THEIR BIOCONTROL ABILITIES. **M.A. El-Naghy, E.M. Fadl-Allah, G.M. Shaban and A.H.A. El-Naggar.** *Department of Botany, Faculty of Science, Minia University, Minia, Egypt. Email: ahmedhetta@yahoo.com*

Single-spore cultures of 40 isolates of *Trichoderma harazianum* Rifai were characterized on the basis of their morphological, physiological and biochemical characters, including growth of the isolates on seven different media. Cultural characteristics such as odour, colour and diffusible pigments were determined on each of the media. All isolates, grown on 2% malt extract agar, were examined by microscopy. Interactions between hyphae of *Trichoderma harazianum* isolates and several soil-borne fungal plant pathogens were determined in dual cultures. Production of trichodermine and trichodermol was also investigated. Secretion of the lytic enzymes CMC-ase, chitinase and β-1,3 glucanase in culture media containing the respective substrates was also determined. Attempt was made to correlate the features studied with the biocontrol efficiency of each isolate, in order to identify the most efficient ones.

1.27 BIOLOGICAL CONTROL OF *FUSARIUM* WILT OF BANANA USING NON-PATHOGENIC *F. OXYSPORUM* ENDOPHYTES. **A. Faber, C. Steinberg and A. Viljoen.** *Grain Crop Institute, Private bag X 1251, Potchefstroom 2520, South Africa. Email: aneenf@arc.agric.za*

Fusarium oxysporum f.sp. *cubense* (Foc) is responsible for *Fusarium* wilt, a disease that caused serious losses to export bananas in Central America in the mid-1900's. Non-pathogenic isolates of *F. oxysporum*, found in disease-suppressive soils, were reported to suppress *Fusarium* wilt in greenhouse pathogenicity trials. To study their interaction on banana roots, a non-pathogenic *F. oxysporum* endophtye from banana roots in Kiepersol, South Africa that reduced *Fusarium* wilt incidence by 67% was modified with a red fluorescent protein gene (DsRed-Express), while a pathogenic isolate of Foc was modified with a green counterpart (GFP). The modified and unmodified isolates did not differ in growth and morphological characteristics, or in their virulence to banana plantlets. By using a split-root inoculation system, we showed that the non-pathogenic *F. oxysporum* isolate induced significantly higher levels of cell wall-bound phenolics in non-inoculated banana roots than the water control. Competition is unlikely to be the primary mode of action when the pathogen and non-pathogen were inoculated on the same roots at equal concentrations. Confocal laser scanning microscope observations were unable to prove that the reduced infection of banana roots was the result of induced resistance, as neither the pathogen nor the non-pathogen was able to infect banana roots in the absence of wounds. Whether the non-pathogenic *F. oxysporum* isolates would protect bananas by means of induced resistance after wounding and following abiotic stress needs to be further investigated.

1.28 EFFICACY OF DIFFERENT COMMERCIAL BIOCONTROL AGENTS ON *FUSARIUM* WILT OF LETTUCE. **G. Gilardi, G. Martano, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy. Email: giovanna.gilardi@unito.it*

Fusarium wilt of lettuce, caused by *F. oxysporum* f.sp. *lactucae*, has emerged as a major production problem in several lettuce-producing areas in Italy. Disease management is complicated by the limited availability of registered fungicides and by the intensive cropping systems adopted by local growers. The causal agent is seed transmitted, making soil disinfestation only partially effective. The only partial efficacy of chemical control measures and the reduced availability of resistant cultivars of commercial interest have encouraged the evaluation of biological control agents against this disease. Four biocontrol strains of *Fusarium oxysporum* (Microsan, Fo 23, Fo 251/2 and Fo MSA 35) three *Trichoderma*-based formulations (Rootshield, Remedier and *Trichoderma* TV1) and one product based on *Streptomyces griseoviridis* (Mycostop) were applied, by mixing into the soil, six days before transplant, at the same time as artificial inoculation of the pathogen. Susceptible cultivars to *Fusarium* wilt were used in different trials carried out under glass at 28-32°C. The commercial formulations were applied at the dosages suggested by their distributors, and the experimental Fo MSA 35, formulated as talc, at 2-4 g/l of soil. In the presence of high disease pressure, the best levels of control as well as increased growth response, were shown by Rootshield, followed by Fo MSA 35. Fo *IF 23* sometimes caused reduction in biomass. Mycostop partially reduced *Fusarium* wilt at dosages of 0.05-0.1 g/l. The results show that biological control can play a role in the management of *Fusarium* wilt of lettuce in a sustainable agriculture system.

1.29 EFFECT OF BIOLOGICAL CONTROL AGENTS ON SOME COTTON SEEDLING DISEASES. **M.E.M. Hasan, Mona, M.S. Mikhail, K.K. Sabet, H.M. Kenawy and K.K. Kasem.** *Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt. Email: maggie2000_eg@yahoo.com*

Testing different strains of plant growth-promoting rhizobacteria (PGPR) *Bacillus subtilis*, *B. polymyxa*, *Azospirillum* sp. and *Pseudomonas putida* showed significant reduction in linear growth of pathogenic fungi (*Rhizoctonia solani*, *Fusarium oxysporum* and *F. oxysporum* f.sp. *vasinfectum*). *P. putida* was the most effective strain, giving the highest reduction. Treatment of cotton seeds with PGPR strains before sowing reduced pre- and post-emergence damping-off and increased plant survival compared with untreated controls in infested soil. The highest percentage of surviving plants was found in treatments with *P. putida* and mixed strains 77.5 and 72.5% respectively. Also application of PGPR strains showed an increase in seedling growth (fresh and dry weight and plant height) compared with untreated controls in

infested soil, and caused changes in macro- and microelement content in both plants and soil.

1.30 MANAGEMENT OF CHARCOAL STUMP ROT DISEASE OF TEA WITH BIOCONTROL AGENTS. **D.K. Hazarika and A.K. Phukan.** *Krishi Vigyan Kendra, Napaam, Tezpur, Assam, India. Email: dkhazariak@yahoo.co.in*

Tea is a beverage of world importance, and India is the leading producer. Within India, Assam is the natural habitat of tea and produces more than 60% of Indian tea. Charcoal stump rot caused by *Ustulina zonata* (Lev.) Sace is one of the most common primary root diseases of tea throughout north-east India. The pathogen is soil-borne and can attack and kill the tea plant at any stage of growth. Agrochemicals are extensively used in the plantations for managing diseases, insect pests and weeds. These agrochemicals can be toxic to non-target organisms and can also withstand the vigorous process of manufacture to end up in the consumer's tea cup. This health hazard needs attention from agriculturists and environmentalists, and search for alternate methods of tea husbandry and maintaining sustainability of soils becomes imperative. Exploitation of soil micro-organisms offers an attractive alternative to the use of chemicals; microbial agents are economical, eco-friendly, safe to handle and perceived to be less damaging to the environment. Keeping this in view, an experiment was conducted at Assam Agricultural University to manage charcoal stump rot with biocontrol agents. All the antagonistic microorganisms tested, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma koninjii*, *Gliocladium virens* and *Pseudomonas fluorescens*, assayed by dual-culture and culture-filtrate methods, were found effective in inhibiting the mycelial growth of *U. zonata*. The inhibitory activity of autoclaved culture filtrate was much less than that of filter-sterilized culture filtrates. Tea seedlings inoculated with these antagonists by root-dip and soil-drench methods in pots of contaminated soil significantly reduced plant mortality and increased plant growth. Maximum reduction in plant mortality with highest plant growth and shoot and root dry weight was recorded in seedlings inoculated with *T. harzianum* and *P. fluorescens*.

1.31 INTEGRATED MANAGEMENT OF BLACK PEPPER FOOTROT. **G.M. Hegde, H. Hegde and T. Ganapathi.** *Krishi Vigyana Kendra, Uttar kannada, Sirsi, UAS, Dharwad, Karnataka, India. Email: kvkuks@gmail.com*

Foot rot of black pepper (*Phytophothora capsici*), is a serious disease which occurs during the south west monsoon (June -September) in south India. The disease threatens the crop, causing yield losses of up to 100% and many farmers have had their fields wiped out. Therefore to manage the disease various integrated treatments were tried during 2006-07. The treatment: spray and drench with metalaxyl MZ at 1.25 g/l (June) – spray with *Pseudomonas fluorescens* at 10ml/l (July, August) – spray with potassium phosponate at 3 ml/l (August) and soil application of neem cake (2 kg/vine) + *Trichoderma harzianum* (50g/vine) has resulted in lowered disease incidence (5.12%) and increased yield (10.13 q/ac) vis-à-vis 26.25% and 7.45 q/ac in normal farmers' practice. Hence, *P. fluorescens*, which is inexpensive and environmentally safe, can be used as a component in the management of foot rot of black pepper.

1.32 INTEGRATED MANAGEMENT OF RHIZOME ROT OF GINGER. **G.M. Hegde, H. Hegde and J. Vishwanath.** *Krishi Vigyana Kendra, Uttara kannada, Sirsi, UAS, Dharwad, Karnataka, India. Email: kvkuks@gmail.com*

Rhizome rot (soft rot) of ginger caused by *Pythium aphanidermatum* has become a major menace and has proved to be the most predominant among the rhizome rot pathogens, causing yield loss up 60%. Among various integrated management practices tried at our institute, the best treatment was: soil solarization + rhizome treatment with metalaxyl MZ at 0.3 % + soil application of *Trichoderma harzianum* at 10 kg along with 1 t/h FYM + soil application of Eupatorium. This gave least disease incidence (4.9%) and maximum yield (7848.20 kg/ha). The second best treatment was soil solarization + rhizome treatment with Metalaxyl MZ at 0.3 % + soil application of *T. harzianum* at 10 kg along with 1 t/h FYM + copper oxychloride at 0.3 % drenching (45 DAP) immediately after the onset of disease. This gave 6.2% disease incidence and yield of 7502 kg/ha compared to 21.07 % and 3102.10 kg/ha in control plots.

1.33* BIOLOGICAL CONTROL OF PIERCE'S DISEASE IN THE VINEYARD WITH A BENIGN STRAIN OF *XYLELLA FASTIDIOSA*. **D.L. Hopkins and C.M. Thompson.** *University of Florida, MREC, 2725 Binion Road, Apopka, FL 32703, USA. Email: dhop@ufl.edu*

There is currently no control for Pierce's disease (PD), caused by *Xylella fastidiosa* subsp. *piercei*, in susceptible grapevines. In previous greenhouse tests, benign strains of *X. fastidiosa* subsp. *piercei* provided cross protection against virulent PD strains in susceptible *Vitis vinifera* cultivars. In vineyard tests, one of these benign strains, EB92-1, inoculated into 'Cabernet Sauvignon' provided control of PD for 10 seasons; whereas, untreated vines died within 4 years. This strain was injected only once into the 2nd or 3rd internodes of young grape plants in the spring of the first season. Vineyard evaluations of this biological control strain are underway in the research center vineyard and in commercial vineyards in different locations in the southern U.S. and with various grape genotypes. Treated 'Chardonnay' vines have remained free of PD in 2 commercial vineyards in central Florida for 3 years. In other commercial vineyards, EB92-1 appears to be controlling PD in the cultivars, 'Cabernet Sauvignon', 'Cabernet Franc', 'Chenin Blanc', 'Mourvedre', 'Seyval Blanc', and 'Vidal Blanc'. In the research center, treated American cultivars 'Chambourcin' and 'Cynthiana' also are free of PD. The control seems to result from an induced resistance, since the agent does not have to be present in the part of the vine challenged by the pathogen to attain control. Biological control with this benign strain of *X. fastidiosa* has the potential to provide control of Pierce's disease in commercial vineyards in Florida and other areas where PD occurs.

1.34 OPTIMISING BIOLOGICAL CONTROL OF *SCLEROTINIA* IN NEW ZEALAND KIWIFRUIT. **S.M. Hoyte, P.A.G. Elmer and J. Taylor.** *HortResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand. Email: shoyte@hortresearch.co.nz*

BOTRY-Zen® is a biological control agent (BCA) whose active ingredient (*Ulocladium oudemanseii*, isolate HRU3) colonises necrotic floral and leaf tissues, and has been shown to suppress both *Sclerotinia sclerotiorum* and *Botrytis cinerea* in kiwifruit. The efficacy of applying BOTRY-Zen® twice during flowering against

Sclerotinia flower blight and fruit scarring has ranged from 61-78% and 34-75% respectively, over two-year trials and is often significantly less than that achieved using the fungicide, iprodione. Attempts have been made to increase the cost-effectiveness of BOTRY-Zen® by comparing one and two applications, different application rates (6-12 kg/ha), different surfactants (Du-Wett™, Actiwett™ and Flexceed™) and through broadening the mode of action by combining BOTRY-Zen® with different BCAs at reduced rates. Applications of BOTRY-Zen® at 8 and 10 kg/ha (with Du-Wett™) and at 6 kg/ha (with Actiwett™), applied once at 80-90% flowering, gave equal disease control to two applications of BOTRY-Zen® at 6 kg/ha (with Du Wett™) each applied at 30% and 90% flowering. During 2005-07, other BCAs (*Trichoderma atroviride*, *Epicoccum nigrum* and *Coniothryium minitans*), that had been selected based on suppression of *S. sclerotiorum* ascospore infection of kiwifruit petals in laboratory bioassays, were evaluated in field trials to determine whether suppression of *Sclerotinia* could be enhanced by applying tank mixtures of BCAs. Several combinations of BOTRY-Zen® with other BCAs were shown to provide equal, but not superior disease control of *Sclerotinia*, compared with BOTRY-Zen® alone. The best practice use of BOTRY-Zen® against *Sclerotinia* is discussed.

1.35 BIOLOGICAL PROTECTION AGAINST FUNGAL DISEASES OF CEREALS. **J. Hýsek and M. Vach.** *Crop Research Institute, v.v.i., Drnovská 507, 16106 Praha 6 - Ruzyn, Czech Republic. Email: hysek@vurv.cz*

We used biopreparations based on antagonistic microorganisms for the treatment of cereals (winter wheat and spring barley). The preparations were: Supresivit (*Trichoderma harzianum*), Trianum P (*T. harzianum*), Polyversum (*Pythium oligandrum*) and Ibefungin (*Bacillus subtilis*). The applications were: a) seed-treatment, b) mixture with mineral fertilizer and c) spray. On treated plots we diagnosed less infestation with phytopathogenic fungi such as *Fusarium* spp., *Septoria* spp., *Drechslera* spp. and *Rhynchosporium* sp. Seed-treatment was the most effective, and least effective was spraying the plant surface.

1.36 BIOCONTROL OF BLACK SCURF IN POTATO BY TREATMENT OF POTATO SEED PIECES WITH *PYTHIUM OLIGANDRUM*. **S. Ikeda, M. Shimizu, A. Shimizu, H. Tahakashi and S. Takenaka.** *Hokkaido, Prefectural Tokachi Agricultural Experiment Station, Memuro, Hokkaido 082-0071, Japan. Email: ikedasc@agri.pref.hokkaido.jp*

The biocontrol activity of *P. oligandrum* (PO) against black scurf of potato caused by *Rhizoctonia solani* (AG-3) was evaluated when potato seed pieces were treated with oospore suspensions of PO. Seed pieces with black scurf sclerotia were soaked for a few seconds in the oospore suspension at 10^4 oospores/ml or 10^5 oospores/ml, and they were air dried. When PO populations attached to the surface of seed pieces were determined, the densities of attached oospores in treatments of 10^4 /ml and 10^5/ml were about 500/cm^2 and about 7400/cm^2 just after soaking, and they decreased to about 100/cm^2 and about 1000/cm^2 after air drying, respectively. These PO-treated seed pieces had significantly reduced disease severity on sprouts, compared with the control. Confocal laser scanning microscopic observation with immuno-enzymatic staining showed that PO hyphae colonized the sclerotia and established close contact with *R. solani* by means of coiling around the hyphae present on the surface of the seed piece. We also used a potato tuber disk assay to determine the ability of PO to induce resistance. The PO-treated tuber disks showed enhanced resistance to *R. solani* and reduction in disease severity. Northern hybridization assay indicated the enhanced expression of defense-related genes in potato such as 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase, lipoxygenase and basic PR-6 genes. These results suggest that biocontrol of black scurf by PO involves mycoparasitism and induced resistance.

1.37 DISRUPTION OF ULTRASTRUCTURE AND CYTOSKELETON IS INVOLVED IN BIOCONTROL OF THE DAMPING-OFF PATHOGEN *APHANOMYCES COCHLIOIDES* BY *LYSOBACTER* SP. STRAIN SB-K88. **M.T. Islam.** *Department of Crop Sciences, University of Göttingen, Division of Plant Pathology and Plant Protection, Grisebachstrasse 6, 37077 Göttingen, Germany. Email: tofazzalislam@yahoo.com*

The rhizoplane bacterium *Lysobacter* sp. SB-K88 suppresses damping-off disease in sugar beet and spinach caused by *Aphanomyces cochlioides* through plant colonization and antibiosis. Interaction of the pathogen and SB-K88 on potato dextrose agar revealed remarkable curly growth, excessive branching, and inhibition of the pathogen's mycelial growth. We elucidated the mode of antagonism of SB-K88 by detecting changes in ultrastructure and organization of the cytoskeletal filamentous actin (F-actin) network in the affected *A. cochlioides* hyphae and zoospores. Transmission electron microscopy revealed significant ultrastructural alterations including invagination of membranes, disintegration or necrosis of cell walls, accumulation of excessive lipid bodies, electron-dense enlarged vacuoles and degradation of cytoplasm in the excessively curled and branched hyphae induced by SB-K88. Confocal laser scanning microscopy confirmed the role of SB-K88 metabolites in severe disruption of the organization of F-actin networks in both hyphae and zoospores of *A. cochlioides*. An inhibitor of actin polymerization, latrunculin B, also similarly disrupted F-actin organization in the hyphae and zoospores. These results suggest that the inhibitory effect of *Lysobacter* sp. SB-K88 on *A. cochlioides* is likely due to a combination of F-actin disruption and ultrastructural alterations in the zoospore and hyphae.

1.38 *BOTRYTIS CINEREA* DISEASE MANAGEMENT THROUGH THE USE OF ORGANIC MULCHES IN VINEYARDS. **M.A. Jacometti, S.D. Wratten and M. Walter.** *The Horticulture and Food Research Institute of New Zealand (HortResearch), P.O. Box 51, Lincoln, New Zealand. Email: mwalter@hortresearch.co.nz*

Four organic mulches [anaerobic *marc* (pomace), aerobic *marc*, grassand paper] were tested to enhance the activity of soil flora and fauna below vines, to disrupt *Botrytis cinerea* disease lifecycle and improve the vines' resistance to the pathogen in a commercial New Zealand vineyard (2003-2006). Biological diversity (bait lamina probes) and microbial activity (Biolog Ecoplates), moisture and temperature were monitored at the soil-mulch interface. *Botrytis* sporulation and debris degradation rate were assessed at flowering and at leaf plucking. At harvest, yield and *B. cinerea* severity in bunches were determined. Canopy density, leaf nutrients, berry skin strength, and berry nutrients (sugars and phenolics) were measured. Resistance of berries to *B. cinerea* was assessed for ripe, surface sterilised and artificial inoculated fruit. All mulches led to a reduction in *B. cinerea* sporulation. This reduction was significantly correlated with elevated

rates of vine debris decomposition and increased soil biological activity. All treatments gave a reduction in *B. cinerea* sporulation, an increase in vine debris degradation and the two *marc* and paper treatments gave an increase in activity of soil organisms ($P<0.05$). Nutrient levels and carbon/nitrogen ratios were also affected in these treatments. These changes to soil and vine characteristics increased grape skin strength by up to 10% and sugar concentrations by 1.2-1.4 °Brix. The severity of *B. cinerea* bunch infections in mulch-treated vines was reduced to below the economical threshold (5% of bunches infected).

1.39 FATE AND ACTIVITY OF FUNGAL BIOCONTROL AGENTS (BCAS) ON STRAWBERRY. B. Jensen, B. Andersen, U. Thrane, D.F. Jensen, K.F. Nielsen and J. Larsen. *Department of Plant Biology, Thorvaldsensvej 40, 1871, Frederiksberg C. University of Copenhagen, Denmark. Email: bje@life.ku.dk*

Fungal BCAs against grey mould are being used at flowering in strawberries. The objective of an ongoing project is to study the fate and activity of selected BCAs from application until harvest and consumption. The activity of BCAs based on *Trichoderma harzianum* and *Clonostachys rosea* against the grey mould pathogen, *Botrytis cinerea*, was examined on flowers using strains with reporter genes encoding either GFP or DsRed fluorescence. Approximately 70% of both *C. rosea* and *B. cinerea* conidia germinated on flower tissue while only 20% of *T. harzianum* conidia germinated within 24 hours at 20°C. Dual inoculation of the fungi showed that *C. rosea* significantly reduced both *B. cinerea* and *T. harzianum* germination on flowers. In addition, *C. rosea* significantly reduced grey mould symptoms. The fate of BCAs after application to the flowering plants, approximately 10^4 CFU/flower, was studied in field trials. Quantification of the BCAs on berries developed from inoculated flowers showed that less than 160 CFU/berry of either *T. harzianum* or *C. rosea* were recovered one month later. High density (worst case) inoculation of berries with *T. harzianum* and *C. rosea* showed no increase in CFU density on the berries after four day incubation at 20°C. Sub-samples of these berries will be analysed for presence of microbial metabolites. Data on fate and activity of BCAs on strawberries both pre-harvest and post-harvest will aid risk assessment of BCAs by pointing out the kind of assessment studies needed to ensure safe use of BCAs.

1.40 PASTURE SEED ADDITIVE – A PROTOTYPE *TRICHODERMA*-BASED BIO-INOCULANT FOR ENHANCED SEEDLING EMERGENCE AND PASTURE GROWTH IN NEW ZEALAND. D.R.W. Kandula, A. Stewart, J. McDermid and J.S. Hunt. *National Centre for Advanced Bio-Protection Technologies, P.O. Box 84, Lincoln University, Lincoln, New Zealand. Email: kandulaw@lincoln.ac.nz*

Soil-borne fungal diseases are a major constraint affecting seedling emergence, pasture establishment and subsequent productivity/plant persistence in New Zealand pastures. Extensive laboratory and glasshouse screening of more than one hundred isolates of *Trichoderma* spp. against several pathogens (*Rhizoctonia solani*, *Sclerotinia trifoliorum*, *Pythium ultimum*, *Fusarium culmorum*) identified a cohort of isolates which suppressed damping-off diseases and promoted plant growth of different pasture species. Ten *Trichoderma* isolates (*T. atroviride* LU132, LU140, LU584, LU633, LU634; *T. virens* LU540, LU547; *T. koningii* LU713; *T. hamatum* LU740; *T. viride* LU644) were tested further as granule and seed-coat formulations in small-scale field trials. Evaluation of a prototype pasture seed additive (PSA) product formulated as a prill containing a combination of *T. atroviride* isolates in several large scale research and on-farm trials significantly improved seedling emergence by 20-44% and increased pasture dry matter (in repeated assessments) by 10-40% relative to untreated controls. *In vitro* bioassays revealed the compatibility of PSA with several plant protection chemicals and fertilisers and on-going experiments are aimed at integrating PSA with other farming practices.

1.41 INDUCTION OF NEW FUNGICIDE-TOLERANT ISOLATES AND INTRASPECIFIC FUSION OF *TRICHODERMA ATROVIRIDE* USING PROTOPLAST TECHNOLOGY. J. Kandula, M. Carpenter and A. Stewart. *National Centre for Advanced Bio-Protection Technologies, P.O. Box 84, Lincoln University, Canterbury, New Zealand. Email: Kandulaj@lincoln.ac.nz*

Trichoderma atroviride LU132 is a biocontrol agent against *Botrytis* mould of grapes and strawberries and has been commercialised in New Zealand as the product Sentinel®. Uptake of the product would be greater if it could be used as part of an integrated disease management strategy with the fungicide Switch which has Chorus and Maxim as its active ingredients. Increased tolerance to Switch was induced in LU132 by exposing protoplasts to x4 field concentrations of the fungicide. One stable fungicide tolerant mutant 4XF2 was selected and compared against the wild type for biocontrol ability in a detached strawberry necrotic leaf assay. 4XF2 was equally effective in controlling *Botrytis cinerea* and had similar growth characteristics to the wild type LU132. Quantitative analysis of antifungal metabolite 6 pentyl alpha pyrone (6PAP) production revealed retention of very high level of 6PAP in the mutant as well.

1.42 INFECTION OF DIFFERENT FUNGAL GENERA WITH TWO *ROSELLINIA NECATRIX* MYCOVIRUSES BY INOCULATION OF PROTOPLASTS. S. Kanematsu, A. Sasaki, Y. Oikawa, M. Onoue, T. Ito, K. Suzaki, K. Yoshida and N. Matsumoto. *Apple Research Station, National Institute of Fruit Tree Science, National Agriculture and Food Research Organization, Morioka 020-0123, Japan. Email: satokok@affrc.go.jp*

The host range of mycoviruses has not been well documented because their transmission is generally limited to the intercellular route through hyphal fusion. To elucidate potential hosts of RnPV1-W8 (*Partitiviridae*) and MYRV3-RnW370 (*Mycoreoviridae*) from *Rosellinia necatrix* (Xylariales), we inoculated fungal protoplasts with the viruses. Virus particles were purified from isolates W8 and W370 of *R. necatrix*, and inoculated to protoplasts of *Diaporthe* sp. (Diaporthales). Infection of RnPV1 and MYRV3 was confirmed by the presence of dsRNA genomes from regenerated mycelia of *Diaporthe* sp. Colony morphology of infected isolates was slightly changed from their virus-free, parental isolates. Both viruses were transmitted vertically through hyphal fusion in *Diaporthe* sp., though horizontal transmission was not observed. We also confirmed infection of *Cryphonectria parasitica, Valsa ceratosperma* (Diaporthales), and *Colletotrichum gloeosporioides* (Sordariomycetes, inc. sed.) with RnPV1. RnPV1 was vertically transmitted in one of 30 single conidial isolates of *C. gloeosporioides.* MYRV3 infected *C. parasitica* and *V. ceratosperma*, but these fungi were often cured of MYRV3 infection during subculture as was the case with *Diaporthe* sp. Isolates of *Diaporthe* sp, *C. parasitica* and *V. ceratosperma* isolates harboring MYRV3 showed reduced virulence on Japanese pear twigs, apple fruits and apple twigs, respectively. In conclusion, artificial infection

with RnPV1-W8 and MYRV3 could extend their host range within the Sordariomycetes, though virus-host fungus interactions seem to be unstable in new combinations.

1.43 ENHANCING EFFICACY OF BIO-FORMULATIONS BY MIXING ANTAGONISTS FOR THE MANAGEMENT OF SHEATH BLIGHT AND LEAF FOLDER OF RICE. D. Kaur, P.V. Rao and R.S. Singh. *Department of Plant Pathology, Punjab Agricultural University, Ludhiana 141004, India. Email: ramassingh@rediffmail.com / ramassingh7@yahoo.com*

Trichoderma harzianum (Th38) and *Pseudomonas fluorescens* (C7R12) were highly antagonistic to *Rhizoctonia solani* (rice sheath blight), and *Beauveria bassiana* (Bb20) was antagonistic to *Cnaphalocrosis medinalis* (rice leaf folder). All three antagonists were compatible with each other. Talc-based formulations were developed singly and in combinations. Treatments were given as seedling root dip (SRD), foliar spray (FS) and combination of SRD+FS. Incidence and intensity of sheath blight and leaf folder were estimated. In the greenhouse, 53.7, and 38 % blight incidence and intensity were observed with SRD of a mixed formulation of the three bioagents as compared 80.7 and 72 % in controls. FS with mixed formulation gave 50.3 and 36.5 % disease incidence and intensity as compared 79 and 72.5 % in controls. FS also gave 6.5 eggs, 1 larva and 2.2 % leaf folder damage/pot while 20.5 eggs, 12.7 larvae and 20.5 % leaf damage were found in the control. SRD+FS with mixed formulation showed minimum 47.7 and 35 % disease incidence and intensity as compared to 80 and 73.5 % in controls. In field conditions, SRD, FS and SRD+FS with mixed formulation showed 41, 40.2 and 38 % disease intensity as compared 76, 76 and 75.5 % in controls. Individually, Bb20 was highly effective against leaf folder. Hence, the use of mixed formulations of the three bioagents should protect rice crops against both biological constraints.

1.44 STUDIES ON DIAGNOSIS AND BIOLOGICAL CONTROL OF GRAPEVINE CROWN GALL. A. Kawaguchi, K. Inoue, H. Sawada and H. Nasu. *Agricultural Experiment Station, Okayama Prefectural General Agriculture Center, Akaiwa-city, Okayama 709-0801, Japan. Email: akira_kawaguchi@pref.okayama.lg.jp*

Crown gall of grapevine, caused by tumorigenic *Agrobacterium vitis,* is the most important bacterial disease of grapevine throughout the world. To develop a practical method to identify tumorigenic and nonpathogenic *A. vitis* rapidly and simultaneously, multiplex PCR using the pTi- and pRi-specific primer set VCF3/VCR3 and the species-specific primer set Ab3-F3/Ab3-R4 was developed. This multiplex PCR system can be used to identify isolates collected from crown galls or immature crown galls of grapevine. Screening tests of nonpathogenic *A. vitis* as biological control agent were carried out, and nonpathogenic *A. vitis* strain VAR03-1 was selected as biological control agent against grapevine crown gall. In a test with a 1:1 cell ratio of pathogen/nonpathogen on stems of tomato, sunflower, and grapevine, this strain was effective in reducing the incidence of gall formation on grapevine and reduced gall size. The strain was bacteriocinogenic, causing an inhibition zone against tumorigenic *A. vitis* strains on YMA medium. In biological control tests, using the method of planting grapevine seedlings in soil infested with some tumorigenic strains, pre-treatment of grapevine roots with strain VAR03-1 (adjusted to 10^9 cells/ml and soaked for 1 h) reduced percentage of galled plants and controlled crown gall of grapevine seedlings. In a field test, furthermore, pre-inoculation of grapevine roots with strain VAR03-1 reduced percentage of galled plants and controlled crown gall of grapevine seedlings. Thus, we obtained evidence that VAR03-1 was an effective bacterial agent to control grapevine crown gall using this method.

1.45 CONTROL OF PLANT DISEASES BY *OXYPORUS LATEMARGINATUS* EF069 PRODUCING VOLATILE ANTIFUNGAL COMPOUNDS. H.Y. Kim, S. Lee, G.J. Choi, Y.H. Choi, K.S. Jang, N.D. Sung and J.-C. Kim. *Chemical Biotechnology Research Center, Korea Research Institute of Chemical Technology, Korea. Email: kjinc@krict.re.kr*

In an attempt to develop mycofumigant biofungicides for control of storage and soil-borne diseases, 75 isolates of endophytic fungi were obtained from healthy tissues of red pepper and trees in the family Lauraceae, and tested for their fumigant activities against the phytopathogenic fungus *Botryris cinerea*. Among them, only two fungi designated as CF016 and EF069, isolated from the stems of cinnamon (*Cinnamomum loureirii*) and stems of red pepper (*Capsicum annum* L.), respectively, produced volatile antifungal compounds. CF016 and EF069 were identified as *Nodulisporium* sp. and *Oxyporus latemarginatus,* respectively, on the basis of their morphological characteristics and ITS-5.8S ribosomal DNA sequences. Volatile compounds of EF069 and CF016 inhibited the mycelial growth of all the plant pathogenic fungi tested. The volatile gases produced by *O. latemarginatus* EF069 effectively controlled grey mold on apples and *Rhizoctonia* disease on *Phalenopsis* sp. In addition, EF069 suppressed the development of Chinese cabbage clubroot by 33% and radish damping-off by 85% under growth chamber conditions. In field trials, EF069 was highly effective in controlling clubroot of Chinese cabbage. The volatile antifungal compound produced by EF069 was identified as 5-pentyl-2-furaldehyde. Further studies on large-scale fermentation, formulation and field trials in various conditions are needed in the development of this new microbial fungicide using *O. latemarginatus* EF069 producing volatile antifungal compounds.

1.46 EVALUATION OF CROSS-PROTECTION BY AN ATTENUATED STRAIN OF *CHINESE YAM NECROTIC MOSAIC VIRUS* IN CHINESE YAM. T. Kondo, K. Kasai, K. Yamashita and M. Ishitani. *Aomori Green BioCenter, Aomori Prefectural Agriculture and Forestry Research Center, 221-10 Yamaguchi, Nogi, Aomori 030-0142, Japan. Email: tooru_kondo@pref.aomori.lg.jp*

Chinese yam necrotic mosaic virus (CYNMV) is the most widespread virus on Chinese yam cv. Nagaimo plants in Japan and causes serious yield losses. To apply cross-protection to Nagaimo plants, an attenuated strain of CYNMV, designated KM3, had been selected from CYNMV isolates obtained in the field. Here we report evaluation of the utility of KM3 in the control of CYNMV severe strains in two field sites tested over a three-year period. A reverse transcription-polymerase chain reaction-restriction fragment length polymorphism (RT-PCR-RFLP) was used to discriminate KM3 from other CYNMV isolates in the field. So far, KM3-infected plants developed only a mild mosaic or necrotic mosaic in a small number of leaves during the growing period in open fields. No other CYNMV isolates were detected from KM3-infected plants by RT-PCR-RFLP. On the other hand, the CYNMV infection rate of uninoculated plants increased year by year; in the third year reaching 100% in one field. The decrease in tuber yield for plants infected with KM3 was only 0-6% less than that for plants grown from virus-free seed tubers, while that for plants infected with CYNMV occurring in the field was 14.4-18.7%. These results indicate that Nagaimo plants infected with

KM3 were protected from subsequent infection with CYNMV occurring in the field, and avoided significant tuber yield loss.

1.47 BIOLOGICAL CONTROL OF PRE- AND POSTHARVEST DISEASES IN MANGO. **L. Korsten, K. Zeeman and E. Arrebola-Diez.** *Dept. Microbiology and Plan Pathology, University of Pretoria, Pretoria, 0002, South Africa. Email: lise.korsten@up.ac.za*

The growing global demand for fresh tropical and subtropical fruit of high quality and without pesticide residues necessitates the development of alternative approaches to disease control. As it is highly perishable, mango fruit destined for export is subject to various pre- and postharvest diseases. These pathogens can currently be effectively controlled with fungicides, but new pesticide legislation in developed countries has forced industry particularly in developing countries to evaluate alternative products. The antagonist *Bacillus licheniformis* has been shown to effectively control the mango preharvest pathogens *Xanthomonas campestris* pv. *mangiferaeindicae* and *Colletotrichum gloeosporioides*, causing mango bacterial black spot and anthracnose respectively. The antagonist was most effective when applied in integrated field sprays, and showed some potential disease control, as well as decreased sunburn, when applied to a plastic cap with a woolly base covering the fruit. The postharvest diseases soft brown rot (*Botryosphaeria* spp.) and anthracnose could also be controlled in commercial packhouse dip treatments or when applied in an integrated program as a wax-based product. Several modes of action have been shown for the antagonist when tested against the various pathogens, such as competition for nutrients and space, and production of lipopeptides belonging to the fengycin and surfactin families. Additional molecular and electron microscopic studies confirmed the strain identity and antagonist-pathogen interaction on the host surface. The general viability of biocontrol systems and efficacy for export consignments are discussed.

1.48 POTENTIAL FOR BIOLOGICAL CONTROL OF JAPANESE KNOTWEED IN EUROPE USING PHYTOPATHOGENIC FUNGI. **D. Kurose, N. Furuya, Y. Inoue, R.H. Shaw, D.H. Djeddour, H.C. Evans, M. Matsumoto, M. Takagi and K. Tsuchiya.** *Laboratory of Plant Pathology, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan. Email: daikuro@agr.kyushu-u.ac.jp*

Japanese knotweed (*Fallopia japonica*, Polygonaceae) is a plant of Japanese origin which has now spread globally, causing major problems in Europe and north America where there is an urgent need for sustainable control measures. The biological control potential of the phytopathogenic fungi collected from Japanese knotweed in Japan was investigated with CABI Europe-UK. Surveys across Kyushu island revealed that Japanese knotweed is host to a number of fungal diseases and three of these have been prioritized: two types of rust and a leafspot species. Rusts of the genus *Puccinia* have a very good track record in weed biocontrol tending to be highly co-evolved and specific to their host. *Puccinia polygoni-amphibii* var. *tovariae* Authur was found in the field from August to December causing severe damage to all stages of knotweed. However, the most impressive pathogen was found to be a highly damaging and ubiquitous leafspot which was restricted to Japanese knotweed in the field and occurred from June to December. The incidence and development of the leafspot disease was observed at two permanent sites, and laboratory studies were used to identify the causal agent, which was found to be a *Mycosphaerella* sp. and elucidate its life cycle, which passes only through ascospores with no asexual stage (anamorph) involved. In the field, the disease is widespread and severe, suggesting that this pathogen holds great potential as a biological agent providing the host range is restricted, as predicted by the early results of host-range testing.

1.49 LYSR HOMOLOG EYSR IN *AGROBACTERIUM VITIS* STRAIN E26 AFFECTS THE BIOCONTROL OF GRAPEVINE CROWN GALL. **J.Y. Li, D.P. Liu, Y.H. Wang, J.H. Wang and H.M. Wang.** *Department of Plant Pathology, China Agricultural University, Beijing 100094, P.R. China. Email: lijinyun@cau.edu.cn*

Grapevine crown gall, caused by *Agrobacterium vitis*, is an economically important disease causing extensive damage in most viticulture regions worldwide. One nontumorigenic *A. vitis* strain, E26, can prevent crown gall infection when applied to wounds on grapevines prior to inoculation of tumorigenic strains. ME27, a Tn5 mutant of E26, is reduced in its biocontrol ability and does not significantly differ from the wild type strain of E26 in phenotypes of producing agrocin, growth rate in minimum medium and on grapevine shoot explants. Sequence analysis revealed that ME27 has a mutation in a gene (*eysR*) encoding a LysR-type transcriptional regulator (LTTR). The deduced EysR protein has characteristic helix-turn-helix (HTH) and LysR-substrate binding domains. ME27 is fully complemented with cloned eysR. The same biocontrol ability of complemented ME27 and E26 is observed. A region of about 3.5 kb flanking *eysR* was sequenced and compared with homologous regions of *A. tumefaciens* C58 and *A. vitis* S4 genomes. Gene order and homology are highly similar between the species. To determine whether *eysR* alone is responsible for the decrease of biological control, site-directed mutants of genes immediately downstream of *eysR* are being tested. Additionally, Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was used to determine whether *eysR* regulates the expression of genes downstream of itself.

1.50 ANTAGONISTIC POTENTIAL OF ENDOPHYTIC FUNGI AGAINST SEEDBORNE PATHOGENS. **T.D. Martins, L.C. Assumpção, V.C. Frare, M.H.D. Moraes and J.O.M. Menten.** *ESALQ/USP – Av. Pádua Dias, 11, CP 09, 13418-900, Piracicaba-SP, Brazil. Email: martins@esalq.usp.br*

Our objective was to evaluate the antagonistic potential of endophytic fungi isolated from soybean seeds, against the seedborne pathogens *Fusarium oxysporum, Rhizoctonia solani* and *Phomopsis* sp. We used 5mm discs of the endophytic fungi and the pathogen on Petri dishes of PDA medium, on opposite sides. The control treatment was placement of a 5mm disc of the respective fungi, alone, at the center of the Petri dishes. The experimental design was totally randomized, with four repetitions. The Petri dishes were incubated at 20±2°C for seven days in alternating cycles (12h light/ 12h darkness), and we then measured colony growth inhibition and the presence of an inhibition halo. *Penicillium* spp. produced an inhibition halo, while the *Trichoderma* spp. analyzed showed hyperparasitism. In this case, we used May and Kimati's (1999) modified scale to assess endophytic growth on the plant pathogen colonies.

1.51 MICROSCOPIC ANALYSIS OF TOMATO ROOT COLONIZATION BY *PYTHIUM OLIGANDRUM* AND *RALSTONIA*

SOLANACEARUM. **A. Masunaka, K. Nakaho, H. Takahashi and S. Takenaka.** *National Agriculture Research Center for Hokkaido Region, Memuro-cho, Hokkaido 082-0071, Japan. Email: masunaka @affrc.go.jp*

Pythium oligandrum (PO), a non-pathogenic soil-inhabiting oomycete, is well-known as a biocontrol agent of damping-off and root diseases. PO has many biocontrol properties, such as mycoparasitism, antibiosis, competition, and induction of plant defense reactions. Recently our group found that PO was able to suppress bacterial wilt caused by *Ralstonia solanacearum* (RS) in tomato. To improve our understanding of interactions between PO and RS during biocontrol, we developed an immuno-enzymatic staining procedure for PO and used an RS strain carrying the GFP reporter gene to differentially and simultaneously visualize them using confocal laser scanning microscopy. Forty eight hours after inoculation, RS was found to colonize wounding sites of root tips in both control plants (not treated with PO) and PO-treated plants. Approximately 1 week after inoculation, the longitudinal colonization of RS developed from wounding sites to the top of stem in control plants. On the contrary RS development was markedly suppressed in PO-treated tomato; the longitudinal colonization of RS was observed in some secondary roots, but not in primary roots. The colonization of PO was mainly observed on the surfaces of basal parts of secondary and primary roots by attaching to root hairs, but not the elongation zones and the axils of emerging or developed roots. These microscopic observations indicate that direct competition for infection sites is unlikely to be main motive force, and induced resistance seems to be the main mechanism in the reduction of bacterial wilt by PO.

1.52 PLANT-MICROBE INTERACTIONS IN THE BIOLOGICAL CONTROL OF DRY ROOT-ROT OF CHILLI INCITED BY *RHIZOCTONIA SOLANI* AG-3. **K. Mathur.** *Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur 313001, Rajasthan, India. Email: kusum.mathur@rediffmail.com*

Disease suppression through biocontrol agents may involve the induction of resistance responses in the host. Local strains of the fungal biocontrol agent *Trichoderma* spp. (*T. harzianum* Jh-2, *T. aureoviride* DG-5, *T. longibrachiatum* D-1 and *T. viride* ITCC 1433, effectively suppressed the dry root rot pathogen *Rhizoctonia solani* AG3 *in vitro* and also in chilli (*Capsicum annum* L. and *C. frutescens* L) rhizospheres in the field. Soil application of *Trichoderma* spp. (1×10^5 spores/ml) caused enhanced accumulation of sesquiterpenoid phytoalexin capsidiol in roots of four chilli cultivars with a significant cultivar × *Trichoderma* spp. interaction. Soil application of *Trichoderma* spp. also resulted in increased levels of superoxide radical, hydrogen peroxide and catalase in chilli roots. However, the changes in the levels of other components of the ROS scavenging system, *viz.*, superoxide dismutase, ascorbate, ascorbate-peroxidase and glutathione were not consistent with the level of protection achieved. The *Trichoderma* spp. induced chitinase and β-1,3 glucanase enzymes in chilli roots. These results suggested that in addition to antagonism, *Trichoderma* spp. suppressed *R. solani*-incited root rot through phytoalexin synthesis and systemically acquired resistance (SAR). Drenching with *T. harzianum* (1×10^5 spores /ml) in chilli roots induced re-iterative bursts of hydrogen peroxide (H_2O_2) generation in leaves within 24 h of application. Leaf inoculation with spore suspension led to a similar response in distant leaf tissues within the same time frame. Systemic induction of PR proteins in response to biochemical agents appears to be mediated by these re-iterative micro bursts of H_2O_2 and micro-HR.

1.53 BIOLOGICAL CONTROL OPTIONS FOR *SONCHUS* SPP. IN AUSTRALIA. **K. McCarren and J. Scott.** *CSIRO Entomology, Private Bag 5, PO Wembley WA 6913, Australia. Email: kathryn.mccarren@csiro.au*

Sowthistles, *Sonchus oleraceus* and *S. asper* (Asteraceae), are widespread invaders of disturbed ecosystems in Australia. *S. oleraceus* is a major weed of Australian cropping systems, especially those of south east Queensland and northern NSW, its incidence having increased over the past two decades. Surveys of these weeds throughout Australia have found two organisms on *Sonchus* spp. that are very damaging; an undescribed species of eriophyid mite (*Aceria* sp.) causing extensive leaf-rolling damage, and a rust fungus, *Miyagia pseudosphaeria*, causing leaf dieback. Both appear to be highly specialized species closely associated with their host, *S. oleraceus*. Both organisms are specific to *Sonchus* spp., neither infecting *Actites megalocarpus* (Sonchinae subtribe) nor other closely related species within the tribe Lactuceae under glasshouse no-choice trials or field conditions. The interactions of these two organisms with the only native sowthistle species in Australia, *Sonchus hydrophilus*, are being investigated to establish the origins of these organisms and likely impact, both in natural and cropping ecosystems. In the Australian context, both could be considered for a conservative approach to biological control.

1.54 THE INFLUENCE OF ABIOTIC FACTORS ON *TRICHODERMA* BIOLOGICAL CONTROL AGENTS. **K.L. McLean, J.M. Steyaert, S.D. Card and A. Stewart.** *National Centre for Advanced Bio-Protection Technologies, P.O. Box 84, Lincoln University 7647, Canterbury, New Zealand. Email: Stewarta@lincoln.ac.nz*

One of the limitations to widespread uptake of biocontrol as a disease management strategy is a lack of understanding of the complex interactions between biocontrol agents and abiotic factors. The influence of environmental parameters such as rainfall and temperature on the efficacy of *Trichoderma* biocontrol agents used in the horticultural sector (*Trichoderma atroviride* for control of onion white rot and *T. hamatum* for control of *Sclerotinia* lettuce drop) in New Zealand is reasonably well established. Recent research has focused on understanding the interactions between *Trichoderma* and soil type and pH in an effort to minimise the inconsistent performance of biocontrol agents. Pot and field trials determined that *T. atroviride* protection was optimal in peat and clay soils whereas *T. hamatum* did best in clay and silt/sand soils. Addition of spent mushroom compost or poultry manure to the different soil types also had an impact on *Trichoderma* populations with *T. atroviride* preferring the organic amendments in volcanic but not sandy soils. *T. hamatum* showed the opposite trend with higher populations in clay and sandy soils but not volcanic soils. A similar pattern was observed for both *Trichoderma* species with the application of nitrogen. In detailed lab-based assays, small changes in pH caused significant changes in mycelial growth and conidial development of *T. atroviride* and *T. hamatum*. *T. atroviride* preferred pH 4 whereas the optimum for *T. hamatum* was pH 3. This information will enable the identification of soil types and environments that different *Trichoderma* species will work in.

1.55 EFFICACY OF GARLIC AND ALLAMANDA TABLETS IN CONTROLLING DISEASES OF VEGETABLES. **M.B. Meah, M.S. Islam and H.P. Seal.** *IPM Lab, Dept of Plant Pathology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh. Email: bmeah@yahoo.com*

Total extracts of garlic and allamanda, and their separated components differentialy inhibited the growth and sporulation of *Phomopsis vexans*, *Sclerotium rolfsii*, *Phytophthora capsici*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Fusraium oxysporum*. Seeds treated with both garlic and allamanda tablets germinated faster and in significantly higher percentage than untreated seeds. Both the tablets retained disease inhibition efficacy up to 90 days after preparation. Seed germination varied from 78-100% against *P. vexans* and 80-93% against *S. rolfsii* for different age and concentration of the tablet solution. No damping off or seedling blight or tip over were observed in the tray soil sown with tablet-treated seeds, against 4-5% damping off and 2-3% seedling blight in untreated seeds. Higher spray concentration (1:1) performed better than the lower one (1:2). *Phomopsis* blight of eggplant, leaf anthracnose, die-back of twig and fruit infection in citrus, number of galls in carrot and pea were reduced through use of allamanda tablets. Allamanda tablet spray reduced mosaic and leaf curl by 46 and 37% respectively in tomato. Combinations comprising soil treatment with *Trichoderma harzianum* CP followed by spraying of allamanda tablet yielded the highest return (BCR = 3.96). Use of apparently healthy seeds in combination with seed treatment with garlic/allamanda tablet solution could be a nice option for healthy seedling production. Carbonyl and nitride groups were detected in the separated components, and complete structure elucidation is in progress.

1.56 MECHANISMS OF BIOCONTROL OF THE CONIFER PATHOGEN *HETEROBASIDION* BY *PHLEBIOPSIS GIGANTEA*. **A.C. Mgbeahuruike, M. Karlsson and F.O. Asiegbu.** *Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, SLU, Box 7026, SE-75007 Uppsala, Sweden. Email: anthony.mgbeahuruike@mykopat.slu.se*

Phlebiopsis gigantea has routinely been used for the biocontrol of the conifer pathogen *Heterobasidion annosum* s.l., but the mechanism of control is not properly understood. In the present work, we studied the growth and interaction of *Heterobasidion parviporum* and *P. gigantea* in both carbon-rich (Hagem) and low-carbon media (Norkrans). Laccase and wood degradation abilities were measured on 64 isolates. The data were analysed using multiple regression, where correlations between different variables were tested. Results showed that high growth rate in carbon-rich media correlated with high growth rate in xylan ($p = 0.028$) and cellulose ($p = 0.072$) which are both low-carbon Norkrans media. High growth rate on sawdust, a lignin carbon source, correlated with high growth rate in ferulic acid, a lignin precursor ($p = 0.046$) and in xylan, a hemicellulose ($p = 0.006$). High wood degradation ability and high lacasse production correlated with high antagonistic ability on sawdust with p values of 0.037 and 0.015 respectively, whereas high antagonistic ability in Hagem media correlated with high antagonistic ability in xylan ($p = 0.027$) but was negatively correlated with growth rate in ferulic acid ($p = 0.039$). Additionally, to gain a better understanding of the molecular basis for the differences in antagonistic ability and growth, RT-PCR, is being used to evaluate the expression levels of a subset of genes (hydrophobin, cyclophilin etc) from the isolates. The results will be presented and discussed.

1.57 A *FUSARIUM*-BACTERIA INTERACTION FOR BIOCONTROL. **M. Moretti, D. Minerdi, G. Gilardi, C. Barberio, M.L. Gullino and A. Garibaldi.** *AGROINNOVA, University of Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy. Email: marino.moretti@unito.it*

Among the soilborne pathogenic fungi *Fusarium oxysporum* is very dangerous for a large number of plants. This species includes strains that can penetrate roots but do not invade the vascular system or cause disease, and could be interesting for biocontrol. *F. oxysporum* strain MSA 35 was isolated from naturally suppressive soil in Italy. It was found associated with a bacterial population that was examined by electron microscopy, fluorescence *in situ* hybridization (FISH) and a 16S rRNA gene clone library. The bacterial community adheres to the external surface of hyphae and conidia and is dominated by Gammaproteobacteria. Alphaproteobacteria and Betaproteobacteria are also present. MSA 35 deprived of bacteria is no longer antagonistic against Fusarium wilt on lettuce plants and becomes pathogenic. Molecular analysis showed that MSA 35 belongs to the *forma specialis lactucae*. The reasons why a pathogenic *Fusarium* becomes non-pathogenic in association with bacteria and acquires antagonistic activity are currently under investigation. Semi-quantitative RT-PCR analyses indicated that putative genes involved in *F. oxysporum* virulence such as pectate lyase, polygalacturonase, MAP kinase and class V chitin synthase are not expressed in bacteria-associated MSA 35 but they are in the absence of bacteria. This appears to be the first report of a bacteria-fungus interaction in which a bacterial consortium completely changes the behaviour of a fungus.

1.58 NEW MECHANISMS USED BY *PSEUDOMONAS* BACTERIA IN BIOLOGICAL CONTROL OF THE TAKE-ALL FUNGAL PATHOGEN OF WHEAT. **M. Nayudu, C. Samundsett, R. Kaur, A. Franklin, M. Koeck, T. Murphy, H. Millar, S. Khan, K. Groom, J. Singh, J. Macleod and Y. Zhang.** *School of Botany and Zoology, Faculty of Science, Australian National University, ACT 0200, Australia. Email: murali.nayudu@anu.edu.au*

Biological control of the take-all fungal pathogen *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas* bacteria depends on bacterial colonization of wheat roots and production of an anti-fungal agent. *Pseudomonas* strain AN5 (*Ps.* str. AN5) is a non-fluorescent isolate from Australia which, in field trials, increased wheat yield by up to 20% by controlling take-all. The mechanisms involved in the production by *P. fluorescens* biocontrol bacteria of the anti-fungal agents 2,4-diacetylphloroglucinol or phenazine-1-carboxylic acid, which suppress the take-all pathogen, have been well characterized. *Ps.* str. AN5 does not produce these anti-fungal agents but produces the sugar acid D-gluconic acid in suppression of the take-all pathogen. Gluconic acid is the first anti-fungal agent to be identified which is a simple sugar, and hydrophilic in nature compared to the hydrophobic benzene-based anti-fungal compounds discovered previously. We have identified, cloned and sequenced the genes responsible for sugar acid production by *Ps.* str. AN5. Using supernatant extracts we have shown that a protease produced by *Ps.* str. AN5 attacks the take-all fungal hyphae. This seems to be the key factor in take-all biocontrol by *Pseudomonas*. We have shown that *Ps.* str. AN5 wheat root colonization is a specific process in which the *Pseudomonas* induces erosion zones leading to the bacteria colonizing the epidermal and cortical regions. Two transposon mutants have been identified which are impaired in the process of root colonization. The nature of these new bacterial processes and the *Pseudomonas* genes identified that are responsible for take-all biocontrol will be outlined.

13.1 ASSESSMENT OF THE EFFECT OF PESTICIDES ON WEEVILS FOR CONSERVATION OF KOLA NUTS IN CAMEROON. **M.A. Ngoungoure.** *ICRAF-African Humid Tropic, P.O. Box 16317, Yaoundé, Cameroon. Email: amanjeli@yahoo.fr*

The efficiency of traditional methods in reducing post-harvest kola nut weevil infestation was assessed in the ICRAF-AHT nursery. The study was aimed at identifying and estimating post-harvest losses, investigating the effect of healthy/unhealthy fruits on nut infestation, and assessing the efficiency of products used by farmers against kola nut weevil infestation (Actellic 50 EC solution, 500 gm/l of pyrimiphos methyl, Malagrain DP5 powder, 5% Malathion and neem *(Azadirachta indica* seed extracts). Kola nuts were stored in baskets following a split-split plot experimental design in randomized complete blocs with four replicates. Each basket was considered as an experimental unit, and contained either 500 infested or 400 healthy kola nuts. Nuts were manually sorted out every month and Actellic solution, Malagrain powder and neem seed extracts applied after one, three and four months respectively to kola nuts depending on remanence duration. Over a four month storage period, 70.98% of losses were caused by weevil infestation (most commonly *Balanogastris kolae*) while 5.65% of losses were due to drying and rot. The kola nuts were not influenced ($P = 0.992$) by the initial health of the kola pods, but were highly affected ($P < 0.001$) by the products applied. Actellic 50 EC was found to be the most efficient (0.28% losses). The results are discussed with a view to assessing the use of a range of organic products for kola nut preservation by farmers in Cameroon.

13.2* PARASITIC APTITUDE OF *PENICILLIUM RESTRICTUM*. **R. Nicoletti, M. De Stefano and A. Carella.** *CRA – Tobacco Experimental Institute, Via Vitiello 108, 84018 Scafati, Italy. Email: rosario.nicoletti@entecra.it*

Although quite widespread in soil and often recovered in association with plant pathogenic fungi, *Penicillium* species have seldom been reported as parasites. Isolates of the monoverticillate species *P. restrictum* were recovered from the rhizosphere of healthy lupin plants growing in a field severely damaged by crown and root rot incited by a number of soil-borne fungi. These isolates were able to inhibit mycelial growth of *Pythium ultimum* and *Rhizoctonia solani* AG-2-1 and AG-4 in vitro, and adpressed growth of hyphae together with presumptive parasitic interactions were detected in dual cultures with the same pathogens on minimal media. Further observations on this previously unreported parasitic behaviour of *P. restrictum* were made by scanning electron microscopy, and included other plant pathogenic fungi, such as *Fusarium* spp., *Macrophomina phaseolina*, *Phytophthora nicotianae*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii* and *Thielaviopsis basicola*. Clear evidence of hyphal penetration by haustorium-like structures was documented, demonstrating the capacity of *P. restrictum* to develop parasitically. Its rhizosphere competence and possible ecological role as a biological control agent against soil-borne fungal pathogens are considered.

13.3 ECOLOGICAL FITNESS OF PLANT-BENEFICIAL *BACILLUS SUBTILIS* STRAINS IN SOIL: INFLUENCE OF RHIZOSPHERE-SPECIFIC PARAMETERS ON SURFACTIN SYNTHESIS. **V. Nihorimbere, P. Fickers, P. Thonart and M. Ongena.** *Unité de Bioindustries, Faculté des Sciences Agronomiques, B-5030 Gembloux, Belgium. Email: ongena.m@fsagx.ac.be*

Rhizobacteria referred to as "plant growth-promoting rhizobacteria" contribute to the biological control of plant pathogens. They enhance root development by producing phytohormones or by inhibiting pathogens through the synthesis of various compounds. In the case of *Bacillus subtilis* strains, cyclic lipopeptides such as surfactin may be closely involved in biocontrol activity. In vivo surfactin production is thus crucial but is influenced by various rhizosphere-related growth conditions. In this study, the LacZ reporter system served to monitor the expression of the *psrfA* gene in strain BGS3 in parallel with surfactin production. Population densities of about 10^7 cfu/g roots were measured on tomato root seedlings inoculated with BGS3 and low but significant surfactin gene expression was observed. Various carbon sources were individually tested, and high levels of both gene expression and surfactin production were observed on substrates that are the most abundant in exudates. Other results also showed that strain BGS3 used aerobic as well as anaerobic conditions to express surfactin and to produce lipopeptides. The influence of growth rate of BGS3 cells was also assayed in a chemostat-type bioreactor, and the lowest growth rate value tested ($0.1\ h^{-1}$) led to the highest surfactin gene expression level. This work appears to be the first study on the influence of rhizosphere parameters on expression of *Bacillus* biocontrol determinants, and our results suggest that efficient surfactin synthesis may occur in cells developing very slowly on roots in poorly aerated soils where available substrates for growth mainly derive from plant exudates.

13.4 GENETIC DIVERSITY OF BACTERIA ANTAGONISTIC AGAINST SHEATH BLIGHT AND BAKANAE RICE DISEASE REVEALED BY RAPD. **M. Niknejad Kazempour, S.M. Tabatabaei and N. Hasanzadeh.** *Depatment of Plant Pathology, Faculty of Agriculture, University of Guilan, Rasht, Iran. Email: nikkazem@yahoo.fr*

Nineteen antagonistic bacterial strains were isolated from rice field contaminated by *Rhizoctonia solani* and *Fusarium moniliforme*. The antagonistic strains were identified based on morphological, biochemical, physiological and pathogenic characteristics, total cellular protein profiles (SDS-PAGE) and PCR with specific primers. The strains antagonistic to *R. solani* included B4, B6, B17, B18, B22, B24, B41 and B42 and those antagonistic to *F. moniliforme* included F1, F6, F12, F15, F16, F18, F21, F24, identified as *Pseudomonas fluorescens*. Out of fifteen RAPD primers, five were selected based on their ability to amplify all antagonistic strains. The nineteen strains were grouped based on RAPD analysis. Intra-population gene diversity and distributed genetic diversity were assessed by POPGENE software. Nei's genetic distance for A, B and C populations was 0.22, 0.28 and 0.29 respectively. RAPD analysis revealed that strains B18 and F12 formed a distinict group compared to other strains and showed maximum similarity with strains B24 and F18. Other strains were placed in separate subgroups. Strains B41 and B6 showed maximum genetic distance. Molecular analysis using the PCR-based RAPD method is thus useful to differentiate such strains at the intra-specific level.

13.5 PHOSPHATE-SOLUBILIZING RHIZOBACTERIA MEDIATE PLANT GROWTH PROMOTION AND *FUSARIUM* WILT SUPPRESSION IN TOMATO. **S.R. Niranjana, P. Hari Prasad, N.M. Carmen and H.S. Prakash.** *Department of studies in Applied Botany and Biotechnology, University of Mysore, Manasagangotri, Mysore 570006, India. Email: srn@appbot.uni-mysore.ac.in*

The role of phosphate-solubilizing rhizobacteria (PSRB) in improving health of tomato plants was investigated in the present study. Forty three isolates of PSRB were obtained from native tomato rhizospheres from different parts of Karnataka. Among these isolates, 33 were found to be positive for solubilizing both inorganic and organic forms of phosphorus. Isolate PSRB21 showed preferential ability to solubilize different forms of phosphate, under *in vitro* conditions. It was found to solubilize sodium phytate initially; when this substrate became scarce it began to produce organic acids which led to decrease in pH of the medium. The phytase activity was found to gradually decrease as the pH increased. In greenhouse studies, tomato seedlings treated with all the selected isolates showed increased plant height, root length, fresh weight, dry weight and P content, compared to uninoculated controls. When the pH and available phosphorus in rhizosphere soil samples of 30 day-old-seedlings were analyzed, the pH was not much varied but the available phosphorus was high in plants raised form seeds bacterized with PSRB21. Even though PSRB21 showed increased plant growth and highest available phosphorus in rhizosphere soil, it failed to protect tomato seedlings against *Fusarium* wilt disease. The isolate PSRB21 which was found antagonistic to *Fusarium oxysporum* reduced wilt incidence (21%) when compared to the control (82%) under greenhouse conditions.

13.6 BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* INFESTING PEANUT BY THE NEMATODE-TRAPPING FUNGUS *DACTYLARIA BROCHOPAGA* UNDER FIELD CONDITIONS. **E.M.A. Noweer.** *Plant Pathology Department, National Research Center, Dokki, Egypt. Email: enoweer@hotmail.com*

Control of root-knot nematode under field conditions by using the nematode-trapping fungus *Dactylaria brochopaga* (Drechsler, 1937) alone or combined with yeast, molasses and vermiculite is reported. The results revealed that the highest reduction in number of nematode larvae per kg soil and the highest reduction in number of root galls per plant (92%) was achieved when applying *D. brochopaga* with yeast, molasses and vermiculite. The data revealed that the yield kernels per plant were significantly increased ($P \leq 0.05$ and/or $P \leq 0.01$) in all the *D. brochopaga* treatments compared to the untreated control treatment.

13.7 EFFECT OF BIOLOGICAL FUNGICIDES ON *BOTRYTIS CINEREA* INFECTION, AND FRUIT-SET OF BLUEBERRIES, BOYSENBERRIES AND BLACKCURRANTS. **F.O. Obanor, B.J. Smith, K.S.H. Boyd-Wilson, G.I. Langford, J.T. Smith, M. Goodwin, P. Harris-Virgin, B. Donovan and M. Walter.** *The Horticulture and Food Research Institute of New Zealand Ltd., P.O. Box 51, Lincoln, New Zealand. Email: fobanor@hortresearch.co.nz*

The fungus *Botrytis cinerea* causes flower blight in blackcurrant (*Ribes nigrum*) and blueberry (*Vaccinium* spp.) and grey mould in boysenberry (*Rubus* hybrid). The potential of biological fungicides (BFs) for *Botrytis* control has been investigated for these berries in laboratory and field studies using brush application (bee-vector simulation) and spray application. The effect of BFs on fruit set was also studied by simulating bee-vectored application. Studies were conducted on blueberry in the USA (2007) for seven BFs, and on blackcurrant and boysenberry in NZ (2006/07) for three BFs at 10 and 100% product rates. Products were *Bacillus subtilus* (Serenade®), *Gliocladium catenulatum* (Prestop® WP), *Pseudomonas syringae* (Bio-Save™ 10LP and 11LP), *Streptomyces griseoviridis* (Mycostop®), *S. lydicus* (Actinovate® SP), and unlabelled yeast (USA); and *B. subtilus* (Serenade®), *Trichoderma atroviride* (Sentinel®) and *Ulocladium oudemansi* (Botry-Zen®) (NZ) in comparison with fluodoxinil + cyprodinil (Switch®) (USA and NZ). Fruit set (%) in blackcurrant was not affected ($P = 0.144$) by the fungicide treatments. However, in boysenberry and blueberry fruit set was reduced ($P < 0.05$) by two of the fungicides at the 100% product rate. In blackcurrant and boysenberry, all products suppressed *Botrytis* infection of flowers in the laboratory and field tests. The least amount of disease ($P < 0.05$) was obtained in flowers treated with fluodoxinil + cyprodinil followed closely by *T. atroviride.* Blueberry data are currently being analysed. The bee-simulated brush applications of BFs (100%) generally achieved 10-100 times the active ingredient loading on the flower styles compared with conventional spray applications. However, method of application did not affect the *Botrytis* control potential on berry flowers.

13.8 ETIOLOGY AND BIOLOGICAL CONTROL OF BANDED LEAF AND SHEATH BLIGHT OF MAIZE. **Y.S. Paul and V. Sood.** *Department of Plant Pathology, CSKHPKV, Palampur HP-176062, India. Email: yspaul@hillagric.ernet.in*

BLSB (banded leaf and sheath blight) has become more destructive with the popularization of hybrid maize varieties in Himachal Pradesh. Maize-growing areas of the state were surveyed to work out the status of the disease. A total of 33 isolates of *Rhizoctonia solani* causing BLSB were collected, and maintained as pure cultures. Based on detailed cultural, morphological, and cytological studies, the isolates were placed in 11 cultural groups (C1-C11). Anastomosis grouping of the isolates was done following the clean-glass and charcoal PDA method with international tester isolates obtained from Sneh and Shiego Japan. The isolates were found to belong to two groups (AGI-IA and AG I-IA/IB). For genetic characterization RAPD analysis was done and the isolates fell into 7 genetic groups (G1-G7). Two *Trichoderma* isolates (HB15 and HB17) out of six isolated from parasitized mycelium and sclerotia were found to be the most effective against the pathogen using the dual culture method. HB15 was selected for field experiments because of its fast growth and better sporulation. It was found to be significantly superior to commercially available bioagents tested in field experiments at Palampur. Seed-and-soil application of HB15 integrated with chopping of lower leaves was found to be the most effective in management of BLSB of maize. Seed-and-soil application of HB15 was observed to be superior as it reduced horizontal spread of the disease.

13.9 SEED AND SOIL TREATMENT WITH BACTERIAL ANTAGONISTS IN SUPPRESSING *SCLEROTIUM* ROOT ROT OF SOYBEAN. **S. Prathuangwong, S. Kriphon, R. Sangprathoom and S. Kasem.** *Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, 50 Paholyothin Rd., Chatuchuck, Bangkok 10900, Thailand. Email: agrsdp@ku.ac.th*

Three known biocontrol bacteria are *Bacillus amyloliquefaciens* KPS46, *Paenibacillus* sp. SW01/4, and *Serratia marcescens* Spt360, which have been reported to protect various plants from multiple diseases. Five new strains isolated from soybean rhizospheres in this study include NB423, NB419, NP2031, KB404, and SB305 are notable for their effect on root rot reduction of soybean caused by *Sclerotium rolfsii*. In laboratory experiments, all strains tested except Spt360 inhibited mycelial growth of the pathogen, and KPS46 and SW01/4 were better at inhibition than

the new strains. Strains KPS46 and KB404 also showed significantly highest seed germination of 100%, followed by NB419, Spt360 and SW01/4 respectively. In the greenhouse, soybean seeds cv. SJ4 were sown into *S. rolfsii* infested soil, and treated with individual antagonists, or with *Rhizobium* and antagonist, or boosted soil (soil drenching) with antagonist, 7 days after sowing. The result revealed that seed treatment with antagonist alone did not give increased seed germination but reduced disease severity, compared with antagonist plus rhizobium seed treatment. Treatment of seeds with SW01/4, or SW01/4 and rhizobium, and both treatments were again boosted soil with SW01/4 were highest increase in 80% seed germination and 74% disease severity reduction. SW01/4 also enhanced shoot height and root length of soybean seedlings more than all strains tested under pressure of *Sclerotium* root rot endemicity trials. It is evident from present and prior studies that *Paenibacillus* sp. SW01/4 has the potential to control both root and foliar diseases of soybean.

13.10 IDENTIFICATION AND CHARACTERIZATION OF A NEW BIOCONTROL AGENT IN THE GENUS *LYSOBACTER*. **G. Puopolo, A. Raio and A. Zoina.** *Università di Napoli "Federico II", Via Università, 100, 80055 Portici, Italy. Email: puopolo@unina.it*

Extracellular lytic enzymes are used by biocontrol agents to attack plant-pathogen cell walls. A Gram-negative bacterial isolate named PG4 was selected as it showed high lipase, protease and chitinolytic activities *in vitro*. Analysis of its 16S rDNA sequence allocated PG4 to *Lysobacter*, and its 16S rRNA sequence clustered with the sequences of *L. antibioticus* and *L. gummosus*. At the same time, the Biolog Test was used to determine its physiological profile. PG4 was tested *in vitro* against a broad range of fungi and bacteria, and showed strong inhibitory activity that can be attributed to the production of diffusible antibiotic compounds. Antibiotic production was drastically reduced by growing PG4 on King's B medium (KB) but was restored by the addition of Fe3+. Adhesion assays in 96-well polystyrene plates were used to evaluate biofilm production; the ability of PG4 to form biofilm was correlated with entry into stationary growth phase except when the bacterium was grown on KB. The strain was tested for the control of some fungal pathogens *in vivo*. The strong reduction of plant mortality confirmed the potential of *Lysobacter* sp. strain PG4 as a biological control agent.

13.11 LOOKING FOR NEW BIOCONTROL AGENTS WITHIN A COLLECTION OF FLUORESCENT PSEUDOMONADS. **G. Puopolo, A. Raio and A. Zoina.** *Università di Napoli "Federico II", Via Università, 100, 80055 Portici, Italy. Email: puopolo@unina.it*

Agricultural soils can host bacterial strains able to interfere with pathogenesis by protecting plant roots from fungal attack. Several *Pseudomonas* spp. produce a wide range of antibiotic compounds that can limit the growth of fungal pathogens. In this work, fluorescent pseudomonad strains isolated from different soils were identified according to a polyphasic approach. Molecular identification of the isolates was achieved by phylogenetic analyses of *recA* gene, while physiological profiles were evaluated by using API20NE and Biolog tests. All bacterial strains were characterized for antagonism by scoring the production of lytic exoenzymes, siderophores and antibiotics and by evaluating *in vitro* the inhibition of mycelial growth of four plant-pathogenic fungi. The ability to colonize plant surfaces was also assessed using swimming and swarming motility tests. The production of quorum-sensing signals was investigated, by using several bioreporter strains such as *Chromobacterium violacearum* strain CV026 and *Agrobacterium tumefaciens* strain NTL4. Five strains were evaluated for the control of *Fusarium oxysporum* f.sp. *lycopersici* and *F. oxysporum* f.sp. *radicis lycopersici* on tomato plantlets by using a predictive *in vivo* approach. Only those strains able to produce antibiotics and quorum-sensing signals performed well in protection of tomato plantlets.

13.12 INHIBITION OF PLANT PATHOGENS BY *BACILLUS SUBTILIS* BJ037. **J. Qiu, W. Liu and D. Zhao.** *Institute of Plant & Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, P.R. China. Email: qiujiyan@yahoo.com.cn*

Chemical pesticides are mainly used to control plant disease, and the chemicals have serious negative influence on the environment. Development and application of biological pesticides are more and more the concern of scientists and farmers in China. *Bacillus subtilis* BJ037 was isolated from the soil of a vegetable field in Beijing, China and cultures of it have been found to inhibit plant pathogens broadly and efficiently. The antagonistic ability of BJ037 was tested in vitro in pot tests in the greenhouse. When fifteen pathogens were tested on PDA, strain BJ037 displayed broad-spectrum antagonistic activity to all. BJ037 broth filtrate was able to inhibit mycelium growth of *Fusarium oxysporum* f.sp. *conglutinans*, *F. oxysporum* f.sp. *cucumerinum* and *F. oxysporum* f.sp. *niveum*. In the greenhouse BJ037 broth showed excellent biocontrol of *F. oxysporum* f.sp. *conglutinans*, which causes yellow wilt on cabbage, and the highest control efficiency reached 96.25%. Also plant roots treated with BJ037 broth, and 3 days later inoculating the pathogen, showed both low disease index and low infection rate. In contrast, the control rate of *F. oxysporum* f.sp. *conglutinans* on cabbage in the spring season was around 70% to 80% after treating with BJ037 broth by the method of drenching or dipping, after transplanting, and a further three times during the growing season.

13.13 PURIFICATION AND SOME PROPERTIES OF ANTIFUNGAL SUBSTANCES FROM ANTAGONISTIC *BACILLUS* SP. BJ-6. **Z.G. Ren, Z.P. Liu, Q.X. Shang, X.Y. Zhao, S.H. Liu and Y.M. Wei.** *Department of Plant Science and Technology, Beijing Agricultural College, Beijing 102206, P.R. China. Email: yanminwei@yahoo.com.cn*

The BJ-6 strain of *Bacillus* sp. was isolated from the rhizosphere of an apricot tree in Beijing, China. It showed strong antagonistic activity to 10 species of plant pathogens tested, i.e. *Valsa mali*, *Physalospora piricola*, *Monilinia laxa*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Alternaria solani*, *Alternaria brassicae*, *A. mali*, *Fusarium oxysporum*, and *Verticillium dahliae*. The strain could produce antifungal substances which were purified from BJ-6 culture broth by ammonium sulfate precipitation at different saturations from 20% to 90%. The crude antifungal substances precipitated at 30%-40% saturation were the most inhibitory, and the width of the inhibition zone with *V. mali* was 20 mm. Further purification showed that there were two absorption peaks (P_1 and P_2) at 280 nm after the crude substances (precipitated at of 30%-40% saturation) were passed through gel chromatography with Sephadex G-150. The P1 fraction had strong antagonistic activity, while the other (P_2) did not. P1 showed one protein band after 15% SDS-polyacrylamide gel electrophoresis, and its molecular weight was determined to be 10 kDa. It was also found

that the protein still had antifungal activity after being treated at 121 ºC for 20 min at pH 7, and could cause deformation of the spores, inhibit spore germination, and induce abnormal mycelium of *A. mali*. Thanks to Academic Human Resources Development in Institutions of Higher Learning under the Jurisdiction of Beijing Municipality (PXM2007-014207-044536).

13.14 THE ROLE OF HEXOSAMINIDASE IN THE GROWTH PROMOTION OF LETTUCE BY *TRICHODERMA HAMATUM* GD12. L.S. Ryder and C.R. Thornton. *Biosciences, Geoffrey Pope Building, Stocker Road, Exeter, Devon, EX4 4QD, UK. Email: L.S.Ryder@exeter.ac.uk*

Trichoderma species are important agents in the biocontrol of root pathogens and are attractive alternatives to synthetic pesticides. In addition to biocontrol properties, certain strains display plant-growth-promotional activities. While the involvement of chitinases in the biocontrol activities of *Trichoderma* species has been studied in depth, little attention has been paid to the role of chitinases in the 'nutritional chitinolysis' of *Trichoderma* spp. A rhizosphere-competent strain of *T. hamatum* (strain GD12) was recently isolated that elicits increases in the root and photosynthetic biomass of lettuce other plant species during growth in low nutrient status plant growing media such as peat-based composts. High levels of hexosaminidase activities occur during saprotrophic colonization of peat by GD12 suggesting that the fungus is mobilizing hexosamines present in the medium. Consequently, we set out to determine the role that hexosaminidases might play in saprotrophism of the fungus and whether mobilization of hexosamines could account for the plant-growth-promotion witnessed during *Trichoderma* GD12-plant interactions. We targeted the gene encoding the extracellular enzyme hexosaminidase (Hex) using primers based on the *T. harzianum exc2y* sequence, and amplified a 1.9 kb fragment of the gene. Strains disrupted in hexosaminidase production were developed using an insertional mutagenesis strategy and preliminary phenotypic analysis demonstrates that a *DHex* mutant displays enhanced plant growth-promotion and biocontrol abilities. The mechanism for this phenomenon is currently being determined.

13.15 DETERMINATION OF FACTORS INFLUENCING BLAST FIELD RESISTANCE IN RICE POPULATIONS, USING MULTIVARIATE ANALYSIS. H. Sabouri, A. Rezaei, M. Kavousi and M. Katouzi. *Gilan, Golsar, Bolvar Gilan, 188 street, No. 10, P.O. Box 41668-13515 and Azad Universiy of Tabriz, College of Agriculture, Tabriz, Iran. Email: s_katuzi@yahoo.com*

In order to study the relationship between morphological and phenological traits and blast field resistance in Iranian rice populations, an F2 population derived from a cross between TAM (susceptible) and KHZ (resistant) was constructed. The genetic material involved 192 F3 families, each derived from bagged seeds of a single F2 plant. Positive correlations were observed between blast score and panicle weight, flag leaf area, panicle length and panicle exertion. Negative correlation also was observed between blast score and unfilled grains. The result of path coefficient analysis for this population showed that flag leaf area (length times width) was the most important character in blast score. Stepwise regression for blast score revealed that panicle length and panicle exertion explained most of the observed variation. Factor analysis revealed five factors which explained 77.24% of the total variation. The first factor, named panicle structure, was related to physiological sinks and source. The second factor was a component of length. Increase of the first two factors would increase blast resistance.

13.16 INTRODUCTION OF SOME IMPORTANT ANTAGONISTIC BACTERIA AFFECTING DAMPING-OFF OF CANOLA IN IRAN. S. Sarani, A. Sharifi Tehrani, M. Ahmad Zadeh and M. Javan Nikkhah. *1-Zabol University, Pajouheshkadeh Keshavarzi 2, 3 and 4, Plant Protection Dept., College of Agriculture, Tehran University, Iran. Email: saranisistani@gmail.com*

Three hundred ninety five bacterial isolates were collected from canola root and rhizosphere in Golestan, Mazandaran, Guilan and Tehran provinces. The antagonistic effect of the bacteria on *Rhizoctonia solani* was studied using dual culture assay. The results showed that 60 isolates could inhibit the growth of fungi on PDA medium. On the basis of the biochemical, physiological and morphological tests, isolates Pf41, Pf51, Pf411 and Pf412 were identified as *Pseudomonas fluorescens*, isolate Bu1 as *Burkholderia cepacia*, isolates B1, B2, Bs44 and B6 as *Bacillus subtilis*, and S44 and str45 as *Streptomyces* sp. Studies on the biocontrol mechanism showed that the isolates produced antibiotics and volatile metabolites that prevented mycelial growth. Also the isolates produced some of antimicrobial metabolites including hydrogen cyanide, protease and a siderophore. The isolates significantly reduced disease compare with the control, although none of the isolates were able completely to prevent disease occurrence. The isolates applied as soil treatment had a significantly higher disease control compared to seed treatment. The isolates considerably reduced disease under greenhouse conditions.

13.17 CORRELATION BETWEEN ANTIFUNGAL METABOLITE PRODUCTION OF ANTAGONISTIC BACTERIA AND BIOLOGICAL CONTROL OF *RHIZOCTONIA SOLANI*, CAUSAL AGENT OF CANOLA DAMPING-OFF. S. Sarani, A. Sharifi Tehrani, M. Ahmad Zadeh and M. Javan Nikkhah. *Zabol University, Pajouheshkadeh Keshavarzi, Iran. Email: saranisistani@gmail.com*

Seven strains of bacteria were studied for their ability to suppress damping-off of canola (*Brassica napus*) caused by *Rhizoctonia solani*, including four bacterial species: *Pseudomonas fluorescens* (strains P1, P2 and P3), *Burkholderia cepacia* (strain Bu1), *Bacillus subtilis* (strains B1 and B2) and *Streptomyces* sp.(strain S1). Isolate P3 produced the greatest inhibition in vitro and in greenhouse tests. The various strains had significantly different effects on disease reduction compared with the control. All isolates were able to colonize canola roots in the absence of *R. solani*. P3 was the strongest colonizer. Results of studies on the biocontrol mechanism showed that isolates produced antibiotics and volatile metabolites that prevented mycelial growth. Also the isolates produced some of antimicrobial metabolites including hydrogen cyanide, protease and siderophore. Research showed that in many cases there was no significant correlation between in vitro antibiotic production and effect of bacteria under greenhouse conditions. A significant correlation between the production of other antimicrobial metabolites such as siderophore, hydrogen cyanide and protease was not observed. But there was a relationship between inhibition of fungal growth in vitro, reduction of disease under greenhouse and farm conditions, production of volatile metabolites and colonization.

13.18 FLUORESCENT PSEUDOMONADS MEDIATE RESISTANCE IN RICE PLANTS AGAINST LEAF-FOLDER AND SHEATH ROT DISEASE. **D. Saravanakumar, N. Lavanya, K. Muthumeena and R. Samiyappan.** *Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimibatore 641 003, India. Email: agrisara@rediffmail.com*

A survey was conducted to isolate the fluorescent pseudomonads from different ecosystems of southern India and they were assessed for their growth promoting activity on rice, and relative efficacy against leaf-folder pest and sheath rot pathogen *in vitro*. The results showed significant performance by *Pseudomonas fluorescens* strains Pf1, TDK1 and PY15 when compared to other strains. A combination of strains Pf1, TDK1 and PY15 was effective in reducing sheath rot and leaf-folder incidence in rice plants compared to application of individual strains under glasshouse and field conditions. A biochemical and molecular study on induction of PR proteins (chitinase and beta1,3-glucanase) and defence enzymes (phenylalanine ammonia lyase, scavengers of ROS, lipoxygenase) revealed a greater accumulation of defence molecules in rice plants treated with *P. fluorescens* strains Pf1+TDK1+PY15 against sheath rot and leaf-folder infestation. Further, the application of these *P. fluorescens* strains significantly increased rice yield compared to untreated controls under glasshouse and field conditions. The present study revealed the probable influence of a mixture of *P. fluorescens* strains on antagonism, plant growth promotion and induced systemic resistance (ISR) in enhancing disease resistance in rice plants against sheath rot disease and leaf-folder attack.

13.19 THE EFFECTS OF *BACILLUS PUMILUS*, ISOLATED FROM WHEAT RHIZOSPHERE, ON RESISTANCE IN WHEAT SEEDLING ROOTS AGAINST THE TAKE-ALL FUNGUS. **E. Sari, H.R. Etebarian and H. Aminian.** *Department of Plant Protection, Abourayhan Campus, University of Tehran, P.O. Box 11365/4117, Tehran, Iran. Email: etebar@chamran.ut.ac.ir*

The aim of this study was to verify that induced resistance is another mechanism through which *Bacillus pumilus* 7km can suppress *Gaeumannomyces graminis* (Sacc.) von Arx and Oliver var. *tritici* Walker (Ggt). Plant growth-promotion activity of *B. pumilus* 7km and its effect on disease severity of take-all were also evaluated. Soil was drenched with *B. pumilus* 7km, and disease severity, root and shoot fresh weights and root and shoot heights were measured. The activities of soluble peroxidase (SPOX), ionically cell wall-bound peroxidase (CWPOX), β-1,3-glucanase, β-1,4-glucanase and total phenolic compounds were also determined. The results indicated that disease severity in the bacterized roots was significantly less than the pathogen control roots. Also this isolate promoted root length, and root and shoot fresh weights, compared with the healthy control plants. Wheat plants treated with *B. pumilus* 7km showed increased presence of SPOX, CWPOX, β-1,3-glucanase, β-1,4-glucanase and phenolic compounds in bacterized roots challenged with the pathogen. In this treatment, maximum SPOX, β-1,3-glucanase and β-1,4-glucanase activities were recorded on day 4, and CWPOX activity on day 8. Maximum total phenolic concentration was recorded on day 6. The results suggest that the inhibitory effect of *B. pumilus* 7km on take-all may be related to its ability to enhance defence responses in wheat roots.

13.20 VIRAL TRANSFECTION BY PROTOPLAST FUSION BETWEEN SOMATICALLY INCOMPATIBLE ISOLATES OF *ROSELLINIA NECATRIX*. **A. Sasaki, S. Kanematsu, H. Nakamura, T. Shimane and K. Yoshida.** *Plant pathology Research Team, National Institute of Fruit Tree Science, National Agriculture and Food Research Organization (NARO), Independent Administrative Institution, 2 Fujimoto, Tsukuba, Ibaraki 305-8605, Japan. Email: sasaatu@affrc.go.jp*

The white root rot fungus, *Rosellinia necatrix* isolates W8 and W287 are both weakly virulent, harboring multiple dsRNA segments (L1, L2 and M) that differ in size and signal intensity. M dsRNA in isolate W8 is a member of the *Partitiviridae* (RnPV1-W8). The effect of these dsRNAs has not been clear because virus-free and somatic compatible isolates of W8 and W287 were not available. Here, a viral transmission protocol into somatically incompatible isolates was developed. Protoplasts were prepared from virus-infected isolates (W8 and W287) and virus-free isolates (W37 and W563) transformed with the bacterial hygromycin B resistance gene. Protoplasts prepared from virus-infected and virus-free isolates were fused by the polyethylene glycol-mediated method. Regenerated mycelia were selected for hygromycin B resistance, and the presence of viral dsRNAs was confirmed. W37 and W563 transfected with W287 dsRNAs contained L1 and L2 dsRNAs. When isolate W37 was transfected with W8 dsRNAs, they were all present, but in the case of W563, four types of transfection pattern were observed (M dsRNA only, L1 and L2 dsRNAs, L1 and M dsRNAs, and all three together). Infected dsRNAs in W37 and W563 were transmissible via hyphal anastomosis to their virus-free, respective counterparts. Virus-transfected and virus-transmitted strains showed the same DNA banding pattern by UP- and ISSR-PCR as the original virus-free isolates. Virus-transfected strains with all or L1 and M dsRNAs from W8 indicated growth reduction and reduced virulence, but this was not the case with strains transfected with dsRNAs from W287.

13.21 FERMENTATION: A KEY DETERMINANT OF SUCCESSFUL BIOCONTROL PRODUCT DEVELOPMENT. **D.A. Schisler, C.A. Dunlap, P.J. Slininger, S. Zhang, M.J. Boehm and M.A. Jackson.** *USDA-ARS, National Center for Agricultural Utilization Research, Peoria, Illinois USA. Email: David.Schisler@ars.usda.gov*

Biological control of plant pathogens has enormous potential for solving intractable disease control problems, yet much of this potential remains unfulfilled. Our research addressed a serious impediment to bringing effective biocontrol products to the marketplace the lack of adequate methods for mass producing these agents with superior efficacy and amenability despite the stresses of commercial production. *Cryptococcus flavescens* OH 182.9 reduces *Fusarium* head blight (FHB) severity on wheat by as much as 60% and deoxynivalenol in grain by nearly 30% in field experiments. The carbon loading and C:N ratio of fermentation media were optimized to enhance OH 182.9 product survival and efficacy. We further discovered that prolonged cold adaptation (28 h) of colonized culture broth at 15 C enhanced the storage stability and efficacy of an OH 182.9 product. Cold adaptation improved liquid hyperosmotic shock tolerance and altered the temperature dependence of osmotic shock tolerance. Fluorescence anisotropy and force curves from atomic force microscopy suggested that cold adaptation significantly altered the membrane properties of OH 182.9. In other studies, the feasibility of obtaining the efficacy advantages of microbial mixtures by conducting bi- and tripartite, mixed-strain yeast fermentations was assessed. Growth curves of each component strain were determined by plating on a melezitose-based medium. A co-culture of OH 182.9

and C. *aureus* OH 71.4 that reached equivalent cell densities significantly reduced (32%) FHB disease severity in multiple greenhouse experiments. The possibility of obtaining superior efficacy and cost benefits with mixed-strain fermentation products justifies further evaluation of this approach.

13.22 EFFECT OF HIGH-DENSITY APPLE PLANTINGS ON THE SOIL MICROFLORA AND ITS INTERACTION WITH *DEMATOPHORA NECATRIX* AND *SCLEROTIUM ROLFSII*, CAUSING ROOT ROT DISEASES. Satish K. Sharma, D.K. Kishore and K.K. Pramanick. *IARI Regional Station (Cereals & Horticultural Crops), Amartara Cottage, Cart Road Shimla 171004, Himachal Pradesh, India. Email: satishsharma_27@yahoo.com*

The apple rhizosphere is rich in soil microflora; however, organic matter, increased soil moisture, root activity, and frequent cultural practices influence microbial activity in the soil ecosystem. Microbial populations under different high density apple plantings were studied and evaluated against the soil-borne pathogens *Dematophora necatrix* and *S. rolfsii* under *in vitro* and *in vivo* conditions. The relative abundance of various microorganisms increased with increasing plant densities (S1-S5) and decreased with increasing soil depth. The population of bacteria, actinomycetes, fungi and *Azotobacter* was 25.44-13.05×10^7, 11.55-9.05×10^5, 9.43-6.16×10^4 and 5.38-1.94×10^4 c.f.u /g soil, respectively. The *in vitro* growth inhibition by different antagonistic *Trichoderma* spp. ranged from 64.36 to 92.12% for *D. necatrix* and 33.20 to 67.70% for *S. rolfsii*. Some promising biocontrol agents under *in vitro* conditions evaluated in pots against white root rot revealed that four *Trichoderma viride* isolates were more effective in restricting the mortality of plants in the range of 6.25 to 12.5% as compared to 37.5% in the control, and 25 % with *Trichoderma harzianum*. However, 81.25% of plants showed disease symptoms in the control, 50% with *Azotobacter* and 37.50% with *Trichoderma hamatum*. A few of these micro-organisms are being further evaluated for management of these pathogens in a nursery. In conclusion, high microbial densities in high-density plantings of apple not only help in the healthy growth of plants but also help in combating diseases like root rot through their antagonistic activity.

13.23 ROLE OF FUNGICHROMIN IN CONTROL OF TOMATO LATE BLIGHT BY *STREPTOMYCES* PMS-702 FORMULATION. H.-D. Shih and J.-W. Huang. *Division of Plant Pathology, Agricultural Research Institute, Wufeng, Taichung 41301, ROC. Email: tedshih@wufeng.tari.gov.tw*

Previous studies showed that fungichromin produced by *Streptomyces padanus* Baldacci strain PMS-702 was an effective antibiotic for control of damping-off of cabbage caused by *Rhizoctonia solani*. Laboratory and field tests indicated that *Streptomyces* PMS-702 formulation was also effective in reducing tomato late blight caused by *Phytophthora infestans*. Four compounds, sterol glycoside, daidzein, fungichromin and an unknown compound 4 were obtained from culture filtrate of *S. padanus* PMS-702. Among them, fungichromin was most effective in reducing sporangium production and zoospore release from sporangia. Further tests showed that both culture filtrate and fungichromin were effective in inhibiting sporangial production, sporangial germination, zoospore release from sporangia and cytospore germination of *P. infestans*. These effects were negatively correlated with the dilution of culture filtrate and fungichromin. Observations under the light microscope and scanning electron microscope indicated that fungichromin was able to incite substantial plasma agglutination, cell malformation and cell collapse of the treated sporangia and zoospores of *P. infestans*. It also caused zoospore membrane rupture and plasma leakage. The minimum inhibitory concentration of fungichromin against zoospore release (over 90% inhibition) was 5 ppm. These results suggested that fungichromin played an important role in the mode of action of PMS-702 formulation for controlling tomato late blight.

13.24 ENDOPHYTIC *STREPTOMYCES* SP. MBCN152-1 AS A BIOCONTROL AGENT OF *ALTERNARIA BRASSICICOLA* ON CABBAGE PLUG-SEEDLINGS. M. Shimizu, S. Nakasuji, M. Kubota and K. Nishi. *Graduate School of Bioresources, Mie University, Kurimamachiya, Tsu 514-8507, Japan. Email: shimizu@bio.mie-u.ac.jp*

A seed-borne pathogen *Alternaria brassicicola* causes serious damage on plug-seedlings of cabbage through the growing season in Japan. In this study we isolated and screened endophytic actinomycete strains for biocontrol of *A. brassicicola* on cabbage plug-seedlings. Ninety-eight strains of endophytic actinomycetes were isolated from surface-sterilized cabbage plants (*Brassica oleracea* var. *capitata*). In order to evaluate their biocontrol activities, cabbage plug-seedlings pretreated with mycelial suspension of each strain were spray-inoculated with conidial suspension of *A. brassicicola*. *Streptomyces* sp. strain MBCN152-1 was found to give the highest protection level of all the strains, hence this strain was selected as a candidate. Cabbage seeds artificially infested with *A. brassicicola* were grown for 2 weeks in artificial soil blended with a spore suspension of MBCN152-1. Disease incidence was completely suppressed. Scanning electron microscopy revealed that mycelia of this strain actively grew on the cuticle surface of seedlings as well as beneath it in places. MBCN152-1 did not antagonize any fungal pathogens tested on agar media, indicating that antibiosis may not be a key factor for biocontrol ability and that other factors such as induced resistance and/or nutritional competition may be involved in this suppressive capability. From these results, we consider that *Streptomyces* sp. MBCN152-1 has a high potential in commercial application as an alternative to chemical fungicides. Further studies are in progress to elucidate the biocontrol mechanism.

13.25 MODELLING THE RETENTION OF BOTRY-ZEN® ON GRAPE BUNCHES USING A RAIN SIMULATOR. P. Shorten, P.A.G. Elmer, S.M. Hoyte, M.S. Speirs and T.K. Soboleva. *HortResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand. Email: shoyte@hortresearch.co.nz*

BOTRY-Zen® is a biological control agent whose active ingredient (*Ulocladium oudemanseii*, isolate HRU3) effectively colonises necrotic floral tissues, thereby suppressing *Botrytis cinerea* within winegrape bunches. In 2005-06, application of BOTRY-Zen® during flowering was followed by significant rainfall. The subsequent assessments indicated that the colonisation of grape bunches by *U. oudemanseii* was substantially reduced compared to previous seasons. BOTRY-Zen® use in New Zealand vineyards is increasing and the relationship between rainfall, potential loss of the biocontrol product and subsequent tissue colonisation is not clear. A rainfall simulator was used to determine the rate at which *U. oudemanseii* conidia were washed from exposed detached grape bunches (cv. Chardonnay) under different drying time regimes (1 h and 24 h post spraying) and rainfall intensity (28 mm/h and 40 mm/h). A simple model of this wash-off

process was developed that assumed the rate of conidial wash-off was proportional to the number of conidia on the bunch and the intensity of rainfall. This exponential decay model was fitted to the number of conidia remaining on bunches using least squares, and explained the data well (r^2=0.98). Drying time and rainfall intensity both had a significant effect on the retention of *U. oudemanseii* conidia by affecting the rate of wash-off and the total number of conidia retained on the bunches. The implications of the effects of rainfall on persistence and subsequent disease control with BOTRY-Zen® are discussed.

13.26 EFFECT OF DIFFERENT WETTING CYCLES ON DISPLACEMENT OF *FUSARIUM PSEUDOGRAMINEARUM* FROM CEREAL RESIDUES BY FUNGAL ANTAGONISTS. **D.P. Singh and D. Backhouse.** *Botany, SE&RS, UNE, Armidale, NSW 2351, Australia. Email: dsingh3@une.edu.au*

Fusarium pseudograminearum (Fp) is a stubble-borne fungus that causes crown rot in wheat. Different wetting and drying cycles were explored to test their effects on the efficiency of antagonists in displacement of Fp from stubble. The inoculum of antagonists (*Trichoderma harzianum*, *Fusarium nygamai*, *Fusarium equiseti*, *Alternaria infectoria*) and control (bran inoculum without antagonist) was grown on wheat bran for 5-10 days, air-dried overnight and ground in a sterile mortar with a pestle. Barley straw pieces precolonized with Fp were inoculated with all antagonists using 1.3% methylcellulose solution as adhesive. Straw pieces were placed erect on a spiked metal plate into a square plastic pot. An automated misting system was used to provide a mist frequency of 3s every 10 min for 4, 7 and 10 hours wetness duration per day. Misting stopped at 11am to allow straw to dry before the start of the next cycle. Straw pieces were assessed for displacement after 1, 2 and 3 weeks using an index based on proportion of length of the straw from which Fp could be isolated. In the 4 hr misting cycle *T. harzianum* displaced Fp strongly at 3 weeks. *F. equiseti* and *F. nygamai* showed moderate displacement and *A. infectoria* very low displacement. Increasing the wetting duration did not increase displacement. Even short durations of wetness were sufficient to allow displacement to occur.

13.27 NICHE OVERLAP RELATIVE TO NUTRIENT UTILIZATION PATTERNS OF *FUSARIUM PSEUDOGRAMINEARUM* AND ANTAGONISTS. **D.P. Singh and D. Backhouse.** *Botany, SE&RS, UNE, Armidale, NSW 2351, Australia. Email: dsingh3@une.edu.au*

*Fusarium pseudograminearum (*Fp) is a stubble-borne fungus that causes crown rot in wheat. The hypothesis that the antagonists *Trichoderma harzianum* (Th), *Fusarium nygamai* (Fn), *Fusarium equiseti* (Fe) and *Alternaria infectoria* (Ai) will have overlapping nutrient use profiles with Fp was tested. Growth of the fungi on 95 substrates in BIOLOG FF MicroPlates was compared. The correlation coefficients for utilization patterns of all 3 Fusarium spp. were similar ranging from 46-57%. However Th and Ai had only 17-20% similarity to Fp. We examined growth on mono- and polysaccharides found in straw as sole carbon sources. Galacturonic acid favoured growth of Fp, while Th grew best on arabinose, mannose and xylose. However, different sugars or polysaccharides had only a small effect on antagonism. Different nitrogen sources (urea, nitrate, ammonium) had a significant effect on growth, antagonism, and displacement of Fp from straw, especially by Th. Urea and nitrate favoured Th whereas ammonium favoured Fp. Applying N to Fp-colonized straw enhanced displacement of the pathogen, both when Th was applied as an antagonist and by the natural microflora of soil. Urea and nitrate were much more effective than ammonium. Niche overlap may be important in competition by related fungi, but was less important for strong antagonists like Th.

13.28 INTEGRATED CONTROL OF POWDERY MILDEW ON APPLE BY THE MYCOPARASITE *AMPELOMYCES QUISQUALIS* AND PLANT PRODUCTS. **K.P. Singh, A. Singh, D.C. Dimri and J. Kumar.** *Department of Plant Pathology, College of Forestry & Hill Agriculture, G B Pant University of Agriculture & Technology, Hill Campus, Ranichauri 249 199, Tehri Garhwal, Uttarakhand, India. Email: kps60@rediffmail.com*

The presence of *Ampelomyces* spp. was quantified in samples of naturally occurring powdery mildew fungi collected in appkle orchards from 10 sites of Tehri Garhwal District, India, between 2003 to 2006. *Ampelomyces* pycnidia were found in 34% of the samples. Maximum mycoparasitism was recorded in the cultivar 'Mollies Delicious' followed by 'Bakingham'. The incidence of *Ampelomyces* spp., determined as the proportion of samples containing intracellular pycnidia, varied between 0 and 24.7% in *P. leucotricha* of apple. Maximum powdery mildew growth inhibition (63.54%) was observed after treatment with 10^6 spores ml^{-1} of *A. quisqualis* followed by 52.61% at 10^5 ml^{-1} and minimum (22.45%) at 10^3 ml^{-1} spore concentration, in polyhouse conditions in plastic pots. The antagonistic effect of *A. quisqualis* and plant products (Ajoene, Neemazal) against *P. leucotricha* were further assessed under nursery conditions. In one experiment, spores of *A. quisqualis* alone, significantly decreased disease incidence and severity. However, plant products alone or integrated with the mycoparasite showed significant effects on incidence and severity of powdery mildew. The maximum protection (89.2%) was obtained in alternated sprays at 2 week intervals of *A. quisqualis*, Ajoene and Neemazal treatment followed by mixture of Ajoene and Neemazal (100+250µg ml^{-1}) and *A. quisqualis* once every 4 weeks. The integrated spray schedule improved protection from powdery mildew without affecting further development of the apple seedlings.

13.29 POTENTIAL OF BIOCONTROL AGENTS IN CONTROLLING POTATO SCURF AND RICE SHEATH BLIGHT. **N. Singh, S. Ahmed and S.K. Maan.** *Department of Plant Pathology,Punjab Agricultural University, Ludhiana 141004, Punjab, India. Email: narindermutti@yahoo.com*

Forty one percent of soil samples from different districts of Punjab contained *Pseudomonas fluorescens* whereas 21.20% and 19.50% contained *Trichoderma harzianum* and *T. viride*. Twenty six isolates of *P. fluorescens* were isolated, identified and morphologically characterized. Ten strains each of *T. harzianum* and *T. viride* were also isolated and identified. *P. fluorescens* isolate BP49-4 showed the maximum inhibition zone i.e. 7.0 and 8.0 mm in interaction studies with *Rhizoctonia solani* causing potato black scurf and rice sheath blight respectively. Among the ten isolates of *T. viride*, isolate SK 13-1 was the best, showing 7.0 and 6.5 mm inhibition zones of the respective disease agents. With *T. harzianum* culture filtrate the colony diameter was minimum i.e. 9 and 10 mm with *R. solani* causing potato black scurf and rice sheath blight. The bioefficacy of these antagonists was also tested in the field. Foliar sprays of *P. fluorescens* reduced rice sheath blight up to 37%, increasing the yield up to 7.88%. Seed treatment with *T. viride* strain SK 13-1 of scurf-infected potato tubers

gave a disease index of 21% compared to 58.92% observed in controls, increasing the yield by 3.2%. Treatment with *T. harzianum* of scurf-infected seed potato tubers gave a disease index of 16.31% as compared to 98% observed in controls. The increase in yield over controls was 4.8%.

13.30 EFFICACY OF MIXED BIOFORMULATION OF *TRICHODERMA HARZIANUM* AND *PSEUDOMONAS FLUORESCENS* FOR MANAGEMENT OF FOLIAR BLIGHT OF WHEAT. R.S Singh, S.A. Untoo, N. Singh and S.K. Mann. *Department of Plant Pathology, Punjab Agricultural University, Ludhiana 141004, India. Email: ramassingh@rediffmail.com*

Trichoderma harzianum (Th38) and *Pseudomonas fluorescens* (Pf2), potential antagonists of various plant pathogens were found compatible with each other. Talc-based single and mixed formulations were developed and evaluated against foliar blight of wheat (*Drechslera* and *Alternaria* spp.) on variety HD 2329 in a local in field experiment. Treatments were given as seed treatment (ST) @ 6g/kg seed and a combination of ST + one or two foliar sprays (FS) @ 6g/l. Seed germination, plant growth and disease intensity were recorded. Levels of 66.3-77.5%, 66.9-73.4% and 70.9-73% seed germination were observed after seed treatments of Th38, Pf2 and Th38+Pf2 respectively as compared to 68.7% and 56.2% with treatment of Raxil and in controls. Maximum plant growth and minimum disease incidence were observed with the mixed formulation. Minimum disease intensity, 27.9%, 25.2% and 25.1% were recorded with ST, ST + 1 spray, and ST + 2 sprays with mixed formulation, respectively as compared 30.4% and 41% with Raxil and the control. A similar data trend was recorded on a local variety. Minimum blight intensity 35%, 32.7% and 27.4% were observed with ST, ST + 1 and ST + 2 sprays respectively with mixed formulation, as compared 33.3% and 43.8% with Raxil and the control. Overall, the mixed formulation treatments were the best with maximum plant stands, better growth and minimum disease intensity. Application also enhanced yield 33.6%.

13.31 AFLP ANALYSIS OF ANTAGONISTIC STRAINS OF *METSCHNIKOWIA PULCHERRIMA* FOR BIOLOGICAL CONTROL OF POSTHARVEST DISEASES IN APPLE. D. Spadaro, A. Garibaldi and M.L. Gullino. *AGROINNOVA, University of Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy. Email: davide.spadaro@unito.it*

Seven strains of the yeast *Metschnikowia pulcherrima*, isolated from the carposphere of apples cv. Golden delicious, showed biocontrol capability against *Botrytis cinerea* and *Penicillium expansum*. PCR-RFLP of the 18S+ITS rDNA was tested as a rapid and easy way to identify yeast species. The efficacy of these strains was compared with that of nineteen other *M. pulcherrima* strains isolated from different sources in different geographical regions. The strains were more effective in the control of *B. cinerea* than of *P. expansum*, after storage for 28 days at 4°C, with a mean reduction of pathogen growth to 30.0% and 49.3% of the control, respectively. *M. pulcherrima* strains of different origin were shown to have antagonistic properties. Strain 3043 isolated from grape must offered the best control of both *B. cinerea* and *P. expansum*. To assess the genetic diversity of *M. pulcherrima*, the RAPD and AFLP techniques were used. With six RAPD primers 33 polymorphic bands were obtained, while 729 polymorphic bands were obtained with six AFLP primer pairs. The genetic distances obtained by AFLP were mapped on a dendrogram. Strains isolated in different locations with high genetic diversity could have similar biocontrol potential. One primer pair, such as McaEaa or McgEat, were highly informative and sufficient to describe the genetic distances among the strains. AFLP fingerprints could be used to develop STS markers specific for strains, to improve identification and monitoring of the biocontrol agent in the environment.

13.32 BIOLOGICAL CONTROL OF SUNFLOWER NECROSIS VIRUS DISEASE USING TWO DIFFERENT FORMULATIONS OF PLANT GROWTH-PROMOTING MICROBIAL MIXTURES. K. Srinivasan, M. Jayaprakashvel and N. Mathivanan. *Biocontrol and Microbial Metabolites Lab, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, India. Email: seenu_cas@rediffmail.com*

Sunflower necrosis virus disease (SNVD) severely affects sunflower cultivation in India with disease incidence up to 90%. Biological control is the best option for managing SNVD as no other effective method is available. In the present study, powder and liquid formulations of *Bacillus licheniformis* MML2501 (Bl), *Streptomyces fradiae* MML1042 (Sf), *Pseudomonas aeruginosa* MML2212 (Pa) and *Bacillus* sp. MML2551 (Bsp) were developed individually and evaluated as mixtures of three (Bl + Pa + Bsp) or four cultures (Bl + Sf + Pa + Bsp) against SNVD in the field. These formulations remarkably enhanced plant growth and reduced SNVD, thereby increasing seed yield. SNVD reduction was estimated as up to 51.4% and 40.9%, using the four-cultures mix as liquid or powder, respectively, compared to the control. Further, it significantly increased the yield parameters more than the other treatments. The liquid four-culture formulation gave an additional seed yield of 936 kg/ha with an additional income of 212 €/ha whereas the talc formulation gave an additional seed yield 840 kg/ha with an additional income of 190 €/ha compared to control. The results of field experiments clearly showed that both the four-culture formulations can be used for the effective control of SNVD.

13.33 BIOLOGICAL CONTROL OF *BOTRYTIS* DISEASES BY THE ANTAGONISTIC FUNGUS *TRICHODERMA ATROVIRIDE*. A. Stewart, S.D. Card and J.S. Hunt. *National Centre for Advanced Bio-Protection Technologies, P.O. Box 84, Lincoln University, Canterbury, New Zealand. Email: stewarta@lincoln.ac.nz*

Trichoderma atroviride LU132 was effective against *Botrytis* grey mould on strawberry and grapevines in laboratory, glasshouse and field tests. *T. atroviride* significantly suppressed *B. cinerea* sporulation on detached strawberry leaves and whole plants by up to 85%. This was equal to the control given by the fungicide fenhexamid. Across all field trials, *T. atroviride* gave suppression of *B. cinerea* sporulation on leaves and stamens by 52-100% and increased yield of strawberry fruit by up to 36% more than the control. A wettable powder formulation of *T. atroviride* was applied to grapevine var. Chardonnay at 80% capfall prior to *B. cinerea* challenge. Further trials tested different times of application of *T. atroviride* on var. Sauvignon Blanc and levels of bunch rot disease were compared to the untreated control and a standard fungicide spray programme. *T. atroviride* consistently reduced *Botrytis* bunch rot incidence in grapevines across all trials compared to the control (65-98%). The biocontrol agent gave equal or better control than the standard fungicide programme used at each site. *T. atroviride* was shown to protect the strawberry and grape tissues from *B. cinerea* colonization by

competitive exclusion and suppression of secondary inoculum production. Agrimm Technologies Ltd has commercialized *T. atroviride* LU132 for control of *Botrytis* grey mould on grapes. Sentinel® is formulated as a wettable powder for spray application at 5% and 80% capfall, pre-bunch closure and veraison. Sentinel® is registered for use on grapes and tomatoes in NZ with label claim extensions currently being sought for strawberry and berryfruit.

13.34 EFFECT OF MICROBIAL MIXTURES ON THE CONDITION OF VEGETABLE CROPS UNDER MULTIPLE-PATHOGEN STRESS. **M. Szczech and B. Kowalska.** *Research Institute of Vegetable Crops, Konstytucji 3-Maja 1/3, 96-100 Skierniewice, Poland. Email: mszczech@inwarz.skierniewice.pl*

A serious problem with biocontrol agents is their inconsistent performance. However, a community of active microorganisms with diverse mechanisms of disease suppression may enhance the reliability of control. In this work several PGPR bacteria exhibited different modes of action (antagonism, plant growth stimulation) and antagonistic *Trichoderma harzianum* strain PBG were selected for use in combination to control multiple soil-borne pathogens of tomato and cucumber. In greenhouse experiments a potting medium was infested with a cocktail of three pathogenic isolates of *Fusarium* spp. and *Rhizoctonia solani*. Two types of bioassay were performed. In one, the seeds were sown in infested medium and treated with suspensions of single biocontrol agents or of their mixtures. The influence of combined microorganisms on seedlings number, and on their growth in the stressful conditions was studied. For other bioassays, transplants treated with the mixtures or separate components were produced in "clean" medium and then planted in medium infested with the pathogens. The condition of the plants and the effect of the mixtures on the appearance and severity of the diseases were observed during the vegetation period. The yield was estimated and compared to controls and to the yield of treated plants grown simultaneously in non-infested potting medium. At the end of the experiments the degree of colonisation of the plant roots by pathogenic fungi was measured. It was found that the microbial mixtures, especially in the case of tomato, increased plant stands, and some of the combinations enhanced the yield of both plants in the medium infested with multiple pathogens.

13.35 BIOLOGICAL CONTROL OF BLUE MOULD OF APPLE BY *TRICHODERMA* ISOLATES. **F. Tabebordbar, H.R. Etebarian, N. Sahebany and H. Rohany.** *Department of Plant Protection, Abourayhan campus, University of Tehran, P.O. Box 11365/4117,Tehran, Iran. Email: f_tabe58@yahoo.com*

In the present study, the antagonistic effects of 11 isolates of *Trichoderma* were evaluated as potential biological control agents for blue mould of apple caused by *Penicillium expansum*. *Trichoderma* isolates inhibited the mycelial growth of *P. expansum* in dual culture by 55.16 to 69.26%. Volatile metabolites emitted from all isolates inhibited growth of *P. expansum* and the inhibitory effects of *T. harzianum* (T.301.11, T.401.4) were greater compared with the other isolates. Cell-free metabolites of *T. viride* (T.193, T.301.4) and *T. harzianum* (T.301.11, T.404.8) inhibited the pathogen by 58.21, 81.67, 87.06 and 88.36%, respectively. The concentration of pathogen and antagonist for inoculation were 1×10^5 cfu/ml and 1×10^7 cfu/ml, respectively. The antagonists significantly reduced decay area caused by *P. expansum* at 23ºC and 5ºC. *T. harzianum* (T.192) at 23ºC and *T. harzianum* (T.404.8) and *T. viride* (T.193) at 5 ºC had the best effect on decay reduction.

13.36 IMPROVEMENT OF BIOCONTROL AGENT VIABILITY DURING FORMULATION BY INDUCTION OF HEAT TOLERANCE. **N. Teixidó, T.P. Cañamás, M. Abadias, R. Torres, C. Solsona and I. Viñas.** *IRTA, UdL-IRTA centre, XaRTA-Postharvest, 191, Rovira Roure Av., 25198 Lleida, Catalonia, Spain. Email: neus.teixido@irta.cat*

The yeast *Candida sake* CPA-1 is an effective biocontrol agent (BCA) against major postharvest diseases on pome fruits. Optimal formulation is necessary to successfully bring BCAs into commercial use. Unfortunately, most of microorganisms are very sensitive to the drying involved in formulation. The aim of this study was to induce tolerance in *C. sake* cells by mild heat treatment in order to enhance its viability during drying processes, in this specific case during spray-drying. The possible role of synthesis of heat shock proteins (HSPs) and accumulation of sugars and sugar alcohols was also determined. First, 30ºC and 33ºC were selected as temperatures optimal for inducing thermotolerance in *C. sake* cells during incubation in molasses-based liquid medium. Mild heat was applied at different growth phases. Both temperatures induced thermotolerance, tested by exposure to lethal shock at 40ºC. Cells adapted at 33ºC in early or mid stationary phases showed survival values after spray-drying significantly higher than unadapted cells. However, viabilities were not high enough to be suitable for commercial use. Furthermore, it was shown that HSPs, sugars and sugar alcohols were not directly responsible for induced thermotolerance in this yeast. In conclusion, it is possible to induce thermotolerance in biocontrol agents, and this approach can be used to improve viability of cells during formulation.

13.37 BIOCONTROL OF *BOTRYTIS* BUNCH ROT IN GRAPEVINE USING *CANDIDA SAKE*. **N. Teixidó, T.P. Cañamás, R. Torres, C. Solsona, E. Cases, J. Usall and I. Viñas.** *IRTA, UdL-IRTA centre, XaRTA-Postharvest, 191, Rovira Roure Av., 25198 Lleida, Catalonia, Spain. Email: neus.teixido@irta.cat*

Bunch rot caused by *Botrytis cinerea* is an important disease of grapevine. There is increasing interest in the use of biocontrol agents (BCAs) and other environmentally safe methods alternative to chemical fungicides. Biosuppression of *B. cinerea* in vineyards using BCAs has been inconsistent, compared with results in controlled conditions, due to the variable field environment. Research has been directed towards developing formulation strategies capable of enhancing the activity of BCAs. Stress adaptation methods of *C. sake* have been developed to improve performance under field conditions and survival during formulation. Cells adapted to mild heat showed greater thermotolerance than unadapted cells. In this three-season study, different preparations of *C. sake* cells were applied at flowering, pea sized berries, veraison and before vintage in order to evaluate level of *Botrytis* control at harvest. Strategies used included liquid formulation, mild heat adaptation and additives. Results indicated that applications of different preparations of *C. sake* resulted in colonisation of bunches under field conditions. Population sizes of *C. sake* supplemented with the food film Fungicover showed survival rates significantly higher than for other treatments. No improvements were observed in survival rates with different formulations and heat-adaptation strategies. In general, yeast treatments significantly reduced the incidence of *B. cinerea* with respect to the con-

trol. *C. sake*+Fungicover treatment were the most effective, with rot reduction levels of 90%. These results demonstrated the potential of *C. sake* to control *Botrytis* bunch rot and the importance of improving BCA formulations.

13.38 ECOLOGY OF FUNGI ASSOCIATED WITH OAK POWDERY MILDEW, *ERYSIPHE ALPHITOIDES*. **E.T. Topalidou and M.W. Shaw.** *School of Biological Sciences, The University of Reading, Whiteknights, Reading RG6 6AU, UK. Email: etopalidou@gmail.com*

Powdery mildew (*Erysiphe alphitoides)* on oak (*Quercus robur)* was extremely commonly intimately associated with several other fungi of which *Trichoderma* sp., *Acremonium* sp. and *Leptosphaerulina* sp. – the commonest – were successfully isolated on artificial media, but also often including *Tilletiopsis* sp. and *Phoma/Ampelomyces*. The three symbionts isolated had no directly visible effects on young oak leaves. Symbionts were applied to oak leaves, previously inoculated with powdery mildew, singly and in combinations of two. The treated oak leaves were covered with permeable transparent bags to reduce entry of external micro-organisms. Visual assessments of powdery mildew were made every week, and when leaves were about 80%-100% covered with powdery mildew a final assessment was made microscopically. *Leptosphaerulina* sp., which is not otherwise a common hyperparasite of powdery mildews, inhibited mildew; *Acremonium* sp. increased powdery mildew severity; the severity on leaves treated with *Trichoderma* sp. was similar to control leaves, although sporulation was reduced. Effects differed with leaf age; inhibition of powdery mildew on very young leaves was difficult. *Leptosphaerulina* appeared to reproduce exclusively by ascospores from small pseudothecia; these were discharged at relative humidities down to 54%. This suggests that these fungi may interact to limit reproduction of oak powdery mildew to the very early part of the season, but also to limit one another's effects. Oak powdery mildew must form an environmental reservoir of these agents, and *Leptosphaerulina* sp. was active against other, commercially important, mildews such as *Sphaerotheca macularis* which causes strawberry powdery mildew.

13.39 PCR-BASED CHARACTERIZATION OF ANTIBIOTICS-PRODUCING *PSEUDOMONAS FLUORESCENS* ISOLATES AS POTENTIAL BIOCONTROL AGENTS. **K. Tsuchiya, S. Sugisawa, N. Someya, T. Yoshida, H. Sawada, M. T. Noguchi and N. Furuya.** *Kyushu University, 6-10-1, Hakozaki, Fukuoka 812-8581, Japan. Email: kentsuch@agr.kyushu-u.ac.jp*

Collections of *Pseudomonas fluorecens* isolated from the plant rhizosphere varied in spectra and intensity of antagonism to pathogens such as *Gaeumannomyces graminis* var. *tritici* (Ggt) and *Rhizoctonia solani* (Rs), suggesting that various antibiotic substances were involved. The strains were screened by PCR-based detection of the genes encoding synthesis of antibiotic substances 2,4-diacetylphloroglucinol (Phl), pyrrolnitrin (Prn), and pyoluteorin (Plt) by using specific DNA primers. Presumed DNA amplification bands produced were confirmed by Southern hybridization with standard strains. Some strains were positive for one (Phl), others for two (Phl, Prn), and the remaining strains were positive for all (Phl, Prn, Plt). Production of Phl and Prn was identified as expected by TLC. Strains that produced multiple substances did not always show higher disease suppression ability than those producing single substances, suggesting amount of active compound against a kind of target pathogen. Some strains such as LRB3W1 also produced IAA, which may combine promising biocontrol agents (BCAs) with growth promotion. Alginate gelation of wheat seeds, together with treatment with BCAs suppressed take-all of wheat more effectively than direct treatment of seeds with those strains alone. The method may help to increase fixation of BCAs to plant roots. To analyze the behavior of BCA-producing strains on wheat seeds and roots, we engineered the strain to express a green fluorescent protein (Gfp) constitutively, and we then confirmed that the target strains moved along the geminating root(s) from the seed and colonized them.

13.40 CAN FILTRATION HELP TO ELIMINATE *PHYTOPHTHORA* SPP. FROM RECYCLING WATER IN COMMERCIAL NURSERIES? **T. Ufer, M. Posner, H.-P. Wessels and S. Werres.** *Federal Biological Research Center for Agriculture and Forestry, Institute for Plant Protection in Horticulture, Messeweg 11/12, 38104 Braunschweig, Germany. Email: s.werres@bba.de*

The production of hardy ornamental nursery stock in containers is a profitable and increasing part of the horticultural industry in Germany. The plants are cultivated outdoors on special areas where the surplus water from irrigation and natural precipitation is collected via drainage systems and stored in reservoirs for reuse. From epidemiological studies in commercial nurseries it is well known that plant pathogens of the genus *Phytophthora* can be present in the water and in the sediment of these water recycling systems and that they can be easily spread with contaminated irrigation water. In a four year project three designs of slow sand filtration and one lava grain filtration system (Shieer Bio-Filter®) were tested in commercial nurseries to eliminate the pathogens from recirculation water. Samples were taken at three dates during the season (May, August, October) starting in August 2003 (after the first four months of filter operation) ending in October 2006, and at four places in the recycling system (runoffs, retention reservoirs, filter effluents, clean water reservoirs) of each nursery. All four filtration systems eliminated *Phytophthora* spp. successfully and produced sufficient quantities of water for nursery production. The maximum annual quantity of water demanded by the nurseries ranged from 30,000 m^3 up to 163,000 m^3.

13.41 *PYTHIUM OLIGANDRUM* BIOCONTROL IN THE RHIZOSPHERE: INFLUENCE ON FUNGAL POPULATION DYNAMICS. **J. Vallance, G. Le Floch, F. Déniel, J.J. Godon and P. Rey.** *LBEM (EA 3882), ESMISAB, Université de Bretagne Occidentale, Technopôle Brest-Iroise, 29280 Plouzané, France. Email: jessica.vallance@univ-brest.fr*

In this study, fungal populations and their dynamics were investigated in relation to the introduction of the biocontrol agent *Pythium oligandrum* in the rhizosphere of tomato plants grown in greenhouses. *P. oligandrum* is known to display antagonistic activities against several species of pathogenic fungi including *Pythium* group F, which are ubiquitous tomato root minor pathogens in soilless cultures. *P. oligandrum* can also produce an elicitor of plant defence named oligandrin. One limitation to its use is the lack of persistence of the fungus in the rhizosphere. The research was done by inoculating roots of tomato plants grown in hydroponic culture with three selected strains of *P. oligandrum* displaying distinctive traits. These strains were then monitored over time to evaluate their persistence and their effect on the microflora. The strains were detected by real-time PCR at high rates

throughout the 8-month experiment but did not seem to influence the root distribution of other *Pythium* species, especially *Pythium* group F. Inter simple sequence repeat (ISSR) analysis of *P. oligandrum* isolates collected at the end of the growing season, showed that 90% of them belonged to only one of the three selected strains. Single-strand conformational polymorphism (SSCP) and cloning/sequencing showed that introduction of the antagonistic fungus has only a slight influence on the native fungal community and its dynamics.

13.42* BIOLOGICAL CONTROL OF *SCLEROTINIA SCLEROTIORUM* USING *TRICHODERMA HARZIANUM* AND SILICON. D.D. Visser, P.M. Caldwell and N.W. McLaren. *Discipline of Plant Pathology, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa. Email: caldwellp@ukzn.ac.za*

Sclerotinia sclerotiorum (Lib.) de Bary, the causal organism of *Sclerotinia* stem rot (SSR) has become a major pathogen of soybeans in South Africa (SA), particularly in the wetter growing areas. *In vitro* dual culture bioassays were performed to identify possible bio-control mechanisms of EcoT® and Eco77® against hyphae and sclerotia of *S. sclerotiorum*. Ultrastructural studies using ESEM revealed that mycoparasitism is possibly the mode of action as initial signs of hyphae of EcoT® and Eco77® coiling around hyphae of *S. sclerotiorum* were observed. Surface colonization of sclerotia by hyphae of EcoT® and Eco77® and enzymatic degradation was also observed. The effect of Eco77® together with silicon (Si), was tested *in vivo* against *S. sclerotiorum*. Soybean plants were inoculated using the cut stem method. Si (100 ppm), in the form of potassium silicate, was applied in irrigation water to the soil weekly from the V1-V5 stage. Eco77® was sprayed onto plants 2 days before inoculation. Plants treated with Eco77® had a significantly lower rate of disease development (0.382) compared to plants not treated with Eco77® (0.568), regardless of the application of Si. Similarly, plants treated with Eco77® had a significantly lower number of sclerotia (1) compared to plants not treated with Eco77® (3). The significantly lower rate of disease development coupled with a significant reduction in sclerotia showed that Eco77®, and not Si, was responsible for reducing the severity of SSR. A strong positive correlation between rate of disease development and the number of sclerotia produced (R^2= 0.88) was observed.

13.43* DETECTION AND QUANTIFICATION OF *PSEUDOMONAS FLUORESCENS* BIOCONTROL STRAINS BY A STRAIN-SPECIFIC REAL-TIME PCR APPROACH IN THE FIELD. A. von Felten, G. Défago and M. Maurhofer. *Plant Pathology, Institute of Integrative Biology, ETH Zürich, 8092, Zürich, Switzerland. Email: andreas.vonfelten@agrl.ethz.ch*

Pseudomonas fluorescens strains F113, CHA0 and Pf153 are well known biocontrol agents that suppress soil-borne pathogens on different host plants mainly by producing antimicrobial metabolites. These strains are also known to solubilize inorganic phosphates and produce plant-growth hormones, therefore they could also have an effect in plant growth promotion. In order to study biocontrol performance, persistence and proliferation of the three *P. fluorescens* strains under field conditions, unequivocal strain identification and a rapid method for monitoring their population dynamics is needed. A promising approach is based on the sequence-characterised amplified regions (SCAR) technique, which has been extensively used for different organisms. SCAR markers in combination with real-time PCR can then be used for quantitative monitoring of these specific strains in field trials. First results will be presented. Authors acknowledge the support of the Micromaize project 6th FWP, funded by the European Commission.

13.44 CLONING AND FUNCTIONAL CHARACTERIZATION OF THE GENES RELATED TO WHEAT RHIZOSPHERE COLONIZATION IN *BACILLUS CEREUS*. Y. Wang, X.H. Gu, W. Jiang, Q. Wang and R.H. Mei. *Department of Plant Pathology, China Agricultural University, Beijing, P.R. China. Email: wangqi @cau.edu.cn*

Rhizosphere inhabitants interact intricately with the plant host. *Bacillus cereus* 905 isolated from wheat rhizosphere colonized wheat rhizospheres with large population sizes. In this work, we obtained the flagellin gene and the chemotaxis *cheA* gene by PCR, and acquired mutants lacking the chemotaxis *cheA* gene or the flagellin gene by homologous recombination. Using biological methods, it was found that the mutants colonized wheat roots at a lower rate than the wild type. The role of superoxide dismutases (SODs) of *B. cereus* 905 in surviving in the wheat rhizosphere was also analyzed. Two genes, *sodA-1* and *sodA-2* encoding two distinct manganese SODs (MnSODs), were isolated from the bacterium. Three mutant strains have been created, each lacking either *sodA-1*, *sodA-2* or both. Analysis of these mutant strains revealed differences in SOD transcription and enzymatic activity. MnSOD2, encoded by sodA-2, plays a more important role in antioxidative stress. MnSOD1, the product of *sodA-1* gene, is expressed at a lower level. The function of the two MnSODs appears to be essential in colonization of wheat rhizosphere. In conclusion, the *cheA* gene, flagellin gene, and *sodA* genes of *B. cereus* B905 play important roles in surviving in the wheat rhizosphere.

13.45 BACTERIA COLONIZING MELON FRUIT SURFACE ACT AS BIOCONTROL AGENTS TO POSTHARVEST DISEASE PATHOGENS. Y. Wang, L. Xu, Y. Mastuda and H. Toyoda. *School of Life Science, East China Normal University, Shanghai 200062, P.R. China. Email: lxu@bio.ecnu.edu.cn*

The present study was conducted to select antagonistic bacteria that superficially colonize melon fruits and to clarify their suppressive functions against fungal pathogens, aiming at developing practical biocontrol agents for post-harvest diseases of melon fruits. Bacteria colonizing melon fruits were obtained by printing fragmented pericarps onto LB culture medium. Antifungal activity was screened by pair-culturing with fungal pathogens, and identified on the basis of DNA sequence alignments and conventional bacterial characteristics; one was identified as *Bacillus amyloliquefaciens* EXWB3, and the others *B. subtilis* EXWB1, EXWB2 and EXWB4. Four isolates expressed significant antifungal activity to eight postharvest pathogens, *Botrytis cinerea*, *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus niger*, *Trichothecium roseum*, *Penicillium* sp. and *Cladosporium* sp. Of the antagonistic isolates tested, EXWB1 was most effective, suppressing conidial germination of *B. cinerea*, *A. alternata* and *F. oxysporum* and inducing darker cell walls and abnormal conidial shape. The isolate secreted some bio-surfactants, which helped the sprayed bacteria to stick to the hydrophobic fruit surface. Melon fruits treated with EXWB1 retained high levels of sugar, vitamin C and organic acids even after inoculation with *A. alternaria* and *F. oxysporum*. Additionally, EXWB1-26 treatment suppressed the respiration increase and ethylene production that were typical in

pathogen-inoculated fruits, and limited both fungal growth and expansion of the necrotic lesions produced by pathogen inoculation. Thus, the present study provides a promising biological agent to suppress fungal pathogens causing postharvest market diseases of fruit crops.

13.46 RESEARCH ON *PENICILLIUM OXALICUM* ISOLATES. **T. Wenhua, A. Turok, W. Qi and X. Yang.** *Depart. of Plant Pathology, China Agricultural University, Haidian, Beijing 100094, P.R. China. Email: wenhuatang@vip.sohu.com or tangwh@cau.edu.cn*

Hundreds of isolates of *Penicillium oxalicum* were obtained from soil and roots of wheat on selected medium for phosphate-dissolving micro-organisms. Several isolates showed ability to promote wheat seedling growth in pot experiments. The reasons for this were examined. Content of active phosphate increased in soil for some isolates, particularly in soil amended with phosphorite powder. Some isolates, while promoting plant growth, did not increase active phosphate content significantly. However, active phosphate content in the fungus body was significantly increased. Analysis of phytohormone concentrations in fermented cultures of the isolates showed that IAA content was higher in acidic conditions. Resistance induced by fermented cultures of *P. oxalicum* (*P-o- 41*) was determined on *Xanthi*^{N} tobacco with the method of separating treated leaves and leaves inoculated with TMV. Results showed that reduction rate of spots comparing with CK (water treatment) was significant. No significant difference was found comparing with treatment by 38 ppm BTH. In experiments on control of wheat powdery mildew conducted in the greenhouse, disease incidence reduction was significant, but not for control of common root rot caused by *Bipolaris sorokiniana*. Controlling take-all with *P. oxalicum* culture filtrate was tested in field trails. The results showed that disease incidence was reduced and yield increased. This project was supported by national 863 project 2006AA10A211.

13.47 *BACILLUS THURINGIENSIS* B24-14 AND ITS FORMULATIONS FOR CONTROLLING *MELOIDOGYNE INCOGNITA*. **T. Wenhua.** *Dept. of Plant Pathology, China Agricultural University, Beijing, P.R. China. Email: wenhuatang@vip.sohu.com*

Meloidogyne incognita is a nematode causing widespread disease in China, particularly in vegetable-growing areas. A bacterial isolate, B24-14, was isolated from Gobi desert soil located in Gansu province. The bacterium was identified as *Bacillus thuringiensis* based on analysis of 16SrDNA and Bergey Manual system. β-exotoxin produced by B24-14 was obtained by distillation of alcohol graded deposition. Nematocide effectiveness of the β-exotoxin was determined on *Bursaphelenchus xylophilus*. Responding to 8 h treatment of β-exotoxin (4 mg/ml) nematode motility reached 93.75%, Lc 50=574 μg/ml. The bacterium was cultured in beef extract and peptone medium on a shaker at 37°C for 36 h. Solid preparations were made by evaporation of the culture broth at high pressure. The effect on controlling root knot caused by *M. incognita* of preparations was evaluated by pot experiments in the greenhouse. Dosage of both preparations used in the testing was comparable. Experiments lasted for 40 days. Numbers of root knots were recorded and compared. The results showed that disease index was reduced by treatments with both preparations. The efficacy compared with non-treated CK reached 89.5% and 72.4% respectively with the same dosage. This project is supported by national 863 project 2006AA10A211.

13.48 VARIETAL, CULTURAL AND CHEMICAL MANAGEMENT OF STRIPE RUST ON WHEAT IN EASTERN AUSTRALIA. **P. Wilkinson.** *Department of Primary Industry & Forestry, Leslie Research Station, Toowoomba, QLD 4350, Australia. Email: peter.wilkinson@dpi.qld.gov.au*

The re-emergence of stripe rust as a disease of significance across the whole of the Australian grain growing regions has required evaluation of cultural and chemical control options for this disease. There is a need for an improved understanding of the value of various levels of resistance to stripe rust in commercial wheat varieties in the northern wheat producing regions of eastern Australia. Resistant varieties of wheat present the best long term option for management of this disease. There was a greater disease response to timing of pesticide application then to registered dosages.

13.49 ISOLATION OF *DIAPORTHE* AND *PHOMOPSIS* FROM SOYBEAN PLANTS IN ONTARIO AND BENEFITS OF SEED TREATMENTS. **A.G. Xue, M.J. Morrison, E. Cober, Y. chen and J.X. Zhang.** *Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada (AAFC), 960 Carling Avenue, Ottawa, ON, K1A 0C6, Canada. Email: axue@agr.gc.ca*

Diaporthe-Phomopsis complex, caused by *Diaporthe phaseolorum* var. *caulivora*, *D. phaseolorum* var. *sojae*, and *Phomopsis longicolla*, is an important disease of soybean. To determine the predominant causal species in Ontario, 2,700 stem tissues, 2,700 pod tissues, and 9,000 seeds from diseased plants were collected at nine locations each year from 2002 to 2004. A total of 17,280 isolates of the pathogens were recovered, of which 73% were from stems, 23% from pods, and only 3% from seeds. *P. longicolla* was the predominant species (41% of isolates), followed by *D. phaseolorum* var. *caulivora* (37%), and *D. phaseolorum* var. *sojae* (22%). We used *Diaporthe* and *Phomopsis* infested seeds, 10 formulated fungicides and 2 bioagents to evaluate disease at two sites each year from 2002 to 2004. Significant increases ($P \leq 0.05$) in emergence and yield were observed for 8 and 2 of 11 seed treatments in 2002, 7 and none of 19 treatments in 2003, and all of 18 treatments in 2004. All 19 treatments in 2003 and 13 out of 18 treatments in 2004 reduced root rot severity. These treatments increased emergence by 2 to 10%, and yield by 7 to 19%, and reduced root rot severity by 20 to 79%. Results of this study provide evidence that fludioxonil, difenoconazole, triazolinthion, and trifloxystrobin protect soybean from seed-borne *Diaporthe* and *Phomopsis* and increase plant emergence and yield, and that the effectiveness of these fungicides may be enhanced when used in combination with the bioagents.

13.50 PRELIMINARY STUDY ON BIOLOGICAL CONTROL OF ANTAGONISTIC BACTERIUM CE ON PEACH BROWN ROT. **H.Q. Yang, Y.M. Wei, Q.X. Shang, X.Y. Zhao, S.H. Liu and Z.P. Liu.** *Department of Plant Science and Technology, Beijing Agricultural College, 102206 Beijing, P.R. China. Email: liuzhengping8882000@yahoo.com*

The bacterial strain CE was isolated from soil of a peach orchard. The antagonism of CE against *Monilia fructigena* was tested using different methods. CE had shown antagonism against hyphal growth of *M. fructigena*. After incubation for five days, the width of the inhibition zone was 9.0 mm and the hyphae of *M. fructigena* were seen light microscopy to be abnormal. Peach fruits were disinfected with 2% sodium hypochlorite for 2 min,

washed 5 minutes with tap water, and air-dried prior to wounding. A uniform 5 mm deep × 10 mm wide wound was made at the equator of each fruit using a sterile dissecting needle. CE was inoculated on the wound of each fruit and then after 12 h inoculated with *M. fructigena*. The controls were inoculated with *M. fructigena* only. All peaches inoculated only with *M. fructigena* showed disease symptoms the day after inoculation, 56% of those inoculated with *M. fructigena* after CE showed symptoms the fifth day after inoculation. Antifungal proteins produced by CE were obtained from liquid culture after 48h dark incubation, and were isolated by PAGE (12% resolving gel and 5% stacking). About 15 different bands were resolved. We cut out the smallest band from PAGE and crushed it, then added phosphate buffer from which the small protein was extracted by centrifugation for 20 minutes at 6,000 rpm. This protein showed antagonism against hyphal growth of *M. fructigena*. Thanks to Academic Human Resources Development in Institutions of Higher Learning under the Jurisdiction of Beijing Municipality (PXM2007-014207-044536).

13.51 SUPPRESSION OF INVADING PATHOGENS THROUGH A BENEFICIAL PLANT-BACTERIA ASSOCIATION BETWEEN RICE AND *RHIZOBIUM*. **Y. Yanni, F. Dazzo and R. Mishra.** *Sakha Agricultural Research Station, Kafr El-Sheikh, 33717, Egypt. Email: yanni244@hotmail.com*

A total of 19 field trials in the Nile delta clearly established the potential use as biofertilizers of selected strains of *Rhizobium* that naturally colonize rice roots and can enhance crop production while reducing the need for N-fertilizer. A major multinational network of investigators was created to obtain a broad-based understanding of the physiology of this association. Although our contributions improved our understanding of such beneficial associations, complete information is still very far. Our studies showed that rhizobial inoculation of rice may trigger enhanced production of phenolic acids involved in defence reactions during pathogenic ingress. HPLC analysis of different rice plant parts after inoculation with two *Rhizobium* strains as well as *Rhizoctonia solani*, the causative agent of rice blast disease, revealed the induction of phenolic acids such as gallic, tannic, ferulic and cinnamic acids which mediate defence responses against phytopathogens that cause various devastating diseases. The exact mechanism used by the *Rhizobium* to alter the phenolic profiles is still not clear but bacterial endophytes are reported to exert their beneficial effect in two possible ways: 1) by extensive colonization of internal plant tissues and suppression of invading pathogens by niche occupation, antibiosis or both; and 2) by colonization of the root cortex, where they stimulate general plant systemic defences/resistances. It is possible that endophytic rhizobia employ the same mechanisms to protect plants and promote their growth while colonizing their root tissues and enhance plant growth and crop performance.

13.52 BACTERIA AND CHITINASE GENES IN VERMICOMPOST WITH ANTIFUNGAL ACTIVITY. **M. Yasir, C.O. Jeon and Y.R. Chung.** *Division of Applied Life Sciences, EB-NCRC, Gyeongsang National University, Jinju 660-701, Republic of Korea. Email: yrchung@gnu.ac.kr*

Paper sludge supplemented with dairy sludge composted by earthworms (vermicompost) has shown antifungal activity against plant pathogens. The antifungal mechanism was elucidated in terms of bacterial and chitinase gene diversities of the vermicompost compared to the fresh sludge. The bacterial diversity was examined by culturing and molecular methods. Clone libraries of the 16S rRNA gene were constructed from the genomic DNA isolated from both samples. The resulting 252 clones were analyzed by ARDRA using tetrameric restriction enzymes (*HhaI* and *HaeIII*) and the selected 133 clones were sequenced. The sequences from the fresh sludge fell into major lineages of the domain bacteria, *Proteobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Actinobacteria* and *Firmicutes*. In vermicompost, the abundance of various taxa was different, mainly *Bacteroidetes* from the fresh sludge. The DGGE analysis showed a consistent shift of the bacterial community from *Proteobacteria* to *Bacteroidetes* in the fresh sludge to vermicompost. The chitinase gene population was investigated by using degenerate primers. More than 100 colonies among 300 colonies isolated from both samples were randomly sequenced for 16S rRNA gene analysis. A high population of *Actinobacteria* was found in vermicompost, while almost equal numbers of *Actinobacteria* and *Proteobacteria* were in the fresh sludge. The population of chitinolytic and antifungal bacteria active against *Rhizoctonia solani*, *Colletotrichum coccodes*, *Pythium ultimum* and *Phytophthora capsici* was higher in vermicompost than the fresh sludge. Chitinolytic isolates were particularly active against target fungi.

13.53 PURIFICATION AND BIOLOGICAL ACTIVITY OF AN ANTIFUNGAL PROTEIN FROM *BACILLUS SUBTILIS* BS-916. **L. Yongfeng, L Fan, C. Zhiyi, Z. Jie, Z. Mingguo, S. Fuping, L. Youzhou and N. Yafeng.** *No. 50 Zhongling street, Nanjing, P.R. China. Email: Liuyf@jaas.ac.cn*

Strain Bs-916 of *Bacillus subtilis* was isolated from rice field soil and used in controlling rice sheath blight and rice false smut in the field. We discovered that Bs-916 secretes an antifungal protein, and we purified this protein using the Amersham AKTA Explore system. The crude proteins of Bs-916 were eluted through a DEAE Sepharose Fast Flow column and three peaks were found; only the second fraction (B) had antifungal activity. After fraction B peaks were pooled and eluted in a Phenyl Sepharose 6 F.F. column, two peaks were obtained, only the second (E) having antifungal activity. When the fraction E peaks were pooled and eluted in hydroxyapatite columns, there were also two peaks and the last peak (G) exhibited antifungal activity, and gave only one protein band in SDS-PAGE. It was named Bacisubin, and its size was estimated as 41.9 kDa compared with standard proteins. Bacisubin had high antifungal activity against *Rhizoctonia solani*, *Magnaporthe grisea Sclerotinia sclerotiorum*, *Alternaria oleracea*, *A. brassicae* and *Botrytis cinerea*. Bacisubin appeared to inhibit mycelial nutrition. The mycelial apex appeared distorted and enlarged, with rupture and cell wall thickening in presence of Bacisubin. However Bacisubin did not show protease activity or protease inhibitory activity, but exhibited some ribonuclease activity and hemagglutinating activity.

13.54 RESEARCH ON BIOLOGICAL CONTROL OF RICE BLAST. **Y.N. Yoon, D.H. Kim, B.C. Lee, S.J. Hong, Y.K. Hong and S.T. Park.** *Plant Enviroment Division, Yeongnam Agricultural Research Institute, NICS, RDA, Milyang, 627-803, Republic of Korea. Email: yoonyn@rda.go.kr*

Rice blast, caused by the fungus *Magnaporthe grisea* Barr, is the most important constraint to rice production worldwide. In this study, we selected biological control bacteria against rice blast, and tested their control effect through contraposition cul-

ture. In total 18 bacteria were tested, and 12 were selected that gave effective control of rice blast. Among these bacteria, BAC06-10-15-3 had a competition effect and BAC06-10-24-2 did not have a competition effect but depressed melanin formation. The two bacteria were shown to be effective in control of melanin formation against rice blast. In glasshouse tests, these bacteria were shown to be very effective as compared to the control. We will continue the search for biological control bacteria and select those that are effective.

13.55 EFFECT OF ANTIFUNGAL CYCLOPEPTOLIDE FROM *ULOCLADIUM ATRUM* ON CONTROL OF *BOTRYTIS*-INCITED DISEASES AND POWDERY MILDEWS IN GREENHOUSE CROPS. S.H. Yu, E.M. Kwon and B.-S. Yun. *Department of Applied Biology, Chungnam National University, Daejeon 305-764, Korea. Email: shunyu@cnu.ac.kr*

The saprophytic fungus *Ulocladium atrum* Preuss is a promising biological control agent for *Botrytis cinerea* in greenhouse and field crops. Recently we isolated an antibiotic peptide from *U. atrum*. Based on extensive spectroscopic analysis, its structure was identified as a cyclopeptolide with a high portion of N-methylated amino acids. The compound exhibited specific and potent antifungal activity against *B. cinerea*, *Alternaria alternata* and *Magnaporthe grisea* with an MIC of 1, 11 and 33.3 μM, respectively, and moderate activity against *Colletotrichum acutatum* and *C. gloeosporioides* with and MIC of 100 μM. The compound also inhibited conidial germination and germ tube growth of *B. cinerea* in a dose-dependent manner. In greenhouse experiments, the compound effectively controlled *Botrytis* grey mould of tomato and strawberry at 20 μg/ml, and powdery mildew of cucumber, strawberry and paprika at 2 μg/ml.

Concepts in chemical control

31.1 FITNESS OF ANILINOPYRIMIDINE-RESISTANT STRAINS OF *BOTRYTIS CINEREA*. G.A. Bardas, C.K. Myresiotis and G.S. Karaoglanidis. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O. Box 269, 54124, Thessaloniki, Greece. Email: gkarao@agro.auth.gr*

The fitness of anilinopyrimidine-resistant isolates of *Botrytis cinerea*, collected from vegetable crops in Greece during 2005, was investigated. Fitness parameters measured were mycelial growth, spore production *in vitro* and *in vivo*, virulence, percentage spore germination and ability of the resistant isolates to compete in four pairs with sensitive isolates. Measurements of the fitness components in individual isolates showed high variability within both sensitivity groups, in all but virulence fitness components tested. As a group, resistant isolates showed significantly lower ($P<0.05$) mycelial growth and virulence. The resistant isolates showed higher ($P<0.05$) spore production *in vivo* but there was no difference ($P>0.05$) between the two sensitivity groups in spore production *in vitro* and in percentage spore germination. However, the correlation to test if there is any relationship between the values of each fitness component and the level of cyprodinil sensitivity of each isolate was not significant ($P>0.05$) for all fitness components, except spore production *in vivo*. This absence of significance suggests that development of resistance did not affect the fitness of the resistant isolates. Competition of the resistant vs sensitive isolates was isolate-dependent, since in two of the isolate pairs the resistance frequency decreased significantly after five culture or disease cycles while in the remaining two pairs resistance frequency increased significantly after five disease cycles or remained stable for one pair after five culture cycles on artificial nutrient media.

31.2 SEPTORIA LEAF SPOT, TAN SPOT AND BROWN RUST: FUNGICIDE EFFICACY IN SWEDEN. G. Berg, G. Gustafsson and C. Lerenius. *Swedish Board of Agriculture Box 12 SE-230 53 Alnarp, Sweden. Email: gunilla.berg@sjv.se*

Septoria leaf spot, caused by *Mycosphaerella graminicola* (anamorph *Septoria tritici*) is one of the dominating wheat diseases in Sweden and northern Europe. Control of the disease mainly depends on fungicides but also on varieties with good disease resistance. In Sweden there are only very few azoles approved, propiconazole, prochloraz and prothioconazole, which makes the issue of declining field performance very important to investigate. Epoxiconazole is not approved in Sweden. Results from field trials will be presented. Best control of Septoria leaf spot was obtained with prothioconazole. Even metconazole and difenoconazole mixed with propiconazole performed well, but these products have not been used in Sweden. Prochloraz performed better than propiconazole. Tan spot (*Drechslera tritici-repentis*) is also an important wheat disease in Sweden and the efficacy of fungicides on tan spot have been investigated in field trials. Prothioconazole and propiconazole performed well. Even metconazole and propiconazole mixed with trifloxystrobin or difenoconazole showed good control. The field performance of fungicides to brown rust (*Puccinia recondita*) were investigated in two trials. The best fungicide was the strobilurine pyraclostrobin, but metconazole also performed well. Propiconazole showed insufficient control and prochloraz was very bad. Prothioconazole is widely used in cereals, especially winter wheat, in Sweden and there are concerns for resistance management. There is a great need for effective mixture partners to build a reliable anti-resistance strategy.

31.3 SENSITIVITY OF *PHAEOSPHAERIA NODORUM* TOWARDS FUNGICIDES IN SWEDEN. E. Blixt, A. Djurle, J. Yuen and Å. Olson. *Department of Forest Mycology and Pathology, P.O. Box 7026, Swedish University of Agricultural Sciences, Uppsala, Sweden. Email: eva.blixt@mykopat.slu.se*

Various kinds of fungicides have been used for several years to control foliar diseases of wheat. So far no reports have been published on fungicide resistance in *Phaeosphaeria nodorum*, causing leaf and glume blotch in wheat. Two other leaf-spotting fungi, *Mycosphaerella graminicola* and *Pyrenophora tritici-repentis,* have developed resistance towards fungicides based on strobilurins and triazoles. The aim of this study was to investigate the sensitivity of *P. nodorum* towards azoxystrobin, propiconazole, prothioconazole and cyprodinil. *P. nodorum* isolates were collected in 2003-2005 from five fields in three regions of Sweden. Two isolates collected in 1990 were used as reference. The fungus was grown on MEA containing six concentrations of the fungicides. Preliminary results indicate varying sensitivity to propiconazole and prothioconazole to concentrations up to 10 ppm. *P. nodorum* isolates were also able to grow on media containing 1 ppm cyprodinil or 100 ppm azoxystrobin, in contrast to the two reference isolates that showed drastically reduced growth even at 1 ppm azoxystrobin. Primer pairs were designed for sequencing the relevant areas containing possible resistance genes and the results of these studies will be discussed.

31.4 EVOLUTION OF THE CYP51 GENE IN *MYCOSPHAERELLA GRAMINICOLA*: EVIDENCE FOR RECOMBINATION AND SELECTIVE SWEEPS. **P.C. Brunner, F.L. Stefanato and B.A. McDonald.** *Plant Pathology, Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland. Email: patrick.brunner@agrl.ethz.ch*

The goal of this study was to elucidate the evolutionary mechanisms by which *CYP51*-based fungicide sensitivity has evolved in *Mycosphaerella graminicola* over space and time. To accomplish this, we sequenced and compared a portion of the *CYP51* gene encompassing the main mutations associated with sensitivity towards DMI fungicides. The *CYP51* gene showed an extraordinary dynamic shift consistent with a selective sweep both in space and time. No mutations associated with increased resistance to azoles were found in non-European populations. These mutations were also absent in the oldest collections from Europe, whereas they dominated in recent European populations. Intragenic recombination was identified as an important evolutionary process in populations affected by high fungicide selection, suggesting the creation of novel alleles among existing mutations as a potential source of novel resistance alleles. We propose that *CYP51* mutations giving resistance in *M. graminicola* arose only locally (perhaps in Denmark or the UK) and were then spread eastward across Europe through wind-dispersed ascospores. We conclude that recurring cycles of recombination coupled with selection due to the widespread use of azole fungicides will increase the frequency of novel mutants or recombinants with higher resistance. Long-distance gene flow due to wind-dispersal of ascospores will move the resulting new alleles to new areas following the prevailing wind directions. A selective sweep favoring haplotypes with various coding mutations at the target site for azole fungicides during the last 5-10 years is the most likely cause of the decrease in sensitivity reported for many azole fungicides in the same period.

31.5 CHARACTERISATION OF MECHANISMS CORRELATED WITH REDUCED AZOLE SENSITIVITY IN *MYCOSPHAERELLA GRAMINICOLA*. **H.J. Cools, B.A. Fraaije, T.P. Bean and J.A. Lucas.** *Plant Pathology and Microbiology Department, Rothamsted Research, Harpenden AL5 2JQ, Hertfordshire, UK. Email: hans.cools@bbsrc.ac.uk*

Mycosphaerella graminicola causes Septoria leaf blotch, the most important foliar disease of winter wheat in the UK. Control of *M. graminicola* currently relies on azole (imidazole and triazole) fungicides. In the absence of alternative curative fungicides, the dependence on azole chemistry has prompted concerns amongst growers and the agro-chemical industry that resistance could develop, compromising control of this pathogen. Recent field studies have shown a decline in the efficacy of a number of azoles against *M. graminicola*, although the most active compounds still remain effective. We have previously reported mutations in the gene (CYP51) encoding the target for azoles, the sterol 14α-demethylase, accumulating in less sensitive isolates and differentially selected by different azole treatments in *M. graminicola* populations. Interestingly, isolates of *M. graminicola* with the same CYP51 sequence often have a wide range of sensitivities to the most effective azoles, suggesting a contribution of mechanisms other than target site change to the final phenotype. Here we report on the impact of CYP51 mutations on azole sensitivity and intrinsic CYP51 enzyme activity. Furthermore, we discuss studies determining the contribution of mechanisms other than target site change to the final azole sensitivity phenotype in *M. graminicola*.

31.6* RESISTANCE OF *PLASMOPARA VITICOLA* TO QOI FUNGICIDES: ORIGIN, DIVERSITY AND FITNESS. **M.F. Corio-Costet, D. Lafarge, M.C. Dufour, P. Abadie, F. Delmotte, W.J. Chen, L. Douence, J. Jolivet and F. Martinez.** *INRA, UMR Santé végétale, INRA-Bordeaux, ISVV, IFR 103, BP 81, 33883, Villenave d'Ornon, France. Email: coriocos@bordeaux.inra.fr*

Since its introduction in Europe, *Plasmopara viticola* (grapevine downy mildew) has been controlled by chemicals, but resistant strains of the pathogen have emerged regularly, i.e. general resistance to Qoi fungicides. Analysis of the cytochrome *b* gene revealed a single mutation (G143A) as a major mechanism factor conferring QoI resistance. Phylogenetic analysis of mitochondrial DNA fragments (2281 pb) allowed the detection of four major haplotypes (IS, IR, IIS, IIR) belonging to two distinct clades, each of which contained a different QoI resistant allele [1]. The prevalence of mitochondrial haplotypes and resistant alleles was assessed by characterizing 839 isolates collected in 21 localities in a Bordeaux vineyard without QoI treatment. The most frequent haplotypes (IR, IS) were found in 74% of *P. viticola* populations. Resistant-allele frequencies ranged from low (0) to very high (0.75) with an average value of 0.29. At least two independent events led to the emergence of QoI resistance. By combining microsatellite markers [2] and selected markers, a temporal genetic structure of *P. viticola* populations was obtained in three localities in Bordeaux. The genetic variability was low and the genotypic richness was high. Based on determination of the fitness index (Fic), QoI-resistant strains did not exhibit a cost and often tended to have good fitness. To assess these results, competition assays with different mixtures of sensitive and resistant strains using biological and molecular (Q-PCR) tests were done. [1] Chen *et al.*, 2007, Appl. Environ. Microbiol. 73: 5162-5172; [2] Delmotte *et al.*, 2006. Molecular Ecology Notes, 6, 379-381.

31.7* MOLECULAR CHARACTERIZATION OF MUTANTS OF *BOTRYOTINIA FUCKELIANA* RESISTANT TO FUNGICIDES. **R.M. De Miccolis Angelini, C. Rotolo, W. Habib and F. Faretra.** *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola, 165/A, Bari, Italy. Email: milvia.demiccolis@agr.uniba.it*

Acquired resistance to fungicides is an important challenge in modern chemical control of plant diseases. *Botryotinia fuckeliana* (de Bary) Whetz. (*Botrytis cinerea* Pers.), the causal agent of grey mould on numerous economically important crops, is universally recognized to be a pathogen at high risk of developing resistance to chemicals. The genetic basis of resistance to dicarboximides, hydroxyanilides and carboxamides was investigated. Nucleotide sequence analysis of the genes encoding the target proteins of each class of fungicides showed that: 1) point mutations in the two-component histidine kinase (*Daf1*) gene resulting in amino acid substitutions in the protein sequence, were associated with different levels of resistance to dicarboximides; an alternative amino acid change at the same position (I365S or I365B) was detected in low-resistant strains, whereas a double substitution (Z369P and B373S) and two different replacements (G357B or G446S) were identified in moderate- and high-resistant mutants, respectively; 2) substitutions of one of three different amino-acid residues (G5V or G5S, P409L, and T45I), caused by single nucleotide polymorphisms in the 3-keto reductase (*ERG27*) gene, were displayed by laboratory mutants resistant to the hydroxyanilide fenhexamid; 3) three nucleotide substitutions in the highly conserved regions of the iron-sulfur protein of succinate dehydrogenase were detected by sequencing the four protein subunit genes from laboratory mutants resistant to the carboxam-

ide boscalid. Two possible amino acid replacements (P225L or P225F) in the second cysteine-rich cluster (S2) were found in high resistant mutants, whereas a specific amino acid change (H272Y) in S3 was associated with a low level of resistance.

31.8* IMPACT OF GRAPEVINE DOWNY AND POWDER MILDEW DIVERSITY ON THE EFFICACY OF PHOSPHONATE DERIVATIVES DESCRIBED AS STIMULATORS OF PLANT DEFENCES. M.C. Dufour, J. Bouscaut and M.F. Corio-Costet. *INRA, UMR Santé végétale, INRA-Bordeaux, ISVV, BP 81, 33883 Villenave d'Ornon, France. Email: coriocos@bordeaux.inra.fr*

Phosphonates are well known to possess powerful antifungal activity, and the fungicide fosetyl-Al (*O-ethyl phosphonate)* is known to exert both a direct effect on the pathogen and an indirect effect via stimulation of host defences. In this study, the efficacy of two phosphonate derivatives fosetyl-Al and a foliar fertilizer (PK2) was tested on different genotypes and phenotypes of grape downy mildew (*Plasmopara viticola*) and powdery mildew (*Erysiphe necator*). We quantified the level of gene expression of proteins involved in potential plant defence pathways (PAL, STS, CHIT4c, PGIP, PIN, GLU, CHS, CHI, PR1, LOX, LDOX) by RT-Q-PCR. The gene expressions induced after treatments with phosphonate derivatives were compared to that found after elicitation with benzothiadiazole (BTH), which mimics salicylic acid in natural systemic acquired resistance. Phosphonate derivatives were efficient against powdery mildew depending on genetic group (A or B), and group B strains were four times more sensitive to fosetyl-Al than group A strains. Furthermore, downy mildew strains showed differences in sensitivity to phosphonate derivatives depending on their phenotypes (resistant or sensitive to fungicides). Phosphonates exerted effects on the expressions of CHI, PR1, GLU and PAL genes. We discuss the role of diversity of the two pathogens on the efficacy of these chemical compounds and also the effective stimulation of grapevine plant defences.

31.9 DISEASE MANAGEMENT IN TOMATO USING SAFE LEVELS OF CHEMICALS. G. Ganeshan and D. Sharma. *Division of Plant Pathology, Indian Institute of Horticultural Research, Hessaraghatta Lake P.O., Bangalore 560089, India. Email: gg@iihr.ernet.in*

In India tomatoes are grown widely over in an area of 520,000 hectares, producing 7,420,000 tonnes (FAO 2004). The crop suffers from many diseases, with frequent application of pesticides. This results in accumulation of pesticide residues in the produce, making it unsafe for consumption besides affecting the environment. The present paper is focused on generating information on residues of pesticides used in the control of tomato diseases. Several fungicides and insecticides used in the management of tomato diseases were evaluated to find out whether their applications (single or multiple) resulted in persistence of harmful residues at harvest. Triademifon (1g/l), difenaconazole (0.15ml/l), carbendazim + mancozeb – combination product (2g/l), propineb (2.5g/l), metalaxyl+mancozeb (2g/l), mancozeb (2.5g/l), copper oxychloride (3g/l) and chlorothalonil (2.5g/l) against fungal diseases and imidacloprid, acephate, dimethoate, dichlorvos, fipronil against insect vectors transmitting viral diseases, were evaluated. The residue estimations were carried out following application of these pesticides at appropriate doses. The fungicide residues on tomato fruits harvested 3 and 5 days after the last application was ND – 1.70 ppm. Based on the residue persistence of the above fungicides and their prescribed permissible levels (MRL), dinocap, difenconazole, propineb, mancozeb, copper oxychloride and chlorothalonil were found safe to be used (Single and multiple applications). Triademifon, tebuconazole and metalaxyl + mancozeb – combination product, were found to be unsafe, while carbendazim + mancozeb application was safe only at 5 days after last application was made. Among the insecticides imidacloprid, acephate, dimethoate and dichlorvos were found safe.

31.10 INTEGRATED MANAGEMENT OF FUNGICIDE-RESISTANT PLANT PATHOGENS. L.V. Gangawane. *Soil Microbiology and Pesticides Laboratory, dept. of Botany, Dr.B.A. Marathwada University, Aurangabad, India. Email: gangawawne@cyberpoint.in*

When resistant varieties and cultural practices are inadequate to manage the pathogens of different crops, use of chemicals becomes essential. It is estimated that nearly 35,000 formulations involving about 1500 basic chemical ingredients are in the world market. In India consumption of pesticides during 2006 was more than 39,000 tonnes and 128 pesticidal compounds have been registered under the pesticide act 1968. However, many examples of fungicide resistance have been reported from USA, Israel, Japan, Australia, Europe and also from South East Asia. In India, fungicide resistance in plant pathogens of various crops is documented and therefore management of this situation becomes essential. Our laboratory has reported nearly 20 pathogens resistant to different fungicides. Amoung these *Gloesporium ampelophagum* (anthracnose of grapes) against carbendazim and *Plasmopara viticola* (downy mildew of grapes) against aluminium phosetyl are acute problems. Resistance against these fungicides has been evaluated, and integrated managements have been worked out using mixtures of other agrochemicals having different modes of action and use of plant extracts. This has helped to reduce the selection pressure of fungicides, and sometimes 90% –100% control of these diseases has been observed.

31.11 STUDIES ON THE BIOLOGICAL CHARACTERISTICS OF METALAXYL-RESISTANT MUTANTS OF *PHYTOPHTHORA BOEHMERIAE*. Z. Gao, F. Chen, Y. Pan and R. Qi. *College of Plant Protection of Anhui Agricultural University, Hefei 230036, P. R. China. Email: gzm@ahau.edu.cn, gaozhimou@126.com*

Phytophthora boehmeriae Sawada is an economically important plant-pathogenic oomycete causing blights of cotton and ramie in China. Metalaxyl has been one of the most effective fungicides for controlling plant-pathogenic oomycetes including *P. boehmeriae.* Some biological characteristics and their inheritance in metalaxyl-resistant (Mt^r) mutants of *P. boehmeriae*, obtained by metalaxyl-induction on LBA medium, were studied i*n vitro*. The results showed no obvious differences between the Mt^r mutants and their wild-type parents in temperature for mycelial growth, zoosporangium production, sensitivity to malachite green and pathogenicity to cotton seedlings. However, oospore production of the mutants was much lower than that of their parents. Growth rate and colony morphology of Mt^r mutants and their parents all displayed obvious variation or separation, suggesting that the two characters were inherited non-uniformly in their single zoospore and oospore progenies. At the same time, the homothallic character of Mt^r mutants was inherited consistently as well as that of the wild-type isolates in asexual and sexual progenies. The results suggested that the Mt^r mutants of *P. boehmeriae* were

strong in natural fitness, and could develop into a metalaxyl-resistant population easily in a short time as long as the Mt^r mutants were initially produced. The results are valuable for risk evaluation, monitoring and control of resistance of *P. boehmeriae* to metalaxyl.

31.12 STUDIES ON GENETIC DIVERSITY IN RESISTANCE OF *PHYTOPHTHORA BOEHMERIAE* TO METALAXYL. Z. Gao, F. Chen, Y. Pan and R. Qi. *College of Plant Protection, Anhui Agricultural University, Hefei 230036, P.R. China. Email: gzm@ahau.edu.cn, gaozhimou@126.com*

Metalaxyl-resistant (Mt^r) mutants of *Phytophthora boehmeriae* Sawada were obtained by chemical mutagenesis, and the inheritance of resistance of these mutants to metalaxyl was studied *in vitro*. Wild-type and metalaxyl-sensitive (Mt^s) isolates from different localities and host plants were used for the tests. The results showed that there were three patterns of inheritance of resistance of mutants in *P. boehmeriae* to metalaxyl as follows: (1) The metalaxyl-resistant character was steadily inherited in single-zoospore progenies but separated into Mt^r and Mt^s in the ratio 3:1 in the first single selfed oospore generation; (2) The Mt^r character was steadily inherited in both single-zoospore and single-oospore progenies; (3) The Mt^r character was unsteadily inherited, i.e. it reverted to Mt^s or showed continuous variation in successive zoospore progenies. This suggested that the mutation of resistance of *P. boehmeriae* to metalaxyl might occur in the nuclei or in the cytoplasm, and the Mt^r character of the pathogenic oomycete might be controlled by a single dominant nuclear gene, or by steadily inheritable or variably inheritable mitochondrial genes in the cytoplasm. The results also indicated that among different mutants from the same isolate there might be obvious differences in the genetic background of mutation of resistance to metalaxyl. It appears that there is complex diversity in the inheritance of resistance in *P. boehmeriae* to metalaxyl. The results will be useful in monitoring and management of resistance of *P. boehmeriae* to metalaxyl.

31.13 FUNGICIDE MIXTURES FOR CONTROL OF *MYCOSPHAERELLA GRAMINICOLA* ISOLATES WITH REDUCED SENSITIVITY TO DMIS. J. Godwin, R. Boutellier, C. Chassot, U. Hugelshofer, H. Sierotzki and U. Gisi. *Syngenta Crop Protection AG, Research Biology, 4332 Stein, Switzerland. Email: jeremy.godwin@syngenta.com*

Mycosphaerella graminicola (anamorph *Septoria tritici*) is the causal organism of septoria tritici leaf blotch, the most commercially damaging foliar disease of wheat in N.W. Europe. Chemical control of this disease is currently primarily based on azoles, members of the sterol 14α–demethylase inhibitor group of fungicides (DMIs), and significant reductions in sensitivity leading to reduced field performance have been reported in the last few years. Mutations in the CYP51 gene, encoding the sterol 14α–demethylase target protein, and changes in the expression of efflux proteins have been associated with these reductions in sensitivity. Furthermore, molecular genetics studies in combination with in vitro plate assays have identified six different genotype groupings within the N.W. European population, each of which has a different phenotype in terms of sensitivity to a defined set of azole fungicides. The current study builds on this by examining different commercial fungicide products for control of representative isolates from these genotype groupings on whole plants under carefully controlled growth chamber conditions. The data generated in this study highlight the benefit of applying a multisite inhibitor fungicide, such as chlorothalonil, in mixture with azoles in order to give robust control of *S. tritici*, irrespective of the DMI sensitivity profile of the field population. Studies also demonstrated that mixtures of some azoles can interact synergistically against *S. tritici* isolates with reduced sensitivity to DMIs. These data are of interest both from a mechanistic standpoint and for control of this disease under commercial conditions.

31.14 INTELLIGENT CHEMICALS FOR INTEGRATED PEST AND DISEASE MANAGEMENT. S. Gomathinayagam, K. Mathivanan and Lalithakumari. *Faculty of Agriculture and Forestry, University of Guyana, Berbice campus, Guyana. Email: drrekhagoms@yahoo.co.in*

Modern chemical fungicides play a vital role in controlling various fungal diseases in crop plants. The introduction of systemic fungicides has significantly improved crop productivity. The benzimidazole group is the most important, and is used in preventive as well as curative fungicides for several crop diseases. Spraying of carbendazim significantly reduced sheath blight in rice with commensurate increase in grain yield. The ergosterol biosynthesis inhibitors, peneconazole and biloxazole effectively inhibit mycelial growth and sporulation in fungal pathognes. Modern systemic fungicides are not only potent fungitoxicants but also plant growth regulators, thereby protecting plants under stress conditions. Benomyl, carbendazim, baycor and calixin exhibited significant protective effect against methyl isocyanate toxicity in crop plants. Probennazole, a systemic chemical inhibited the bacterial multiplication in rice leaves and its applocation significantly induced defence-related enzymes such as peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and tyrosine ammonia lyase, which are responsible for host resistance thereby reduced the baterial leaf blight in rice. No development of resistance to probenazole was reported. Fungicides showed differential activity on soil microorganisms in different types of soils, directly related to soil texture and microbial populations. Interestingly, the addition of fungicides alone or in combinations selectively stimulated certian beneficial soil microbes. Integration of carbendazim with biocontrol agent, *Trichoderma viride* considerably reduced root disease in cotton.

31.15* SOYBEAN RUST: DISEASE CYCLE AND EFFICACY OF AZOLES AND STROBILURINS. I. Haeuser-Hahn, A. Mehl, U. Steffens and K. Tietjen. *Bayer CropScience AG, Alfred Nobel Str. 50, 40789 Monheim, Germany. Email: Isolde.Haeuser-Hahn@bayercropscience.com*

Soybean rust (*Phakopsora pachyrhizi*) has turned out since 2003 to be very devastating in Brazil. The disease cycle starts with uredospore germination and mainly direct penetration. In most cases an appressorium develops at the end of the germ tube. After penetration of the hyphal tube in the underlying epidermis hyphae grow intercellularly and rapidly colonize the mesophyll. After five to seven days under optimal conditions, urediae are formed, and after nine days these liberate new uredospores for a longer period of time. The mode of action of strobilurins and azoles on the disease cycle of soybean rust is shown in scanning electron micrographs. Strobilurins such as Flint directly inhibit uredospore germination whereas azoles such as Tebuconazole efficiently inhibit mycelial growth. Up to now, several sensitivity monitoring methods have been checked for their applicability to

soybean rust. For strobilurins, because of their mode of action, a spore germination test seems to be most suitable. Radioactive label incorporation studies showed that within the first 6 hours of germination no significant sterol biosynthesis could be detected. Because of the late onset of fungal sterol biosynthesis during germination a spore germination test turned out to be inapplicable for sensitivity monitoring of DMIs and a detached leaf assay was necessary. Preliminary data show that until now no sensitivity changes occurred in the case of both fungicide classes, strobilurins and DMIs.

31.16 OXOLINIC ACID-RESISTANCE MECHANISM OF IN VITRO MUTANTS OF *BURKHOLDERIA GLUMAE* AND THEIR SURVIVAL FITNESS ON RICE PLANTS. **Y. Hikichi, Y. Maeda, K. Ohnishi and A. Kiba.** *Laboratory of Plant Pathology & Biotechnology, Kochi University, Kochi, Japan. Email: yhikichi@cc.kochi-u.ac.jp*

Oxolinic acid (OA)-resistance in field isolates of *Burkholderia glumae,* a causal agent of bacterial grain rot, depends on the amino acid substitutions at position 83 in the GyrA (GyrA83). Among *in vitro* spontaneous mutants from OA-sensitive *B. glumae* strain Pg-10 (Pg-10), we selected OA-resistant mutants which were produced at the rate of 5.7×10^{-10}. The nucleotide sequences of the quinolone resistance-determining region in GyrA showed that Gly81Asp, Gly81Cys, Asp82Gly, Ser83Arg, Asp87Gly and Asp87Asn substitutions in GyrA occurred in these OA-resistant mutants. Introduction of each amino acid substitution to GyrA of Pg-10 resulted in OA-resistance, similarly to *in vitro* OA-resistant mutants having the responsible amino acid substitutions. Though the Asp82Gly recombinant grew *in vitro* less than the parent strain, other recombinants grew *in vitro*, similarly to Pg-10. When rice plants at booting were inoculated with the bacteria, the population of Pg-10 in spikelets increased to 7.0×10^6 cfu/g by 5 days after flowering. Though the population of Ser83Arg recombinant in spikelets increased to 3.8×10^5 cfu/g, growth of other OA-resistant recombinants in spikelets was reduced significantly, compared to that of Pg-10. When spikelets at flowering were treated with OA, only the Ser83Arg recombinant grew vigorously in spikelets, causing bacterial grain rot. These results suggest that only OA-resistant *B. glumae* with GyrA83 substitutions among the GyrA-mutants retains the ability to survive on rice plants, especially when OA is applied. Therefore, GyrA83 substitution is commonly responsible for OA-resistance for *B. glumae* field isolates.

31.17* DISRUPTION OF THE *BOTRYTIS CINEREA* GENOME HIGHLIGHTS THE POSSIBILITY THAT NON-PROTEIN CODING REGIONS MAY OFFER NOVEL FUNGICIDE TARGETS. **D.W. Hollomon, P.M. Wood and T. Joseph-Horne.** *Biochemistry Department, School of Medical Sciences, Bristol University, Bristol, UK. Email: D.W.Hollomon@bristol.ac.uk*

As part of our studies to identify how various branch pathways interact with the core mitochondrial respiratory pathway in filamentous plant pathogenic fungi, we cloned an external alternative NAD(P)H dehydrogenase (ANADH) gene from the grey mould fungus, *Botrytis cinerea*. To determine the function of this gene during early infection and spread, we attempted a "Knock-out" using the haploid *B. cinerea* strain B0510, and a disruption cassette carrying the hygromycin resistance gene. Several stable hygromycin-resistant transformants were obtained, but PCR and Southern blots confirmed that the resident ANADH gene had not been disrupted. Yet all transformants sporulated very poorly, grew more slowly *in vitro* than the parent, and were non-pathogenic. Genome walking outward in both directions from the hygromycin-resistance gene identified the insertion of the disruption cassette in an open reading frame of 106 amino acids, which was at least 2 kb in either direction from any protein sequence recognised in a BLAST search. Recent analysis of several eukaryotic genomes (Gingeras, 2007, Genome Research, 17: 582-690) has identified the widespread occurrence of RNA transcripts with apparently little protein-coding capacity, and their role in the regulation of development. We will report our experiments, using RT-PCR, to see if RNA transcription occurs where the ANADH disruption cassette has inserted in the Botrytis genome. This work highlights the fact that apparent non-coding regions of fungal genomes may regulate proteins associated with cell function and/or pathogenicity, which may offer possible novel fungicide targets.

31.18 EFFECTS OF FUNGICIDES ON CONTROL OF *LEPTOSPHAERIA MACULANS* AND *L. BIGLOBOSA* (PHOMA STEM CANKER) IN OILSEED RAPE. **J.R. Hood, Y.J. Huang, N. Evans, S. Rossall, M. Ashworth, B.D.L. Fitt.** *Rothamsted Research, Harpenden AL5 2JQ, Herts, UK. Email: yong-ju.huang@bbsrc.ac.uk*

Phoma stem canker is a damaging disease of oilseed rape caused by a *Leptosphaeria* species complex, *L. maculans* and *L. biglobosa*. Stem cankers caused by *L. maculans* are more damaging than those caused by *L. biglobosa*. Control of severe stem canker in the UK depends on the use of fungicide. Understanding the sensitivity of *L. maculans* and *L. biglobosa* to fungicide is important for effective control of phoma stem canker. This poster reports work on sensitivity of *L. maculans* and *L. biglobosa* to flusilazole and effectiveness of fungicides on control of the disease in field experiments. Results from *in vitro* assays of sensitivity to flusilazole indicate that isolates of *L. biglobosa* were less sensitive than isolates of *L. maculans*. There was also large variation between isolates of the same species. In winter oilseed rape field experiments to investigate effects of early and late applications of fungicides on control of the disease done in the 2005/06 and 2006/07, there were significant differences between treated and untreated plots. In 2005/06, the early spray (29 Oct.) was more effective in control of phoma leaf spots, while the late spray (29 Nov.) was more effective in control of stem canker. In 2006/07, the early spray (13 Oct.) was more effective than late spray (13 Nov.) in control of both leaf spots and stem canker. For the two cultivars used in the field experiment, there was no difference in phoma leaf spot incidence in autumn but there was a difference in severity of stem canker at harvest.

31.19 OCCURRENCE OF STROBILURIN-RESISTANT ISOLATES OF *PSEUDOCERCOSPORA VITIS*, CAUSAL FUNGUS OF GRAPEVINE LEAF BLIGHT IN JAPAN. **K. Inoue, N. Koya and A. Kawaguchi.** *Agricultural Experiment Station, Okayama Prefectural General Agriculture Center, Akaiwa-city, Okayama, 709-0801, Japan. Email: kouji_inoue3@pref.okayama.lg.jp*

The strobilurin fungicides, kresoxim-methyl and azoxystrobin, have been used to control ripe rot, downy mildew and leaf blight of grapevine for more than ten years in Okayama Prefecture, Japan. Failing efficacy of strobilurin fungicides to leaf blight was lately observed in some vineyards. Seventy seven isolates of *Pseudocercospora vitis* collected from ten vineyards in

Okayama Prefecture were tested for sensitivity to a strobilurin fungicide in 2006. The MIC of azoxystrobin against mycelial growth in presence of 5mM *n*-propyl gallate was more than 400ppm in 61 isolates. A single point mutation (G143A) in the cytochrome *b* gene was found in the resistant isolates tested. Control efficacy of azoxystrobin against resistant isolates was very low in an inoculation test using pottedgrapevine plants. Most of these strobilurin-resistant isolates were also resistant to benzimidazole. These results show that failure of strobilurin fungicides to prevent leaf blight was caused by occurrence of strobilurin-resistant isolates. This appears to be the first report of strobilurin-resistant isolates of *Pseudocercospora vitis*.

31.20 DEVELOPMENT OF AN ENZYMATIC ASSAY FOR MEASURING PHOSPHITE. **N. Jardine, J.A. McComb, G.E.StJ. Hardy and P.A. O'Brien.** *Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA 6150, Australia. Email: N.Jardine@murdoch.edu.au*

The systemic fungicide phosphite is increasingly being used to treat oomycete plant diseases in horticultural crops and native ecosystems around the world. The effects of phosphite application on a plant are variable. Resistance depends on the species, the time of application and the method of application. Very little is known about how phosphite is transported and stored within plants, or how these processes are affected by the physiological status of the plant. This is mainly due to the unavailability of a simple, efficient method for measuring the concentration of phosphite. We have developed an enzymatic assay that enables phosphite to be quantified as an alternative to high-performance liquid chromatography or gas chromatography. Phosphite is oxidised to phosphate by a modified phosphite dehydrogenase. NADH formed in the reaction reduces p-iodonitrotetrazolium violet (INT) via an intermediate electron carrier to produce a stable red formazan that can be measured spectrophotometrically at 490 nm. This method is being further developed to quantify phosphite in plant-tissue samples. Dried plant material is powdered and resuspended in water. Phosphite is separated from the plant extract solution by dialysis and determined with the phosphite dehydrogenase assay. The method is rapid and inexpensive and could potentially be developed to measure the phosphite concentration within plant samples in the field.

31.21 ECONOMIC THRESHOLDS FOR *ALTERNARIA* LEAF BLIGHT INFECTION (*ALTERNARIA* SP.) OF FIELD-GROWN SESAME. **B.R. Kim, K.S. Han, Y.S. Choi, E.S. Yang, J.R. Son and S.Y. Yu.** *BioEnvironment Division, Chungnam Agricultural Research and Extension Services, Yesan, Chungnam 340-861, Republic of Korea. Email: amali@hanmail.net*

Alternaria leaf blight caused by *Alternaria* sp. is one of the most serious diseases affecting sesame in tropical, subtropical and temperate regions. This disease occurs under high humidity and low temperature. Our study was conducted to develop economic thresholds for field cultivation of sesame according to disease-incidence levels from 0 to 40%.The yield losses were 4.2%, 15.3%, 17.1%, and 28.2%, with disease incidence levels of 10%, 20%, 30%, and 40%, respectively. In cases of early disease occurrence, the control threshold (CT) estimated was 16.3%.

31.22 RAINFASTNESS AND LONG LASTING ACTIVITY – KEY REQUIREMENTS IN SPRAYS FOR THE EFFECTIVE CONTROL OF OOMYCETE PATHOGENS IN GRAPES, POTATOES AND VEGETABLES. **G. Knauf-Beiter, D.W. Bartlett and F. Huggenberger.** *Syngenta, Crop Protection Biological Research Centre, CH-4332 Stein, Switzerland. Email: gertrude.knauf-beiter@syngenta.com*

Mandipropamid is a new active ingredient with high intrinsic activity against Oomycete pathogens in grapes, potatoes and vegetables. Biokinetic studies show that a substantial proportion of the active ingredient binds immediately to the epicuticular wax of plant surfaces. As the surface spray deposit has dried, adsorption to the wax layer protects the active ingredient from being washed off by rain. These properties explain the consistently excellent rainfastness of mandipropamid-based products observed in different laboratory and field tests on leaves of potatoes, grapes and vegetables as well as on grape bunches. In field/laboratory tests, potato leaves of different sizes were marked at the time of fungicide application in the field, and at different intervals, marked leaves were removed from the field plants and inoculated with *Phytophthora infestans* in the laboratory. The results of these tests show that mandipropamid fully protects expanding leaves from disease infection. The stability of the surface deposit and the distribution of the active ingredient with growing leaf tissue can explain these results. From the surface deposit and the material adsorbed to the epicuticular wax, small amounts of active ingredient migrate progressively into the plant tissue. Due to the high intrinsic activity of mandipropamid, the amount taken up into the plant tissue is sufficient to provide good translaminar activity and curative disease control during the incubation period. Rainfastness and long-lasting activity are key requirements for reliable preventive control of Oomycete pathogens under field conditions.

31.23 OVEREXPRESSION OF EFFLUX TRANSPORTERS LEADS TO MULTIDRUG RESISTANCE IN *BOTRYTIS CINEREA* FIELD STRAINS. **M. Kretschmer, A-S. Walker, M. Leroch, M. Wiwiorra, H-J. Schoonbeek, P. Leroux, S. Fillinger, M. de Waard and M. Hahn.** *Phytopathology, Department of Biology, P.O. Box 3049, University of Kaiserslautern, Kaiserslautern, Germany. Email: kretschm@rhrk.uni-kl.de*

Botrytis cinerea is a ubiquitous necrotrophic ascomycete which can infect more than 230 plant species. It causes high losses in economical important crops like grapes, tomatoes or strawberries. To control *Botrytis* several applications per year of different fungicides are used. Common fungicide resistance phenotypes are due to mutations of fungicide target sites or increased degradation. Another type of resistance, called multidrug resistance (MDR), known from cancer cells or microbial infections, leads to resistance against different drugs and is correlated with overexpression of ABC- or MFS-transporters. In French vineyards, *B. cinerea* strains with a MDR phenotype were observed for the first time 15 years ago. MDR strains constitute an increasing population which already accounted, in 2005 and 2006, for more than 50% of the population. In Germany 23-34% MDR strains were found in 2006 and 2007. All strains could be classified into three MDR groups according to their fungicide resistance spectrum. Uptake experiments with ^{14}C-labeled fludioxonil with MDR1 and sensitive strains showed strongly reduced fungicide uptake in MDR1 strains. This was clear evidence for a correlation between MDR and increased efflux transport activity. Gene expression studies identified constitutively overexpressed efflux transporters. In MDR1 strains, an ABC- and in MDR2 a

MFS-transporter was overexpressed. MDR3 strains, as a natural cross of MDR1 and 2, showed overexpression of both the ABC- and the MFS-transporters. Knock-out mutagenesis of the ABC-transporter in MDR1 strains led to complete loss of the MDR1 phenotype, which confirmed a correlation between efflux-transporter expression and MDR.

31.24 COST OF FUNGICIDE RESISTANCE FOR *PLASMOPARA VITICOLA*: FITNESS ASSESSMENT AND SPATIO-TEMPORAL DISTRIBUTION. **D. Lafarge, F. Delmotte, V. Machefer, J. Jolivet,L. Douence, M.P. Latorse, R. Beffa and M.F. Corio-Costet.** *UMR Santé Végétale, INRA-Bordeaux, 71 Ave. E. Bourlaux, BP81- F-33883 Villenave d'Ornon Cedex, France. Email: dlafarge@bordeaux.inra.fr*

Since its introduction from USA to Europe in the late 19th century, *Plasmopara viticola*, the causal agent of grapevine downy mildew, has caused extensive damage in vineyards, where chemical control remains the most efficient management tool. This chemical disease control has led to the selection of resistant strains. To manage these resistance phenomena, it is important to understand how resistant populations appear, spread and survive. One major issue is to evaluate pathogen fitness (ability of strains to spread their genotypes within populations) without selection pressure. The aim of this study was to 1) characterize different physiological parameters of resistant and sensitive isolates of *P. viticola* to fungicides, 2) combine the data obtained to define a fitness indicator, i.e. Composite Index of Fitness (CIF), and 3) classify the *P. viticola* isolates according to their CIF. In addition, we tested direct measurement of competitive fitness by mixing resistant and sensitive strains during several asexual reproduction cycles without fungicide pressure. From these data we can estimate the cost of fungicide resistance in *P. viticola*. We also present data on population genetics linked to resistance phenotypes. For this study, we developed and used neutral (microsatellite) and non-neutral (SNP) molecular markers to assess the genotype of strains and their spatio-temporal distribution.

31.25 INFLUENCE OF TRIFLOXYSTROBIN ON PRIMARY INOCULUM AND PROGRESSION OF SCAB EPIDEMICS ON STONE FRUIT. **N. Lalancette, K.A. McFarland and A.L. Burnett.** *Rutgers University, Agricultural Research and Extension Center, 121 Northville Road, Bridgeton, NJ 08302 USA. Email: lalancette@njaes.rutgers.edu*

Primary inoculum for scab epidemics on peach and nectarine is produced by overwintering twig lesions. This inoculum source accounts for the majority of fruit infection during early-to-mid season. The relatively long latent period of 42+ days for fruit scab limits secondary infection cycles to later in the season. Thus, a reduction in primary inoculum could significantly impact disease development on fruit. To evaluate this possibility, the influence of bloom applications of trifloxystrobin on twig lesion sporulation and subsequent fruit infection was studied in a nectarine orchard during 2005-2007. Treatments consisted of applications made at pink and bloom, bloom and petal fall, petal fall and shuck-split, or petal fall, using 3×3 tree plots arranged in a RCBD with three replicates. Five or six sporulation assessments were made from May through July by removing 10-16 infected twigs from each plot and incubating them for 24 h at 25°C and RH > 95%. Conidial production was estimated using a hemocytometer. Fruit disease development was assessed three times during each season by counting the number of lesions on 25 fruit per plot. Analysis of areas under the sporulation and fruit-disease progress curves indicated that all treatments significantly reduced primary inoculum production and fruit disease severity. The most effective treatments reduced peak sporulation by 82-92% and fruit lesion density by 79-87%. These results indicate that use of anti-sporulant fungicides at bloom is an effective strategy for reducing the impact of primary scab inoculum during the subsequent fruit growth and development period.

31.26 EVALUATION OF POLYMERS FOR COATING BEAN SEEDS. **L. Leandro Pires, C. Bragantini and A. Teramoto.** *School of Agronomy and Food Engeneering, Federal University of Goiás, C.P. 131, Campus II, Goiânia, GO, Brazil. Email: larissa@agro.ufg.br*

Coating seeds of bean (*Phaseolus vulgaris* L.) with polymers promotes a uniform distribuition and better fixation of chemical products on the seed surface. Since species respond differently to coating, one of the objectives of this study was to evaluate the effect of different polymers on the quality of bean seeds (cv. Pérola). The polymers tested (PVA paints, xantan gum and polyvinyl acetate) were coated on the seeds. Germination was slower in coated seeds, particularly with polyvinyl acetate; but coating did not affect germination of samples taken over the storage period. Another experiment was conducted to study the effects of polymer coating on the physiological quality of bean seeds (cv. Aporé). Coating polymers were PVA paints (regular and glossy). Bean seeds were put to germinate immediately and at 1, 2, 3, 4, 5 and 6 days after coating. Seed quality was evaluated through a standard germination test, first count and accelerated aging test. Effects of polymer coating on seed water absorption rate were also evaluated. Coated seeds were placed in water immediately and after 2, 4, and 6 days after coating. Weight gains were checked after 1, 2, 4, 8, 16, 24, 36, 48, 60 and 72 hours of imbibition. Coating with glossy paint slowed the germination process when compared to non-glossy paint. This was probably because the glossy paint restricted water absorption. However, final germination percentage of the seeds coated with both paints was not affected.

31.27* STORAGE OF BEAN SEEDS COATED WITH POLYMERS AND TREATED WITH FUNGICIDES. **L. Leandro Pires, C. Bragantini and A. Teramoto.** *School of Agronomy and Food Engeneering, Federal University of Goiás, C.P. 131, Campus II, Goiânia, GO, Brazil. Email: larissa@agro.ufg.br*

Coating seeds with polymers, associated with other chemicals, is being used to promote better fixation of seed treatment products and, therefore, minimizing toxic hazards. Physiological and sanitary seed quality of bean (*Phaseolus vulgaris* L.) seeds, inoculated with *Colletotrichum lindemuthianum*, coated with polymers and treated with fungicides, were evaluated by germination, vigour, and pathology tests, during four months in storage. Germination of coated and treated seed was not affected during the storage period. Combination of a coating paint and a wettable powder fungicide gave higher germination percentages after two and four months of storage. Fungicides formulated as wettable powder or concentrated suspensions promoted a better control of pathogens in general. The combination of dry powder formulation with a coating substance improved the control of *C. lindemuthianum* at the beginning of storage.

31.28 SUPPRESSING CUCURBIT DOWNY MILDEW OF CUCURBITS. **S. Rideout, C. Waldenmaier and M. Mahovic.** *Virginia Tech, Eastern Shore AREC, 33446 Research Dr., Painter, VA, 23420, USA. Email: srideout@vt.edu*

Cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, is the most damaging disease on cucurbits in Virginia. Recently, management of the disease has become more problematic as the pathogen has developed resistance to QoI fungicides. In addition, performance of particular fungicides on different cucurbit crops have led to questions regarding the homogeneity of P. cubensis populations. Over the past two growing seasons (2006 and 2007), our research group has screened 11 different fungicide regimes across three different cucurbits crop: cucumber (*Cucumis sativus*), cantaloupe (*Cucumis melo*), and pumpkin (*Curcurbita pepo*). Disease severity was greatest in cucumber where only regimes containing cyazofamid and propamocarb hydrochloride have been effective at suppressing levels of disease. QoI fungicides (azoxystrobin and pyraclostrobin) have only offered moderate levels of suppression. In addition, moderate suppression has been realized in plots receiving applications of famoxadone + cymoxanil, cymoxanil, mandipropamid, and chlorothalonil. Results in cantaloupe have mirrored those observed in cucumber. However, in pumpkin trials, mandipropamid offered similar levels of control to cyazofamid and propamocarb hydrochloride. In all trials, plots receiving fungicide applications have exhibited less downy mildew and higher yields compared to the nontreated control. These results indicate that QoI fungicides are no longer effective at controlling levels of cucurbit downy mildew in the United States. In addition, performance of particular fungicides varies across different cucurbit crops.

43.1 FUNGICIDE RESISTANCE IN POPULATIONS OF CUCURBIT POWDERY MILDEW. **A. Lebeda and B. Sedláková.** *Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71 Olomouc-Holice, Czech Republic. Email: ales.lebeda@upol.cz*

A total of 108 cucurbit powdery mildew (CPM) isolates, 78 *Golovinomyces cichoracearum* (*Gc*), 30 *Podosphaera xanthii* (*Px*), were screened for tolerance and/or resistance to the three frequently used fungicides (fenarimol, dinocap, benomyl) in the Czech Republic (CR). The isolates, from 9 regions and 22 districts of CR, were collected in 2001-2004. Fungicide sensitivity was determined by a modified leaf-disc bioassay using five concentrations. Significant differences among fungicides and even between different years were found. Occurrence of resistant and/or tolerant isolates of both powdery mildew species in different locations was observed. Fenarimol (Rubigan 12 EC) showed a high level of effectiveness and all isolates of both CPMs were controlled by the recommended concentration (36 µg a.i./ml). Specific temporal variation in tolerance/resistance was observed. Dinocap (Karathane LC) showed decreasing efficacy. A shift to the tolerant reactions in CPM populations was recognized. Benomyl (Fundazol 50 WP) was totally ineffective, because the majority of isolates screened (88% *Gc* and 97% *Px*) showed resistance to the recommended concentration (250 µg a.i./ml) and tolerant or resistant response to higher concentrations (500 µg a.i./ml and 1000 µg a.i./ml). Differences in sensitivity between *Gc* and *Px* were found. Practically all *Px* isolates (except one from 2003) were resistant, whereas 12% of *Gc* isolates during the years 2001-2003 showed sensitive and/or tolerant reactions. At some locations repeatedly sampled during 2001-2004, variation in tolerance/resistance to all fungicides screened was detected.

43.2 EPIDEMIOLOGY OF *ALTERNARIA* SPP. AND YIELD LOSS IN POTATOES. **J. Leiminger and H. Hausladen.** *Chair of Phytopathology, Life Science Center Weihenstephan, Am Hochanger 2A, 85350 Freising Weihenstephan, Germany. Email: j.leiminger@web.de*

Early blight is one of the major diseases of potatoes and other Solanceae. In most potato-growing areas in Germany, early blight is destructive. The primary damage is due to premature defoliation. Heavy infections, which already start early in the growing season, cause yield losses of 50%. In Europe, the disease is increasingly important and the yield losses are greatly underestimated. Early blight is caused by two pathogens, *Alternaria solani* and *A. alternata*. The occurrence of each pathogen was monitored in Germany over several years as well as over the entire vegetation period. In the laboratory, the fungi can be distinguished on conidial morphology. This is crucial because as Deutermycota, *Alternaria* spp., cannot be distinguished on sexual spores. Up to now it has not been possible to distinguish which of the two species causes the necrotic spot in the field, but by using quantitative PCR we could measure the temporal and spatial distribution of both pathogens in the field. Our results indicate that integration of several factors (such as fungicide strategy, nutrition, host cultivar and irrigation) can be very effective for prevention of early blight. Fungicide treatments appear most effective in control of early blight. We aimed to develop a decision-supporting system (DSS) for fungicide treatments as part of an integrated plant protection system against potato early blight. In this we combined fungicide strategies against early as well as late blight.

43.3 SCREENING SURFACTANTS WITH SYNERGETIC INHIBITION ACTIVITY TO SYP-Z048 ON *BOTRYTIS CINEREA*. **P.F. Liu, F.P. Chen, P. Han and X.L. Liu.** *Dept. of Plant Pathology, China Agricultural University, Beijing 100094, P.R. China. Email: pengfeiliu@cau.edu.cn*

A novel fungicide, 5-(4-chloro phenyl)-2,3-dimethyl-3-(pyridine-3)-oxazoline (SYP-Z048), formulated by ShenYang Research Institute of Chemical Industry, showed extra control of grey mould in field tests. To provide a reference for formulation development and rational administration of the fungicide, surfactants that could increase the inhibition of *Botrytis cinerea* by SYP-Z048 were selected from 18 varieties of eight kinds. It included organic silicon, castor oil, tween, alkyl naphthalene sulphur salt, alkylphenol polyoxyethylene, lignin, nitride ketones and phosphate ester salt. In screening, the best combination of SYP-Z048 and surfactants was measured using three physiologic indexes. They were dry weight of mycelium in liquid culture medium, colony diameter on agar culture medium, and area of mycelial expansion on tomato leaves. The results in vitro such as colony diameter and dry weight of mycelium showed high correlation. Treatment with SYP-Z048 combined with 0201B gave the best inhibition, in terms of colony diameter and dry weight, namely 52.17% and 94.67%, respectively while there was only 22.27% and 13.51% inhibition with SYP-Z048 alone. When cultured on tomato leaves, *B. cinerea* was inhibited the most by SYP-Z048 together with the surfactant Silwet L77. The inhibition rate was 83.69%, more than 40% higher than when surfactant 0201B was used.

43.4 STUDIES ON THE MECHANISM OF NOVEL FUNGICIDE SYP-Z048 AGAINST *BOTRYTIS CINEREA*. **X.L. Liu, P. Han, M. Liu and L. Chen.** *Dept. of Plant Pathology, China Agri-*

cultural University, Beijing 100094, P.R. China. Email: Seedlingxili @yahoo.com

A novel fungicide, 5-(4-chloro phenyl)-2,3-dimethyl-3-(pyridine-3)-oxazoline (SYP-Z048) was developed in China. *In vitro*, SYP-Z048 showed good inhibition of Ascomycetes, Basidomycetes and Deuteromycetes. The EC_{50} values for *Botrytis cinerea* and *Fulvia fulva* were 0.306 and 0.126 μg/ml, respectively. The effect of SYP-Z048 on ergosterol synthesis in *B. cinerea* hyphae was studied using HPLC. We found that SYP-Z048 could significantly inhibit ergosterol synthesis. The levels of ergosterol in hyphae were 1.59 (±0.126) and 0.10 (±0.011) μg/mg dry wt under the treatment dosage of 0.5 and 10.0 μg/ml, respectively. The inhibition rates were 22.24% and 95.12%, which were not very different from the effects of the DMI fungicide tebuconazole. Regression analysis indicated that there was positive linear relationship between logarithm fungicide concentration and odds score of inhibition rate of ergosterol. The effect of SYP-Z048 on different sterols in hyphae of *B. cinerea* was determined by the GC method. The results showed that the ergosterol level in total sterols was 43.91%, 43.74% and 32.41% after SYP-Z048 treatment of hyphae at 0.5, 1.0 and 5.0 μg/ml. Compared to the untreated control, the levels of other sterols also showed various changes. After treatment at 0, 0.5, 1.0 and 5.0 μg/ml SYP-Z048 concentration, the eburicol concentration in total sterols was 2.25%, 7.10%, 9.88% and 12.80%, but the 4,4-dimethylfecosterol concentrations were 0.040%, 0.017%, 0% and 0.006%, respectively. Thus, the site of SYP-Z048 action in *B. cinerea* was focused on the sterol C14 demethylase.

43.5 MECHANISM OF RESISTANCE OF *FUSARIUM FUJIKUROI* TO PROCHLORAZ. **X.L. Liu, Z.H. Zhao, J.R. Fan and O.S. Lund.** *Dept. of Plant Pathology, China Agricultural University, Beijing 100094, P.R. China. Email: Seedlingxili@yahoo.com*

Rice bakanae disease, caused by *Fusarium fujikuroi*, is important in most rice-growing areas of the world. Prochloraz is applied widely to control this disease in China. Prochloraz-resistant mutants were obtained by fungicide adaptation and UV-irradiation. Some biological characteristics of the mutants were studied in the laboratory, and the resistance of all mutants was found to be stable. The resistant mutants could grow at levels of 0.5μg/ml prochloraz, at which the mycelial growth of sensitive strains was inbihited completely. Based on the dose response of resistant mutants, the concentration of 0.5 μg/ml could be considered as discriminatory to distinguish sensitive strains from resistant mutants. The metabolism and absorption of prochloraz in mycelia of sensitive strains and resistant mutants were determined by HPLC and gas chromatorgraphic (GC) methods. The analysis showed that the effect of ATP-binding cassette transporter genes was one of the resistant mechanisms of *F. fujikuroi* to prochloraz. However, the metabolism of prochloraz by mycelia was not correlated with resistance. The 1523bp sequence of gene cyp51 from a sensitive strain and resistant mutants with different resistance levels was cloned and compared. The results showed that resistance to prochloraz was not due to mutation in cyp51. Analysis of cyp51 mRNA, measured by real-time PCR showed that over-expression of this mRNA should be the potential resistance mechanism of *F. fujikuroi* to prochloraz.

43.6 DETECTION OF RESISTANCE TO DIMETHACHLON AND CARBENDAZIM IN *SCLEROTINIA SCLEROTIORUM* IN JIANGSU PROVINCE. **H.X. Ma, X.J. Feng, C.J. Chen and M.G. Zhou.** *College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China. Email: mgzhou@njau.edu.cn*

Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum* (Libert) de Bary, is one of the most devastating diseases of rape in China, causing yield losses from 10% to 80%, and decline in oil quality. Chemical control remains the main way to reduce the incidence of *S. sclerotiorum*. Carbendazim (a benzimidazole) has been used since the early of 1980s and dimethachlon (a dicarboximide) replaced benzimidazoles in some regions after the carbendazim failure in 2001 and 2002. Monitoring was done by collecting the pathogen's sclerotia from rotted stems in Jiangsu province of China, where about 700 thousand ha of oil rape are planted, since 2005 in order to study the resistance of *S. sclerotiorum* to dimethachlon and carbendazim by the method of discrimination dose. Sensitivity tests showed that the ratio of isolates resistant to carbendazim was increased all over the areas monitored. The resistance level of these isolates was very high. The ratio of isolates resistant to carbendazim was different among regions in Jiangsu; it reached 50.0% in Zhenjiang and Nantong city, but was only 3.0% in Wuxi and Changzhou city. There were only 6.0% of dimethachlon-resistant isolates in the tested population in Yancheng, and the resistance level was low about 10 folds to sensitive. No dimethachlon-resistance was found in other regions. Furthermore, the strains resistant to dimethachlon had positive cross resistance to other dicarboximide fungicides such as iprodione and procymidone.

43.7 EFFICACY OF FUNGICIDES APPLIED TO THE SOIL FOR MANAGEMENT OF *PHYTOPHTHORA* ROOT AND CROWN ROT ON CHILE PEPPERS. **M.E. Matheron and M. Porchas.** *Yuma Agricultural Center, University of Arizona, Yuma, Arizona, U.S.A. Email: matheron@ag.arizona.edu*

The oomycete pathogen *Phytophthora capsici* can cause extensive losses in pepper plantings. Studies were initiated to evaluate and compare several new and existing fungicides for their ability to protect pepper plants from *Phytophthora* crown and root rot when applied to the soil. In 2005 and 2006, soil was collected from within the root zone of chile pepper field plants infected with *P. capsici*. Five parts of this soil was thoroughly mixed with 2 parts sand in a large container, then dispensed into a series of 0.5-liter plastic pots. A bell pepper seedling (approximately 8 cm tall) was transplanted into each pot, and the soil in each container was then drenched with 200 ml of a solution containing one of the chemical treatments. Plants were maintained in a greenhouse for approximately 2 months. Additional applications of materials to the soil were made after 1-month in 2005 and after 3- and 6-weeks in 2006. Average survival time for pepper plants grown in infested soil not treated with a fungicide was 5 and 29 days in 2005 and 2006, respectively. On the other hand, in both years the survival of plants in soil treated with cyazofamid, fluazinam, fluopicolide, dimethomorph, mandipropamid, and fenamidone + propamocarb did not differ significantly from plants grown in soil not containing *P. capsici*. These ingredients were all effective for management of root and crown rot on pepper plants when applied as a soil drench in these trials.

43.8 DMI SENSITIVITY STATUS OF EUROPEAN *SEPTORIA TRITICI* POPULATIONS AND RELEVANCE OF CYP51 TARGET MUTATIONS. **A. Mehl, K. Stenzel and K. Tietjen.** *Bayer CropScience AG, Research Biology Fungicides, Building 6240, Alfred-Nobel-Str. 50, D-40789 Monheim, Germany. Email: andreas.mehl@bayercropscience.com*

During the last decade fungicides used to control cereal

pathogens have mostly contained QoI and SBI compounds inhibiting mitochondrial respiration or sterol biosynthesis, respectively. Resistance towards QoI fungicides has developed and spread quite fast, based on a single nucleotide polymorphism known as G143A target mutation in several cereal pathogens such as *Erysiphe graminis* f.sp. *tritici, E. graminis* f.sp. *hordei or Septoria tritici*, therefore chemical control in cereals now relies predominantly on the use of DMI fungicides. Today's resistance research or sensitivity-monitoring focuses on azoles and in particular on performance towards the most important wheat pathogen in northwestern Europe, *S. tritici*. In this context, several individual mutations and combinations of these mutations within the azole target site (cyp51) have been reported to be important for azole efficacy. Latest investigations to be presented show the current sensitivity status of European *S. tritici* strains as well as different correlations between selected cyp51 mutations identified within isolates and in vitro efficacy of studied DMI fungicides. The practical relevance of these findings will be discussed as well as consequences for solid-resistance management strategies.

43.9 BEHAVIOR OF CYAZOFAMID DEPOSITS ON NEWLY DEVELOPING POTATO LEAVES. **S. Mitani, T. Kanza, J. Testers and H.T.A.M. Schepers.** *Central Research Institute, Ishihara Sangyo Kaisha, Ltd., Shiga, Japan. Email: s-mitani@iskweb.co.jp*

Cyazofamid is a selective fungicide highly effective against a limited number of important diseases including potato late blight caused by *Phytophthora infestans*. To evaluate the behavior of cyazofamid on newly developing potato leaves, [^{14}C] labeled cyazofamid was applied to young potato leaves and around vegetative growing points. Leaves were sampled after various growth periods and investigated by autoradiography and combustion of the tissues to determine the amount of cyazofamid. When 266 µg/ml cyazofamid (equivalent to practical use rate, 80 ga.i./ha, water volume 300 l/ha) was applied to young leaves, expansion of the labeled zone was observed after 20 days incubation. When cyazofamid was sprayed around the vegetative growing points or on the unfolded young leaves, cyazofamid was detected on the newly developing potato leaves which were invisible or unfolded at the time of treatment. The amount of cyazofamid required to control late blight was actually present on the newly developed leaves. This study supports previous indications that cyazofamid resulted in the best protection of newly developing potato leaves at the beginning of the growing season when crop growth rate was high.

43.10 MONITORING AND METHODS FOR TESTING THE SENSITIVITY TO MELANIN BIOSYNTHESIS INHIBITOR (MBI-D, R) AND QOI INHIBITOR (STOROBILURIN) OF *MAGNAPORTHE GRISEA* ISOLATES. **N. Nakamura, H. Fukuda, K. Uchida, K. Sou and T. Takeda.** *Pesticide Research Department, ZEN-NOH Agricultural R&D Center, 5-5-1 Higashi-yahata, Hiratsuka, Kanagawa 254-0016, Japan. Email: nakamura-nobuhiro @zennoh.or.jp*

Rice blast caused by *Magnaporthe grisea* is a highly destructive disease of Japanese rice crops. It has often inflicted tremendous damage since the dawn of rice cultivation in Japan. To counteract the disease, melanin biosynthesis inhibitor (MBI) and storobilurin have been widely used, giving excellent results. The sensitivity of *M. grisea* to MBI-D (target scytalone dehydratase), MBI-R (target hydroxynaphthalene reductase) and storobilurin (target QoI) fungicides was examined. Single-spore isolates collected in 821 fields in Japan from 2004 to 2007 were tested by mainly *in vitro* assays. Rice plants in the 2nd leaf stage (cv. Koshihikari) grown in cell-trays were sprayed with carpropamid (MBI-D; 100 ppm and 10 ppm), tricyclazole (MBI-R; 100 ppm and 10 ppm) and azoxystorobin (QoI; 100 ppm, 10 ppm and 1 ppm) in each of three replications. After inoculation, MBI-D resistant *M. grisea* isolates were distinguished clearly by making protective value to the histogram. The cause of the MBI-D resistance is known to be a point mutation resulting in the change Val75Met in scytalone dehydratase, the primary target of MBIs. Furthermore, we established a simple diagnosis method by TaqMan Probe assay using Real-time PCR. Analysis for mutation in the scytalone dehydratase gene could be completed within a day. From 2004 to 2006, MBI-D-resistant isolates were noted in various fields. Their distribution has expanded from south to north in Japan. MBI-R and storobilurin-resistant isolates have not so far been observed. The data of 2007 are now being evaluated.

43.11* MOLECULAR CHARACTERIZATION AND DIAGNOSIS OF DMI-RESISTANCE IN *CERCOSPORA BETICOLA*. **D.C. Nikou, A.A. Malandrakis, J.G. Vontas, A.N. Markoglou and B.N. Ziogas.** *Laboratory of Pesticide Science, Agricultural University of Athens, Greece. Email: ziv@aua.gr*

Strains *of C. beticola* with high and moderate resistance levels to epoxiconazole were isolated from sugarbeet fields in Serres, Veria, Xanthi and Orestiada, Greece, heavily sprayed with triazoles for a number of years. Cross-resistance studies showed that epoxiconazole-resistant strains were less sensitive to the triazole flutriafol but not to the benzimidazole benomyl and the carboxamide boscalid. A small reduction in sensitivity to the QoIs pyraclostrobin, azoxystrobin and fenamidone was observed in a few mutant strains. Study of phytopathogenic fitness did not show any adverse effect by the resistance mutations; most mutants retained their resistance levels even after four generations on fungicide-free medium. The C-14 demethylase (CYP51) gene, the target site of DMI fungicides, was isolated from mutant and wild-type strains using primers designed on conserved sequences and RACE techniques. A number of different mutations, possibly associated with resistance in some strains, were identified. Transcriptional levels of the C-14 demethylase gene were also compared between resistant and wild-type strains using real-time PCR. Overexpression of the C-14 demethylase gene, which was observed in most resistant strains, seems the most possible mechanism of resistance to DMIs in *C. beticola*. Moreover, a neutral mutation in the coding region of C-14 demethylase was obsereved in all resistant strains and permitted the development of a molecular probe for fast and accurate identification of resistance to DMIs in *C. beticola* populations from Greece. This research was co-funded by EU and Ministry of National Education and Religious Affairs.

43.12 ESTIMATION OF THE COST-EFFECTIVENESS OF FUNGICIDE APPLICATION FOR CONTROL OF FUNGAL DISEASES OF WHEAT AND BARLEY. **I. Paul and F. Calitz.** *Agricultural Research Council Small Grain Institute, P.O. Box 3507, Matieland, 7600, Stellenbosch, Republic of South Africa. Email: pauli@arc.agric.za*

Small grains, such as wheat and barley, are staples forming a major part of the world's food supply. However, fungal diseases reduce yields. Host plant resistance is relied on as a method of control, but some sources of resistance have become ineffective,

and in South Africa few breeding programs focus on developing new resistant cultivars. Control of fungal diseases, therefore, relies on the application of fungicides. Fungicides are applied at an early stage, and at a later stage of the small grain plant's development. Fungicide application is expensive, and to sustain production it should be applied in a cost-effective manner. In 2006 and 2007 field trials were conducted to determine the cost-effectiveness of fungicides applied either alone, or together with a seed treatment, to control the prevalent fungal diseases of barley and wheat. Two cultivars of wheat or barley were treated with fungicides at early, late, or both early and late stages. The effect of a high-dose seed treatment was also tested. The profitability of fungicide application was calculated as the total profit from the percentage increase in yield obtained with a specific treatment, minus the total costs associated with the application of the fungicides and/or seed treatment. A correctly timed single application of fungicide was generally found to be more profitable than double fungicide applications. Yield response to the application of a seed treatment varied. Overall a better quality grain was obtained when fungicides were applied.

43.13 BIOLOGICAL MODE OF ACTION OF CAA FUNGICIDES AGAINST *PHYTOPHTHORA INFESTANS* AND *BREMIA LACTUCAE*. **A.E. Rubin, U. Gisi and Y. Cohen.** *Bar Ilan University, Israel. Email: ycohen@mail.biu.ac.il*

Four carboxylic acid amide (CAA) fungicides, mandipropamid (MPD), dimethomorph (DMM), benthiavalicarb (BENT) and iprovalicarb (IPRO) were examined for their effects against the oomycetes *Phytophthora infestans* and *Bremia lactucae*. All were highly inhibitory, with significant differences in intrinsic activity, to spore germination. In *P. infestans*, CAAs did not affect zoospore discharge, motility, viability, or encystment, but all prevented germination of cystospore and sporangia. Cystospores which failed to germinate were often swollen. Electron microscopy showed a large vacuole inside such cystospores. Removal of CAAs after 1 hour of exposure allowed spores to germinate and infect. As low as nM concentrations of MPD were sufficient to inhibit spore germination but mM concentrations were required to inhibit colonization or sporulation *in planta*. In *B. lactucae*, all CAAs inhibited spore germination in vitro or on the leaf surface. MPD was more effective than DMM or BENT whereas IPRO was least effective. CAAs enabled (unlike in *P. infestans*) the emergence of minor germ-tubes but prevented their growth. CAAs induced no disruption of F-actin microfilament organization in the inhibited spores. CAAs applied to lettuce plants after spore germination inhibited penetration and infection. Curative application was effective at ≤3 hpi but not at ≥18 hpi. CAAs (except IPRO) applied to upper leaf surfaces inhibited spore germination on the lower surface and hence reduced infection. BENT and DMM were more effective in suppressing sporulation than MPD or IPRO. CAAs were highly effective in suppressing epidemics of late blight in potato and downy mildew in lettuce.

43.14 BIOEFFICACY OF FUNGICIDES, BIOAGENTS AND BOTANICALS FOR MANAGEMENT OF EARLY BLIGHT (*ALTERNARIA SOLANI*) OF TOMATO. **A. Sataraddi, H. Virupakshaprabhu, M.M. Jamamdar and N.P. Ningaraddi.** *Department of Plant Pathology College of Agriculture, Bijapur, India. Email: arunsataraddi@yahoo.co.in*

Early blight of tomato caused by *Alternaria solani* is a major disease in Karnataka. The efficacy of eight systemic and three non-systemic fungicides was tested against conidial germination of *Alternaria solani.* Among systemic fungicides, maximum inhibition was recorded with 0.05% difenconzate followed big hexaconzazole, propicanazole and thiophanate methyl. At higher concentrations (0.075 and 0.1%) all inhibited conidial germination completely. Among non-systemic fungicides maximum inhibition was recorded with mancozeb at all three concentrations tested. A further four systemic, two non-systemic and one botanical were evaluated for their efficacy in disease control under field conditions. Difenconazale at 0.05% was found to be the best fungicide, giving least disease incidence (8.2%) followed by propiconazole.

43.15 WHEAT LEAF RUST IN EUROPE IN 2007: EFFICACY OF FUNGICIDES AND STUDIES ON THEIR BIOLOGY. **M. Semar, D. Strobel and G. Stammler.** *BASF Aktiengesellschaft, D-67117 Limburgerhof, Germany. Email: martin.semar@basf.com*

In 2007, most European wheat growing regions were highly affected by *Puccinia triticina*, the causal agent of leaf rust. Fungicide programmes based on azoles with high intrinsic anti-rust activity provided effective disease control. QoI fungicides also contributed significantly to the control of rust and led to remarkable yield increases – especially when long-lasting efficacy was required. Under high disease pressure in specific trials, yield gains of up to 28 dt/ha compared to untreated controls or 10 dt/ha compared to products with low anti-rust activity were observed with single treatments. Since outbreaks like that of 2007 lead to a strong selection pressure, the sensitivity of *P. triticina* towards triazoles and QoIs was monitored intensively. The epidemic in 2007 will be considered in relation to epidemiology, fungal pathogenicity and fungicide sensitivity in order to provide insights for future fungicide programmes.

43.16 EVALUATION OF FUNGICIDE RESIDUES IN CHILLI AT HARVEST FOR SAFE PRODUCE. **D. Sharma and G. Ganeshan.** *Indian Institute of Horticultural Research, Hessaraghatta Lake P.O., Bangalore 560089, India. Email: debisharma@gmail.com*

Chilli, (*Capsicum annum* L.) is an economically important crop grown throughout the year in India, for both in fresh green as well as red ripe dry versions. A long-duration crop of more than six months, it suffers from a number of diseases, the most important being anthracnose, leaf spot, powdery mildew, *Alternaria* blight, bacterial wilt and chilli leaf curl. A number of fungicides are used for control of these diseases, often near maturity, so there is a risk of toxic residues remaining on the crop at harvest. The present work was done to determine whether such fungicide application result in persistence of harmful residues on the crop at harvest. We also looked for 'IDM friendly' fungicides which are less persistent and can form a part of the chemical control module of an integrated disease management package for chilli. Green chilli fruits ready to harvest were collected at 3 and 5 days after the last foliar spray treatment of any fungicide, and its residues were analyzed by previously standardized GLC/HPLC methods. Residues of thiophanate methyl, mancozeb, iprodione and propineb were found to be within permissible levels prescribed by Codex Alimentarius (FAO/WHO) at harvest while those of tebuconazole, carbendazim, propineb, difenconazole, tricyclazole and triademifon were above the permissible levels prescribed. No detectable residues of any of these fungicides were found in dry red chilli analyzed a minimum of 30 days after the last spray of any fungicide.

43.17 MECHANISMS OF REDUCED SENSITIVITY TO DMI FUNGICIDES IN PLANT PATHOGENS. **H. Sierotzki, C. Chassot, S. Oehring, R. Frey and U. Gisi.** *Syngenta Crop protection AG, Research Biology, Schaffhauserstrasse, 4332 Stein, Switzerland. Email: helge.sierotzki@syngenta.com*

DMI fungicides inhibit the C-14-demethylase in the ergosterol biosynthesis pathway of fungi. The enzyme is encoded by the *cyp 51* gene and has cytochrome P450 activity. Use of DMI fungicides has selected isolates with reduced sensitivity to DMI's. Possible mechanisms are point mutations in *cyp 51*, over-expression of the target enzyme, and efflux pumps decreasing fungicide concentration in fungal cells. Moreover, variants of *cyp 51*, designated A and B, exist in some species. Many plant pathogens have adapted to DMI fungicides by reduced sensitivity, but the mechanism of resistance has been studied in more detail for only a few species: In *Mycosphaerella graminicola*, 9 mutations in *cyp 51* have emerged over the past 10 years, correlating with reduced sensitivity. Mutations in *cyp 51* were also correlated with reduced sensitivity in *Erysiphe necator* and *Blumeria graminis* f. sp. *tritici*. Over expression of *cyp 51* has been detected in pathogens like *Venturia inaequalis*, *Blumeriella jaapii* and *Penicillium expansum*, whereas in *Botrytis cinerea*, *Mycosphaerella graminicola* and *Monilinia fruticola* over-expression of efflux pumps may explain decreased sensitivity. No information is available about possible reasons for decreased sensitivity for *Mycosphaerella fijiensis*, *Septoria nodorum*, *Blumeria graminis* f. sp. *hordei*, *Cercospora beticola*, *Venturia nashicola*, *Pyrenophora teres*, *P. tritici-repentis* and *Rhynchosporium secalis*. Sequences of *cyp 51* from reduced-sensitivity isolates will be presented for the latter three pathogens.

43.18 MANAGEMENT OF *EUTYPA* DIEBACK IN GRAPEVINES BY APPLYING FUNGICIDE TO PRUNING WOUNDS USING COMMERCIAL SPRAY EQUIPMENT. **M.R. Sosnowski, A.P. Loschiavo and T.J. Wicks.** *South Australian Research and Development Institute, GPO Box 397, Adelaide 5001, Australia. Email: sosnowski.mark@saugov.sa.gov.au*

Eutypa dieback is caused by the fungal ascomycete *Eutypa lata*, and is a serious disease of grapevines worldwide. It contributes to vineyard decline by reducing growth and yield and eventually kills vines. Vines are infected by airborne ascospores, which enter the vascular system through pruning wounds, germinating in the xylem vessels and colonising woody tissue. Fungicides such as carbendazim, biocontrol agents and physical barriers such as paints and pastes applied to wounds with a paint brush can control eutypa dieback but in large-scale viticulture this is not economically viable due to labour costs. This study compared the efficacy of carbendazim applied at 2.5 ml active ingredient/l to pruning wounds using two commercial spray machines, a SARDI fan sprayer (output 596 l/ha) and a Hardi air-assisted sprayer (366 l/ha), with manual paint brush application for the control of *E. lata* infection. Over two consecutive seasons carbendazim applied by paint brush provided 96-100% control of infection. Application with the SARDI fan sprayer provided 92-100% control and the air-assisted sprayer provided 50-77% control. Trials are currently underway to optimise water output rates and spray deposition on a range of different spray machines. Spray application of pruning wound protectants has potential to improve control of eutypa dieback on large large-scale vineyard plantings and could also be used for other fungicides, biocontrol agents and alternative products effective against *E. lata*.

43.19 FREQUENCY OF CYP51 HAPLOTYPES OF *MYCOSPHAERELLA GRAMINICOLA* IN EUROPE. **G. Stammler, M. Carstensen, D. Strobel and M. Semar.** *BASF Aktiengesellschaft, D-67117 Limburgerhof, Germany. Email: gerd.stammler@basf.com*

Demethylation inhibitors (DMIs) are efficient fungicides for the control of *Mycosphaerella graminicola*, the most important cereal pathogen in Northern Europe. After the recent spread of QoI resistance in this area, the continued efficacy of DMI fungicides became controversial. In extensive Europe-wide monitoring studies started in 2001, a slight shift to lower sensitivities to DMIs was initially observed. In 2006 and 2007 this shift had stabilised. Mutations in the target gene of CYP51 (14α-demethylase) are considered as one reason for sensitivity changes. Amino acid sequence analysis of CYP51 of many isolates from different regions showed substitutions or deletions at different positions representing 19 amino acid exchanges or deletions in total. The haplotypes could be grouped in different classes (R-types). These classes were characterised by the presence of different combinations of amino acid exchanges at positions 136, 379, 381, 459, 461 and deletions at positions 459, 460 (461, 462), respectively. The frequency of the single mutations and the different combinations of mutations (R-types) at one geographical location were analysed by pyrosequencing. The data showed that different haplotypes are present within one geographical location. The haplotype characterised by the I381V mutation and the 459/460 deletion with and without the A379G mutation is most frequent in all intensive wheat growing regions in Europe. The mutation V136A is of minor importance. Sensitivity analyses employing more than 1000 isolates revealed a broad range of sensitivities within one R-type. In summary, this indicates a limited contribution of the R-type to DMI sensitivity.

43.20 BETA-TUBULIN GENE MUTATIONS IN BENZIMIDAZOLE-RESISTANT ISOLATES OF *FUSARIUM ASIATICUM* IN JAPAN. **H. Suga, T. Nakajima, K. Kageyama and M. Hyakumachi.** *Life Science Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan. Email: suga@gifu-u.ac.jp*

Fusarium asiaticum is one of the primary pathogens of *Fusarium* head blight of wheat and barley in Japan. Benzimidazole fungicides are widely used to control the disease, and in Japan benzimidazole-resistant isolates of *F. asiaticum* have been detected since 2004. It was reported that mutations in the beta-tubulin gene confer benzimidazole resistance in other ascomycetes. However, no mutation was detected in the beta-tubulin gene, FG09530.1 of the resistant isolates. We analyzed the genome of 30 progenies from crossing between benzimidazole-sensitive and resistant isolates with 49 sequence-tagged markers. Two markers, VNTR987 and VNTR1065, were linked to benzimidazole resistance and they were located at ca. 5 M base far from FG09530.1 in chromosome 4. Another putative beta-tubulin gene, FG06611.1, was detected from these marker regions by using the genome sequence database of *F. graminearum*. FG06611.1 encodes 447 amino acids and has homology to FG09530.1 with 59.7 and 76.7 % in the nucleotide and amino acid sequences respectively, although only 313 amino acids were indicated as encoding the protein of FG06611.1. Several mutations were found in FG06611.1 in the benzimidazole-resistant isolates and these included a mutation at codon positions 167 or 200, that were known to confer benzimidazole resistance in other ascomycetes. These results indicate that mutations in FG06611.1 confer benzimidazole resistance in *F. asiaticum* in Japan.

43.21 STATUS AND MANAGEMENT OF METALAXYL RESISTANCE IN THREE OMYCETE PATHOGENS IN PUNJAB STATE, INDIA. **T.S. Thind, S. Goswami and C. Mohan.** *Department of Plant Pathology, Punjab Agricultural University, Ludhiana, India. Email: thind_ts@yahoo.co.in*

Metalaxyl-based fungicides are frequently used by farmers to manage oomycete pathogens on various crops. Recently, many cases of inferior disease control have been reported by growers. Investigations were carried out to monitor changes in sensitivity levels of three pathogens viz. *Phytophthora infestans* (potato late blight), *P. parasitica* (citrus foot rot) and *Pseudoperonospora cubensis* (cucurbit downy mildew) to metalaxyl. In all, 22 sporangial populations of *P. infestans*, 15 of *P. cubensis* and 42 isolates of *P. parasitica* were studied using standard sensitivity assays. Five populations of *P. infestans* showed ED_{90} values between 50 and 100 µg/ml with resistance factor (RF) of 12.5, while 8 populations of *P. cubensis* possessed ED_{90} values ranging between 30-50 µg/ml with RF between 6.0 and 10.0. A majority of *P. parasitica* isolates showed higher tolerance levels with 6 isolates having ED_{90} values of > 100 and RF of > 20.0. Resistant populations/isolates were equally pathogenic with profuse sporulation. Cross resistance studies indicated good inhibitory effects of cymoxanil, dimethomorph, azoxystrobin, mandipropamid, famoxadone, iprovalicarb and fenamidone against both resistant and sensitive strains of the three pathogens. These compounds have new modes of action. In resistance-affected fields, formulations of cymoxanil and mancozeb (Curzate M-8), dimethomorph (Acrobat) and azoxystrobin (Amistar) showed promising control of potato late blight and cucumber downy mildew. Curzate M-8 also controlled citrus foot rot when applied as a soil drench in orchards suffering from metalaxyl resistance. Such novel-action compounds hold promise as alternatives where metalaxyl resistance to these pathogens is a problem.

43.22 EVOLUTION OF QOI RESISTANCE IN *PLASMOPARA VITICOLA* OOSPORE POPULATIONS COLLECTED IN ITALY. **S.L. Toffolatti, M. Prandato, L. Serrati, H. Sierotzki, U. Gisi and A. Vercesi.** *Istituto di Patologia Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. Email: silvia.toffolatti@unimi.it*

QoI resistance in *P. viticola* was first detected in France and Italy in 1999. Molecular and biological assays have been carried out since 2000 in order to provide reliable methods of detecting and quantifying resistance. QoI resistance of oospore populations, collected in October in vineyards located in nothern and southern Italy, was evaluated by both their germination rate on azoxystrobin-amended medium and the quantification of mutant alleles (G143A) in DNA extracted from the oospores. The methods correlated and can be easily used to test QoI resistance. Due to the spontaneous presence of resistant strains and/or migration from surrounding vineyards, resistance rates below 10% were found in samples collected from vineyards never treated with QoI. Particularly high rates, about 90%, were detected following the application of five to six treatments with QoI in the same season, while lower percentages, about 30%, characterized the vineyards treated with QoI in mixture with partners belonging to different cross-resistance groups. A progressive decrease in resistance rates was observed in vineyards where QoI applications were completely suspended or reduced in number for at least one season, but a strong increase was also found as QoI treatments were resumed in the following season. Full recovery of sensitivity can therefore be obtained in vineyards having high levels of resistance, but particular care should be taken where numerous QoI treatments, especially if not in mixture, were applied in previous years.

43.23 FUNGICIDE APPLICATION AND THE MINIMISATION OF RISK. **F. van den Bosch and D. te Beest.** *Rothamsted Research, Harpenden AL5 2JQ, UK. Email: frank.vandenbosch@bbsrc.ac.uk*

According to various sources, fungicides are overused and a reduction in fungicide dose would be economically justified. Although this common wisdom has been around for several years it has not influenced farming practice nor has it influenced advisers. The motivation for using a larger total dose than seems justified has always been reduction of the risk of suffering major losses in years when the epidemic is severe. It is however not known what aspect of risk in relation to income (gross margin) the farmer/adviser is seeking to minimise. This makes it impossible to evaluate the justification for the total dose used and, more importantly, makes it impossible to search for fungicide-use strategies that increase economic and environmental gains. Here we introduce a new approach to calculate the relation between fungicide use and economic gross margins that does take account of the risk of severe losses in an adverse year. Applying this approach to data on *Septoria tritici* epidemics over the last 5 years we have been able to explain and understand what elements of risk farmers and advisers are seeking to minimise. Elaborating this, we investigated the effect of crop resistance rating on the dose that minimised risk. Further, we extend the method to calculate minimum risk dose for treatments targeted at multiple diseases and take a look at insurance policies and the reduction of fungicide use.

43.24 MANAGING *BOTRYTIS CINEREA* IN STRAWBERRY UNDER HIGH DISEASE PRESSURE. **M. Walter, O.E. Timudo-Torrevilla, K.S.H. Boyd-Wilson and G.I. Langford.** *The Horticulture and Food Research Institute of New Zealand (HortResearch), P.O. Box 51, Lincoln, New Zealand. Email: mwalter@hortresearch.co.nz*

In Auckland, the main strawberry growing region in New Zealand, climatic conditions result in consistently high *Botrytis cinerea* risk, with generally one or more infection events per week. A total of 24 chemical and biological fungicides were evaluated in the laboratory and in the field for control of *Botrytis* strawberry fruit infections during this 4-year research project (2003-2007). The importance of latent *Botrytis* infection occurring at flowering *v.* infection resulting from surface contamination at or during harvest was investigated. Based on laboratory leaf, flower and fruit assays, ten chemical and two biological fungicides were selected for field trials. The two biological fungicides were inconsistent in controlling *Botrytis* in the field. The most consistently performing chemical fungicide was fludioxonil + cyprodinil, which also gave some reduction of leak by delaying the onset of leak rots. Good *Botrytis* control was also achieved with boscalid + pyraclostrobin as well as with fenhexamide and captan applied as a tank mix, although data for the latter are only available for one season (2006/07). Captan, when used alone did not reduce *Botrytis* strawberry flower and fruit infections in the laboratory and field. Strawberry fruit rots were caused by latent infections occurring during flowering as well as by fruit surface contamination at or during harvest. Monitoring of fruit rots during picking and grading showed that *Botrytis* fruit rots doubled as a direct result of fruit handling during harvest. This coupled with the constant high *Botrytis* disease pressure explains the difficulty in producing *Botrytis*-free fruit.

43.25 OPTIMAL TIMING OF FUNGICIDE APPLICATION ON *FUSARIUM* HEAD BLIGHT AND MYCOTOXIN ACCUMULATION IN CLEISTOGAMOUS BARLEY IN JAPAN. **M. Yoshida, T. Nakajima, M. Arai, F. Suzuki and K. Tomimura.** *National Agricultural Research Center for Kyushu Okinawa Region (KONARC), 2421 Suya, Koshi, Kumamoto 861-1192, Japan. Email: ymegu@affrc.go.jp*

Fusarium head blight (FHB), caused by several *Fusarium species, primarily F. graminearum* Schwabe, is a widespread and destructive disease of barley and wheat, infecting spikes and reducing grain yield and quality. Moreover, *Fusarium* species that cause FHB produce mycotoxins such as deoxynivalenol (DON) and nivalenol (NIV), which are toxic to humans and animals. Fungicide application is one measure available to reduce the risk of FHB and mycotoxin contamination in grain. The stage at or near anthesis is generally thought to be optimal for fungicide application for barley, as well as for wheat. However, we have found that the most critical time for *F. graminearum* infection and mycotoxin accumulation in barley differs among cultivars. Whereas chasmogamous (open-flowering) cultivars were most susceptible at anthesis, cleistogamous (closed-flowering) cultivars showed good resistance at anthesis but became relatively susceptible after 'spent' anther extrusion. Therefore, we evaluated the effect of the timing of fungicide application on FHB and mycotoxin (DON and NIV) accumulation in cleistogamous barley in Japan. Thiophanate-methyl fungicide was applied at different developmental stages, from before anthesis to 30 days after anthesis (DAA), under artificial inoculation conditions in the field in which inoculum spores were provided throughout the test period. As expected, the optimal timing for chemical control of FHB and mycotoxin accumulation was the time around the beginning of spent anther extrusion, rather than at anthesis. Later application, as late as 30 DAA, was also effective in reducing mycotoxin accumulation, although it was not effective in reducing disease levels.

43.26 ASSESSMENT OF THE RISK OF *PHYTOPHTHORA MELONIS* DEVELOPING RESISTANCE TO FLUMORPH. **S.S. Zhu, X.H. Lu, Q.X. Meng and X.L. Liu.** *Dept. of Plant Pathology, China Agricultural University, Beijing 100094, P.R. China. Email: seedling@cau.edu.cn*

Flumorph is a new systemic carboxylic acid amide (CAA) fungicide, developed in China for the control of Oomycete pathogens. It is highly active against members of the Peronosporaceae and the genus *Phytophthora* but not *Pythium*. In this study, we isolated 108 strains of *Phytophthora melonis* from infected cucumber plants and, after RAPD analysis, selected eight strains with different genotypes to assess the risk of their developing resistance to flumorph. The results showed that the resistance risk was various among the eight strains. Mutants of *P. melonis* with moderate or high level of resistance (with resistance factor range from 3-900) were only isolated with or without UV mutagenesis from three strains. This to our knowledge is the first report indicating the existence of genetic and biochemical potential for the development of resistance to CAA fungicides in *Phytophthora*. The fungicide-resistant mutants of *P. melonis* were not compromized in stability, mycelial growth and pathogenicity, but sporulation was affected in some mutants. Cross-resistance studies indicated that there is cross-resistance among flumorph, dimethomorph and iprovalicarb. But these CAA fungicides have no cross-resistance with the Q_o-inhibiting fungicide azoxystrobin, Q_i inhibitors cyazofamid, the cyanoacetamideoxime fungicide cymoxanil, and the phenylamide fungicide metalaxyl. Our results showed that the mechanisms of resistance of the pathogen to flumorph, dimethomorph and iprovalicarb may be similar, and these fungicides may also act by a similar mechanism against the target pathogen. Combining the above results and the characteristic that *P. melonis* is a soilborne pathogen, we thought that the risk of developing resistance can be classified as moderate.

Crop biosecurity

17.1* CROP BIOSECURITY IN SLOVENIA: STRATEGIES AND IMPLEMENTATION. **J. Boben, G. Urek, S. Radisek, N. Mehle, T. Dreo, S. Sirca, M. Pirc, M. Zerjav, M. Virscek Marn and M. Ravnikar.** *National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia. Email: jana.boben@nib.si*

In recent years crop biosecurity has been recognized as an important focal point in national as well as European security strategies. Infection of host plants, deliberate or accidental, presents a threat to local economies, thus causing economic, environmental as well as social damage in affected areas. Slovenia has recognized crop biosecurity as an important part of national strategy. Awareness is being raised through the dissemination of results of research projects. Our aim was to prepare a list of plant pathogenic microorganisms, important for Slovenia and to develop a quick and simple method for performing pest risk analysis (PRA). Extensive lists of possible plant pathogens on economically important plants for Slovenia were compiled. The pathogens could be screened using the system developed for quick PRA, adapted (Schaad *et al.*, 2006, Phytopathology, 96: 616-621). Our system was developed by careful selection of criteria that determine the importance of the pathogen. Appropriate weighting was assigned to each criterion. The system was tested on different plant pathogens from fungi and nematodes to viruses, bacteria and phytoplasmas. Results obtained are numerical and can be used to rank plant pathogens according to their relevance in a certain area and give information about where to focus attention in the field. Performance of PRA can be greatly facilitated using this system and can be used for rapid assessment of large numbers of pathogens.

17.2 A SURVEY OF *ALLIUM* DISEASES IN NEW ZEALAND. **J.D. Fletcher, R.A. Lister, P.J. Wright, S.L.H. Viljanen-Rollinson, M.T. Andersen and T. Wei.** *New Zealand Institute for Crop & Food Research Limited, PB 4704 Christchurch, New Zealand. Email: fletcherj@crop.cri.nz*

To determine if pests on the regulated list but until now undetected were present in New Zealand we completed a preliminary survey of viruses, bacteria and phytoplasmas in a representative group of 18 crops of *Allium* spp. in Auckland and Marlborough in the summer of 2004-05. For each crop the entire field was walked in a 'W' pattern, and plants showing symptoms of bacterial, fungal and phytoplasma disease were collected. One hundred leaves were randomly collected for virus assays and to estimate virus incidence. Bacteria and fungi were identified from laboratory assays, phytoplasmas from PCR assay and viruses using ELSA and PCR. None of the *Allium* crops in Auckland and Marlborough were infected with phytoplasmas or regulated bacteria. Bacterial species detected included *Pseudomonas marginalis, Erwinia carotovora*, and *Pseudomonas viridiflava*. Onion white rot (*Sclerotium cepivorum*) was observed in one Auckland garlic crop and an onion crop with incidences of 1-3% and 5% respectively. In Marlborough, white rot was observed in a shallot and a garlic

crop (5%) along with *Alternaria porri*, *Penicillium* spp., *Aspergillus* spp. and *Puccina allii,* all at low incidence rates. The survey confirmed the presence of all *Allium* viruses previously recorded in New Zealand, and in some cases on new hosts. In particular, for the first time in New Zealand we detected the regulated *Grapevine virus A* in *A. cepa, A. chinense, A. ascalonicum* and *A. sativum;* Shallot mite-borne latent virus in *A. cepa, A. ascalonicum* and *A. sativum*; and *Shallot yellow stripe virus* in *A. cepa*. Further work is continuing to confirm the suspected observations of regulated Onion mite-borne latent virus and SJOLV.

17.3 INJURIOUSNESS OF *ZARBUS TENEBRIOIDES ELONGATUS* IN AZERBAIJAN. J. Guliyev. *State Phytosanitary Control Service, Ministry of Agriculture, Baku, Azerbaijan. Email: pqasaze@mail.ru*

Wheat is the most important crop in Azerbaijan, and of high priority is protection from plant pests, the most dangerous of which is *Zarbus tenebrioides elongatus* Men. Standard methods and original approaches were used in field studies. An experiment of 8 allotments in 0.25 m^2 squares in fivefold replicates was implemented. We studied the injury caused by larvae at the edges of special barriers like boards for preventing migration of larvae, placed at a depth of 40-50 cm. The results on injuriousness of larvae and adult beetles were processed statistically. It was shown that one larva decreases the productivity off seed to 5.3 g, and one beetle to 2.7 g. The economic threshold of injuriousness of the pest is defined as the combined damage caused by larva and beetle. It was 1.6-2.2 of larvae per 1 m^2 on normal fields, and 0.8-1 on seed sowings.

17.4* DEMONSTRATING PEST FREEDOM - A QUANTITATIVE APPROACH. N.E.B. Hammond, P.A.J. Martin, D.C. Hardie, C. Hauser and S. Reid. *CRC National Plant Biosecurity & School of Veterinary and Biomedical Sciences, Murdoch University, Western Australia, Australia. Email: N.Hammond@crcplantbiosecurity.com.au*

Demonstration of pest freedom is an important part of the phytosanitary processes surrounding global trade, with many countries requiring declarations of pest free areas before accepting commodities from exporting countries. Claims of pest freedom are often not based on physical survey data, but on expert opinion, together with the fact that the pest has not been recorded in the area of interest. These claims, although they may be based on scientific evidence, are generally not transparent and are becoming less acceptable to trading partners. Surveillance data can provide substantial information for supporting claims of freedom. We have applied a quantitative approach to evaluating complex surveillance data (derived from both targeted and general surveillance activities) for the demonstration of pest freedom. This approach is based on scenario tree analysis of the surveillance systems and utilises Bayesian methods for incorporating prior knowledge of pest status in the analysis. It allows quantitative estimates of confidence in freedom to be derived from surveillance that is targeted for the pest in question and which therefore does not meet the criteria for a representative survey. This method provides transparency in the process of demonstrating pest freedom, providing a clear description of the surveillance system. The applications and limitations of this approach will be discussed.

17.5* CREATION OF A NATIONAL TRAINING PROGRAM IN CROP BIOSECURITY FOR FIRST DETECTORS IN THE U.S. G. Holmes, H. Beck and G. Snyder. *Dept. Plant Pathology, NC State University, Box 7616, Raleigh, North Carolina, 27695, USA. Email: gerald_holmes@ncsu.edu*

As part of ongoing efforts to protect US Agriculture from invasive species of pathogens, insects and weeds, USDA's Cooperative State Research, Education, and Extension Service (CSREES) supported a project to develop a training program for First Detectors (e.g., crop consultants and county agents). The goal of the training is to equip First Detectors to detect and report new findings of high-risk pests and Select Agents. An instructorless, online training program was developed and launched in Dec 2007. Advantages of online training over face-to-face training include: consistency, availability and speed. There are six training modules, the content for which was collected from existing instructor-led trainings and from subject matter experts from the National Plant Diagnostic Network, land grant universities, and from other field professionals. The six modules are: 1. Mission of the NPDN; 2. Monitoring for High-risk Pests; 3. Diagnosing Plant Problems; 4. Submitting Diagnostic Samples; 5. Photography for Diagnostics; 6. Disease & Pest Scenarios. Each module takes 30-45 minutes to complete and contains interactive features, practical examples and a 10-question learning assessment. The training program uses a content management system (Lyra) and an open source learning management system (Moodle) as the technological backbone. A Flash-based web interface displays the finished training in a web browser. These three systems manage the learning content, track and manage the learners, and display the content on the internet.

17.6* BETTER DIAGNOSTICS AND SAFER TRADE: THE PLANT DIAGNOSTIC NETWORKS. C. Lapaire Harmon and T.M. Momol. *Department of Plant Pathology, University of Florida, 1453 Fifield Hall, Gainesville, FL 32611 USA. Email: clharmon@ufl.edu*

Trade and tourism support global economies and local markets. Each also has the potential to be a route for introduction of new organisms that could devastate crops and cripple agriculture. Although international trade occurs on a daily basis, a lack of capacity for in-country diagnostics prior to export is an impediment to safe trade. The National Plant Diagnostic Network (NPDN) was developed in 2002 in the United States as a response to crop biosecurity questions; other countries have since used the NPDN as a model. The International Plant Diagnostic Network (IPDN) is one international organization working toward safer agriculture and trade through accurate, consistent and timely diagnostic efforts. NPDN communication and diagnostic protocols are available to NPDN diagnosticians and other diagnostic personnel globally as a step towards safer international trade. Additionally, training in diagnostic techniques and communication protocols on a regular basis increase capability and diagnostic capacity and develop interpersonal connections that form a supportive network of diagnostic personnel across the country. The development and use of standard operating procedures (SOPs) for techniques and communication encourages trust between entities and allows for scientifically-based trade decisions. Knowledge of current diseases and new introductions may enable countries to protect their agricultural industries and encourage early detection, accurate diagnosis, training, and enhanced communication as a means of risk management.

17.7 INTEGRATED MANAGEMENT PRACTICES FOR TOMATO LEAF CURL DISEASE AND WHITEFLY VECTOR POPULATIONS IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL.). **M.S. Patil and B. Anjaneya Reddy.** *Department of Plant Pathology, University of Agricultural Sciences, Dharwad 580005, Karnatak, India. Email: chinmayi3@rediffmail.com*

Tomato suffers severely from whitefly-transmitted disease caused by *Tomato leaf curl virus* (ToLCV), a geminivirus in the genus *Begomovirus*. The disease is characterized by curling and twisting of leaves followed by marked reduction in leaf size; plants look pale and stunted resulting in a bushy appearance. The incidence of the disease in tomato-growing areas ranged from 17-100 per cent with yield loss exceeding 90 per cent. Presently there are no effective viricides available, therefore, the present study was undertaken to assess integrated management practices including the use of insecticides, plant extracts and viricides to manage the whitefly (*Bemisia tabaci* Genn) and the disease. Field experiments were laid out during Summer 2005 with 12 treatments in 3 replications. ToLCV was identified through ELISA, PCR using ToLCV-specific primers, electron microscopy and cloning and sequencing of the coat protein gene. The results showed that among different treatments, a combined treatment of nylon net covering the tomato nursery beds for 25 days, followed by four sprays, *viz*., imidachloprid (0.005%), triazophos (0.15%), thiomethaxam (0.05%) and EcoNeem (0.5%) at 15 day intervals after transplanting to the main plots showed 23.3% disease incidence with 0.3 average number of whiteflies per plant whereas, the control plots recorded 60.3% incidence with 3.2 whiteflies per plant. The data suggested that adopting integrated approaches along with sprays of insecticides and ecofriendly plant extracts could be used effectively to manage the vector and the disease in tomato.

17.8* VIRUSES IN KIWIFRUIT: ARE THEY A PROBLEM? **M.N. Pearson, D. Cohen, R. Chavan and A.G. Bluin.** *School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand. Email: m.pearson@auckland.ac.nz*

In 2003 *Apple stem grooving virus* was discovered in *Actinidia* accessions from China, being held in quarantine in Auckland. Subsequent examination of kiwifruit germplasm from the same source has detected two additional viruses, a ~300 nm rigid rod related to *Ribgrass mosaic virus* (*Tobamovirus*) and a 700-750 nm flexuous virus related to *Citrus leaf blotch virus* (*Flexiviridae*). Currently these viruses have not been reported from commercial kiwifruit crops in New Zealand or elsewhere. The biological properties of the viruses from kiwifruit and their phylogenetic relationships with similar viruses from other plants will be described, and the possible implications for germplasm importation and commercial kiwifruit production will be discussed.

17.9 A 5-PLEX REAL-TIME qPCR ASSAY FOR QUARANTINE DETECTION AND IDENTIFICATION OF *TILLETIA INDICA*. **M-K. Tan and A. Ghalayini.** *EMAI, NSW Dept. of Primary Industries, Private Mail Bag 8, Camden, NSW 2570, Australia. Email: mui-keng.tan@dpi.nsw.gov.au*

A new molecular test has been developed to help in the surveillance of Karnal bunt, a disease which produces bunted grains with rotting fish smell. Karnal bunt is caused by *Tilletia indica* and is the subject of strict quarantine regulations by many wheat growing countries including Australia. Any incursion would cause severe disruption to Australia's international wheat trade and consequent huge losses in export markets. The protocol involves the release of DNA from spores, enrichment of DNA from *Tilletia* species and a final 5-plex real-time quantitative PCR assay to detect, identify and distinguish *T. indica* and other commonly occurring *Tilletia* species (*T. walkeri*, *T. ehrhartae*, *T. horrida*, and a *Tilletia* group comprising *T. caries*, *T. trabutii* and *T. fusca*) in wheat grains. This fluorescent molecular tool has a detection sensitivity of 5-10 spores and thus bypasses the germination step in the current quarantine protocol required for confirmation when only a few spores have been found. The single–tube 5-plex real-time assay contains five dual-labelled species-specific probes and associated species-specific primer pairs for the 5 target *Tilletia* species. The 5 different amplification products are quantitated and identified simultaneously by 5 different fluorescence spectra in a real-time instrument with at least 5 channels (Rotorgene 6000, Corbett Research, Australia). This protocol with its increased level of detection sensitivity and the technology to multiplex 5 fluorescent real-time PCR assay in one tube will greatly reduce workload and reagent costs and enables the implementation of an economically sustainable and effective Karnal bunt surveillance program.

17.10 EVALUATION OF *BACILLUS THURINGIENSIS* STRAINS AND THEIR MUTANTS. **K. Thakur.** *College of Agriculture Nagpur, India. Email: kuldeept2001@yahoo.com*

Bacillus thuringiensis Berliner is used worldwide as biological pest-control product against Lepidopteran insects, especially *Helicoverpa armigera*, an important pest on cotton, pigeonpea, green gram and chickpea in the Vidarbha region of Maharashtra. In the past, only entomological aspects i.e. insecticidal activities were studied at length. The present investigation was carried out to study pathological aspects of newly isolated Bt isolates from this region with the following objectives: isolation, cultural, physiological, serological, characterization of protein, mutagenic effects, toxicological studies of mutants, plasmid separation and toxicity studies of insecticidal crystal protein for industrial standardization. Twenty-one Bt isolates were obtained, each from one soil sample, one from dead larvae of *H. armigera* and one from the USA. Using artificial diet at 50:l and 100:l concentration, ten Bt isolates were selected for bioassay. Maximum larval mortality 19 was observed in $5AM_3$ followed by $4D_1$ (14) standard strain at 50:l concentration.

17.11 SURVEY OF WESTERN AUSTRALIAN WHEAT FOR *TILLETIA INDICA*-LIKE USTILOSPORES. **D.G. Wright, P. Goldsmith, D. Caper and S.J. McKirdy.** *Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth, WA 6151, Australia. Email: dwright@agric.wa.gov.au*

Tilletia indica is a pathogen of wheat that causes a partial bunt, called Karnal bunt, so far not recorded in Australia. It would cause severe disruption to Australia's international wheat trade if it were introduced. Spring weather conditions throughout much of the Australian grain growing areas are suitable for the development of Karnal bunt. There are other species of *Tilletia* that can be found in grain samples and are morphologically similar to *T. indica*. *T. walkeri* causes a partial bunt of ryegrass and is very closely related to *T. indica*. *T. ehrhartae* causes a bunt of veldt grass. Over the last ten years, the Department of Agriculture and Food Western Australia (DAFWA) has undertaken to survey wheat grain collected by Co-operative Bulk Handling (CBH), after harvest, for

the presence of *Tilletia*-like ustilospores. Each year, approximately 200 samples of wheat (500 g each) were collected from 10-20 receival bins. Samples were tested using the selective sieve wash test. *T. indica* was not detected in any of the samples but other *Tilletia* species were detected, including *T. walkeri* (Geraldton and Kwinana), *T. ehrhartae* (Albany, Esperance, Geraldton and Kwinana), *T. caries* (Albany, Geraldton and Kwinana) and *T. laevis* (Albany and Kwinana). Annual ryegrass and Veldt grass are common weed species in Western Australia. They appear to be contributing ustilospores in commercial wheat grain samples during normal wheat production operations. Increased knowledge and improved identification techniques for *Tilletia* species provide the necessary levels of assurance of grain survey results that facilitate international grain trade.

Disease management in organic farming

28.1 ORGANIC MANAGEMENT OF DISEASE IN ORCHARDS WITH 'NEWER' APPLE CULTIVARS. L. Berkett, M. Garcia, R. Moran, H. Darby, R. Parsons, J. Hayden, T. Bradshaw, S. Kingsley-Richards and M. Cromwell. *Dept. of Plant & Soil Science, University of Vermont, 105 Carrigan Drive, Burlington, VT 05405, USA. Email: lorraine.berkett@uvm.edu*

Although there is significant interest in organic apple production in the New England region of the USA, there are very few certified organic orchards, in part, because of disease challenges associated with the predominant cultivar grown in the region (i.e., 'McIntosh'). However, recent shifts in consumer preference for 'newer' cultivars have led to the planting of different apple cultivars which have different disease susceptibility. A long-term research project was initiated in 2006 to examine the opportunities and challenges of organic apple production within two production systems growers are using to change to new cultivars: planting a new orchard with young trees purchased from a nursery and/or "top-grafting" an established, older orchard to new cultivars. Cultivars being studied in replicated plots in each orchard system include: 'Zestar!', 'Ginger Gold', 'Honeycrisp', 'Macoun', and 'Liberty', a scab-resistant cultivar. Both orchard systems are being managed with approved, organic practices and materials. Standard foliar and fruit disease assessments for apple scab, caused by *Venturia inaequalis*, and other diseases are being conducted to determine differences in disease incidence and severity among the cultivars. Based on initial foliar disease assessments, 'Honeycrisp' appears more resistant to apple scab than the other scab-susceptible cultivars 'Zestar!', 'Ginger Gold', and 'Macoun', but appears more susceptible to cedar apple rust, caused by *Gymnosporangium juniperi-virginianae*, than 'Liberty', 'Macoun', and 'Zestar!'. This research is on-going and will document over time the economic costs, returns, and risks associated with these five cultivars being grown under organic production practices within the two orchard systems.

28.2 TRANSMISSION OF *DRECHSLERA TERES* FROM SEED TO SEEDLINGS IN RELATION TO SOIL ORGANIC CONTENT. G. Brodal, K. Drægni and B. Henriksen. *Bioforsk-Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskolevn 7, N-1432 Ås, Norway. Email: guro.brodal@bioforsk.no*

Healthy seed is important for successful plant production, and organic cereal production must rely on management of seed-borne diseases without synthetic seed treatment chemicals. Inoculum thresholds, i.e. definitions of the acceptable levels of seed-borne inocula, are needed for organic cereal production. In general, significantly lower infection frequencies of seed-borne diseases are recorded on seedlings than on seed. The aim of the work presented was to compare transmission of *Drechslera teres* from barley seeds to seedlings in soils with different organic content. Experiments with two seed lots (93% and 94% *D. teres*, respectively) were carried out in greenhouse with 8 different soils collected in different cereal-growing regions. 100 seeds with 3 replicates from each seed lot were planted in each soil type and placed for emergence at 12°C. Percent emergence and percentage seedlings with symptoms of net blotch were recorded at BBCH 10-12. The net blotch frequency as average for the two seed lots ranged from 11 to 21% in the different soils. Higher content of organic materials resulted in lower infection frequencies. There was a correlation between the amount of organic materials in the soil and % net blotch ($r = -0.83$, $P = 0.009$). Experiments will be repeated with less infected seed lots.

28.3* EVALUATION AND MANAGEMENT OF PLANT PATHOGENS ON ORGANIC FARMS. G.K.N. Chhetry and N. Thingujam. *Department of Life Sciences, Manipur university, Canchipur 795003, Imphal, India. Email: gknc2004@yahoo.co.in*

Tribal farmers in India's North-East use traditional mixed cropping exclusively in organic farming. Three systems are adopted for self sufficiency and efficient management of fungal diseases. Initially slash and burn is practiced in the first year crop followed by tilling without organic manuring and sowing of traditional seed varieties. As the fertility of the soil declines, amendment with farm yard manures either singly or in composite forms provide 70% disease control compared with modern agricultural systems. Organic manures both natural and farmyard seem to provide enough nutrition to enable crops to resist fungal disease, particularly brown spot of upland rice (*Helminthosporium oryzae*), anthracnose of bean (*Colletotrichum lindemuthianum*) and frog eye leaf spot of chilli (*Cercospora capsici*). In addition to cultural techniques, application of locally available medicinal plant extracts provides 80% disease control, compared with chemical application. Further amendment of soil with paddy husks prior to farm yard manure amendment two weeks before the sowing of traditionally maintained pure-bred bean varieties provides almost 95% control of anthracnose compared with chemical-based agriculture. The diversity of pathogens and crop practices in traditional agro-ecosystems and detailed technical know-how of disease control strategies will be reported in this paper. In conclusion, different disease control strategies are needed, as disease developement in mixed copping organic farming systems is different although the farming system is organic.

28.4 IMPROVED YIELDS IN NEW ZEALAND KUMARA (SWEET POTATO, *IPOMOEA BATATAS*) BY PLANTING VIRUS-FREE CLONES. P.J. Fletcher, J.D. Fletcher, B.C. Simpkin, R.R. Simpkin and G.W. Simpkin. *New Zealand Institute for Crop & Food Research Limited, Private Bag 4704, Christchurch, New Zealand. Email: fletcherj@crop.cri.nz*

In New Zealand there has been no clear evidence of the yield, quality, or economic benefits of planting clonally selected, virus-free kumara. However, over many seasons kumara growers have observed and selected two superior performing clones (5E and 5W) from their cv. 'Owairaka Red' crops. These virus-infected clones were tissue cultured, then subjected to an *in vitro* virus

elimination protocol. Virus-free glasshouse-grown cuttings were raised and planted in a field trial in December 2006 in the Tangowahine district, Dargaville, in Northland, New Zealand. The trial was planted using 10 un-rooted, 15 cm-long cuttings in each of the four treatments (5E virus-free, 5W virus-free, 5E virus-infected, 5W virus-infected) with a control treatment of virus-infected, non selected, field-propagated material. The trial was harvested after 24 weeks; all tubers from each plant were counted and weighed before yield and mean tuber weights were calculated for each plant and analysed. There were significant differences in the yields and numbers of tubers between the treatments. Virus-free plants had a significantly greater yield than virus-infected plants and, furthermore, those from clone 5E had a greater yield than those from 5W. Although there was no significant effect of virus infection on tuber number, there was a difference between the clonally selected and control treatments. In conclusion, this experiment demonstrates that yield is higher from virus-free, clonally selected kumara cutting material. A repeat experiment, using the progeny from this trial, will determine whether the gains have been maintained.

28.5 REACTION OF DIFFERENT ROCKET CULTIVARS TO *FUSARIUM OXYSPORUM* F.SP. *RAPHANI*. G. Gilardi, G. Chen, A. Garibaldi and M.L. Gullino. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: giovanna.gilardi@unito.it*

Serious losses occur in rocket, both cultivated (*Eruca vesicaria*) and wild (*Diplotaxis tenuifolia*) infected by *Fusarium oxysporum* in north-western Italy. Isolates of *F. oxysporum* from both sources have been identified by pathogenicity tests on different cruciferous hosts and by molecular tools as belonging to the *formae speciales raphani* and *conglutinans*. The pathogen is seed transmitted and at present spreading in several rocket-producing areas in northern Italy. Disease management is complicated by the limited availability of registered fungicides on this crop, so the possibility of using resistant varieties is interesting. Forty-four cultivated and wild rocket varieties obtained from different seed companies in Italy were tested in order to evaluate their susceptibility to *F. oxysporum* f. sp. *raphani*. The trials were carried out under glass by dipping roots in a conidial suspension of two strains of *F. oxysporum* f. sp. *raphani* (code Fus Ruc 9A and Fus Ruc 13/03). Fus Ruc 9A was found to be the more virulent, and the majority of rocket varieties were susceptible or highly susceptible to it. Some rocket varieties showed resistance to Fus Ruc 13/03. The cultivars of *D. tenuifolia* used in our trials were generally less susceptible to *Fusarium* wilt than those of *E. vesicaria*. The data obtained indicate that only a few of the rocket varieties available in Italy are resistant to *F. oxysporum* f. sp. *raphani*. This suggests the need for intensive breeding in order to select resistant varieties.

28.6 SEASON-LONG PHOTOSYNTHETIC ELECTRON TRANSPORT IN TART (SOUR) CHERRY: EFFECT OF COPPER FUNGICIDES. B.R. Gruber, E.L. Kruger, L.R. Rens and P.S. McManus. *Department of Plant Pathology, 1630 Linden Drive, University of Wisconsin-Madison, Madison 53706, WI, USA. Email: brgruber@plantpath.wisc.edu*

Copper fungicides are currently being investigated as an organically-approved management option for control of *Blumeriella jaapii*, the fungus which causes leaf spot disease of tart cherry. While it has been shown that copper controls leaf spot of tart cherry, foliar application of copper is often associated with severe leaf discoloration. Also, there are published reports detailing the ability of copper ions to perturb photosynthetic electron transport in vitro. Because photosynthetic electron transport is essential to the leaf's ability to fix atmospheric carbon, copper fungicides must be proved non-toxic to economically valuable plants before their application. The purpose of the current study was to assess the effect of copper fungicides on tart cherry photosynthetic electron transport (Jmax, μmol quanta m^{-2} s^{-1}) during the final stage of mesocarp maturation. Photosynthetic gas exchange measurements were used to estimate Jmax, with corrections for mesophyll conductance, in mature tart cherry trees during the 2007 growing season. We found no significant ($P = 0.13$) decrease in Jmax during mesocarp maturation (6 July – 21 July 2007) in trees sprayed with copper fungicides, after regressing estimates of Jmax on time. There were also no significant relationships between time and Jmax for trees sprayed with synthetic fungicides ($P = 0.12$) or unsprayed trees ($P = 0.28$). Thus, while providing sufficient protection against infection by *B. jaapii*, foliar applications of copper fungicides did not significantly perturb photosynthetic electron transport in tart cherry in this 1-year study.

28.7* A SINO-ITALIAN PROJECT FOR TRANSFERRING ORGANIC FARMING TECHNOLOGIES TO CHONGMING ISLAND. M.L. Gullino, A. Garibaldi, G. Minuto, M. Pugliese, G. Shen, L. Huang, X. Qian and C. Clini. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: marialodovica.gullino@unito.it*

Chongming island is the third biggest Island of geographical China after Taiwan and Hainan. A few kilometers from Shanghai, Chongming is turning out to be the first Ecological Recreational Island of China. Production of organic food for the local market is promoted as a means to link higher income to environmental protection. A Sino-Italian project, supported by the Italian Ministry for Land and Sea Environment and the Shanghai Municipal Government, has been designed to convert the conventional high-pollution agricultural system into green and organic farming systems. The technical and economical feasibility of biological control, integrated pest management, grafting on resistant rootstocks, diagnostic kits for plant pathogens, and the use of biodegradable mulching films and drip irrigation combined with fertirrigation were evaluated over two years of field trials on pear, pumpkin, waxy corn, soybean, tomato and watermelon. In the pear orchard, the dosage of pesticides and fertilizers was reduced by 27% and 47% respectively compared to the conventional agricultural system, mantaining the same yields. With waxy corn, pesticides dosage was reduced by 69% while yield increased by 6%.

28.8 POTENTIAL USES OF FODDER RADISH TO CONTROL *MELOIDOGYNE HAPLA* IN ORGANIC FARMING. J. Hallmann, F. Rau and H. Buck. *Federal Biological Research Centre for Agriculture and Forestry, Institute for Nematology and Vertebrate Research, Toppheideweg 88, 48161 Munster, Germany. Email: j.hallmann@bba.de*

In organic farming, yield losses due to soil-borne diseases and pests are often considered only to be a problem during the first 5 years after conversion, but should be no problem in well-managed, long-term organic farms. However, this does not seem to apply to plant damage caused by plant-parasitic nematodes in organic farming in Germany, where problems often start several

years after conversion. This is especially the case for the root-knot nematode *Meloidogyne hapla* in vegetable production. To control *M. hapla*, various uses of fodder radish (*Raphanus sativus*) were explored: (1) selection of cultivars with high resistance and their growth as green manure, (2) growth of fodder radish as a catch crop which involves plant incorporation after 5-6 weeks, and (3) fodder radish used as biofumigation with incorporation at flowering stage after approx. 8 weeks. Results showed that listed fodder radish cultivars varied a lot in *M. hapla* reproduction rates from highly susceptible to almost resistant (Pf/Pi < 0.2). The highest degree of resistance was achieved by cv. 'Commodore'. If grown as a green manure crop 'Commodore' significantly reduced the population density of *M. hapla*. As a catch crop, fodder radish caused a decline in *M. hapla* densities up to 90% on commercial farms, regardless whether a susceptible ('Siletina') or resistant cv. ('Commodore') was grown. If used for biofumigation, control of *M. hapla* was achieved with cultivars that did not propagate the nematode. In conclusion, fodder radish provides several options for control of *M. hapla* in organic farming.

28.9 HEALTH CONDITION OF WHEAT GRAINS IN ORGANIC FARMING IN ITALY. **A. Infantino, G. Avantaggiato, G. Aureli, A. Belocchi and A. Santori.** *C.R.A. - PAV, Centro di Patologia Vegetale, Via C.G. Bertero 22, 00156 Rome, Italy. Email: alessandro.infantino@entecra.it*

The incidence of the most important pathogenic species present on durum and bread wheat grains produced in Italy under organic farming has been evaluated. A total of 349 samples (234 of durum and 115 of bread) from experimental plots cultivated in 2003-2005 have been analyzed by the deep-freezing blotter test. On a sub-set of samples, deoxynivalenol (DON) was measured by both ELISA and HPLC. Fungal species associated with Fusarium head blight (FHB), like *Microdochium nivale* and *Fusarium* spp., were among the most frequently isolated. Only 8 samples (2.3%) showed incidence of both species higher than 15%. Within *Fusarium* spp., incidence of *F. poae* showed an increasing trend in the three years, while incidence of DON-producing species (*F. graminearum* and *F. culmorum*) varied within the years. The two methods of DON measurement were highly correlated. In all samples analyzed, DON values were always under the threshold of 1750 ppb fixed for durum wheat and of 1250 ppb for bread wheat. Correlations between DON and the incidence of DON-producing species were positive, but never exceeding 0.6. The overall results showed good sanitary status of wheat grains in Italy, probably due to the climatic conditions unfavourable to FHB during the three-year period considered. Monitoring the incidence and distribution of the pathogenic fungi present on wheat grains is important for implementation of control strategies, and for choosing the varieties and environments most suited for organic wheat farming.

28.10 THE EFFECT OF REMOVING SOURCES OF PRIMARY INFECTION ON BACTERIAL LEAF BLIGHT OF RICE. **J.H. Kim, K.K. Lee, D.H. Kim, J.S. Choi, T.H. Noh and D.G. Lee.** *Jeollabuk-do Agricultural Research and Extension Services, Iksan 570-704, Republic of Korea. Email: sunflower@jbares.go.kr*

The effect of removing sources of primary infection for bacterial leaf blight on rice was evaluated in fields infested with *Xanthomonas campestris* pv. *oryzae* at Iksan, Korea. The presence or absence of four primary sources of infection including weeds in a nearby a rice field, rice stubble in a diseased field, irrigation water contaminated with the pathogen, and a seed bed located in a habitual disease occurrence area were examined for their effects on rice bacterial leaf blight. Among the primary infections, avoidance of seed bed contamination was the most effective way to suppress the disease in field. The removal of nearby weeds and of rice stubble in a diseased field, and avoidance of contaminated irrigation showed 71.6%, 55.4% and 69.3% reduction in disease, respectively. Removal the primary infection sources was found to be more effective in reducing disease severity than chemical control. It would be a good alternative to reduce disease occurrence and increase yield.

28.11 EFFECT OF LEAF SPOT AND ANTHRACNOSE OCCURRENCE ON BLACK RASPBERRY FRUIT QUALITY. **K.K. Lee, J.H. Kim, J.R. Lim, S.W. Choi, D.H. Kim, J.S. Choi and W.H. Lee.** *Jeollabuk-do Agricultural Research and Extension Services, Iksan 570-704, Republic of Korea. Email: sunflower@jbares.go.kr*

Leaf spot and anthracnose occurred on black raspberry grown in Gochang, Jeongup, and Iksan areas of Korea in 2006-2007. Anthracnose of black raspberry appeared as small dark brown circular spots gradually enlarging to form cylindrical dark brown lesions. The leaf spot symptoms on leaves were greenish black, circular to angular spots on the upper leaf surfaces. Leaf spot and anthracnose caused by *Colletotrichum* spp. and *Pseudocercospora rubi* on black raspberry caused decreased fruit quality. There was a close correlation between level of disease on leaves and weight of fruit, fruit diameter, and fruit sugar content ($r=0.98^{**}$ $r=0.95^{**}$, r=0.70, respectively) in the field. Therefore leaf spot and anthracnose have a negative effect on black raspberrry fruit quality. The regression equation between disease severity and fruit quality showed $Y=-0.0193X+2.036$, $R^2=0.91$, $Y=-0.069X+18.33$, $R^2=0.97$, $Y=-0.033X+10.734$, $R^2=0.49$ respectively.

28.12 IMPROVEMENT OF RICE BLAST RESISTANCE USING INTERPLANTING. **I.S. Oh, B.R. Kim, J.H. Roh, M.K. Kim and S.S. Han.** *Crop Environment and Biotechnology Division, National Institute of Crop Science, RDA, Suwon 441-857, Republic of Korea. Email: ohinseok@rda.go.kr*

Interplanting involves growing two different cultivars in an area at the same time. This procedure is adopted for chemical-free control of rice blast disease. In this study, mixed interplanting, with no distinct row arrangement, was investigated at Hongsung county in Chungnam province. Results showed that interplanting did not enhance resistance to leaf blast at the maximum tillering stage. However, the cultivars Samdukbyeo and Gopumbyeo, when interplanted, showed enhanced resistance, with 50.8% of panicle and neck blast. Cultivars Hopyeongbyeo and Nampyeongbyeo showed decreased panicle and neck blast at two ratios of mixed interplanting with 48.6% and 61.8%. 'Hopyeongbyeo' and 'Nampyeongbyeo', when interplanted, showed highly enhanced blast resistance. Furthermore, the yields and qualities of mixed interplanting cultivars were better than single planting cultivars. Results of this study suggest that interplanting methods have an effect on the resistance to panicle blast and on rice grain qualities.

28.13* SOIL SUPPRESSIVENESS AND FUNCTIONAL DIVERSITY OF THE SOIL MICROFLORA IN ORGANIC FARMING SYSTEMS. **J. Postma and M.T. Schilder.** *Plant Research Interna-*

tional BV., Post Box 16, 6700 AA Wageningen, The Netherlands. Email: joeke.postma@wur.nl

Arable fields of 10 organic farms from different locations within the Netherlands were sampled in three consecutive years. The soil samples were analysed for disease suppressiveness against *Rhizoctonia solani* AG2.2IIIB in sugar beet, *Streptomyces scabies* in radish and *Verticillium dahliae* in oilseed rape. In addition, a variety of microbial, chemical and physical soil characteristics were assessed. All data were correlated by multiple regression and multivariate analyses. Significant differences in soil suppressiveness were found between the fields for all three diseases. Multiple regression indicated a significant positive correlation between suppressiveness against *Rhizoctonia* and the numbers of antagonistic *Lysobacter* spp., whereas % active fungi and bacterial biomass correlated negatively. Grass-clover stimulated *Rhizoctonia* suppression as well as the presence of antagonistic *Lysobacter* spp. (*L. antibioticus* and *gummosus*) in clayey soils. *Streptomyces* suppression correlated positively with higher numbers of antagonistic *Streptomyces* spp., % active fungi and bacterial population size. The presence of antagonistic *Streptomyces* spp. correlated with high fungal/bacterial biomass. *Verticillium* suppression was only measured in 2004 and 2005, since suppressiveness was not consistent between the years. Nevertheless, a significant correlation with pH, potential nitrogen mineralization and lower bacterial biomass was found. Bacterial and fungal PCR-DGGE profiles did not, in general, significantly correlate with disease suppression. The most significant factors explaining the composition of the dominating bacterial and fungal populations were % lutum, pH, C/N-quotient, and numbers, diversity and activity of bacteria. Additionally, the % organic matter and years of organic farming were significantly explanatory for the bacterial population.

28.14 EFFECT OF COPPER-BASED FUNGICIDES ON FRUIT QUALITY OF TART (SOUR) CHERRY. **L.R. Rens, B.R. Gruber and P.S. McManus.** *Department of Plant Pathology, 1630 Linden Drive, University of Wisconsin-Madison, WI 53706, USA. Email: rens@wisc.edu*

Blumeriella jaapii causes cherry leaf spot (CLS), a serious disease of tart (sour) cherry in North America and Europe. Copper-based fungicides control *B. jaapii* but have been associated with phytotoxic symptoms on the leaves of sprayed trees. We compared the phytotoxic effects of copper fungicides to the damage caused by CLS in terms of fruit quality. Fresh cherry fruit mass and brix (g of sucrose per 100 g of H_2O) were compared for trees that were either sprayed with copper fungicides, sprayed with synthetic fungicides, or not sprayed, during the final stage of mesocarp maturation (14 June – 20 July) in 2007. On 14 June, there were no significant differences between fungicide treatments for fruit mass ($P = 0.68$) or for brix ($P = 0.17$) measurements. However, on the last sample date, 20 July, copper-sprayed trees had significantly lower fruit mass compared to the non-sprayed controls ($P = 0.002$) and synthetic-fungicide-sprayed trees ($P = 0.005$). Also, on 20 July, copper-sprayed trees had significantly lower ($P = 0.02$) brix measurements than did synthetic-fungicide-sprayed trees. However, there was no difference in brix measurements between copper-sprayed and non-sprayed trees ($P = 0.20$). In summary, this 1-year study demonstrated that copper fungicides, despite their sufficient control of *B. jaapii* infection, could be having a detrimental effect on fruit mass and fruit sucrose content. Mechanisms explaining copper's negative effect on fruit mass and fruit sucrose content, such as amount of visible leaf damage or photosynthetic processes, are being determined.

28.15 EFFICACY TESTING OF NOVEL ORGANIC FUNGICIDES AND ELICITORS FOR CONTROL OF *PLASMOPARA VITICOLA* IN GRAPEVINE: FROM THE LAB TO THE FIELD. **L. Tamm, T. Amsler, B. Thürig, and H.-J. Schärer.** *Research Institute of Organic Agriculture, FiBL, CH-5070 Frick, Switzerland. Email: Lucius.tamm@fibl.org*

Downy mildew (*Plasmopara viticola*) may cause substantial losses in organic viticulture. Copper is still widely used for downy mildew control. However, copper use is criticized due to its ecotoxicological profile. As a result, its use has been restricted to 6 kg/ha and year in the EU. Depending on country, the maximum annual load is limited to 3-4 kg/ha. In the future, copper use may be phased out altogether and alternative substances are therefore urgently needed. Within the EU-funded REPCO project, novel organic fungicides and elicitors against downy mildew on grapevines were evaluated on grapevine seedlings indoor under controlled conditions and in a second stage in a screening vineyard under field conditions in Frick, Switzerland. Test substances included plant extracts, bio-control agents and simple chemicals. The test substances included commercial formulations as well as crude formulations at very early developmental stage. From more than 100 products tested under controlled conditions, 30 were further examined under field conditions. All of the new products tested under field conditions showed at least partial efficacy. Five novel substances have been identified which show the potential to become viable alternatives to copper use. We will report results from trials conducted in 2004-2007.

28.16* ECONOMICALLY IMPORTANT POTATO TUBER DISEASES IN ORGANIC FARMING IN LATVIA. **I. Turka.** *Univ. of Agriculture, Liela 2, Jelgava, LV 3001, Latvia. Email: Inara.Turka@llu.lv*

In Latvia, organic potatoes could be grown on a larger scale than at present. Restrictions on use of mineral fertilisers and pesticides on organic farms determine potato yield and quality, but late blight caused by *Phytophthora infestans* (Mont.) de Bary is also a major risk. Increased variation in *P. infestans* populations in Europe have made the situation even worse. Organic farmers are therefore mainly growing early potatoes to successfully overtake the late blight infection. On many farms, soil-borne pathogens especially are a real disaster. Black scab *(Rhizoctonia solani* Kühn) common scab (*Streptomyces scabies* Güss) and silver scab (*Helminthosporium solani*) are the main economically important potato tuber diseases. Thus in biological farming, production levels of potato products will be lower than on conventional farms. Organic farmers are growing cultivars resistant to powdery scab, and no data has been obtained that powdery scab is causing quality losses of tubers as has been found in other European countries. Biological means of control have not given positive results against these pathogens. One approach is to use high-tech solutions to reduce the negative environmental risk associated with high input use. An alternative approach is the use of different management practices and training of farmers. In conclusion, our research suggests finding out if organic potato prices are high enough to offset the costs of seed and production practices, and whether it is possible to obtain an adequate income from the work contributed.

28.17 DEVELOPMENT OF A SPRAY PROGRAM FOR APPLE WITH REDUCED FUNGICIDE APPLICATION IN KOREA. **J.Y. Uhm, D.H. Lee, D.H. Kim and H. Woo.** *School of Applied*

Biology and Chemistry, Kyungpook National University, Daegu 702-701, Korea. Email: jyuhm@knu.ac.kr

In Korea, due to frequent rain during the apple growing season, especially in one month of the rainy season, the disease problem is very serious. If fungicides are not used at all, more than 90% of the fruit may be rotten and almost all the leaves may drop before harvest. Most apple growers spray fungicides 14 to 16 times in each growing season. Among the diseases of economic importance, white rot is most serious, as cv. Fuji that is highly susceptible to this disease accounts for more than 70% of apples produced. In addition to white rot, *Marssonina* blotch and bitter rot also occasionally cause considerable damage. In the course of work to reduce fungicide application, two important clues to control white rot were found. One is that the infected fruit can be effectively cured by the application of ergosterol biosynthesis inhibitors (EBIs), especially tebuconazole, in early or mid August regardless of the time of infection, and the other one was that some fungicides, whether they were systemic or not, confer high post-infection activity against white rot. By applying these two facts, basic spray programs with 25-day spray intervals were developed which can control white rot efficiently. The program was modified several times to improve control against bitter rot and *Marssonina* blotch. It is now widely accepted by apple growers in Korea. The fungicide spray frequencies were reduced to five, six and seven times in one cropping season for early-, mid- and late-season varieties, respectively.

28.18 MICROBIALLY ENRICHED COMPOST FOR PLANT HEALTH MANAGEMENT IN ORGANIC CULTIVATION. **N.W. Zaidi and U.S. Singh.** *Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar 263145, India. Email: najamwzaidi@yahoo.co.in*

Trichoderma harzianum (TH) and *Pseudomonas fluorescens* (PsF) are promising biocontrol agents for managing plant diseases. They both colonize cow dung and farm yard manure (FYM) very well. At 32°C and 30-40% moisture content, populations of TH and PsF in colonized cow dung may go as high as ~10^{11}cfu/g and ~10^{13} cfu/g in air-dried compost, respectively, under both sterilized and unsterilized conditions. Because these agents can grow under a wide range of temperature (20 to 32 ˚C) and moisture (20 to 50% of air-dried compost), TH and/or PsF can be used by farmers to colonize their compost either directly in compost pits or after decomposition of FYM. Populations of these agents in samples received from farmers were quite high (> 10^{8}cfu/ g air-dried sample). When added during vermicomposting, PsF multiplies very well (~10^{12} cfu/g) and also enhances the earthworm population. TH may colonize cocoons and inhibit the release of earthworms. Composts colonized by TH and/or PsF act as both pesticide and fertilizer. Bioagent-colonized FYM significantly improved the growth of seedlings (tomato and okra) mainly because of improved nutrition. There was an almost 6-fold increase in water-soluble humic matter content in colonized FYM. Similarly, concentrations of most of the micronutrients were significantly higher in bioagent-colonized FYM. Use of such FYM significantly improved plant health and yield in organically grown onion, chickpea, soybean, tomato, sugarcane and vegetable pea in the field. Bioagent-colonized FYM/vermicompost is being widely used in general agricultural practice by a large number of organic farmers in Uttarakhand state and in adjoining states.

DISEASE MODELS, EPIDEMIOLOGY

8.1 *FUSARIUM* SPP. AND *MICRODOCHIUM NIVALE* POPULATIONS ON WINTER WHEAT IN FLANDERS, IN RELATION TO PRESENCE OF MYCOTOXINS. **K. Audenaert, F. De Witte, R. Van Broeck, K. Messens, M. Höfte and G. Haesaert.** *Department Biological Sciences and Landscape Architecture, Ghent University College, Ghent, Belgium. Email: kris.audenaert@hogent.be*

Head blight of wheat caused by various *Fusarium* spp. and *Microdochium nivale* (*Fusarium nivale*) can result in significant yield losses. Furthermore, *Fusarium* spp. can produce various mycotoxins such as deoxynivalenol (DON) and zearalenon (ZEA). Maximum levels for both mycotoxins have been set by the European Commission since their presence can cause severe health problems particularly for pigs and chickens. The presence of *Fusarium* and mycotoxins is mainly the result of the infection of wheat plants by the pathogen in the field. This presence is favoured by recent agricultural practices such as reduced tillage, incorporation of crop residues and the increased importance of maize in culture rotation systems. In addition, *Fusarium* infections are also promoted by prolonged periods of rain during wheat anthesis. The objective of our study was to gain insight into the geographical composition of the *Fusarium* spp. population throughout Flanders. In 9 locations, corresponding to the major wheat growing areas in Flanders, 12 winter wheat races were grown under similar conditions in 3 replicates and evaluated for *Fusarium* symptoms. In parallel, DON levels were measured using competitive ELISA. Finally, the population composition was characterized using species-specific PCR to distinguish the major *Fusarium* spp. and *M. nivale*. Results of this survey will be presented and discussed.

8.2 DISEASES OF OILSEED RAPE - CURRENT SITUATION IN LATVIA. **B. Bankina, O. Balodis and Z. Gaile.** *Latvia University of Agriculture, Institute of Soil and Plant Sciences, Liela- 2, Jelgava, LV 3001, Latvia. Email: Biruta.Bankina@llu.lv*

The area of oilseed rape has increased dramatically during last ten years in Latvia. Therefore development of diseases becomes one of the most important risk factors for its cultivation under conditions of intensive management in Latvia. Studies of the peculiarities of rape disease epidemiology are necessary for developing economically and biologically reasonable systems of disease management. Assessments and first investigations of winter rape diseases were started in production fields during autumn 2005. Snow mould (*Typhula* spp.), clubroot (*Plasmodiophora brassicae*), downy mildew (*Peronospora brassicae*), and mildew (*Erysiphe cruciferarum*) were observed in some cases, but these are not economically important at present. Blackleg (phoma stem canker) caused by *Leptosphaeria* spp., sclerotinia stem rot (*Sclerotinia sclerotiorum*) and alternaria blotch, (*Alternaria* spp.) have been the most important diseases in Latvia during recent years. Blackleg and alternaria blotch were observed every autumn on the young leaves, but severity did not exceed 2%. Incidence of alternaria was 48-99% on the pods depending on year, variety and field. It is necessary to clarify harmfulness of this disease. The main yield losses were caused by damage to stems. Incidence of blackleg was 5-61% and 60-96% during harvesting in 2006 and 2007 respectively. Sclerotinia stem rot was not observed in 2006, but spreading of this disease was very high (8-78%) in 2007 depending on varieties and field. Research is continuing to clarify the economic importance of these diseases and the details of pathogen development under Latvian conditions.

8.3* INOCULUM AND CLIMATIC FACTORS DRIVING EPIDEMICS OF *BOTRYTIS CINEREA* IN NEW ZEALAND VINEYARDS. **R.M. Beresford, P.N. Wood, D.C. Mundy and G.N. Hill.** *HortResearch, Mt Albert Research Centre, Private Bag 92169, Auckland 1142, New Zealand. Email: rberesford@hortresearch.co.nz*

Disease models that can make accurate predictions of harvest-time severity of *Botrytis* bunch rot in vineyards prior to onset of grape ripening (veraison) are sought to allow more effective planning of fungicide and biocontrol applications and harvesting operations. Fourteen predictive variables were investigated in relation to *Botrytis* disease data from 21 vineyard experiments carried out in three New Zealand regions over three seasons. Of these, two weather variables and one inoculum variable together explained 70% of the variation in logit *Botrytis* severity at harvest. The weather variables were rainfall and infection risk (I) from the "Bacchus" model, both accumulated daily between veraison and harvest. The "Bacchus" model (B. Rengasamy unpublished), relates *B. cinerea* infection risk to temperature (T) for each hour with surface wetness ($I= 84.37-7.238T+0.1856T^2$). The inoculum variable, measured at pre-bunch closure, represented latent *Botrytis* infection within grape berries and was defined as the incidence of sporulating *B. cinerea* on detached berries that had been frozen for 24-48 h, surface sterilised and then incubated in moist trays for 6-10 days. Further research is investigating ELISA- and DNA-based quantification of pre-bunch closure latent *B. cinerea*. Field experiments are investigating the predictive potential of other risk factors, including *B. cinerea*-colonised flower debris, vine canopy density, yeast-assimilable nitrogen in berries and measured or predicted berry sugar content. Mid-season prediction of *Botrytis* risk for fungicide decision-making will require future weather information, derived from either long-range weather forecasts or weather scenarios based on historical probabilities of high-risk weather patterns.

8.4 EFFECT OF TEMPERATURE AND LEAF WETNESS DURATION ON INFECTION OF *COLLETOTRICHUM ACUTATUM*, CAUSAL AGENT OF ANTRACHNOSE ON EVERGREEN AZALEAS. **D. Bertetti, M. L. Gullino and A. Garibaldi.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: domenico.bertetti@unito.it*

Colletotrichum acutatum is the causal agent of anthracnose observed on several cultivars of evergreen azaleas grown in Italy. This pathogen is difficult to eradicate from infected plants, which become unmarketable. Several trials were carried out to evaluate the effects of temperature and leaf wetness duration on development of the disease. Trials were made in growth chambers, using three-month-old plants of the evergreen azalea 'Palestrina', inoculated with a conidial suspension of the pathogen. To evaluate the effect of temperature, inoculated plants were kept in humid plastic bags, at 100 RH, for all the experiments, testing different temperatures: 6, 10, 15, 20 and 25°C. To evaluate the effect of leaf wetness duration on the infection, inoculated plants were kept in humid plastic bags, at 100 RH, for 1, 2, 3, 6, 12 and 48 h; the experiment was carried out at different temperatures: 10, 15, 20, 25 and 30°C. After symptom appearance, the percentage of infected surface was estimated on 40 leaves for each plant. Anthracnose was severe at 15 and 20°C. Symptoms decreased at temperatures outside this range. At 15 and 20°C, the pathogen needed at least 24 h of leaf wetness to develop significant symptoms and 48 h to produce 72 to 78% infection of the leaf surface. No symptoms were observed at 30°C. In all trials the incubation period was rather short: at the most favourable temperatures, first symptoms appeared 48 h after inoculation.

8.5 OOSPORES OF *PHYTOPHTHORA INFESTANS* IN POTATO LEAVES IN LATVIA. **G. Bimsteine.** *Latvia University of Agriculture, Soil and Plant Research institute, LV-3001, Jelgava, Liela 2, Latvia. Email: gunita.bimsteine@llu.lv*

Problems with potato late blight caused by *Phytophthora infestans* (Mont.) de Bary have increased during recent years. This has been explained by the pathogen's ability to form oospores in potato leaves. Consequently the pathogen population has become more aggressive. *P. infestans* oospore formation in Latvia was first reported in the 1980s. Sexual reproduction and formation of oospores could be the reasons for appearance of the stem form. The control of this form is more difficult. Oospores could be found on the field in potato leaves with two or more spots. More potato leaves with two spots were observed at the end of the growing season when disease severity exceeded 50%. Starting in 2002, 215 samples were collected from potato trials fields, conventional fields and from home gardens in different regions of Latvia. The results showed that oospore formation in potato leaves in the field does occur in Latvia. Oospores were found in 80-94% of cases. This means that plant rotation and certification of seed material are the significant measures for potato late blight control, and use of fungicides may not be effective.

8.6 EVALUATION OF A DECISION SUPPORT SYSTEM FOR CONTROL OF STRAWBERRY POWDERY MILDEW IN HUELVA (SOUTH-WESTERN SPAIN). **C. Blanco, B. de los Santos and F. Romero.** *Department of Plant Protection, Centro I.F.A.P.A. Las Torres-Tomejil, 41200 Alcalá del Río, Sevilla, Spain. Email: cesar.blanco.ext@juntadeandalucia.es*

The efficiency of a Decision Support System (DSS) for strawberry powdery mildew control were tested during three seasons on an experimental farm in Huelva (south-western Spain). Two different fungicide application schedules, based on DSS, were compared with the standard strawberry Integrated Production (IP) management. The first schedule consisted of sulphur application, and the second followed the IP management fungicide protocols for the type of fungicide. In both cases, application dates were supported by the DSS. When fungicide was applied following the DSS, a reduction of 20% in frequency of applications was achieved for the three crop seasons, and furthermore the amount of chemical fungicides applied was reduced by 41.6%, when compared to standard IP management. The influence of cultivars and soil disinfestation on the efficiency of DSS was also tested, using three strawberry cultivars ('Camarosa', 'Ventana' and 'Marina') and comparing biosolarized and non-disinfested plots. Statistical analysis showed that DSS performed differently for each cultivar and on disinfested and non-disinfested soil, with higher disease incidence on biosolarized soils.

8.7 SPLASH DISPERSAL OF *PLASMOPARA VITICOLA* PRIMARY INOCULUM. **T. Caffi and V. Rossi.** *Institute of Entomology and Plant Pathology, Sacro Cuore Catholic University, Via E. Parmense 84, 29100 Piacenza, Italy. Email: tito.caffi@unicatt.it*

Primary infections of *Plasmopara viticola* are caused by zoospores originating from oospores that overwinter in leaf litter or in soil. The inoculum is carried from the ground to the grape leaves by splashing rain, but little information exists about the relationships between rainfall, travelling distance and distribution of the inoculum within grapevine canopies. Experimentally, the soil of single curtain-trained plants was uniformly covered with a

powder of two colours: red, under the projection of the canopy on the ground (row); blue, outside this projection (inter-row), in order to mark the splashes from raindrops that fell in these areas. Twelve traps for splashes were placed within the canopy at 3 different heights (40, 80, and 120 cm above the ground) to mimic leaves. Blotting papers were arranged in the abaxial part of the traps, substituted after each rainfall and observed for the number and dimension of the droplets. More than 23,000 droplets were collected (average of 3.9% of the trapping surface covered). The numbers of red and blue droplets were not significantly different, but the former were 1.6 times bigger than the latter. More than 99% of the total droplets were collected at 40 cm above the soil. Rain events lasted 2 to 19 hours, with 1.6 to 64.2 mm of water, 1.3 to 3.8 m/s of wind (max gusts of 30.6 m/s), but these differences did not influence droplets number and distribution significantly. Continuation of these studies will contribute to better understand the relationships between rainfall and primary inoculum of grapevine downy mildew.

8.8 ASCOSPORE MATURATION AND DISCHARGE IN *ERYSIPHE NECATOR*. **T. Caffi, S. Cavagna and V. Rossi.** *Institute of Entomology and Plant Pathology, Sacro Cuore Catholic University, Via E. Parmense 84, 29100 Piacenza, Italy. Email: tito.caffi@unicatt.it*

Ascospores of *Erysiphe necator* are a relevant source of inoculum for spring infections. They form within chasmothecia (formerly named cleistothecia) which develop on the affected grapevine tissue in late summer to autumn, disperse to the bark by rain-splashes, and overwinter. Naturally dispersed chasmothecia were collected at 15-day intervals from ripening to complete leaf fall, in 2005 and 2006. Chasmothecia were placed on the bark of trunk pieces, overwintered outside, and observed weekly for the developmental stage and for discharged ascospores until the end of June. During leaf fall, 56% of chasmothecia had mature ascospores, 26% had immature ascospores, while 18% were unfilled (undifferentiated or empty). During winter (complete leaf fall to bud break) the distribution of chasmothecia in the three classes was 12%, 34%, and 28%, respectively, while after bud break it was 3%, 30%, and 67%, respectively. 35% of ascospores were discharged before complete leaf fall, 7% during winter (especially soon after leaf fall), and 58% after bud break. 55% of the total ascospores discharged before complete leaf fall were released from the earliest group of chasmothecia collected, 38% and 4% from the following groups, while no ascospores were discharged from the latest chasmothecia. These result showed that in northern Italy the development of chasmothecia depends on the period of their formation, resulting in two distinct periods of ascospore discharge in autumn and spring, as previously observed in Australia but not found in the state of New York. These findings may be relevant to disease epidemiology and management.

8.9 EFFECT OF TEMPERATURE AND HUMIDITY ON GERMINATION OF *MONILINIA LAXA*, *M. FRUCTIGENA* AND *M. FRUCTICOLA*. **C. Casals, N. Lamarca, C. Griera, N. Teixidó and J. Usall.** *XaRTA-Postharvest, 191, Rovira Roure Av., 25198 Lleida, Catalonia, Spain. Email: carla.casals@irta.es*

Monilinia spp. are the most important cause of post-harvest decay in peaches and nectarines in the Ebro Valley, Spain. Postharvest losses are typically more severe when relative humidity and temperature are favourable for disease development. The main *Monilinia* species affecting post-harvest stone fruit around the world are *M. laxa*, *M. fructigena* and *M. fructicola*. To evaluate the effect of temperature (0-38ºC) and humidity (a_w: 0.99-0.87) on the lag times prior to germination and germination rates, the three *Monilinia* species were evaluated. The optimum germination conditions were 25ºC and 0.99 relative humidity, respectively, for all the species studied. At 38ºC no species was able to germinate but when the spores were incubated at 0ºC all of them could germinate. In the driest condition studied (0.87 a_w), no species was able to germinate at any temperature, whereas at a 0.90 a_w, spores only germinated at 15, 25 and 30ºC depending on the species studied. In contrast, at 0.95, 0.97 and 0.99 a_w it was possible to find spores germinated for all temperatures tested. Generally, lag times were longer and germination rates were slower when conditions of temperature and a_w were far from the optimum. Knowledge of the germination requirements of this fungus are important in order to understand its behaviour in natural situations, and to predict and to control fungal disease on stone fruits in the field.

8.10 MECHANISMS RELATING ATMOSPHERIC SULPHUR DEPOSITION TO *SEPTORIA* SEVERITY ON WHEAT. **P. Chandramohan and M.W. Shaw.** *School of Biological Sciences, The University of Reading, Reading, RG6 6AS, UK. Email: p.chandramohan@rdg.ac.uk*

In other work a correlation was shown between atmospheric S pollution in England and the relative abundance of *Mycosphaerella graminicola* and *Phaeosphaeria nodorum* on wheat. *P. nodorum* was common during the period of high emissions in the 20th century. Possible causal relationships explaining this correlation include direct effects of acid rain on spore survival and germination; indirect effects of acid rain on the infection process; and indirect effects of S fertilization on the host. Experiments to test these alternatives used three varieties of wheat typical of the early, mid and late 20th century. All fungal isolates were recent. Although isolates varied, *P. nodorum* spores were more sensitive to sulphurous acid than *M. graminicola*; mycelium could grow in very acid media. Infection in simulated acid rain was as effective as in pH 6, for both pathogens. In glasshouse studies using defined nutrients both *M. graminicola* and *P. nodorum* were more severe when sulphates were low, but the relative increase in *M. graminicola* severity was much greater. If the species compete, this could explain the long-term observations, because relatively poorer growth of *M. graminicola* would release niche space for *P. nodorum*. Field studies using plots amended with high and low sulphate and inoculated with *M. graminicola* and *P. nodorum* are in progress. Initial results also show very strong effects on *Puccinia triticina*, which complicates interpretation.

8.11 META-ANALYSIS OF FUNGICIDE EFFICACY TRIALS AGAINST ASIAN SOYBEAN RUST IN BRAZIL. **R.S.C. Christiano, H. Scherm, E.M. Del Ponte, P.D. Esker and C.V. Godoy.** *Department of Plant Pathology, University of Georgia, Athens, GA, USA. Email: scherm@uga.edu*

With the arrival of Asian soybean rust in the Western Hemisphere in 2001, field research to optimize chemical control of this important yield-limiting disease has proliferated. We present a meta-analytical synthesis of the results of 66 fungicide efficacy trials containing >800 entries (specific fungicidal treatments) conducted in Brazil from 2001/02 to 2006/07. Weighted median response ratios for disease severity (R_S) and yield (R_Y) were 0.141

and 1.40, respectively, indicating that, on average, fungicide treatments reduced disease by 85.9% (range: -38.9 to 100%) and increased yield by 40.0% (range: -28.8 to 435%). Response ratios were dependent on disease pressure (expressed as disease severity of the untreated control), being worst under low disease pressure and best under medium (R_S) or high (R_Y) disease pressure. Rank correlation analysis revealed a highly significant inverse relationship between R_Y and R_S for low and high (but not medium) disease pressure. Based on a comparison of the empirical distribution functions between entries with 1 vs. 2 seasonal fungicide applications, 2 applications significantly improved R_S under low and medium disease pressure and R_Y for all three disease pressure classes. For trials that included multiple fungicide classes, pairwise differences in R_S values between fungicide classes were calculated for each trial, and the median differences across trials were tested for deviation from zero. Based on this analysis, DMI fungicides performed significantly better than QoI fungicides. R_S values of DMIs improved significantly in mixture with QoIs but not with benzimidazoles. Differences in performance among specific active ingredients will be discussed.

8.12 DRIVERS AND POSSIBLE CONSEQUENCES OF A CHANGING POPULATION OF *PHYTOPHTHORA INFESTANS* ON THE UK POTATO INDUSTRY. D. Cooke, R.A. Bain, N.J. Bradshaw, A.K. Lees, M.C. Taylor and D.S. Shaw. *SCRI, Invergowrie, Dundee, UK; SAC, Auchincruive, Ayr, UK. Email: david.cooke@scri.ac.uk*

Since 2005 a progressive increase in the A2 mating type of *Phytophthora infestans* has been noted in the United Kingdom (UK). In 2005 the A2 type was found in 38% of reported outbreaks and in 2006 more detailed monitoring indicated that this figure had risen to 65% of 165 outbreaks. In work sponsored by the British Potato Council, the population structure was examined by genotyping almost 1000 isolates from 2006 as well as 300 isolates from the 2003, 2004 and 2005 seasons using Simple Sequence Repeat (SSR) markers. This indicated that the population was dominated by fewer than 10 genotypes and that the increase in the A2 mating type was explained by the dramatic expansion of a single A2 genotype to over 40% of the 2006 population. This genotype has displaced resident genotypes across much of the UK, and several concerns and questions are thus raised. Is there an increased risk of sexual recombination as a result of mixed mating type disease outbreaks, what are the factors that explain such a shift in population, and what are the impacts for disease management? The paper will present findings of studies on pathogen aggressiveness as well as details of the ongoing monitoring in the 2007 season both in the UK and in the context of similar analyses in other European countries. It is proposed that the EUCABLIGHT web site will prove a valuable tool in this latter analysis.

8.13 FACTORS AFFECTING SHORT AND LONG DISTANCE DISPERSAL OF FUNGAL PATHOGENS – CHICKPEA *ASCOCHYTA* BLIGHT AS A MODEL. S.A. Coventry, J.A. Davidson, M.U. Salam and E.S. Scott. *CRC for National Plant Biosecurity, The University of Adelaide, Waite Campus, Adelaide, SA 5064, Australia. Email: steven.coventry@adelaide.edu.au*

Containment and eradication of exotic plant pathogens requires a comprehensive knowledge of epidemiology. Some fungal plant pathogens are spread by both rain-splash and wind, allowing dispersal over long distances and making containment difficult. Plant pathogens already identified in Australia with short and long distance dispersal are being used to evaluate a risk model designed for forecasting disease dispersal. *Ascochyta rabiei*, of world-wide significance in causing *Ascochyta* blight in chickpeas, has both rain-splash and wind dispersed spores, making it a suitable candidate to study the effect of environment on spore dispersal. Field trials were established in 2006 and in 2007 at two sites in south-eastern Australia to study the nature and pattern of spread of *A. rabiei*. Rain was critical in the initiation and continual spread of *A. rabiei* from diseased seedlings in 2006 and from chickpea residues in 2007. In plots of a susceptible cultivar, the prevailing wind was also influential in spread of disease from the central focus. Laboratory experiments to assess the effect of wind speed, rain droplet size and wind-driven-rain on spore dispersal, and studies on the factors affecting viability of conidia, will help to further calibrate the model. This model may provide a template for studying the spread of other diseases and for understanding likely scenarios of plant pathogen incursions.

8.14 AETIOLOGY AND EPIDEMIOLOGY OF *FUSARIUM* HEAD BLIGHT IN THE NORTHERN GRAIN BELT OF AUSTRALIA. P.A.B. Davies and L.W. Burgess. *Faculty of Agriculture, Food and Natural Resources, University of Sydney,NSW Australia. Email: pdav5649@mail.usyd.edu.au*

During a 2005 field survey following an epidemic of *Fusarium* head blight (FHB) of wheat, both *F. graminearum* and *F. pseudograminearum* were isolated from the rachis of FHB-affected stems across the Liverpool Plains in the northern grain belt of Australia. Both crown rot (CR), caused by *F. pseudograminearum*, and FHB occur in this region. In fields where the incidence of CR was high, *F. pseudograminearum* was the dominant FHB pathogen. *F. pseudograminearum* was commonly recovered following the repeated cropping of susceptible winter cereals. In contrast, *F. graminearum* was recovered at high levels even under farming systems that included rotation to non-hosts, long fallowing and cultivation. Therefore, while rotation to non-hosts limited the incidence of FHB caused by *F. pseudograminearum*, it did not appear to affect FHB caused by *F. graminearum*. This suggests that farming systems have an impact on the relative importance of these pathogens. Furthermore, despite the recovery of *F. graminearum* from infected heads, perithecia were absent from residues in affected crops. However, abundant *F. graminearum* perithecia were present on maize residues in the vicinity of two of the survey sites. Long distance dispersal of *F. graminearum* ascospores as demonstrated in North America may explain the source of *F. graminearum* for the outbreak. Ascospore dispersal has not been studied in Australia. Such dispersal would have implications for FHB control, requiring regional inoculum management. Further work is being completed to assess the potential for, and impact of long distance dispersal of ascospores in Australia.

8.15 METHOD OF INOCULATION OF *GUIGNARDIA CITRICARPA* (*PHYLLOSTICTA CITRICARPA*) ON 'PÊRA RIO' SWEET ORANGE FRUIT. T.F. de Almeida, R.F. dos Reis and A. de Goes. *São Paulo State University, Campus of Jaboticabal, Jaboticabal, SP, Brazil. Email: agoes@fcav.unesp.br*

The objective this work was to evaluate the method of inoculation using conidia of *Guignardia citricarpa* on 'Pêra-Rio' sweet orange fruit and to verify the symptom types of citrus black spot expressed. Colonies of *G. citricarpa* were produced on OPDA (orange peel-dextrose-agar) medium, prepared with 200 g of orange peel + 20 g of dextrose + 16 g of agar. Mature sweet orange peel

was triturated using a blender, and 1l of water was added and was filtered through two layers of cheesecloth. The plates were maintained at 25°C for 12/12 h photoperiod for 30 days. The conidia were removed from colonies by adding 10 ml of sterile water, and scraping with a hard brush, then filtering the suspension through two layers of cheesecloth. Ten g/l of sucrose and orange juice (1%v/v) was added to the suspension and the number of conidia adjusted to 10^4 and 10^8 conidia/ml. Fruit measuring 2-2.5 cm of diameter was inoculated using a hand sprayer and enclosed in plastic bags. The plants were maintained in a mist bed for 48 h at 25-27°C, and transferred to the greenhouse. All fruits inoculated showed symptoms typical of CBS approximately 40 days after inoculation. Fruits inoculated with 10^4 conidia/ml showed symptoms of hard spot, whereas 80% of fruit inoculated at 10^8 showed symptoms of false melanose, and 20% of freckle spot.

8.16 NEW INSIGHTS ON THE LIFE CYCLE OF WHITE TIP DISEASE (*PHYTOPHTHORA PORRI*) IN LEEK (*ALLIUM PORRUM*). **B. Declercq, N. Cap, J. De Nies, S. Pollet and M. Höfte.** *Laboratory of Phytopathology, Faculty of Bioscience Engineering, Coupure Links 653, Ghent University, BE-9000 Ghent, Belgium. Email: bart.declercq@ugent.be*

Phytophthora porri is a major disease of leek, causing yield and quality losses in cold and humid conditions, mainly in autumn and winter. On 1 September 2005, a project of Ghent University together with some vegetable research stations began, aiming to develop a predictive model for *P. porri* which will help farmers to spray with more effective timing. To control the pathogen, there is a need to clearly understand its epidemiology. From September 2005 up till now, *P. porri* infestation of leek has been monitored in different fields. It is known that *P. porri* survives the crop-free period by forming oospores which constitute the source of primary inoculum. It is generally believed that oospores initiate infection when they are splashed onto leek plants in a rain shower during the growing season. However, our results indicate that the oospores germinate in the soil during periods of heavy rain and form sporangia, which release zoospores into puddles. There is a strong possibility that primary infection is not caused by splash dispersal of the oospores, but by splash dispersal of the zoospores. Interestingly, oospores were able to germinate in vitro when soil solution was added instead of sterile distilled water. So it is possible that the germination of oospores and subsequently the forming of zoospores is triggered by soil solution or minerals in the soil.

8.17 INTEGRATED CONTROL OF *PODOSPHAERA APHANIS* WITHIN POLYTHENE TUNNELS IN THE UNITED KINGDOM. **J.L.A. Dodgson, S. Parker and A.M. Hall.** *School of Life Sciences, University of Hertfordshire, College Lane, Hatfield, AL10 9AB, UK. Email: jolyond@yahoo.co.uk*

Polythene tunnels have been used by UK strawberry growers since the early 1990's. They protect the ripe fruit, provide early and late season temperatures that are more conducive to strawberry production, and conditions are less suitable for the growth of *Botrytis cinerea*. The use of polythene tunnels has also increased the amount and severity of strawberry powdery mildew infections caused by *Podosphaera aphanis*. Many growers regularly apply fungicides, over 15 times a year in some cases. The epidemiology and development of *P. aphanis* under field conditions has been studied so that the number of fungicide applications applied can be reduced. Analysis of the development of symptoms of *P. aphanis* within commercially managed strawberry tunnels have shown that initial inoculum is either planted into the site on infected plants or overwinters on established plants. Once the tunnels are covered and conditions are suitable for growth and development of the fungus, infection spreads throughout the tunnel. As a result tunnel management has been modified. A rule-based prediction system has been developed that can predict when a site is at highest risk of re-infection by *P. aphanis* inoculum from conidia (asexual spores) after a previous fungicide application. The fungal life cycle is divided into two phases, germination and growth (lag phase). Prediction is based on in-tunnel measurements of temperature, relative humidity and leaf wetness. When the system is implemented growers will only need to apply fungicides when there is a predicted high risk day rather than by following a regular schedule.

8.18 PUTATIVE INOCULUM SOURCES, INFECTION PERIODS AND TEMPERATURE REQUIREMENTS FOR INFECTION OF APPLE FRUIT BY *COLLETOTRICHUM ACUTATUM* IN NEW ZEALAND. **K.R. Everett, O.E. Timudo-Torrevilla, D.C. Mundy, P.W. Shaw, D.R. Wallis, R.W.A. Scheper and P.N. Wood.** *The Horticulture and Food Research Institute of New Zealand Ltd, PB 92169, Mt Albert, Auckland, New Zealand. Email: Keverett@hortresearch.co.nz*

Summer rots of apples in New Zealand are caused most commonly by *Colletotrichum acutatum*. Symptoms of small dark spots appear on fruit in summer (January and February). These lesions can enlarge, become covered with concentric rings of orange spores, and affected fruit can drop prematurely. Buds collected in April 2005 (autumn) and in January 2006 yielded *C. acutatum* from 55-76% of isolations. Few spores were produced on incubated twigs collected from the ground in January 2006 suggesting that twigs were a less important inoculum source. Isolations from sterilised petals and fruit yielded *C. acutatum* from petals in October, and from fruit surfaces in January and February. No *C. acutatum* was isolated from fruit surfaces in December, and only a small number of isolations from fruit sampled in November. Inoculations in the field did not produce symptoms until a mean daily temperature of c. 15°C was exceeded, usually in January and February. Both spore production and infection of fruit were limited at temperatures below c. 15°C on detached fruit in the laboratory. Analysis of water washing from the canopy and rain splash from the ground indicated that the principal source of spores for summer infections is from infected debris (flower remnants and fruit) on the ground. New fruit infections arise once the temperature threshold of 15°C is exceeded in January and February.

8.19 STRATEGIES TO STOP SPREAD OF *LEPTOSPHAERIA MACULANS* (*PHOMA* STEM CANKER) ONTO OILSEED RAPE IN CHINA. **B.D.L. Fitt, B.C. Hu, Z.Q. Li, S.Y. Liu, R.M. Lange, P.D. Kharbanda, M.H. Butterworth and R.P. White.** *Rothamsted Research, Harpenden, Herts., AL5 2JQ, UK. Email: bruce.fitt@bbsrc.ac.uk*

Field experiments in Europe have shown that Chinese winter oilseed rape (*Brassica napus*) cultivars are highly susceptible to the pathogen *Leptosphaeria maculans* (cause of *Phoma* stem canker). Climatic and agronomic conditions in China are suitable for *L. maculans* since the closely related, less damaging, *L. biglobosa* occurs on oilseed rape crops there. Major gene resistance to *L. maculans* is not durable; when introduced into commercial cultivars it is rapidly overcome by changes in pathogen populations.

The threat to Chinese oilseed rape production from *L. maculans* is illustrated by the spread of *L. maculans* into other areas where previously only *L. biglobosa* was present, such as Canada and Poland. Models have been developed to describe the spread (in space and time) of *L. maculans* across Canada, based on survey data collected over a 15 year period in Alberta province. These models have been used to estimate potential spread of *L. maculans* across oilseed rape growing areas of China. Costs of the *Phoma* stem canker epidemic to Canada (crop losses, compensation, survey costs, breeding costs etc.) are being used to estimate the economic/social impact of a *Phoma* stem canker epidemic in China. Short-term strategies to prevent occurrence of severe *Phoma* stem canker epidemics in China include training of extension workers to recognise symptoms of the disease and use of PCR-based diagnostics to detect the pathogen on imported seed. Long-term strategies include introducing durable resistance to *L. maculans* into Chinese oilseed rape cultivars as a component of an integrated disease management programme.

8.20 ORIGIN AND WORLDWIDE SPREAD OF THE ASCOMYCETE *VENTURIA INAEQUALIS*. **P. Gladieux, Z.G. Zhang, D. Afoufa-Bastien, R.M. Valdebenito Sanhueza, M. Sbaghi and B. Le Cam.** *UMR077, INRA, Angers, France. Email: lecam@angers.inra.fr*

Venturia inaequalis is an ascomycete causing apple scab, a disease that has invaded all apple growing regions worldwide. Monitoring and predicting the effectiveness of intervention strategies require knowledge of the origin, introduction pathways, and population biology of pathogen populations. Analysis of the variation of genetic markers offers the potential to retrieve this information. Here, we used microsatellite loci to describe the worldwide population genetic structure of *V. inaequalis*. We show that its centre of diversity is Central Asia, where domestication of apples began 5000-8000 years ago. Geographical partitioning of genetic variation was consistent with large-scale dispersal of the pathogen following its host across all temperate regions of the world. The most likely scenario is that *V. inaequalis* would have entered Europe owing to human trade and migrations along the silk roads and then into newfound lands with the European diaspora. Across the new range, levels of variability point to multiple introductions and all populations displayed signatures of significant post-introduction increases in population size. Most populations exhibited high genotypic diversity and random association of alleles across loci, indicating recombination both in native and introduced areas. The intimate and long-standing relationship between apples and humans makes *V. inaequalis* a model invasive phytopathogenic fungus that has now reached the ultimate stage of the invasion process with a broad geographic distribution and well-established populations displaying high genetic variability, regular sexual reproduction, and demographic expansion.

8.21 LONGEVITY OF SECONDARY SPORIDIA OF FLORET INFECTING *TILLETIA* SPECIES IN LAB AND FIELD ENVIRONMENTS. **B.J. Goates.** *USDA-ARS, 1691 S. 2700 W. Aberdeen, Idaho 83210, USA. Email: Blair.Goates@ars.usda.gov*

Forcibly discharged air-borne secondary sporidia resulting from germinating teliospores of *Tilletia horrida*, *T. indica*, and *T. walkeri* initiate local infection of florets and cause the diseases rice smut, Karnal bunt of wheat, and rye grass bunt respectively. Secondary sporidia are considered to be fragile and short lived, and to require very high humidity to survive longer than a few hours. To examine this, secondary sporidia of these species originating from agar cultures were deposited onto plastic petri dish lids via natural liberation and were air dried and maintained at 10-20% RH at 20-22°C, and at 40-50% RH at 18°C. After various time periods lids were inverted over PDA to determine if sporidia could regenerate. Sporidia commonly regenerated and had produced new secondary sporidia with extensive hyphae 18 hours after lids with dried sporidia were inverted over PDA. Regeneration occurred after up to 30 days at 10-20% RH and 60 days at 40-50% RH. Sporidia initially dried rapidly at 10% RH or dried over 10 hours had no difference in viability. Sporidia of *T. horrida* and *T. indica* dried on petri dish lids and placed 20-25 cm from the soil surface in wheat and barley fields for up to 46-49 days rapidly regenerated over PDA, even after prior diurnal periods that included temperatures above 38°C and relative humidity below 10%. It appears sporidia can survive for extensive dry periods in common field environments and then rapidly regenerate under humid rainy conditions associated with the diseases.

8.22 SURVIVAL OF *DIAPORTHE PHASEOLORUM* VAR. *CAULIVORA* IN CROP RESIDUES. **P.E. Grijalba and A. Ridao.** *Cátedra de Fitopatología, Universidad Nacional de Buenos Aires, Av. San Martín 4453, Capital Federal (1417), Argentina. Email: grijalba@agro.uba.ar*

Stem canker caused by both *D. phaseolorum* var. *meridionalis* and *D. ph*. var. *caulivora* are important diseases of soybeans in Argentina. The objective of this study was to determine the survival ability of *D. ph.* var. *caulivora* in artificially infested straws under field conditions. Complementary attempts were also made in the laboratory to induce the production of anamorphic and teliomorphic stages of both pathogens. Straw pieces of soybean, maize, sorghum, sunflower, potato and wheat were autoclaved, placed in Petri dishes on potato dextrose agar and water agar, and inoculated with a 7-day-old mycelium of each fungus. Cultures were kept at 25±2°C under light with a 12 h photoperiod. Straws artificially infested with *D. ph*. var. *caulivora* were placed in plastic net bags and transferred to an uncropped soil area over the spring season (experimental field in Buenos Aires). *D. ph* var. *meridionalis* produced pycnidia in 10-15 days and perithecia in 35-40 days on both media, whereas *D. ph.* var. *caulivora* produced only perithecia. After 5 months under field conditions, abundant *caulivora* perithecia developed on debris of soybean, maize, wheat and sorghum, whereas pieces of potato and sunflower had disintegrated and no perithecia were observed. These findings suggest that other crops, besides soybean, could maintain alive the inoculum of *D. ph*. var *caulivora* for at least 5 months.

8.23 ANNUAL CHANGES IN OCCURRENCES OF THREE RICE *RHIZOCTONIA* DISEASES IN PADDY FIELDS IN 1996-2006. **Q. Guo, C. Morishima, M. Arakawa and K. Inagaki.** *Faculty of Agriculture, Meijo University, Tempaku, Nagoya 468, Japan. Email: inagakik@ccmfs.meijo-u.ac.jp*

During 11 years from 1996 to 2006, 3 rice sclerotial diseases, i.e., bordered sheath spot caused by *Rhizoctonia oryzae*, brown sclerotium disease (aggregate sheath spot) caused by *R. oryzae-sativae* and brown sheath blight caused by *R. solani* AG-2-2 (IIB) were surveyed in a paddy field (area: 15ha) near Nagoya City, central Japan. In the field, 54 to 70 plots were set up at intervals of ca. 5 m for the survey of disease occurrences which were conducted at the maturation stage (late September) of a late maturing cultivar, 'Aichi no kaori'). Disease occurrence (%) of bordered sheath

spot and brown sclerotium disease in the field was 17.6% and 27.4% on average, ranging from 0 to 61% and from 4 to 85%, respectively, while brown sheath blight was 4.0%, ranging from 0 to 16.7%. As to disease occurrence within the field, bordered sheath spot generally occurred at the center of the field (besides yearly differences at some sites), but the brown sclerotium disease ordinarily occurred at one of four peripheral sites (besides some differences). With bordered sheath spot, there was a high positive correlation (0.618) between mean temperature in July over 11 years and disease occurrence (%), while in brown sclerotium disease there was a negative correlation (-0.573) between precipitation (mm) in August and disease occurrence (%). These results (positional tendencies of disease development in the field and relationship between disease occurrence and atmospheric factors) are considered as important contributions to disease forecasting and control.

8.24 A COMPARISON OF *PODOSPHAERA MACULARIS* AND *P. APHANIS* AND THE ROLE OF CHASMOTHECIA ON STRAWBERRIES. **A.M. Hall, J.L.A. Dodgson and M. Farooq.** *School of Life Sciences, University of Hertfordshire, College Lane, Hatfield, AL10 9AB, UK. Email: a.m.hall@herts.ac.uk*

In the UK strawberries and hops have traditionally been grown in similar areas of the country (eg. Kent, Herefordshire). However older literature does not distinguish clearly between the powdery mildew on strawberries (now *Podosphaera aphanis*) and the powdery mildew on hops (variously recorded as *Sphearotheca macularis, S. humuli* or *P. aphanis*). Since the early 1990s most UK strawberry production has been in polythene tunnels, and strawberry powdery mildew is the most serious disease on these crops. Thus the powdery mildew on hops was seen as a potential threat to strawberry production. The work reported here used both morphological and molecular techniques to distinguish between these two types of powdery mildew and the results suggest that there is no threat to strawberry crops from powdery mildew in nearby hop yards. There are clearly two separate species of powdery mildew. However, work on the development, maturation and survival of the chasmothecia of *P. aphanis* on strawberries and subsequent ascospore release does show that the threat to strawberries is from epidemics initiated in the spring from ascospores released from the *P. aphanis* chasmothecia which have over-wintered on the strawberry crop.

8.25 SURVIVAL OF THE SOYBEAN RUST PATHOGEN IN KUDZU VINES IN FLORIDA, USA. **P.F. Harmon, W.M. Jurick, J.J. Marois and D.L. Wright.** *UF IFAS Plant Pathology, 1453 Fifield Hall, Gainesville, FL 32611, USA. Email: pfharmon@ufl.edu*

Since introduction in 2004, soybean rust caused by *Phakopsora pachyrhizi* has become established in kudzu, *Pueraria lobata*, patches in Florida, USA. Kudzu at six sites at four latitudes in Florida was sampled throughout winter 2006. The inoculum potential of living leaves or leaf litter was estimated at each site by quantifying germination of urediniospores produced from each sample. At all but one site, inoculum fell below levels of detection before disease-favourable weather in spring 2007. Low but detectable levels of viable spores were produced on leaf litter for a short time from multiple sites where kudzu had been defoliated by freezing. Detached leaf assays were carried out in controlled environment chambers under various moisture and temperature conditions to further investigate the potential for urediniospore production from diseased, but dead, leaf material. Time, moisture, and temperature all significantly affected pathogen survival. Under the most conducive conditions for survival, viable spores were produced from detached leaves for the length of the experiment. State-wide monitoring of kudzu since introduction has provided incidence data from several sites. Our data and the incidence data from across the state suggest that recurrence of soybean rust in specific sites where host defoliation occurs, but relatively few hours below freezing accumulate, could be due to small quantities of the pathogen surviving on leaf litter. Additional research is needed to further model pathogen survival so that accurate estimations of initial inoculum may be obtained for the many kudzu sites across the state.

8.26* EPIDEMIOLOGICAL MODELLING OF *PHYTOPHTHORA RAMORUM* INCIDENCE AND SPREAD IN THE UK. **T.D. Harwood, M. Pautasso, X. Xu, M.W. Shaw and M.J. Jeger.** *Division of Biology, Imperial College London, Silwood Park Campus, SL5 7PY, UK. Email: m.pautasso@ic.ac.uk*

The newly discovered zoosporic plant pathogen *Phytophthora ramorum* causes sudden oak death (SOD) in the West Coast of the USA and twig wilt, dieback, stem lesions and leaf necrosis on a range of ornamental species in nurseries and garden centres in the USA and Europe. The pathogen is now known to be present also in the semi-natural environment in the UK and there is a need to forecast the development of this plant epidemic given current knowledge about its spatiotemporal distribution and the structure of the trade network involved in its spread. Spatially explicit modelling of the UK-*P. ramorum* cases during 2003-2006 shows a clear nursery-to-nursery spread within 60 km only for 2003-2004. Theoretical models show that scale-free directed networks have a significantly lower epidemic threshold than local, random and small-world networks even when the number of nodes is only 100, which can be a relevant order of magnitude for the horticultural trade in the UK. Spatial analyses at the landscape level suggest that the disease can spread from nurseries/garden centres to the semi-natural environment and vice versa within 60 km. Analysis of a realistic model of the UK retail and wholesale nursery network in suggests that statutory actions have managed to reduce the spread of *P. ramorum*, but that its spread into the natural environment may have been delayed only slightly.

8.27 EPIDEMIOLOGY OF *ALTERNARIA PANAX* AND FORECASTING DISEASE IN AMERICAN GINSENG. **S.N. Hill and M.K. Hausbeck.** *Department of Plant Pathology, Michigan State University, East Lansing, MI 48814-1311, USA. Email: hillshau@msu.edu*

Alternaria panax, a yearly problem for ginseng growers, incites blighting of the foliage, peduncles, and drupes of cultivated American ginseng (*Panax quinquefolium*). An epidemiological study investigated the influence of air temperature, duration of leaf wetness, rain, and relative humidity on atmospheric conidial concentrations of *A. panax*. Commercial ginseng gardens were monitored in central Wisconsin from mid-May to September for four growing seasons. Hourly concentrations of airborne *A. panax* conidia were enumerated using a Burkard volumetric spore sampler. Fungicides were not applied. Numbers of infected plants were assessed in predetermined portions of the ginseng garden. Disease pressure in the plots was severe, and significant concentrations of *A. panax* conidia were detected in the atmosphere from late May through September yearly. Conidial concentrations were greatest following extended durations of leaf wet-

ness and periods of decreasing relative humidity. A disease forecaster (TOM-CAST), originally developed to predict leaf blight caused by *A. solani* on tomato, was evaluated for management of *A. panax* in commercial gardens. For three years, fungicide sprays initiated by TOM-CAST (using 10 and 15 disease severity value thresholds) were compared with sprays applied at 7- and 10-day intervals. Three fungicide programs were evaluated, and included chlorothalonil alone or in alternation with pyraclostrobin, and pyraclostrobin alternated with copper hydroxide. Although select TOM-CAST treatment programs were comparable to the 7-day schedule in limiting foliar disease, only the 7-day applications adequately protected seed yield and quality.

8.28 SCIENTIFIC VISUALIZATION FOR SIMULATION OF CEREAL CROPS DISEASES. T.Z. Ibragimov. *All-Russia Scientific Research Institute of Phytopathology, Bolshie Vyazemy, Moscow region, 143050, Russia. Email: itz@vniif.rosmail.com*

Development and distribution of plant diseases occurs in a coordinated system of time, space and environment. Thus each component has a dimension. Time is one dimension, space has three dimensions. The greatest dimensional component is the state of the environment which is determined first of all as the state of the host plant and meteorological conditions. These last dimensions can be quite large. For analysis and basic understanding of important factors in arrays of the multivariate data, it is pertinent to use methods of scientific visualization, which "compresses" them in maps suitable for further interactive processing. With application of software OpenDX, visual simulation of developing *Septoria* diseases and other diseases of wheat including multiple pathosystems is possible. Received "visual images" allow us to see and understand features of development of diseases. For simulation of distribution of wheat diseases in time and space, the EpiInfo GIS software has been used. Interactive maps displaying a phytosanitary situation on cereal crops are constructed.

8.29* A META-POPULATION MODEL OF A GENE-FOR-GENE SYSTEM IN A MULTI-LINE VARIETY AND A FUNGAL PATHOGEN. K. Ishiguro. *2-1-9 Kannondai, Tsukuba, Japan. Email: ishiguro@affrc.go.jp*

A lattice-structured model of a multi-locus gene-for-gene system in a disease resistant-multi-line host variety (ML) and a haploid fungal pathogen is described. The model includes evolution of the pathogen population, such as multi-generation asexual reproduction within each epidemic season, migration by spore dispersal between neighboring cells, mutation of the avirulence alleles, and random genetic drift due to over-winter bottlenecks. The mutation and genetic drift processes are considered as stochastic. The ML consists of several host lines, each of which possesses a unique resistance gene. The number of mixed host lines and their mixture ratio are arbitrarily determined in the model. Each cell is assumed as a host crop field. The infection rate of each genotype of the pathogen on ML is determined based on the proportion of susceptible lines in ML. At the start of the simulation runs, one lesion that infects only a single host line was provided a seed, and temporal changes of genotype frequency of the pathogen were calculated. As the number of mixed lines in ML increased, establishment and dominance of pathogen genotypes with a broader virulence range were delayed even without assuming cost of virulence.

8.30 DIURNAL PATTERN OF AIRBORNE SPORES OF *FUSARIUM* HEAD BLIGHT IN THE WEST AUSTRALIAN MEDITERRANEAN ENVIRONMENT. K.W. Jayasena, R. Loughman, J. Bathgate and D.G. Wright and K. Tanaka. *Department of Agriculture and Food, 444 Albany Hwy, Albany, WA 6330, Australia. Email: kjayasena@agric.wa.gov.au*

The fungus *Fusarium graminearum* (teleomorph: *Gibberella zeae*), was first recorded in Western Australia (WA) in 1959 when it occurred as a stalk rot in sorghum. The first known re-occurrence of the fungus was in 2004 in wheat, linked to instances of intensive cropping of winter cereal in sequence after summer millet crops with retained residues. In order to understand the epidemiology of the pathogen, the airborne spores were monitored over millet residue at one site from October 2004 to December 2005 using a 7-day volumetric spore sampler. Weather was also monitored during the period. Ascospores were trapped throughout the year but numbers peaked in September, corresponding to the period of anthesis of cereals planted in winter. During this period only a few macroconidia were trapped. Ascospores were released throughout the day but least numbers were trapped between 10 am to 1 pm. Then there was a gradual increase which peaked during 11 pm and midnight and then decreased to 10 am. Daily mean relative humidity between 72 to 93% and daily mean temperature of 8 to 16ºC were more favourable for ascospore release while rainfall had no direct effect on this. Observations of more ascospores trapped in WA contrast with the finding in northern New South Wales (NSW) where macroconidia were the dominant spore type trapped using susceptible wheat trap plants.

8.31 SPATIAL PATTERN OF *SCLEROTIUM* ROOT ROT AND ITS EFFECTS ON YIELD COMPONENTS IN FALL-SOWN SUGAR BEET CROPS IN SOUTHERN SPAIN. R. Jordán-Ramírez, E. Remesal, R.M. Jiménez-Díaz and J.A. Navas-Cortés. *Instituto de Agricultura Sostenible, CSIC, Apdo. 4084, 14080 Córdoba, Spain. Email: jnavas@ias.csic.es*

The spatial pattern and temporal dynamics of *Sclerotium* root rot (SRR), caused by *Sclerotium rolfsii*, was quantifed in commercial sugar beet fields in southern Spain during two consecutive seasons using heterogeneity and spatial correlation analyses. Overall, ordinary runs analysis indicated a departure from randomness of diseased plant status for adjacent plants within rows. The binomial index of dispersion, interclass correlation and estimates parameters for the binary power law applied to various quadrat sizes suggested aggregation of diseased plants for all plots, and indicated that the degree of heterogeneity was a function of disease incidence. Spatial analysis by distance indices (SADIE) showed a non-random arrangement of quadrats with diseased plants. Spatial pattern was often characterized by the occurrence of several clusters of diseased plants. Increasing clustering over time was evidenced by stronger values of clustering index and increasing size of patch clusters (i.e., clusters contributing to new infections) in SRR assessments made on successive dates. Strongly significant spatial associations were found between the number and size of the spatial aggregates during consecutive disease assessments in each season, suggesting the occurrence of secondary cycles of the pathogen. Sugar beet root yield and sugar content were spatially and negatively correlated with SRR incidence and severity. On the contrary, non-sugar compounds (i.e., Na, P, alpha-amino N) were positively correlated with both disease components. High levels of these compounds have been associated with plant stress, indicating a detrimental effect of SRR to both root yield and industrial quality of *S. rolfsii*-infected sugar beet roots.

8.32 SPATIAL PATTERN OF *SCLEROTIUM* ROOT ROT AND ITS EFFECTS ON YIELD COMPONENTS IN FALL-SOWN SUGAR BEET CROPS IN SOUTHERN SPAIN. R. Jordán-Ramírez, E. Remesal, R.M. Jiménez-Díaz and J.A. Navas-Cortés. *Instituto de Agricultura Sostenible, CSIC, Apdo. 4084, 14080 Córdoba, Spain. Email: jnavas@ias.csic.es*

The spatial pattern and temporal dynamics of *Sclerotium* root rot (SRR), caused by *Sclerotium rolfsii*, was quantifed in commercial sugar beet fields in southern Spain during two consecutive seasons using heterogeneity and spatial correlation analyses. Overall, ordinary runs analysis indicated a departure from randomness of diseased plant status for adjacent plants within rows. The binomial index of dispersion, interclass correlation and estimates parameters for the binary power law applied to various quadrat sizes suggested aggregation of diseased plants for all plots, and indicated that the degree of heterogeneity was a function of disease incidence. Spatial analysis by distance indices (SADIE) showed a non-random arrangement of quadrats with diseased plants. Spatial pattern was often characterized by the occurrence of several clusters of diseased plants. Increasing clustering over time was evidenced by stronger values of clustering index and increasing size of patch clusters (i.e., clusters contributing to new infections) in SRR assessments made on successive dates. Strongly significant spatial associations were found between the number and size of the spatial aggregates during consecutive disease assessments in each season, suggesting the occurrence of secondary cycles of the pathogen. Sugar beet root yield and sugar content were spatially and negatively correlated with SRR incidence and severity. On the contrary, non-sugar compounds (i.e., Na, P, alpha-amino N) were positively correlated with both disease components. High levels of these compounds have been associated with plant stress, indicating a detrimental effect of SRR to both root yield and industrial quality of *S. rolfsii*-infected sugar beet roots.

8.33 CERCBET 3 - A FORECASTER FOR EPIDEMIC DEVELOPMENT OF *CERCOSPORA BETICOLA*. E. Jörg, P. Racca and B. Kleinhenz. *ZEPP-Central Institution for Decision Support Systems and Programmes in Crop Protection, Rüdesheimer Str. 60-68, 55545 Bad Kreuznach, Germany. Email: erich.joerg@dlr.rlp.de*

Cercospora beticola is the most prevalent and damaging fungal disease in German sugar beet growing. Control strategies are based on action thresholds. A model has been developed which forecasts epidemic development (expressed as disease incidence) and signals when action thresholds are exceeded. The plot–specific model, CERCBET 3, uses as input meteorological parameters (temperature, relative humidity), easily accessible agronomic field characteristics and a single recording of *C. beticola* disease incidence. Extensive validation in 2001-2003 showed that CERCBET 3 in 80-95% of cases correctly forecast the dates when thresholds were exceeded. As cultivar diversity in German sugar beet growing is increasing, a module has been included into CERCBET 3 which reflects susceptibility to *C. beticola* by introducing a sporulation factor. In some cases a second or even third fungicide treatment could be necessary to control *Cercospora* leaf spot and so a further module which models fungicide efficacy has been elaborated. *CERCBET 3 is* interactively available for sugar beet growers on the internet platform ISIP, which is provided by the German federal crop protection services.

8.34* FUS-OPT A DECISION-SUPPORT SYSTEM FOR FUNGICIDE SCHEDULING AGAINST *FUSARIUM* HEADBLIGHT. E. Jörg, P. Racca, J. Weinert, A. von Tiedeman and B. Kleinhenz. *ZEPP-Central Institution for Decision Support Systems and Programmes in Crop Protection, Rüdesheimer Str. 60-68, 55545 Bad Kreuznach, Germany. Email: erich.joerg@dlr.rlp.de*

This model includes different processes in the epidemiology of *F. graminearum*. The first is ascospore formation on the before-crop residuals, which can be subdivided in two sub-processes: mycelial growth and build-up of perithecia. The temperature dependence and moisture dependence of both processes was examined. A temperature and precipitation dependent function was developed for calculating the water-content of crop residuals. With this function we could easily determinate the periods when ascospores disperse. In addition the model can show infection rate, which depends on wheat head wetness and temperature. Infections are only possible if the wheat is in a susceptible phase, and the model predicts highest susceptibility during flowering (BBCH 61-69). The model issues a daily infection pressure. These values are summed, only in the susceptibility period, in an infection pressure index (IPI). The IPI is compared to a threshold value in order to predict risk for toxin content at the end of the season and give a recommendation for treatment when the threshold is exceeded. The results of the first model validation were very satisfactory. In 90% of the cases, the model gave the correct recommendation, in 6.5% the model proposed an unnecessary treatment and in only 3.2% of the cases did the model underestimate the end-of-season toxin content.

8.35 SIMPEROTA1/3 - A DECISION-SUPPORT SYSTEM FOR TOBACCO BLUE MOULD DISEASE. B. Kleinhenz, P. Racca and E. Jörg. *ZEPP–Central Institution for Decision Support Systems and Programmes in Crop Protection, Rüdesheimer Str. 60-68, 55545 Bad Kreuznach, Germany. Email: kleinhenz@zepp.info*

Blue mould is the most serious threat to German tobacco crops. In order to efficiently control the disease while minimising the risk of non-tolerable fungicide residue levels on tobacco leaves a decision-support system has been developed which optimises the timing of fungicide treatments. The DSS consists of two models, SIMPEROTA 1, which forecasts the dates of blue mould first appearance and SIMPEROTA 3 which suggests the timing of fungicide applications. Crucial biological processes are included into the models (infection, mycelium growth, sporulation and spore release). Input parameters are temperature, relative humidity and leaf wetness recorded hourly. Validation with data from 2003 and 2006 showed that SIMPEROTA 1 gave satisfying results. The model is suitable for practical use and can be employed for steering or monitoring efforts of extension services and for the timing of the first fungicide treatment. SIMPEROTA 3 gives advice on follow-up treatments and the length of spraying intervals. This model needs to be validated before introduction into practice.

8.36 RESEARCH ON APRICOT DECLINE IN AUSTRIA. A. Krbec and B. Schildberger. *Höhere Bundeslehranstalt für Wein- und Obstbau Klosterneuburg, Abteilung Biologie, Referat Pflanzenschutz, Wiener Straße 74, 3400 Klosterneuburg, Austria. Email: barbara.schildberger@hblawo.bmlfuw.gv.at*

Besides known pathogens such as *Pseudomonas syringae*, *Xanthomonas campestris*, phytoplasmas etc. various fungi and protists have been described as the cause of apricot tree decline worldwide. Screening for fungi of diseased and healthy apricot trees in Austria at the research station of the Federal College and Insti-

tute for Viticulture and Pomology Klosterneuburg has focused on *Phytophtora* spp., *Thielaviopsis basicola*, *Verticillium* spp. and *Valsa* spp. Work with plant tissue such as roots, bark, wood, branches, leaves and fruits and soil has led to the implication of two species of *Phytophtora*, *Verticillium sphaerosporum* and various species of *Fusarium*. The well-known pathogens *F. oxysporum* and *F. solani* were found in all samples and all parts of diseased trees. Five isolates on apricot rootstock *Prunus insititia* St. Julien 'INRA GF 655/2' were tested for pathogenicity by inoculation with spore suspensions. Suspected pathogenic samples were identified by sequenzing of the internal transcripted spacer region and the 5.8 S rDNA.

8.37 EXAMINATION OF FIELD SOILS IN THE NORTHERN ROCKY MOUNTAIN REGION FOR *CERCOSPORA BETICOLA*, USING ELISA AND PCR. **R.T. Lartey, T.C. Caesar-TonThat, B.L. Allen, S. Hanson, W.B. Stevens and R.G. Evans.** *USDA/ARS, Northern Plains Agricultural Laboratory, 1500 North Central Ave., Sidney, MT 59270, USA. Email: robert.lartey@ars.usda.gov*

As an integral part of an ongoing nationwide survey, we examined several fields in the Northern Rocky Mountain Region (Montana, North Dakota, Wyoming, Idaho and Colorado) for *Cercospora beticola*, the causal agent of *Cercospora* leaf spot (CLS) of sugar beet. We used enzyme-linked immunosorbent assay (ELISA) to detect *C. beticola* in soil and confirmed the results with PCR. Soils were randomly sampled from several areas including sugar beet fields, pasture and forest. The sugar beet fields were either under sugar beet or in rotation with other crops such as barley and safflower. Others consisted of fields with no previous history of sugar beet or had never been cultivated (i.e., rangeland, grassland). Soil samples were subjected to ELISA using pre-adsorption *C. beticola* specific antibodies. For PCR detection, DNA was purified from soil using PowerSoil DNA Kit (MO BIO, CA) as per the manufacturer's instructions. The DNA was then subjected to PCR in Extract-N-Amp PCR mix (Sigma Aldrich, St Louis MO) using the *C. Beticola*-specific actin primers CBACTIN959 L (5' GTAAGTGCTGCCACAATCAGAC 3') and CBACTIN959 R (5' TACCATGACGATGTTTCCGTAG 3'). The amplicons were resolved by electrophoresis in 1% agarose gels. Using ELISA and PCR, we detected *C. beticola* in soil at most locations. Contrary to the hypothesis that *C. beticola* exists only where sugar beet is grown, soils testing positive included sites with no production of the known host plants, sugar beet and safflower. This survey has identified areas with *C. beticola* and risk of CLS incidence on susceptible crops even at locations with no history of the disease.

8.38 THE IMPACT OF SOLAR RADIATION ON SPORE SURVIVAL IN *PHYTOPHTHORA INFESTANS*, *BREMIA LACTUCAE* AND *PSEUDOPERONOSPORA CUBENSIS*. **V.H. Le, B. Nordskog, R. Nærstad, D.M. Gadoury and A. Hermansen.** *Bioforsk-Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, N-1432 Ås, Norway. Email: vinh.le@bioforsk.no*

Aerial dispersal of inoculum is critical to the spread of many plant diseases; including potato late blight (*Phytophthora infestans*, Pi), lettuce downy mildew (*Bremia lactucae*, Bl) and cucurbit downy mildew (*Pseudoperonospora cubensis*, Pc). In addition to relative humidity and temperature, spore survival during aerial dispersal is affected by solar irradiation (SI), in particular during long-distance transport at higher altitudes. We evaluated the potential survival of spores in air by placing detached spores of Pi, Bl and Pc on filter paper in either direct sun or shade at time intervals from 0.5 to 3 h (Pi and Bl), or up to 42 h (Pc). The filter papers were then placed in moist chambers for 15 min prior to incubation on pea agar (Pi) or water agar (Bl and Pc) for 24 h, before the viable spores were counted. Spores were considered viable if they grew a germ tube or released zoospores. Preliminary results show that no spores of Pi, Bl and Pc germinated after 1, 3 and 30 h exposure to direct sun, with critical SI doses near 700, 2000 and 8500 Wm^{-2}, respectively. In shade, no Pi spores germinated after 3 h, while spores of Bl and Pc were still viable after 3 and 42 h, respectively. In Norway, the potential for long distance distribution of Pi is therefore restricted, but more likely for Bl and Pc. Further experiments will be done to find the maximum survival time for spores of these pathogens under Norwegian conditions.

8.39 USING MOLECULAR TOOLS TO INVESTIGATE THE ROLE OF TUBER- AND SOIL-BORNE INOCULUM OF *PHYTOPHTHORA INFESTANS*. **A.K. Lees, L. Sullivan, N.A. Williams and D.E.L. Cooke.** *Plant Pathology, SCRI, Invergowrie, Dundee, DD2 5DA, UK. Email: Alison.Lees@scri.ac.uk*

Phytophthora infestans, the oomycete pathogen that causes late blight, infects potato foliage and tubers, reducing yield and quality of ware and seed potato crops. The presence of both the A1 and A2 mating types of *P. infestans* increases the likelihood of the formation of oospores that can survive in soil for many years and germinate to initiate early epidemics. Moreover, sexual reproduction as a result of oospore formation provides the potential for increased recombination within the pathogen population, enhancing the ability to overcome control measures. This study aims to investigate the significance of *P. infestans*-infected seed and oospores as a source of primary inoculum, taking into account recent changes in the UK and European *P. infestans* populations. To develop integrated disease control strategies targeted to specific stages of the disease cycle, information is required on the means of infection from the seed tuber or soil into the growing crop, the relative contribution of different sources of inoculum in causing disease, and the influence that host resistance, other pathogen interactions and climate have on these processes. The investigation of such factors has been facilitated by the use of a quantitative, species-specific, *P. infestans* PCR assay and SSR assays developed at SCRI which allow the detection, quantification and tracking of the pathogen. Examples of studies conducted using these tools are presented.

8.40 KARNAL BUNT OF WHEAT - VALIDATION OF AN EPIDEMIOLOGICAL MODEL IN FARMERS' FIELDS. **S.K. Mann and P.P.S. Pannu.** *Dean PGS, PAU, Ludhiana, Punjab, India. Email: drskmann@yahoo.com*

The epidemiological model which had been developed for forecasting Karnal bunt of wheat, is based on wheat field weather parameters (8-21°C and 80% relative humidity) found favourable for the survival of the infective stage (secondary sporidia) of *Tilletia indica*. The model was validated on farmers' fields in the endemic region of India viz., the Jalandher, Gurdaspur and Hoshiarpur districts of Punjab state. Two-acre fields of 10 farmers in each of these districts were selected and sown with recommended variety PBW 343 as per the cultural practices recommended by Punjab Agricultural university, Ludhiana. The weather conditions were monitored daily and inoculum load of the

pathogen *T. indica* was monitored diurnally in the wheat field for the day and night regimes during the heading period of the crop. The weather conditions were favourable during the heading period and the sporidial inoculum during the day time also remained at threshold levels from ear emergence to heading during 2007. One spray of Tilt 25EC @ 160 ml/200 l water was given to one-acre plots of each farm at all 3 locations. The incidence of Karnal bunt-infected grains in the unsprayed plots was 1.2, 1.26 and 12.02% at Jalandher, Gurdaspur and Hoshiarpur respectively, whereas in the fungicide-sprayed fields, the incidence was 0.01, 0.001 and 0.16% respectively at the 3 locations. The present investigations demonstrated successful management of Karnal bunt, a disease which was of quarantine significance affecting the food grain trade.

8.41 PRODUCTION OF *MYCOSPHAERELLA PINODES* INOCULUM ON WHEAT GRAIN FOR FIELD INOCULATIONS. **M.V. Marroni and S.L.H. Viljanen-Rollinson.** *New Zealand Institute for Crop & Food Research Limited, P.B. 4704, Christchurch, New Zealand. Email: marroniv@crop.cri.nz*

Ascochyta blight of peas is a major disease reducing productivity of pea crops worldwide. In New Zealand, *Mycosphaerella pinodes* is the main causal agent of the disease. A four-year research programme was established in 2004 between the New Zealand pea industry stakeholders and Crop & Food Research which aimed to develop sustainable management practises to increase productivity of New Zealand pea crops. Under this project, fungicide trials required artificial inoculation with *M. pinodes* to ensure the development of ascochyta blight epidemics. Traditional inoculum production methods comprise inoculation of barley grain with mycelial plugs and incubating for approximately 4 to 7 weeks. We aimed to develop a faster method to meet the demands of our large field fungicide trials. Inoculum of *M. pinodes* was produced on wheat grain. Selected isolates from the Crop & Food Research culture collection were cultured in Petri dishes containing PDA for 10-12days at 20°C with a 12 h photoperiod of white light. Wheat grain (200 g) was placed in 1 l beakers, moistened and covered with aluminium foil lids and autoclaved twice at 120ºC for 1 h at 24 h intervals. A liquid suspension was obtained from *M. pinodes* cultures and used to inoculate the wheat grain. Beakers were turned over twice a week to homogenize the contents. Grains were fully colonized after 3 weeks incubation at 20ºC, after which they could be spread onto the field plots. This method resulted in grain being colonized faster than inoculating the grain with mycelium plugs.

8.42 SNPS AND MICROSATELLITES FOR *GROSMANNIA CLAVIGERA*, A PINE PATHOGEN ASSOCIATED WITH THE MOUNTAIN PINE BEETLE. **S. Massoumi Alamouti, C.K.M. Tsui, S. Diguistini, L. Bernier, J. Bohlmann, C. Breuil and R. Hamelin.** *Department of Forest Science, Faculty of Faculty, The University of British Columbia, Vancouver, V6T 1Z4, Canada. Email: clementsui@gmail.com*

The fungal tree pathogen *Grosmannia clavigera* and its bark-beetle associate, the Mountain Pine Beetle, have destroyed over 13 million hectares of lodgepole pine in Canada since 1993. The epidemic is expanding into the north of British Columbia and, in Alberta, towards Canada's boreal forests. Within epidemic areas some trees appear to be more resistant to beetle-fungal attack, and we have showed that pathogenicity varies between *G. clavigera* isolates. Understanding the pathogen's genetic variability in epidemic areas may provide important insights for predicting regional pine mortality, and could help in selecting trees that may be more resistant to the pathogen. Using microsatellites and single-nucleotide polymorphisms (SNPs), we are characterizing genetic variability in populations of *G. clavigera* from different geographic regions and tree hosts, and from both endemic vs. epidemic beetle populations. We have generated a draft genome sequence for *G. clavigera*, and are using this resource to develop the microsatellite markers. We have characterized expressed sequence tags for *G. clavigera*, and are using these data to identify candidate SNPs from epidemic isolates, working with house keeping genes, genes involved in metabolic processes, genes expressed in the presence of tree defense metabolites, and genes with unknown function. Our preliminary analyses show low SNP variability. This is consistent with asexual fungal reproduction in the epidemic areas; and with genetic diversity being restricted both by periodic expansions and collapses of the fungal-beetle populations, and by symbiotic association between the pathogen and its vector.

8.43 TIMING SPRAYS FOR CELERY LATE BLIGHT BASED ON THE DISEASE-PREDICTIVE MODEL TOMCAST. **E. Minchinton, V. Galea, F. Thomson, L. Trapnell and S. Nadesan.** *Department of Primary Industries, Knoxfield, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia. Email: liz.minchinton@dpi.vic.gov.au*

Celery (*Apium graveolens*) is sprayed weekly to control late blight caused by *Septoria apiicola*, resulting in up to 16 fungicide sprays after transplanting. Growers are keen to reduce costs and the public is demanding fewer pesticides. TomCast, a disease-predictive model based on leaf wetness and temperature was evaluated on celery crops in January (summer) and April (autumn) 2005 commencing at transplanting in Victoria, Australia. Weekly sprays were compared with 10, 15, 20 and 25 disease severity values (DSVs) spray thresholds in the summer trial and 10, 12, 15, and 20 DSVs in the winter trial. The summer trial was sprayed with the industry standard, mancozeb + copper and Hortiwett alternating with chlorothalonil, while the winter trial was sprayed with chlorothalonil or at the first appearance of disease, difenoconazole, and thereafter weekly sprays of chlorothalonil. In summer 3, 4 or 5 sprays were avoided before late blight first appeared at week 7 and in winter 6 or 7 sprays were avoided before late blight first appeared at week 9. In both trials late blight appeared at canopy closure. Spraying based on TomCast did not control the disease post-canopy closure as the temperature to commence DSV scoring in TomCast was too high for Australian conditions. TomCast as a decision support tool could be employed in the early stages of crop production prior to canopy closure followed by a systemic spray and thereafter weekly sprays. This strategy theoretically could provide a 2% increase in profit.

8.44 INTEGRATED MANAGEMENT OF APPLE SCAB IN SMEDEREVO COUNTY IN SERBIA. **A. Minuto, M.L. Gullino, A. Garibaldi, G. Aleksić, T. Veljković.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: minuto.andrea@tiscali.it*

Starting in 2005 in the framework of a bilateral project funded by the Italian Ministry of Environment, Land and Sea aimed at reducing the negative impact of disease control methods, the integration of conventional disease control strategies with climate monitoring was tested in Padinska Skela and in Smederevo re-

gion to control apple scab (*Venturia inaequalis* (Cooke) Winter), a serious disease of apples in Serbia. One weather station (*i*Metos) was installed in Padinska Skela (Agroekonomik, 20 ha) and 4 stations were set up in Smederevo (1500 ha). In Padinska Skela, on the basis of climatic conditions, the first ascospore release was predicted on 28/03/2006 and, respectively under laboratory and field conditions (Burkard sampler), ascospores were effectively released on 17 and 28 March. In Smederevo area chemical sprays (triazoles and strobilurins combined or not with copper and/or alchilen bisdithiocarbammates) were carried out every 7-10 days (traditional strategy) or on the basis of disease risk estimated from weather (integrated strategy). Under laboratory and field conditions (Burkard sampler) ascospores were effectively released on 17 and 30 March, while based on weather conditions the first ascospore release was predicted on 4 April 2006 (a delay of 4 days). In Smederevo, where fungicides were sprayed the on the basis of weather conditions or at a fixed times, the severity of infection on leaf and fruit of cv Golden delicious calculated according with Townsend-Heuberger formula was 39 and 72% in untreated plots, 24 and 58% in periodically sprayed plots and 2-5% and 1-5% in plots treated only when weather conditions were really conducive.

8.45 MUESTRAN VERSION 1.0: A FRIENDLY SYSTEM DEVELOPED ON MS EXCEL® TO ASSIST POPULATION SAMPLING. **G. Mora-Aguilera, N. Ruiz and S. Michereff.** *Fitopatología, Colegio de Postgraduados. Montecillo Texcoco Edo. de México, 56230, Mexico. Email: morag@colpos.mx*

In epidemiological studies, it is always assumed that disease intensity has to be assessed in space and/or time to estimate integrative epidemic parameters. It is also recognized that the quality of evaluation determines the final analytical outcome. However the emphasis on quality has strongly relied on the efficiency of scales or diagrams to assess disease intensity, whereas the actual approach required to select those units to be assessed is overlooked. In this work, our goal was to develop a computer-friendly application to assist users to estimate sampling size (n) using spatial attributes, at any specific time, as a means to consider the dynamics of biological populations. This approach uses a graphic display to identify the n-units were the unbiased principle of the sample mean and variance, to μ and σ^2, is satisfied. Although this approach has long been recognized in epidemiology, in MuestraN version 1.0, developed on MS Excel®, three major features enhance its applicability: a) calculations are computer-assisted, requiring only one variable to be included (e.g *severity* or disease counts), b) it extends the unbiased principle for a binomial variable for cases were *incidence* is the target variable, and c) it includes the sampling cost and precision estimates for a string of n_i-units. The precision is estimated, *sensu* Karandinos, for an aggregated or not aggregated spatial condition. A variance-to-mean ratio is also included for purposes of decision making on the aggregation. Application to actual epidemiological data will be presented and discussed.

8.46 EFFECT OF TEMPERATURE AND RAINFALL VARIATIONS ON COFFEE BERRY DISEASE (*COLLETOTRICHUM KAHAWAE*). **J.A. Mouen Bedimo, D. Bieysse, C. Cilas and J.L. Nottéghem.** *Institute of Agricultural Research for Development (IRAD), Foumbot Multipurpose Station, P.O. Box 665, Bafoussam, Cameroon. Email: josephmouen@yahoo.fr*

Arabica coffee production in Africa is highly affected by coffee berry disease (CBD), due to *Colletotrichum kahawae*. This disease is specific to green berries and leads to 60-80% harvest losses under conditions favourable to development of the pathogen. Agricultural practices coupled with chemical control with 8 to 12 annual fungicide applications are known to be very effective against CBD, especially in high altitude regions (>1600 m) where farms sustaining the most damage are found. Temperature and rainfall may be very decisive for development of CBD epidemics. Consequently, an epidemiological study was conducted on smallholders' farms in Cameroon, to assess disease development dependence upon variations of these factors. Cross-correlations between disease severity and climatic parameters recorded weekly over two successive years (2004-2005), showed a significant increase of disease severity depending on decreasing temperatures (minimum or maximum). They also indicated a high variation of disease severity depending on the number of rainy days during berry growth. However, no significant correlation was found with the quantity of rainfall over the two years of observations. Thus, temperatures and rainfall distribution appear to be the key climatic parameters that favour the development of CBD epidemics. These results suggest that temperature and rainfall parameters might be very useful for setting up predictive models enabling optimization of effective control of CBD in areas with high disease incidence.

8.47 EPIDEMIOLOGY OF STOLBUR DISEASE IN THE CZECH REPUBLIC. **M. Navratil, R. Fialova, P. Lauterer, P. Valova, D. Safarova and M. Stary.** *Dept. of Cell Biology and Genetics, Faculty of Science, Palacký University, Šlechtitelů 11, Olomouc, Czech Republic. Email: milan.navratil@upol.cz*

During the last 10 years stolbur disease has spread in the South Moravia region. Recently, the phytoplasma has occurred in most field pepper and tomato crops and causes considerable yield loss. The stolbur disease situation was analysed in Lednice locality, where there are local stolbur epidemics in pepper, tomato and celery. The causal agent '*Ca*. Phytoplasma solani' was detected by PCR and characterized by sequencing the 16SrDNA gene. The phytoplasma was found mainly in *Lycopersicon esculentum*, *Capsicum annuum*, *Apium graveolens*, *Solanum tuberosum*, and *Solanum melongena* as well as in the weeds *Convolvulus arvensis*, *Solanum dulcamara*, *Solanum nigrum*, *Portulaca oleracea*, *Galinsoga parviflora*, *Cirsium arvense*, *Trifolium arvense*, *Calystegia sepium*, *Datura stramonium* and *Amaranthus retroflexus*. Massive incidence of the disease was noted in pepper, tomato and celery crops. It reached in pepper 9.2% in 2006, 14.2% in 2007; in tomato 14.6% in 2006, 8.5 in 2007; in celery 6.7% in 2007. Yield losses reached 50% in infected tomato and 80-100% in infected pepper plants. *Convolvulus arvensis*, *Cirsium arvense*, and *Calystegia sepium* were the primary sources of stolbur infection. Of the known vectors, *Lygus rugulipennis* and *Macrosteles laevis* were predominant in the locality studied. This research was supported by project No. 522/06/0618 of the Czech Science Foundation.

8.48 DISEASES OF WINTER AND SPRING WHEAT IN RUSSIA. **L.N. Nazarova, L.G. Korneva, C.C. Sanin and X.M. Chen.** *All-Russia Research Institute of Phytopathology, Moscow, Russia. Email: vniif@vniif.rosmail.com*

Analysis of damage in a disease complex of winter and a spring wheat showed that crop losses for the period 2001-2005 were 8-19%. A principal cause of loss was intensive development of diseases. Everywhere and annually on crops, leaf rust (*P. triticina*),

septoria leaf blotch (*Septoria tritici*, *S. nodorum*), powdery mildew (*Blumeria graminis*) regularly appeared. A number of the diseases showed a tendency to increase. Yellow leaf spot (*Pyrenophora tritici repentis*) in structure of a pathogenic complex was 20-30%, crop losses up to 30%, a stripe rust (*P. striiformis*), a share fusarium leaf spot (*Fusarium nivale*), common bunt (*Tilletia tritici*) and dwarf smut (*T. controversa*) were found in the North Caucasian region. In Central, Central Chernozem and North Caucasian regions, serious damage was caused by snow mould (*Microdochium nivale*). In epidemic years disease severity reached 60-70%, with destruction in foci of 30%. Alternaria blotch (*Alternaria infectoria*, *A. tenuissima*) was found frequently in the Volga region. In 1997 the spring wheat was 3-4% infected, and in 2005, 30-40%. There was an increase of ergot (*Claviceps purpurea*). Pathogens of wide host-range, able to infect 14 kinds of cereals including wheat, were marked. Disease severities were steady in the Central and Volgo-Vjatski regions in 2003-2004, and cultivars with an *Agropyron repens* gene were especially strongly infected. It is necessary to carry out monitoring of progressing diseases.

8.50 DYNAMICS OF LOCAL ADAPTATION IN A WILD PATHOSYSTEM. C. Neema, J. Franchel and J. Capelle. *Plant Pathology, AgroParisTech, 16 Rue Claude Bernard, 75231 Paris Cedex 05, France. Email: neema@agroparistech.fr*

Co-evolution between a fungal plant parasite, *Colletotrichum lindemuthianum* and its host, the common bean (*Phaseolus vulgaris*) was studied in wild populations. Significant population differentiation for neutral markers and local adaptation of the parasite was concomitantly detected at several scales. Therefore, local adaptation seems to be associated with a reduction of gene flow between geographic units. However, this pattern could be due either to broad meta-population dynamics acting on a regional scale or to local intra-population dynamics. In order to know if local adaptation can arise at a finer scale, we searched for these patterns at a local level in a single host population. We used three hierarchical levels of analysis: groups of plants from a single population, individual plants within those groups and pods within plants. Disease prevalence and fungal virulence were associated with a reduction of host fitness-related traits suggesting that the fungus may exert significant selection pressure in wild populations. The occurrence of local adaptation was determined using cross-inoculation experiments. A higher percentage of compatibility and a shorter incubation period were observed when strains were tested against their plant of origin. Furthermore, microsatellites were used to study local adaptation on a temporal scale. The results showed local adaptation on the individual host plant scale, and indicate that fine-scale dynamics have evolutionary consequences in this pathosystem.

8.51 STUDIES OF RELEASE AND INFECTIVITY OF *PHYTOPHTHORA INFESTANS* SPORES. B.J. Nielsen, J. Grønbech Hansen, H. Pinnschmidt, R. Nærstad, A. Hermansen, V. Hong Le and A. Hannukkala. *Bioforsk, Norwegian Institute for Agricultural and Environmental Research. Plant Health and Plant Protection Division, Norway. Email: ragnhild.naerstad@bioforsk.no*

The existing potato late blight forecast and decision support systems used in the Nordic countries are based on data from the 1930s and the 1950-60s on how temperature and humidity influence *Phytophthora infestans* epidemiology. Influence of weather conditions on sporangium production, release, viability and infection of *P. infestans* was investigated in Denmark, Norway and Finland using Burkard spore traps and exposure of trap plants in field plots in 2005-2007. Large amounts of sporangia were trapped after nights with long periods with high humidity. Sporangia produced in one humidity period were mainly released at the first humidity drop in the morning hours, but there were also some periods with delayed sporangium release. Local new infections occurred mainly during the morning hours when leaves were still wet. On many days sporangia did not survive until the afternoon and the results indicate that conditions for survival of sporangia and infection are major bottlenecks for the spread and development of the disease. These results have important implications for practice. Survival of sporangia and infection must be better integrated into the calculation of "risky days" in late blight forecasting systems.

8.52 INVESTIGATING THE INOCULUM SOURCES FOR DOWNY MILDEWS IN ONION, LETTUCE AND CUCUMBER IN NORWAY. B. Nordskog, D.M. Gadoury, M.B. Brurberg, T.H. Sivertsen, R. Kennedy and A. Hermansen. *Bioforsk-Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, N-1432 Ås, Norway. Email: arne.hermansen@bioforsk.no*

Downy mildews are some of the most important plant diseases in the production of several field vegetable crops in Norway. Disease outbreaks are difficult to predict since disease severity and first appearance of the pathogens can differ substantially between seasons. As part of an ongoing project, the initial sources of inoculum for downy mildews of onion (*Peronospora destructor*), lettuce (*Bremia lactucae*) and cucumber (*Pseudoperonospora cubensis*) was investigated to ensure the use of appropriate control measures for these diseases in Norway. Necrotic leaf tissue from infected plants was examined for the presence of oospores, which have so far been found profusely in lettuce and sparsely in onion, but not in cucumber. Other aspects surveyed were the distribution of spores in air. Spore traps were used to identify both the initial appearance of inoculum, and the presence and numbers of spores over a field. We used real-time PCR to determine daily spore catch. The results were compared to data from parallel spore traps where hourly numbers of spores were counted with a microscope. An attempt to reconstruct an early infection of *P. cubensis* was made by producing trajectories to show where possible sources of infection could have been located in the case of long distance distribution of spores by air. This work will be continued in 2008 and 2009, and the results will be used for better forecasting of downy mildew pathogens in Norway.

8.53 THREE-DIMENSIONAL MODEL OF *EUTYPELLA* CANKER OF MAPLE (*EUTYPELLA PARASITICA*). N. Ogris, D. Jurc, B. Piškur and M. Jurc. *Slovenian Forestry Institute, Department of Forest Protection, Večna pot 2, SI-1000 Ljubljana, Slovenia. Email: nikica.ogris@gozdis.si*

A dissectional study was performed to obtain a three-dimensional model of the *Eutypella* canker. Trunk cross sections were taken at 10 cm intervals. Each cross section was captureded by a digital camera. On each photograph, two contours representing outline of the disc and discoloured wood were drawn and digitized using CorelDRAW®. The contours were exported to solidThinking® where three-dimensional representations of trunk and canker were made. The skin surface-creation tool was used, which creates a surface that fits across a number of cross-sectional curves arranged in space. The results showed that the average volume of discoloured

wood in *Eutypella* canker can occupy more than half of the affected trunk. In cross-sections, it could be observed that the trunk is embraced and enclosed by the canker like a pie. The "pie" effect is caused by the progressive advance of the fungus where the tree is usually unsuccessful in protecting itself. This is also the reason for the ellipsoid shape of the outer outline of the bark necrosis. *Eutypella parasitica* spreads within the tree by the hyphae invading bark tissue and xylem. Longitudinal progress of *E. parasitica* is faster in the xylem than in the transverse direction in the bark tissue. This results in longer internal trunk lesions in comparison to the outer visible necrosis of the bark. Increased annual rings under healthy bark were consistently observed in cross-sections of the trunk where canker is forming. The consequence of this phenomenon is a convexity of the *Eutypella* canker.

8.54 FORECAST OF SOUTH AMERICAN LEAF BLIGHT OF RUBBER TREES. **P. Parizzi, F.X.R. do Vale, L.A. Maffia and E.S.G. Mizubuti.** *Ministério da Agricultura, UTRA-Viç, Vila Gianetti, casa 38, 36570-000, Viçosa, MG, Brazil. Email: pauloparizzi@yahoo.com.br*

South American leaf blight (SALB), caused by *Microcyclus ulei*, is the most important disease of rubber (*Hevea* spp.) plantations in Brazil. To help develop decision tools for disease management, we started studies to model SALB progress and to develop a forecast system. In Viana, Espírito Santo State and Viçosa, Minas Gerais State, SALB severity on different rubber clones was monitored together with meteorological data (air temperature, relative humidity (RH), leaf wetness, rain, and evaporation) during one year. Growth models were adjusted to the severity values, and the Gompertz transformation best fit the experimental data. Disease severity was not correlated with rain, but was significantly ($P<0.05$) and positively correlated with both daily hours of RH ≥ 90% and minimum average temperatures, and negatively correlated with evaporation. In both places, low SALB severity was observed in the periods in which average air temperature was below 20ºC, even when daily leaf wetness duration was more than 10 consecutive hours. Considering all analyses, three linear models best explained the relationship between SALB severity and meteorological variables at both places. These equations are easy to apply under field conditions and are potentially useful for forecasting SALB. However they must also be evaluated at other places where rubber is grown in Brazil.

8.55 CONSTRUCTION OF MAPS ESTIMATING PLANT DISEASE DISTRIBUTION; AN EPIDEMIOLOGICAL SIMULATION APPROACH. **S. Parnell, T.R. Gottwald and F. van den Bosch.** *Rothamsted Research, Harpenden, Herts., AL5 2JQ, UK. Email: stephen.parnell@bbsrc.ac.uk*

The ability to accurately estimate the spatial distribution of plant disease in a landscape is crucial for effective disease management and is a key challenge for regulatory agencies. Resource constraints mean sampling cannot be done at every host location and so estimates must be made about disease incidence at unsampled locations. A key focus in botanical epidemiology has been to estimate the mean incidence of disease in a given host population. These approaches ignore space or incorporate space only implicitly which may lead to an under or over estimate of incidence for diseases characterised by complex spatio-temporal patterns. In this paper we seek to develop a map of disease in a given host population and in doing so explicitly account for space. From a disease map, incidence can be determined at a range of scales. In botanical epidemiology, common solutions to this disease mapping problem have been found in the field of geostatistics. Interpolation techniques (e.g. kriging) are employed to generate continuous statistical models from disease observations at point samples. However, these approaches do not directly relate to the epidemiological characteristics of the target pathogen and fail to account for the influence of heterogeneity in host distribution or disease progression. We use an iterative simulation approach which utilizes information on the underlying host distribution and on spatial complexities in pathogen dispersal and infection to accurately map disease risk at unsampled host locations. In addition, optimization techniques are employed to find sampling configurations which lead to the most accurate mapped prediction.

8.56 THE BRASSICA$_{SPOT}$ MODEL FOR PREDICTING DISEASE RISK EVALUATED FOR WHITE BLISTER CONTROL IN AUSTRALIAN BROCCOLI CROPS. **J.E. Petkowski, E.J. Minchinton, R. Faggian, R. Kennedy and D. Cahill.** *Department of Primary Industries, Victoria, Private Bag 15 Ferntree Gully DC, Victoria 3156, Australia. Email: Joanna.Petkowski@dpi.vic.gov.au*

White blister, a new disease of *Brassica oleracea* in Australia, caused an epidemic on broccoli resulting in up to 100% crop losses during 2002. White blister is caused by the oomycete, *Albugo candida*. The Brassica$_{SPOT}$ disease predictive model (Warwick University, UK) was evaluated to examine the disease aetiology and provide growers with information on the efficacy of the model under Australian climatic conditions. Weather data were collected half hourly by a Model T weather station placed within crops. White blister risk predictions were generated and crop inspections conducted weekly in 24 broccoli crops in 2005-2007. The white blister disease occurred in summer and winter broccoli crops within 7-14 days and 14-28 days, respectively after high risk of infection events were predicted by the model. Disease management using the model was tested in the field on a resistant and a susceptible cultivar in conjunction with fungicide sprays. The unsprayed resistant cultivar had 78% less white blister on broccoli heads compared with the unsprayed susceptible cultivar. Disease incidence on the heads of the susceptible cultivar was significantly lower when sprayed with 2 applications of azoxystrobin based on the model predictions, or 11 weekly sprays of tribasic copper sulphate compared with the unsprayed control. The resistant cultivar sprayed weekly, had significantly lower disease incidence on heads when compared with unsprayed and model-based treatments. A cost-benefit analysis showed that the highest increase in profit was generated by the Brassica$_{SPOT}$ model for the susceptible cultivar but not for the resistant cultivar.

8.57 EFFECT OF TEMPERATURE, LEAF WETNESS AND SPORE AGE ON UREDOSPORE GERMINATION OF *PUCCINIA TRITICINA* AND *P. RECONDITA* F. SP. *SECALIS*. **P. Racca, T. Räder, B. Hau and E. Jörg.** *ZEPP – Central Institution for Decision Support Systems and Programmes in Crop Protection, Rüdesheimer Str. 60-68, 55545 Bad Kreuznach, Germany. Email: racca@zepp.info*

In laboratory trials the germination of older and fresh wheat and rye brown rust uredospores was investigated under different temperature and wetness regimes. The germination rate of older spores was clearly lower than that of fresh spores. For fresh inoculum of *P. recondita* f. sp. *secalis* a minimum temperature of 3.9°C was required for germination, the optimum temperature was 16.8°C and the maximum ca. 30°C. Only one hour of leaf

wetness was sufficient to initiate germination. In the older Inoculum, at least three hours of leaf wetness was necessary to start germination. Minimal temperature was ca. 0°C, optimal temperature ca. 12.8°C and maximal temperature ca. 30°C. With fresh inoculum of *P. triticina*, the first spores germinated after an hour of leaf wetness. The minimum, optimum and maximum temperatures for germination were ca. 0°C, ca. 17.3°C and ca. 30°C, respectively. With fresh wheat brown rust spores, there were only slight differences in germination over the temperature range from 5 to 25°C. With older spores, the first also germinated after an hour of leaf wetness. The minimum, optimum and maximum temperatures were ca. 0°C, ca. 13.9 °C and ca. 30.7°C, respectively. Compared with fresh spores, the older spores clearly differed in their germination in the temperature range 5 to 25°C.

8.58 PUCREC - A DECISION-SUPPORT SYSTEM FOR CONTROLLING LEAF RUST IN WINTER RYE AND WINTER WHEAT. **P. Racca, T. Räder, B. Hau and E. Jörg.** *ZEPP-Central Institution for Decision Support Systems and Programmes in Crop Protection, Germany. Email: racca@zepp.info*

Leaf rust of wheat and rye (*Puccinia recondita* and *Puccinia triticina*) occurs in all over the world. Epidemics can reduce yields of susceptible varieties by more than 25%. At present, for leaf rust of winter rye, there are no practical prognosis models and/or simulation models, with which first appearance can be predicted and/or the epidemic course can be calculated. There are some studies on the epidemiology of rust in winter rye. In climate chambers and field trials, the essential parameters of an epidemic such as spore germination, latent period and infection rate were investigated. Based on this data, a simulation model for leaf rust epidemics was developed. PUCREC simulates the first occurrence of *P. recondita* and *P. triticina* on the different leaf layers, and the increase in disease incidence, and advises when a threshold is overridden and fungicide treatments should be applied. PUCREC needs temperature and leaf-wetness duration as input parameters. The model is also linked to an ontogenesis model (SIMONTO) for the wheat and the rye crop. First validation of the model gave very satisfactory results and the system was used for the first time by the German plant protection services during the 2006 season.

8.59 APPLICATION OF PLANT DISEASE MODELS IN EXTENSION AND PRACTICE – THE GERMAN ADVISORY PORTAL ISIP. **M. Röhrig and R. Sander.** *Information System for Integrated Plant Production (ISIP), Rüdesheimer Str. 60-68, 55545 Bad Kreuznach, Germany. Email: roehrig@isip.de*

Numerous models for plant pests and diseases have been developed in recent years but their application in extension and practice has been limited. Funded by the German Environmental Foundation, the German Plant Protection Services (PPS) started a project in 2001 to close this gap: the Information System for Integrated Plant Production (ISIP, http://www.isip.de) was developed as a universal framework to implement weather-based simulation models on the Internet. Since then, twelve models for pest and diseases in various agricultural crops have been integrated into the system; nine are publicly available and three are in validation. In general, the models have a regional output to give the user an overview of the current risk potential. This output is supplemented by data from field monitoring where available and by a written comment by the regional extension officer of the PPS. Since the extension officer is acquainted with the model, he can interpret its results and derive recommendations. In addition, users can run the models on their own data, and obtain site-specific prognosis. In future, the scope will be widened from agricultural to horticultural crops and on the technical side a Geographic Information System (GIS) will be included. In conclusion, the concept of a 'three-fold decision support' with model results, field data and comment is unique for an agricultural Internet information system and makes ISIP a comprehensive tool for knowledge transfer and decision support in plant protection.

8.60 WEATHER CONDITIONS TRIGGERING ASCOSPORE DISCHARGE IN *VENTURIA PIRINA*. **V. Rossi, S. Giosuè, F. Salinari and R. Bugiani.** *Institute of Entomology and Plant Pathology, Sacro Cuore Catholic University, Via E. Parmense 84, 29100 Piacenza, Italy. Email: simona.giosue@unicatt.it*

A 5-year study (2002-2006) was carried out in two pear orchards in northern Italy, by trapping air-borne ascospores of *Venturia pirina*. Characteristics of 155 ascospore discharge events (hour of the day, duration, ascospore number) and weather conditions of the hours preceding the beginning of the discharge (WD, wetness duration; R, rainfall; Tw, temperature during wetness) were analysed with the aim of better defining environmental conditions favouring ascospore discharge. Ascospore discharge showed a diurnal periodicity, with the 92% of total spores trapped in daylight. Thirty-seven percent of ascospore discharge events were triggered by rainfall, 55% by leaf wetness, while 8% occurred under dry conditions. The probability of ascospore discharge to occur was calculated using a logistic regression procedure with a stepwise selection of the independent variables. The variable 'WD × Tw' was selected as the most influential, while Tw, WD and R were not. The logistic equation provides the probability of an ascospore discharge to occur based on the combination of wetness duration and average temperature during the wet period preceding the beginning of the ascospore discharge. Probability was higher than 0.5 when 'WD × Tw' was higher than 197.5°C×h, while it was 0.9 when 'WD × Tw' was 585°C×h. This result showed that ascospore discharge in *V. pirina* is mainly influenced by wetness and temperature, while in *V. inaequalis* it depends mainly on rainfall. Calculation of the infection periods for controlling pear scab should take account of this difference.

8.61 *PUCCINIA HYSTERIUM*-REGULATED POPULATION DYNAMICS OF *TRAGOPOGON PRATENSIS* IN THE PARK GRASS EXPERIMENT. **N.K.G. Salama and M.J. Jeger.** *Division of Biology, Imperial College London, Silwood Park Campus, Ascot, Berkshire, SL5 7PY, UK. Email: nabeil.salama@imperial.ac.uk*

Tragopogon pratensis within the Park Grass experiment (PGE) at Rothamsted Research, Hertfordshire, UK, has shown population dynamics that resemble an outbreak species. It is hypothesised that this population dynamic is regulated by the presence of the autoecious, demicyclic rust fungus *Puccinia hysterium* which sterilises the host and alters its performance. *SIR*-type models were developed to incorporate density dependency, disease-induced mortality and a host seedbank. These models produce dynamics which either cycle, crash or develop a steady-state. The parameters of the model are obtained from the long-term records of the PGE. The model is applied to dynamics within each of the plots where there are records of *T. pratensis*. Furthermore, analysis has been undertaken to discover the role of the varying nutrient regimes applied to each of the PGE plots, on the host-pathogen interactions and population dynamics within each plot.

8.62 EPIDEMIC CLASSIFICATION OF PHYTOSANITARY SITUATIONS ON CEREAL CROPS USING MATHEMATICAL MODELLING. **S.S. Sanin, J.A. Strizhekozin and X.M. Chen.** *All-Russia Research Institute of Phytopathology, Moscow, Russia. Email: vniif@vniif.rosmail.com*

Most plant protection researchers and experts divide emerging phytosanitary situations into three classes: epiphytoty, moderate development of disease, and yield depression. The known principles and methods for estimating these situations (Van der Plank J.E., Kranz J. *et al.*) do not fully describe the true picture of disease development and the corresponding economic damage. We offer a method of epidemic classification which uses a parameter we call the Epidemiological Hazard Index. This index represents the volume of potential yield losses in the case of weather conditions favoring disease development. Potential yield losses are determined on the basis of phytosanitary observation data using the corresponding mathematical models. Using the proposed method for cereal crops (wheat, barley, and rye), we determined hazard indices (yield losses), corresponding to the classes of disease development. We developed mathematical models for calculating yield losses for some main cereal diseases. Using the proposed classification and corresponding models, epiphytotic frequency, yield losses, and the payback of protective measures can be estimated. A variant of this classification is used for to assess the resistance of cultivars to epidemics, or their ability to resist the development of massive disease outbreaks.

8.63 MODELING SPATIAL DEPLOYMENT OF GENE-FOR-GENE RESISTANCE. **N. Sapoukhina, C.E. Durel and B. Le Cam.** *UMR INRA/INH/UA - Plant Pathology, INRA Angers, BP 60057, F-49071 Beaucouzé, France. Email: Natalia.Sapoukhina@angers.inra.fr*

The problem of the durability of plant resistance has stimulated us to develop a generic model that accounts for evolutionary processes underlying pathogen invasions and spatial/genetic heterogeneity of the invaded plant. Here, we formulate a spatially realistic population-genetic model to study the effects of the genetic and spatial composition of the host population on pathogen invasion through post-immigration recombination. We conclude that spatial patterning of resistant genotypes rather than host genetic composition governs evolutionary and hence invasive pathogen dynamics. It is shown that if virulence is costly, the disease-reducing effect of resistance genes manifests better in random mixtures of susceptible and resistant hosts than in patchy patterns. The model shows that a random mixture of host plants carrying different single resistance genes can replace a host carrying a combination of these genes without loss of efficiency. The approach provides theoretical support for studying rapid emergence and spread of novel pathogen genotypes carrying multiple virulence genes. It can be applied in practice to the design of innovative strategies for the most appropriate deployment of plant resistance genes.

8.64 DEGREE DAYS BASED MODEL FOR PREDICTING THE OCCURRENCE OF *ERYSIPHE POLYGONI* IN *VIGNA MUNGO*. **T. Saravanan, T. Ragavan, Nagalingam S. Venkataraman and V. Subramanian.** *Agricultural Research Station, Kovilpatti. 628501, India. Email: pathsaran75@rediffmail.com*

Powdery mildew caused by *Erysiphe polygoni* DC is one of the most important diseases of *Vigna mungo.* Field experiments and laboratory studies were done to study the effect of degree-days on powdery mildew in *V. mungo*. The conidia of *E. polygoni* were detected at the end of October in years 2002 to 2005, when the crop is at peak vegetative stage, and their number gradually increased till the end of harvest in all four years of observations. The hourly conidial concentration monitored by spore trap was correlated positively with hourly temperature and wind velocity and negatively correlated with hourly RH and leaf-wetness hours. In the correlation analysis, degree-days were found to influence the disease initiation positively during four days prior to initial disease observations in all four years. Morning RH and leaf wetness hours also influenced the initiation positively. A stepwise regression analysis showed that, degree-days had strong significant negative influence on initiation as well as development of the disease, whereas morning RH was found to influence only disease initiation positively along with degree-days. The regression analysis suggested that a quadratic model best explains the relationship of degree days to disease development in all years when compared to other models viz., Logistic, Linear, Richards and Gompertz models. The model developed was validated during 2006 in three locations, and proved its efficiency, with ±6% variation in the predicted disease index.

8.65 DISTRIBUTION OF SYMPTOMLESS *BOTRYTIS CINEREA* INFECTIONS IN WEED PLANTS. **A. Shafia and M.W. Shaw.** *School of Biological Science, University of Reading, Berkshire, Reading RG6 6AS, UK. Email: a.shafia@reading.ac.uk*

Botrytis cinerea can produce serious pre- and postharvest diseases in many crops. The pathogen is known to survive in dead plant tissues and debris but its survival within a healthy plant is less frequently reported. Previous studies have shown that *B. cinerea* can grow throughout roots, stems and leaves of cultivated *Primula* and lettuce as symptomless infection without showing any signs of aggressive infection. In this work, wild plants belonging mostly to Compositae were collected from the University of Reading compound, which covers approximately 130 hectares of mostly coarse pasture. Leaf, stem, flower and root samples selected from symptomless plants randomly collected from across the campus were surface-sterilised with hypochlorite, then ethanol, rinsed with sterile water and plated on Botrytis Selective Media. *B. cinerea* was isolated from leaf, stem, roots and flowers of *Senecio vulgaris* (annual; 18% of samples), *S. jacobaea* (perennial; 16%), *Centaurea scabiosa* (perennial; 39%), *Taraxacum officinale* (perennial/ruderal; 12.5%) and *Cirsium vulgare* (perennial; 6%). It was never found in the perennials *Achillea millefolium* except in dense flower clusters, *Tussilago farfara* or *Bellis perennis* nor in 18-month old crops of *Gerbera* × *hybrida* (which had previously suffered aggressive attacks on leaf bases), but was common in 2-month old lettuce crops (28%). In other plant families, the fungus was isolated from stems and leaves of symptomless wild *Arabidopsis thaliana* (19%) and some populations of *Primula vulgaris* (6%). This may suggests a distinct mode of infection and life-history in some host species. The population genetics of the isolates recovered will be studied.

8.66 APPLE SCAB, CAUSED BY *VENTURIA INAEQUALIS* IN INDIA. **K.P. Singh, J. Kumar and R.K. Prasad.** *Department of Plant Protection, College of Forestry, University of Horticulture & Forestry, Ranichauri 249 199, Tehri Garhwal, Uttarakhand, India. Email: kps60@rediffmail.com*

Apple scab is generally controlled by calendar-based fungicide applications regardless of the presence of ascospores of the causal

fungus, *Venturia inaequalis* (Cooke.) G. Wint. (anamorph *Spilocea pomi*) revealed 2 day (light infection), 1 day (moderate infection) and 1 day (severe infection) delay in symptom expression under orchard conditions. The number of cumulative degree-days for 50 and 95 per cent ascospore discharge was approximately 418 and 792 (R^2 =0.943), respectively for orchards situated at 1900-2200 m asl and > 1182 (95% ascospore discharge; R^2 = 0.967) for orchards situated at >2200 m asl. Thus, seasonal variation of ascospore discharge during experimental years differed at different locations in the region. When comparing PAD levels in different orchards under similar weather conditions, there was a clear relationship between PAD in the spring and the outbreak of scab on fruits and foliage in the autumn in poorly managed orchards. PAD values were 50 times higher in the poorly managed orchards than in the integrated managed orchards. Yield losses during epidemic years in 1996 and 1997 in the region went up to 70 per cent. In a fifteen-year study (1992-2006) conducted in apple orchards in Uttarakhand Himalayas, pseudothecia development started during November-December and progressed steadily when moisture and temperature conditions were favourable. To improve spraying efficiency with reduced fungicide use, reliable scab warnings are helpful. The total infection periods (μMETOS, RSS – 412) were observed in each year periodically from March till August at the Gangothri valley area of Uttarakhand.

8.67 ROLE OF ASCOSPORES IN THE DEVELOPMENT OF *SCLEROTINIA* ROT OF OILSEED BRASSICA. R. Singh and D. Singh. *Department of Plant Breeding, CCSHAU, Hisar 125 004, India. Email: rsb@hau.ernet.in*

Sclerotinia sclerotiorum causes *Sclerotinia* rot of oilseed Brassica. Being a ubiquitous necrotroph pathogen, it is proving a bottleneck and limiting to cultivation of oilseed Brassica. Nowadays, this disease is gaining ground because of airborne inoculum i.e. ascospores. Hence the present study was carried out on ascopores. Mature sclerotia produced apothecia under laboratory conditions in 16 days at 13±3°C. Ascospore germination was recorded at temperatures between 9 and 30°C, highest being 64.66% at 21°C. No infection was obtained on uninjured plant parts except petals after ascospore inoculation. The role of ascospore infection through petals in the development of *Sclerotinia* rot will be discussed.

8.68 A MODELLING FRAMEWORK FOR THE DISPERSAL OF AIRBORNE DISEASES ON DIFFERENT SCALES. Soubeyrand, J. Chadœuf, C. Lannou, M. Höhle, L. Held and I. Sache. *UMR1290 Bioger-CPP, INRA-AgroParisTech, BP01, 78850 Thiverval-Grignon, France. Email: sache@grignon.inra.fr*

Modelling spore and disease dispersal on different time and space scales is a challenge both for the management of several plant diseases of economic importance and for the development of theoretical epidemiology. Most existing models are scale-specific and often do not adequately match the observed over-dispersion of disease. We propose a generic modelling framework based on a limited set of biological assumptions, including stochasticity and parameter-sparse. This framework is suitable to describe the spread of airborne diseases around a primary focus, within a plot and between plots. The key component in the framework is the construction of an infectious potential generated by the disease sources. In addition, at each scale considered, specific characteristics are handled: leaf receptivity at the focus scale, vertical (along with horizontal) spore dispersal at the plot scale, and anisotropy in distance and direction of spore dispersal at the between-plots scale. When fitted to experimental data obtained on each scale either with brown or yellow rust of wheat, each model correctly described the over-dispersion of disease. The model is expected to be easily transferred to other pathosystems involving airborne plant diseases.

8.69 MONITORING OF "*CANDIDATUS* PHYTOPLASMA PYRI" OCCURRENCE IN PSYLLID VECTOR POPULATIONS IN PEAR ORCHARDS IN THE CZECH REPUBLIC – PRELIMINARY REPORT. J. Suchá and L. Talácko. *Research and Breeding Institute of Pomology Holovousy, Czech Republic. Email: sucha@vsuo.cz, talacko.vsuo@seznam.cz*

The frequency of individuals of the psyllid species *Cacopsylla pyri, C. pyrisuga and C. pyricola* infected with '*Candidatus* Phytoplasma pyri' was monitored in a pear orchard managed according to integrated pear production in Eastern Bohemia (Czech Republic). The insects were captured on trees at two to four weeks intervals during the growing seasons of 2006 and 2007. Phytoplasmas in psyllid samples were detected by PCR/RFLP. *C. pyri*, *C. pyrisuga* and *C. pyricola* were all found in the orchard surveyed, but only a low portion of these psyllid species was infected with the phytoplasma in both years. The observations will continue. The research was funded by the NAZV Grant Agency No. QG60123.

8.70 CONIDIAL GERMINATION OF *CORYNESPORA CASSIICOLA* ISOLATES UNDER DIFFERENT CONTINUOUS WET PERIODS. A. Teramoto, L.C. Ferreira, F. Yoshida, L. Leandro Pires, M. Cagnin Martins and M. Gomes da Cunha. *School of Agronomy and Food Engineering, Federal University of Goiás, C.P. 131, Campus II, Goiânia, GO, Brazil. Email: adritera@terra.com.br*

Corynespora cassiicola is the causal agent of target spot of different crops such as cucumber, cotton, soybean, barbados cherry, melon, tomato and others. The importance of target spot in some crops is increasing, and studies for better understanding the pathosystem host-*Corynespora*-environment are necessary. This research had as objective to identify the period of continuous wetting that resulted in best conidial germination. This information is important to prevent the disease in the field when weather conditions are favorable. Isolates of *C. cassiicola* from cucumber, barbados cherry, coffee, cotton, melon, soybean, tomato, and bigleaf hydrangea were tested. The periods of continuous irrigation tested were 1, 2, 4, 8, 10, and 24 hours. Eight hours of irrigation were enough for all isolates to reach maximum sporulation, except for one isolate from cucumber and another from melon, when maximum sporulation was reached in 4 hours and for one isolate from Barbados cherry that required 24 hours.

8.71 HOST SPECTRUM OF *CORYNESPORA CASSIICOLA* ISOLATES. A. Teramoto, L.C. Ferreira, F. Yoshida, L. Leandro Pires, M. Cagnin Martins and M. Gomes da Cunha. *School of Agronomy and Food Engineering, Federal University of Goiás, C.P. 131, Campus II, Goiânia, GO, Brazil. Email: adritera@terra.com.br*

Corynespora cassiicola, widespread in Brazil, is the causal agent of target spot in several crops and attacks several host species of economic importance. Results of cross-inoculation of different isolates of *C. cassiicola* in different host plants are im-

portant for efficient recommendation of crop rotation aiming to reduce inoculum source in the fields. The cross-inoculations were carried out in seedlings of cucumber (*Cucumis sativus*), barbados cherry (*Malpighia emarginata*), coffee (*Coffea arabica*), cotton (*Gossypium hirsutum*), melon (*Cucumis melo*), soybean (*Glycine max*), and tomato (*Lycopersicon esculentum*) using *C. cassiicola* isolates from cucumber, barbados cherry, coffee, cotton, melon, soybean, and tomato. The cucumber plants were susceptible to all isolates except for an isolate from barbados cherry. Cotton and melon plants were susceptible to all isolates. Coffee plants were susceptible to isolates from barbados cherry and soybean. Barbados cherry plants were susceptible to isolates from cucumber and barbados cherry. Soybean plants were not susceptible to isolates from barbados cherry and melon, and tomato plants were susceptible only to the isolate from tomato.

8.72 SYSTEMIC PROGRESS OF *LEPTOSPHAERIA MACULANS* FROM COTYLEDONS TO THE STEM OF *BRASSICA NAPUS*. **R. Travadon, B. Marquer, A. Ribulé, I. Sache, H. Brun, R. Delourme and L. Bousset.** *UMR 1290 BIOGER-CPP INRA - Agro ParisTech, B.P. 01 78850 Thiverval-Grignon, France. Email: sache@grignon.inra.fr*

Phoma stem canker (*Leptosphaeria maculans*) is the most damaging disease of oilseed rape. Leaf infections result in systemic colonization by hyphae, which grow through the lamina, petiole and stem, eventually causing stem cankers and lodging. Sexual reproduction occurs on stem debris, where compatible isolates mate to produce pseudothecia. Information is lacking on how systemic growth of the fungus influences its distribution in planta, and on how polygenic resistance affects fungal ability to reach the stem. This study aimed at (i) evaluating the effect of inoculum load on incidence and severity of stem cankers, (ii) assessing the probability of stem colonization by one isolate inoculated either alone or with a competing isolate, and (iii) assessing the effect of polygenic resistance on fungal growth. In controlled conditions, wounded cotyledons of two double-haploid lines, either susceptible or with a high polygenic resistance, were inoculated with pycnidiospores from two isolates. Cotyledon infection rates and stem canker severity were measured. Presence of the two isolates in stems was revealed with three molecular markers. Stem canker incidence and severity increased with the inoculum load on cotyledons. Infrequently, the fungus was detected in symptomless stems. In co-inoculation experiments, the dominant isolate was mainly detected in the stem. Polygenic resistance significantly reduced the incidence and severity of cankers. Characterizing intraspecific interactions during fungal growth, our work highlights new means of studying the aggressiveness of *L. maculans* isolates and of assessing the level of polygenic resistance in oilseed rape.

8.73 CHARACTERIZATION AND POPULALION DYNAMICS OF *COLLETOTRICHUM* SPP. ON STRAWBERRY. **W. Van Hemelrijck, J. Debode, K. Hauke, M. Maes and P. Creemers.** *Proefcentrum Fruitteelt vzw, Dept. Mycology, Fruittuinweg 1, 3800 Sint-Truiden, Belgium. Email: wendy.vanhemelrijck@pcfruit.be*

Anthracnose of strawberries is an important disease in warm, humid regions around the world. Due to climate change this disease has now spread to more northerly regions. The causal agents of anthracnose on strawberries are fungi in the genus *Colletotrichum.* A complex of species can be responsible: *C. gloeosporioides*, *C. fragariae*, *C. dematium* and *C. acutatum*; the last is known to be the main causal agent of anthracnose fruit rot. As *C. acutatum* is a quarantine pathogen, correct characterization of the attendant species is essential for effective disease management. The aim of our study was to improve knowledge on aetiology and epidemiology of *Colletotrichum* to improve disease management, with limitation of chemical inputs. To this end our main objectives are the isolation and characterization of *Colletotrichum* species present in Belgian strawberry fields and determination of their pathogenicity and genetic diversity. We also aim to gain insight into the role of latent *C. acutatum* infections in anthracnose epidemiology and investigate the influence of fungicides and herbicides on *Colletotrichum* species and on the microbial diversity in the phyllosphere. The first results of this project will be presented. Research subsidized by the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT-Flanders).

8.74 AN EPIDEMIOLOGICAL STUDY OF *STEMPHYLIUM VESICARIUM* ON PEAR IN BELGIUM. **S. Van Laer, W. Van Hemelrijck, M. Höfte and P. Creemers.** *Proefcentrum Fruitteelt vzw, Dep. Mycology, Fruittuinweg 1, 3800 Sint-Truiden, Belgium. Email: stijn.vanlaer@pcfruit.be*

Brown spot on pear caused by *Stemphylium vesicarium* was first detected in Belgium in the region of Haspengouw and Waasland in 2001 and since then every year the number of orchards with Brown Spot has increased. Although most fruit growers experience no big problems to this day, the losses can be very high when infection occurs. A survey among Belgian fruit growers in 2006 revealed that the disease is not equally spread in Belgium. In certain regions the disease is very rare, almost absent. There are reasons to believe this has something to do with the soil or certain soil properties. An epidemiological study carried out in 2005 and 2006 supports this idea. Different orchards were examined in which an infestation gradient occurred; *Stemphylium* infestation was highest in that part of the orchard where tree growth was lowest. Furthermore there seemed to be a relation between the time of senescence and the infestation observed earlier in the season. Trees with a high *Stemphylium* infestation showed earlier or accelerated senescence symptoms. Analysis of the soils in those orchards reveals that soil conditions play a role in determining the sensitivity of the tree to *Stemphylium* infection. A hypothesis will be presented which links climate change and oxidative stress to the presence of the disease. Research subsidized by the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT-Flanders).

8.75 TEMPORAL SPORE DISPERSAL PATTERNS OF GRAPEVINE TRUNK DISEASE PATHOGENS IN SOUTH AFRICA. **J.M. van Niekerk, F. Halleen and P.H. Fourie.** *Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa. Email: phf@cri.co.za*

Infection of pruning wounds by air-borne inoculum has been found to be the most important infection pathway of grapevine trunk disease pathogens such as *Eutypa lata*, *Phaeomoniella chlamydospora* and species of *Phaeoacremonium*, Botryosphaeriaceae and *Phomopsis*. The effect of climatic factors on the spore dispersal patterns of these pathogens in South Africa was largely unknown. A spore trapping experiment was therefore conducted in an 18-year-old Chenin Blanc vineyard. Spore trapping, using a Quest volumetric spore trap and specially developed protocol that allowed identification of morphologically diverse spores, was done for 14 weeks during the grapevine pruning seasons (June to mid-September) of 2004 and 2005. In both years, weather data

were recorded using an automatic weather station. Spores of *E. lata*, *Phomopsis viticola* and Botryosphaeriaceae spp. were trapped in throughout the trapping periods of 2004 and 2005, with higher levels of spores trapped in 2005. Spores of all three pathogen groups were trapped during or after periods of rainfall and/or high relative humidity. In neither of the two years were spores of *P. chlamydospora* or *Phaeoacremonium* spp. trapped. Statistical analysis of the data furthermore indicated that spore event incidences, as well as the number of spores released during a spore event, were governed by rainfall, relative humidity, temperature and wind speed prior to and during the spore events, and qualitative and quantitative spore dispersal models were constructed on these parameters.

8.76 SUSCEPTIBILITY OF GRAPEVINE PRUNING WOUNDS TO INFECTION BY TRUNK DISEASE PATHOGENS IN SOUTH AFRICA. **J.M. van Niekerk, F. Halleen and P.H. Fourie.** *Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland, 7602, South Africa. Email: phf@cri.co.za*

Eutypa lata, *Phaeomoniella chlamydospora* and species of *Phaeoacremonium*, Botryosphaeriaceae and *Phomopsis* have been identified as grapevine trunk pathogens. These pathogens spread via air-borne or water-splashed inocula and infect grapevines mainly through pruning wounds. Temporal pruning wound susceptibility to infection by these pathogens was determined in an 18-year-old Chenin Blanc vineyard. Grapevines were pruned in July and August of 2004 and 2005. The pruning wounds were inoculated with spore suspensions of *E. lata*, *P. chlamydospora*, *Neofusicoccum australe* and *P. viticola* directly after pruning, and 1, 2, 3, 7, 10, 14, 17, and 21 days after pruning. Control treatments consisted of either spraying wounds with sterile water or painting with a non-fungicidal pruning-wound sealant. After 8 months, pathogen incidence in pruning wounds was determined by means of isolations and the data were statistically analysed. It was observed that, irrespective of pruning time, pathogen incidence declined with increasing wound age in both years. The rate of decline was, however, observed to be much slower in 2004 compared to 2005, and wounds remained susceptible for 3 or more weeks after pruning in both years. Wounds made and inoculated during late-winter (August) yielded higher pathogen incidences than mid-winter (July) wounds. Long term protection of pruning wounds with chemical and/or biological agents is therefore essential to successfully manage these pathogens.

8.77 A NEW APPROACH TO MODELLING THE DYNAMICS OF OOSPORE GERMINATION IN *PLASMOPARA VITICOLA*. **A. Vercesi, S.L. Toffolatti, R. Guglielmann and L. Ironi.** *Istituto di Patologia Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. Email: annamaria.vercesi@unimi.it*

Oospores are the only overwintering structures of *Plasmopara viticola*, the causal agent of grapevine downy mildew, and their germination provides the inoculum for primary infections. Macrosporangium formation depends on both climatic factors and endogenous mechanisms, mainly depending on calcium-activation. Confocal microscope observations showed that calcium concentration increases in the oospore cytoplasm during germination. Moreover inhibitors of both external calcium uptake and release of the ions from intracellular reserves inhibit oospore germination. Inversely, calcium ionofores significantly increase the percentage of germinated oospores. Knowledge of the underlying calcium-dependent mechanisms is sufficient to create a phenomenological differential model of the dynamics of the germination process, but uncertainty about functional relationships between variables prevents the quantitative formulation of an ordinary differential model. To solve identification problems of the oospore germination dynamics, we considered an hybrid approach which builds an input-output model upon a qualitative differential one. In outline, the simulation outcomes of the qualitative differential equations were translated by fuzzy rules that automated interpretation of results into an input-output model whose parameters were estimated from experimental data.

8.78 EPIDEMIC DEVELOPMENT OF *ASCOCHYTA* BLIGHT IN PEAS. **S.L.H. Viljanen-Rollinson, V.M. Marroni, R.E. Falloon and R.C. Butler.** *New Zealand Institute for Crop & Food Research Limited, PB 4704, Christchurch, New Zealand. Email: viljanens@crop.cri.nz*

Epidemic development of *Ascochyta* blight (caused by *Mycosphaerella pinodes*) was followed on the process pea cultivar Durango in Canterbury, New Zealand, in the 2006/07 growing season. Treatments of three sowing dates, six fungicide applications and two inoculations were applied in a field trial, the design of which was derived from a Latinised resolvable row and column design, with three replicates. Plots were assessed approximately every 10 days for *Ascochyta* blight severity. Weather data (soil and air temperature, rainfall, leaf wetness) were collected near the trial site. Disease severity and area under the disease progress curve (AUDPC) were greatest in plots not treated with fungicides, reaching 40-55% leaf area infected and AUDPCs of 850 to 1640. Plots treated with a fungicide every 7-10 days had the least disease (less than 15% leaf area infected and AUDPCs of 75-200). In August-sown plots, fungicide treatments applied for 3 consecutive weeks early in the season were associated with less disease than treatments applied later in the season. However, the opposite occurred in plots sown in September or October, where disease severity was lower and AUDPCs were smaller in plots with fungicides applied late in the season than in plots with early applications. Disease was more severe in treatments inoculated on 5 November than those inoculated on 23 November. These results show that monitoring of disease and weather parameters can assist fungicide management of this important disease.

8.79 DISTRIBUTION OF *MONILINIA* SPECIES ON PEACHES AND NECTARINES IN EBRO VALLEY, SPAIN. **M. Villarino, C. Casals, N. Lamarca, J. Usall, J. Segarra, A. De Cal and P. Melgarejo.** *Department of Plant Protection, INIA, Carretera de La Coruña, km 7, 28040 Madrid, Spain. Email: cal@inia.es*

Monilinia laxa and *M. fructigena* were the known causal agents for brown rot in peaches and nectarines in Spain until *M. fructicola* was reported in Ebro Valley, Spain, in 2006. *M. laxa* was isolated in 85-90% of brown rot fruit, followed by *M. fructigena* isolated in 10-15%. Post-harvest losses are typically severe, especially when conditions are favourable for disease development, in some cases reaching 80-85%. To determine the distribution of each *Monilinia* species in Ebro Valley, more than fifty peach or nectarine orchards were selected in five areas in 2006 and 2007. Between 400-500 brown rot fruit were removed from orchards each year. Over 700 isolates were identified by PCR using the molecular specific primers IlaxaS and IlaxaAS for *M. laxa*, IGenaS and IGenaAS for *M. fructigena* and IColaS and IcolaAS for *M. fructicola*. *M. laxa* was the species most isolated, followed by *M. fructicola* and *M. fructigena*. The distributions and epidemic

frequencies of the species were different between areas and years. The spatial relationships will be discussed. In addition, phenotypic characterization of the isolates of each species was also carried out, analyzing their aggressiveness and their resistance profile to fungicides.

8.80 CLIMATIC CONDITIONS AFFECTING LATENT INFECTIONS AND BROWN ROT ON PEACH FRUIT IN THE EBRO VALLEY, SPAIN. M. Villarino, I. Gell, C. Casals, N. Lamarca, J. Usall, J. Segarra, P. Melgarejo and A. De Cal. *Department of Plant Protection, INIA, Carretera de La Coruña, km 7, 28040 Madrid, Spain. Email: cal@inia.es*

Brown rot on peaches in Spain is caused by *Monilinia laxa* and *M. fructigena.* A third species, *M. fructicola* is included in the A2 list of quarantine organisms for Europe. European brown rot is usually initiated in spring as blossom blight from inoculum derived from overwintered mummified fruits, necrotic twigs and buds. Under favourable environmental conditions, blossom blight can progress to twig blight and branch canker, which serve as additional sources of secondary inoculum, and may eventually lead to latent infection of immature green fruit, and pre- and post-harvest brown rot on mature fruit. Post-harvest losses are typically more severe than pre-harvest losses and routinely occur during storage and transport. To evaluate the effect of climatic conditions on incidence of latent infection and brown rot by *Monilinia* spp. in peach and nectarine orchards, seventeen field experiments were performed in commercial orchards located in Ebro Valley, Spain, over six growing seasons from 2002 to 2007. Temperature, relative humidity, wetness duration, rain, and wind velocity were recorded over the crop season. The effect of climatic factors was analysed using a multiple regression model. The analysis indicated that temperature and wetness duration explained the incidence of latent infection and brown rot variation caused by these fungi. No latent infections were developed with T < 8ºC. More than 22 h of W were required in order to observe latent infections when T = 8ºC while only 5 h of W were needed with 25ºC to observe the same effect.

8.81 QUATERNARY CONCEPT OF INTEGRATED PEST MANAGEMENT (IPM) FOR THE CONTROL OF POWDERY MILDEW IN SUGAR BEET. P.F.J. Wolf and J.A. Verreet. *University of Kiel, Institute Phytopathology, Herrmann-Rodewald-Str. 9, Germany. Email: peterfjwolf@aol.com*

In central Europe, powdery mildew may cause as much as10-15% reduction of sugar yield. In order to control the disease, a quaternary concept was developed comprising four elements: The period without disease risk is determined by so-called negative-prognosis (i). First symptoms appear in the period from mid-July to early September. If disease initiation cannot be excluded, analysis of a sample of 100 leaves is advised. The disease scores enable the application of action thresholds (ii). The latter are defined as early stages of the epidemic in order to optimize the efficiency of fungicide treatments. For an initial treatment the threshold is 5% infected leaves. However, incidence in the height of action thresholds does not cause immediate damage. The stage when a sugar beet is effectively damaged is defined by the economic damage threshold (iii). As a consequence, because exceeding the action threshold does not imply immediate yield risk, loss prediction (iv) is required. The loss prediction assesses the likelihood that disease progress will exceed the economic damage threshold at harvest. Loss risk exists when the action threshold is exceeded by mid-August if cultivar susceptibility is low, and by the end of August if susceptibility is high.

8.82 DYNAMICS OF *FUSARIUM* SPORE POPULATIONS IN AIR IN A COMMERCIAL GREENHOUSE IN CANADA. J. Yang, P.D. Kharbanda, R.J. Howard and M. Mirza. *Alberta Research Council, Bag 4000, Vegreville, AB, Canada. Email: jian.yang@arc.ab.ca*

The patterns of macroconidia released by *Fusarium* spp., the causal agent of internal fruit rot of sweet pepper (*Capsicum annuum* L.), were investigated in 2006 and 2007. A Burkard seven-day recording volumetric spore trap was set up in a commercial greenhouse near Lacombe, AB from May 25, 2006 to October 3, 2007. Daily samples were collected and total *Fusarium* macroconidia on a 12 mm^2 slide per hour were counted. Greenhouse conditions, including temperature, humidity and radiation, were recorded. *Fusarium* spores were also trapped onto Nash-Snyder medium (NS), and identified according to the cultural morphology on carnation leaf agar. The monthly pattern of airborne *Fusarium* spores fitted to a polynomial model (R^2 = 0.9213), while the *Fusarium* spore populations over time (day) were distributed in a multimodal pattern. The patterns reflected the biological cycle of the host, the disease cycle and other factors. The populations reached the highest peak in June-July in both years. The *Fusarium* spores in the air had two peaks daily, however, the hourly patterns varied in different months. The total hourly or monthly *Fusarium* spore populations were positively correlated with average air temperature, radiation and humidity. *Fusarium* colonies growing on NS in the pepper greenhouse were identified as *F. lactis* (Pirotta & Riboni) Nirenberg & O'Donnell and *F. proliferatum* (Matsushima) Nirenberg. Compared to data collected in 2004 and 2005, total popultions of *F. proliferatum* had increased in the greenhouse in 2006 and 2007.

8.83 DETECTION OF LATENT INFECTION OF WHEAT LEAVES CAUSED BY *BLUMERIA GRAMINIS* f.sp. *TRITICI* USING NESTED PCR. X. Zeng, Y. Luo, Y. Zhou and X. Duan. *State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, P.R. China. Email: yilinzhou6@yahoo.com.cn*

Wheat powdery mildew (*Blumeria graminis* f.sp. *tritici*, Bgt) is one of the main wheat diseases in China. Three molecular primer pairs were designed based on the internal transcribed spacer (ITS) sequences of the Bgt ribosome, and their species specificity for wheat mildew was confirmed. The primers F1/R demonstrated a higher sensitivity than the other two primer pairs, and could detect as low as 1 pg DNA of Bgt. A nested PCR assay was developed for which an internal and an external primer pair were generated based on the sequence of the PCR product amplified with F1/R. The sensitivity of this nested PCR assay was as good as 0.1 fg DNA of Bgt. The regular and nested PCR approaches were used to detect infections and were compared in sensitivity for detection of latent infection. Generally, the nested PCR could detect latent infection 2-3 days earlier than the regular PCR. The nested PCR assay was also used to detect latent infection levels of naturally and artificially infected leaves collected from wheat fields in Beijing and Shandong. The results demonstrated a high sensitivity and predictability of the nested PCR compared with the regular PCR in estimation of latent infection level of seedlings. This study provided useful tool that can be used to rapidly and accurately monitor latent infections of wheat mildew

in seedlings, benefiting disease prediction. This work was funded by National Natural Science Foundation of China (No. 30771786) and National Key Technology R&D Programmes (No. 2006BAD08A01 and 2006BAD08A05).

8.84 TEMPORAL POPULATION GENETIC DYNAMICS OF *PHYTOPHTHORA INFESTANS* IN GREAT BRITAIN. **J. Zhan, A.K. Lees, D.S. Shaw and D.E.L. Cooke.** *SCRI, Invergowrie, Dundee, DD2 5DA, UK. Email: jiasui.zhan@scri.ac.uk*

Knowledge on temporal population genetic dynamics and the genetic mechanisms driving these dynamics are important in order to understand the evolutionary potentials of plant pathogens over changing environments. We sampled more than 1400 isolates of *Phytophthora infestans* from Great Britain between 2003 and 2006 and assayed these isolates with 11 molecular markers (SSR) and a phenotypic trait (mating type). We found a significant change in the population genetic structure of the GB population of *P. infestans* over the four years: 1) the frequency of the A2 phenotype increased from less than 0.1 in 2003 to more than 0.5 in 2006; 2) only five out of more than 140 genotypes were detected in all four years and a new genotype, which was first detected in 2005, accounted for more than a quarter of the 2006 population; 3) allele frequency in the majority of the 11 SSR loci changed significantly across the survey years and there was evidence of directional increases in genetic differentiation over time. Further analysis revealed that the hypothesis of Hardy-Weinberg equilibrium was rejected in all four populations, suggesting that *P. infestans* in GB exists in a non-random, or asexual state. The implication of our findings for the adaptation of *P. infestans* to changing environments will be discussed.

Diseases of Mediterranean crops

21.1 FIRST RECORD OF *DIPLODIA* DISEASE CAUSED BY *DIPLODIA PHOENICUM* ON DATE PALM TREES IN QATAR. **E.H. Al-Turaihi.** *Agricultural Development Department P.O. Box 1966 Doha, Qatar. Email: al_turaihi@yahoo.com*

Date palm (*Phoenix dactylifera*) is widely planted in Qatar because it is well established in the country and used as an ornamental and/or fruit tree. During the year of 2004, a disease occurred on date palm trees in Doha City. The affected trees showed yellowish/brown streaks 15 cm to about 1 m in length extending along the leaf base and rachis. These symptoms were first observed on the outer fronds and then on the central leaf cluster and terminal bud. The disease rarely caused the death of old palm trees but might cause the death of offshoots. Samples were collected from the infected area of affected trees, cut into 3- to 5-mm lengths, surface-sterilized for 2-3 min in sodium hypochlorite diluted to produce 1% available Cl_2, then placed on potato dextrose agar (PDA) and incubated at 25 °C for 1 week. *Diplodia phoenicum* was isolated from all samples of diseased plants and identified by the production of black pycnidia which extruded unicellular, hyaline spores that became dark and bicellular, measured 22-24µm×10-12µm at maturation. These spores were typical of *Diplodia phoenicum*. It was reported that the fungus usually enters palm tissues through wounds made during pruning or removing the offshoots. This is the first reported observation of *Diplodia* disease caused by *Diplodia phoenicum* occurring on date palm trees in Qatar.

21.2* MOLECULAR CHARACTERIZATION OF MEDITERRANEAN *CITRUS TRISTEZA VIRUS* ISOLATES. **L. Barbarossa and V. Savino.** *Istituto di Virologia Vegetale del CNR, Sezione di Bari, and Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. Email: l.barbarossa@ba.ivv.cnr.it*

Citrus tristeza, caused by *Citrus tristeza virus* (CTV), is the most destructive virus disease of citrus. The virus is usually present in field trees as a mixture or complex of isolates that produce a variety of symptoms in different citrus hosts. CTV is reported from almost all Mediterranean countries and is a serious threat to their citrus industries due to the predominance of sour orange as rootstock. CTV management strategies include eradication, quarantine and certification programs, use of resistant or tolerant rootstocks, or cross-protection with mild isolates, depending upon the incidence and severity of CTV present in a region. Typing of prevailing CTV strains is a key element for predicting disease impact and devising appropriate control strategies suitable to specific regions. At the same time it can provide useful indications for understanding the dynamics of CTV populations in given citrus growing areas. In this study data is presented on the sequence diversity of 30 CTV isolates from several countries of the Mediterranean Basin. Genotypic profiles were determined based on amplification of multiple molecular markers. The coat protein (CP) genes were also sequenced. RFLP profiles, and nucleotide and deduced amino acid sequences were analysed and compared to several CP gene sequences of known CTV isolates. Results showed high isolate variability between and within different countries. The genetic variability of CTV currently present in the Mediterranean region may reflect the sources of viruses introduced as infected budwood and subsequently spread by propagation and aphid vectors, but also suggest that new variants may be evolving from mixed infections.

21.3 MANAGEMENT OF CHICKPEA BLIGHT THROUGH USE OF RESISTANT VARIETIES AND CHOICE OF SOWING DATE. **E. Bazgir.** *Dept. of Plant Protection, Lorestan University, Khorramabad, Iran. Email: bazgir.e@lu.ac.ir*

Lorestan is one of the major provinces of Iran producing chickpea (*Cicer arietinum*). Chickpea blight caused by *Ascochyta rabiei* is prevalent in this area, due to climatic conditions and use by farmers of domestic non-resistant cultivars. In this research two disease-tolorant cultivars, Arman and Hashem and the susceptible cultivar Grit were sown at an early, normal or late date, and the effect on disease was evaluated (disease incidence, disease severity and seed yield). Disease was most prevalent on 'Grit' and with normal sowing date, while on 'Hashem' and 'Arman', disease severity and incidence was less and they produced higher seed yield. We conclude that crop losses caused by chickpea blight may be checked by using these two disease-tolerant varieties in Lorestan province.

21.4* FIRST REPORT OF A '*CANDIDATUS* PHYTOPLASMA MALI' ISOLATE AFFECTING APPLE TREES IN TUNISIA. **M. Ben Khalifa, M. Marrakchi and H. Fakhfakh.** *Laboratoire de Génétique Moléculaire, Immunologie et Biotechnologie, Faculté des Sciences de Tunis, Campus Universitaire, El Manar 2092, Tunis, Tunisia. Email: benkhalifamekki@yahoo.fr*

In Tunisia, apple (*Malus domestica* cvs. Royal Gala and Golden Delicious) with typical symptoms of apple proliferation (AP) in-

cluding early leaf reddening and dropping and secondary shoot growth near the apex have been observed since 1990. In Europe, AP is associated with infection by the phytoplasma '*Candidatus* Phytoplasma mali', previously assigned to the phytoplasma 16Sr X apple proliferation group. DNA was extracted from phloem taken from apples with symptoms of AP. This DNA was used as template in a polymerase chain reaction (PCR), using the universal phytoplasma primer pair P1/P7. All samples from AP-affected trees gave products with the expected size (1.8 kbp). No amplification was obtained with negative controls. Restriction profiles were generated by digestion of PCR products with AluI, RsaI and SspI endonucleases, and were identical to each other and to those of a '*Ca.* Phytoplasma mali' positive control. The RFLP analysis provides evidence of the occurrence in Tunisian apple orchards of '*Candidatus* Phytoplasma mali'. This is the first report of a '*Ca.* Phytoplasma mali' isolate associated with apple proliferation in Tunisia.

21.5 EFFECT OF HOT-WATER TREATMENT ON THE FUNGAL COMMUNITY IN GRAPEVINE NURSERY PLANTS. L. Casieri, V. Hofstetter, K. Gindro and O. Viret. *Station de Recherche Agroscope Changins-Wädenswil ACW, CH-1260 Nyon, Switzerland. Email: Leonardo.Casieri@acw.admin.ch*

Esca is one of the most important causes of vineyard decline in Europe. This complex disease, caused by a consortium of fungi, has been studied over the past decades, and the aetiology and epidemiology of some of the fungi considered responsible are becoming clearer. However, adequate control of the propagation material and effective treatment to control the disease are still lacking. The use of hot water as an eradication measure for contaminated nursery stock has been proposed but there is still a no consensus on the efficacy of such treatments. For instance some authors report complete removal of fungal pathogens after hot water treatment of dormant canes, while others report no differences in vascular discoloration and pathogen isolation between treated and untreated dormant cuttings. Here we show that fungal species can be isolated from nursery plants after hot-water treatment (45 minutes at 50°C). Treated and untreated plants of five different cultivars were analysed; pith and wood, from debarked and surface sterilized plants, were taken from rootstock, pruning wound areas and grafting point, plated on PDA medium and checked regularly for 10 days. After isolation in pure culture, the isolates were identified morphologically and/or by sequencing and blasting the ITS. Preliminary results showed a high fungal diversity in both treated and untreated plants.

21.6 FIELD SURVEY, MORPHOLOGY AND GENETICS OF *VALSA CERATOSPERMA* IN A PEAR ORCHARD IN LOMBARDY, NORTHERN ITALY. B. Cavagna, M. Saracchi and A. Tantardini. *Istituto di Patologia vegetale, Via Celoria 2, 20133 Milano, Italy. Email: marco.saracchi@unimi.it*

In May 2004 a severe outbreak of *Valsa ceratosperma* was found for the first time in Lombardy, causing canker in a pear orchards in Quistello (Mantua). The disease on pear was reported for the first time in Italy in Emilia Romagna, in 2001 and was included in the *EPPO alert list* as a pathogen newly introduced in a EU country. *V. ceratosperma* occurs in China, Japan and Korea where cause cankers on apple and occasionally on pear and quince. In March 2005 the fungus was eradicated from the Lombardy site by removing the infected pear orchard. Since then, pear orchards around the infected area have been monitored, and in June 2005 another small outbreak was discovered in Quistello; since 2006 the development and incidence of the disease has been studied in the infected orchard and a fungal strain (Q1) isolated and for further investigation. The symptoms and fungal morphology found corresponded with those of *Cytospora vitis,* the anamorph stage of *V. ceratosperma*. The Q1 strain was compared to four Japanese strains and two isolates from Emilia Romagna. First results showed that the Italian strains are similar while they differ in morphology and growth rate from Japanese isolates. Further work will be done to compare the Italian and Japanese isolates genetically.

21.7* INTEGRATED MANAGEMENT OF *ASCOCHYTA* BLIGHT OF CHICKPEA. W. Chen, K. McKay, S. Temple and F.J. Muehlbauer. *USDA-ARS, 303 Johnson Hall, Washington State University, Pullman, WA, USA. Email: w-chen@wsu.edu*

Chickpea is produced in the US as an important specialty and rotational crop in three geographic regions (central California, the Pacific Northwest, and the northern Great Plains). *Ascochyta* blight caused by *Ascochyta rabiei* is an economically important and persistent disease in each of the production regions. Integrated management practices have been developed that allow viable chickpea production. Management practices are tailored to each production region because of different cultural practices and different prevailing weather conditions. Chickpea is sown in the spring in the Pacific Northwest and the northern Great Plains, but is sown in the fall in central California. The shift in planting time in California from spring to fall exposes the crop to moist winter and early spring weather, which favors development of *Ascochyta* blight and other diseases. In the Pacific Northwest, the wet, cool spring and early summer weather is conducive to *Ascochyta* blight, whereas the hot and dry summer late in the growing season helps suppress *Ascochyta* blight. In the northern Great Plains, humid weather prevails throughout the growing season where *Ascochyta* blight has to be judiciously managed all season long. The general management practices across the US for control of *Ascochyta* blight include rotation, use of resistant cultivars, and fungicidal seed treatments. Foliar fungicide applications are tailored to different production regions, and are an integral part of the management. Cultivars with improved resistance will help reduce dependence on foliar fungicide applications. These management practices tailored to the different production regions in the US will be presented.

21.8 HISTORICAL REVIEW OF *CITUS TRISTEZA VIRUS* WITH PARTICULAR REFERENCE TO ITALY. M. Davino, A. Caruso, G. Sorrentino, M. Guardo and S. Davino. *Dipartimento di Scienze e Tecnologie Fitosanitarie sez. Patologia vegetale, Università degli Studi di Catania, Via Santa Sofia 100, Catania 95123 Italy. Email: guido.sorrentino@entecra.it*

Citrus tristeza virus (CTV) and Huanglongbing Liberibacter are two of the most destructive diseases of citrus. Over 100 million trees on sour orange rootstock have been destroyed in Brazil, Argentina, California, Florida, Spain, and Venezuela. The virus continues to spread into new areas in Israel, Cyprus, and Central America. Until 1998 CTV was recorded in Italy on only a few trees imported illegally. Now, different foci have been found in Central Italy, in Apulia, Sicily and more recently also in Calabria region. CTV can cause varying field symptoms on different scions and rootstocks. The most severe component is seedling yellows (CTV-SY) discovered in Sicily on Common Tarocco sweet orange grafted on sour orange. The virus is vectored by different aphid

species such as *Toxoptera citricidus* Kirkaldy, *Aphis gossypii* Glover, *A. spirecola* Patch (*A. citricola* Van der Goot), *T. aurantii* Boyer de Foscoulombe and *Myzus persicae* Sulzer in a semi-persistent manner. In this review we report the more important discoveries around the word, as well as all foci recorded in Italy and also the movement of CTV over short distances by aphids and long distances by man.

21.9 EMERGENCE AND SPREAD OF RECOMBINANT NECROTIC PVY VARIANTS (PVY^{NTN}) IN TUNISIA. F. Djilani-Khouadja, H. Fakhfakh, L. Glais, M. Tribodet, M. Marrakchi and C. Kerlan. *Laboratoire de Génétique Moléculaire, Immunologie et Biotechnologie, Faculté des Sciences de Tunis, Campus Universitaire, El Manar 2092, Tunis, Tunisia. Email: fattouma.djilani@laposte.net*

During November and December 2005, different fields and cultivars of late potato crops located in Cap Bon, Jendouba, Bizerte, Manouba (northern Tunisia), Monastir, Mahdia (Sahel of Tunisia) and Kairouan (central Tunisia) were surveyed. Potato leaf samples from 55 plants showing clear *Potato Virus Y* (PVY, *Potyvirus*) symptoms were collected. All samples were first analysed by DAS-ELISA using polyclonal anti-PVY antibodies. All the plants collected were infected by PVY. The PVY isolates were then mechanically inoculated onto *Nicotiana tabacum*. Of the inoculated plants, 30 developed systemic vein necrosis as reported for PVY^{N} strains. In confirmatory tests, 25 isolates reacted in ELISA with PVY^{N}-specific monoclonal antibodies, only one isolate reacted with PVY^{O} antibody; six isolates were co-infected with both PVY^{O} and PVY^{N}. In order to detect specifically the PVY^{NTN} variants, the cause of Potato Tuber Necrotic Ringspot Disease (PTNRD), the 25 PVY^{N} isolates were tested by Immunocapture RT-PCR (IC-RT-PCR) using specific primers. Furthermore, the IC-RT-PCR, designed to identify three recombination breakpoints, showed that all the 21 PVY^{NTN} variants were recombinant in all the three sites. These results confirmed the emergence and spread of the PVY^{NTN}, a new recombinant variant of PVY in Tunisia.

21.10 VIRUSES ON GLADIOLUS, IRIS AND TULIP IN THE CZECH REPUBLIC. G.S. Duraisamy and P. Radovan. *Mendel University of Agriculture and Forestry, Zemedelska 1, 613 00 Brno, Czech Republic. Email: gansels@gmail.com*

The status of *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV) and *Tobacco rattle virus* (TRV) in gladiolus, iris and tulip was investigated by visual examination and by ELISA, which was used to test for the presence of BYMV, CMV and TRV infection in both aerial and underground parts of gladiolus, iris and tulip. Of 262 gladiolus plants, 63, (7%) were infected by BYMV, 29, (4%) by CMV and 2, (7%) by TRV. From 180 plants of iris 1, (1%) was infected by BYMV, 6, (7%) by CMV and 2, (8%) by TRV. Of 28 tulip plants, 28, (6%) were infected by CMV and 7, (1%) by TRV. The present investigation revealed the widespread occurrence of BYMV in gladiolus, and CMV in iris and tulip in the Czech Republic.

21.11 MANAGING GRAPEVINE TRUNK DISEASES, PETRI DISEASE AND ESCA – AN AUSTRALIAN PERSPECTIVE. J. Edwards and I.G. Pascoe. *Department of Primary Industries Victoria, Private Bag 15, Ferntree Gully Delivery Centre, Victoria 3156, Australia. Email: Jacky.edwards@dpi.vic.gov.au*

Grapevine trunk diseases cause decline and death of vines, thus severely limiting the sustainability of Australian vineyards. Little was known about the factors contributory to these diseases, how to prevent them, or how to minimise their economic impact when our research began in 1999. Petri disease, which causes significant losses during vineyard establishment, was shown to be widespread, whereas esca, currently the most serious grapevine disease in Europe, was shown to be rare in Australia. The causal organism, *Phaeomoniella chlamydospora*, is spread from infected mother vines into cuttings, and into newly planted vineyards via infected planting material. Long duration (30 minute) hot water treatment of dormant cuttings was shown to be effective in reducing the risk of producing infected planting material. Best practice protocols for the nursery industry were developed and promoted to encourage production of *P. chlamydospora*-free planting material. Glasshouse studies demonstrated that infected grapevines are more susceptible to water stress. Field trials showed that management practices that reduced stress, such as the use of mulch, reversed the symptoms of decline in infected grapevines. Recently, the number of reports of Petri disease in Australia has dropped considerably, suggesting that industry now has the tools both to prevent the spread and to minimise losses attributable to these diseases as a result of this research.

21.12 INFECTIOUS CLONED CDNAS OF CRINIVIRUSES: MODIFIED CLONED CDNAS FOR *LETTUCE INFECTIOUS YELLOWS VIRUS* AND *CUCURBIT YELLOW STUNTING DISORDER VIRUS*. B.W. Falk, J.A. Lindbo, T.P. Knustad, L.R. Stewart, J. Wang and M. Turina. *Dept. of Plant Pathology, Univ. of California, Davis, CA 95616, USA. Email: bwfalk@ucdavis.edu*

Criniviruses are whitefly-transmitted plant viruses whose genomes are composed of two RNA molecules. A number of criniviruses are significant pathogens of many important crops. One current example is *Cucurbit yellow stunting disorder virus* (CYSDV) which was only recently introduced into the USA and is now a major concern for melon production in the southwestern US. Screening for virus-resistant plant varieties would benefit from efficient, artificial inoculation systems that would avoid tedious whitefly inoculations. To this end we have constructed full-length infectious clones of both *Lettuce infectious yellows virus* (LIYV) and CYSDV behind phage promoters. In vitro transcription of these clones generated RNAs that replicate in protoplasts. Further, we have modified these clones and replaced selected LIYV or CYSDV genes with the green fluorescent protein (GFP) gene. The RNAs from these constructs efficiently replicated in protoplasts and expressed GFP. We added the *Hepatitis delta virus* ribozyme to yield an exact LIYV RNA-1 3' terminus, and placed the LIYV RNA-1 cDNA behind an enhanced 35S promoter to give a putative transcription start at the exact 5' end of LIYV RNA-1. Initial experiments showed infectivity in *Nicotiana tabacum* protoplasts, and in *N. benthamiana* leaves after co-bombardment with a plasmid encoding the p19 suppressor of gene silencing. We are extending these results to develop additional GFP-tagged virus clones that will express all virus genes and will also be deliverable to plants by agroinfiltration.

21.13 PATHOGENICITY OF *PHYTOPHTHORA CINNAMOMI* ON *LUPINUS LUTEUS* GROWING IN MEDITERRANEAN *QUERCUS* GRASSLANDS. P. Fernández, M.S. Serrano, P. de Vita, M.D. Carbonero and M.E. Sánchez. *Patología Agroforestal, Universidad de Córdoba, Spain. Email: ag1sahem@uco.es*

A severe root rot caused by *Phytophthora cinnamomi* has been reported since the early 1990s in southwestern Spain and southern Portugal, leading to widespread mortality of evergreen Mediterranean oaks. *Lupinus luteus* is commonly sown in *Quercus rotundifolia* and *Q. suber* grasslands for animal grazing in this area. Artificial inoculations of four different cultivars of *L. luteus* have demonstrated the high pathogenicity of *P. cinnamomi* on this herbaceous species. In addition, soil samples were taken in slightly to highly infested *Quercus* sites in early spring, when lupin plants sprouted. A second sampling was completed in early summer, when the plants, infected or not by *P. cinnamomi*, were already wilted, ready for summer grazing. Quantification of fungal ufc per gram of soil and comparison among sites and sampling times suggested the potential of *L. luteus* to increase *P. cinnamomi* inoculum levels and consequently to favour the infection of *Quercus* roots.

21.14 CHARACTERIZATION OF *BURKHOLDERIA GLADIOLI* STRAINS CAUSING BACTERIAL ROT OF SAFFRON (*CROCUS SATIVUS*). **M. Fiori and G. Falchi.** *Dipartimento di Protezione delle Piante, Facoltà di Agraria, Università degli Studi, 07100 Sassari, Italy. Email: fiorim@uniss.it*

Burkholderia gladioli is the causal agent of a bacterial disease recently reported in saffron (*Crocus sativus* L.) grown in central Sardinia (Italy). The symptoms were rot of emerging flowers and shoots, and spots on leaves. In the last two years the disease has been particularly harmful, reducing flowering by some 80%. Isolations on nutrient glucose agar from symptomatic plants produced two types of colony. The first type (ten isolates) was round, wrinkled, and yellowish. The second type (fifteen isolates) was round, smooth and colourless. In pathogenicity tests, the twenty five isolates reproduced symptoms on saffron plants, while only the first type of isolate was pathogenic on gladiolus leaves. The twenty five isolates were analyzed with the computerised BIOLOG system, conventional tests and genomic tests such as PCR and PCR-RFLP. BIOLOG, conventional tests and PCR using LP1 and LP4 primers, identified all isolates as *Burkholderia gladioli*. PCR-RFLP analysis using three restriction enzymes (*Alu*I, *Dde*I and *Bss*KI), identified only ten of the isolates (nine wrinkled and one smooth) as *B. gladioli* pv. *gladioli*. Our evidence indicates that other *B. gladioli* forms are also involved in this bacterial rot of saffron, and further studies are under way to verify this.

21.15 CURRENT STATUS OF *TOMATO CHLOROSIS VIRUS* AND *TOMATO INFECTIOUS CHLOROSIS VIRUS* IN SPAIN. **M.I. Font and C. Jordá.** *Instituto Agroforestal Mediterráneo (IAM), Universidad Politécnica de Valencia, Camino de Vera, s/n, 46022 Valencia, Spain. Email: mafonsa@upvnet.upv.es*

Tomato chlorosis virus (ToCV) and *Tomato infectious chlorosis virus* (TICV) are emergent whitefly-transmitted, phloem-limited criniviruses infecting tomato in several countries worldwide. ToCV is transmitted by *Bemisia tabaci* (Gennadius) biotypes A and B, *Trialeurodes vaporariorum* (Westwood) and *T. abutilonea* (Haldeman), whereas TICV is transmitted only by *T. vaporariorum*. In tomato (*Solanum lycopersicum* L.), ToCV and TICV cause leaf interveinal yellowing that often develops into red or necrotic flecks,with brittle and rolled lower leaves. The host range of these viruses includes some important crops and ornamental species. In Spain ToCV was first detected in 2000 infecting tomato crops which had been collected during 1997 in Malaga and Almería (southern Spain). Later, similar symptoms were observed in tomato crops grown in different regions. In 2001 TICV was detected in greenhouse and field tomatoes in the Castellón province (eastern Spain). Now ToCV is prevalent along the southern, eastern and east regions (provinces of Barcelona, Castellón, Valencia, Alicante, Murcia, Almería, Granada, Málaga, Sevilla, Pontevedra, and Badajoz), Balearic (Mallorca), and the Canary Islands (Tenerife and Gran Canaria). TICV only has been detected in the provinces of Castellón, Valencia, Alicante and Murcia, however, surprisingly, during 2005-2007, TICV was not detected infecting tomato crops in Spain. Has been TICV displaced by ToCV in tomato epidemics in Spain?

21.16 A NEW LEAF SPOT DISEASE OF COMMON BEAN IN SPAIN: DIAGNOSTIC TECHNIQUES AND IDENTIFICATION OF RELATED SYMPTOMS. **M.I. Font, M.J. Zanón, A. García, M. de Cara, J. Tello, M.C. Cebrian and C. Jordá.** *Instituto Agroforestal Mediterráneo (IAM), Universidad Politécnica de Valencia, Camino de Vera, s/n, 46022 Valencia, Spain. Email: mjordag@eaf.upv.es*

During November 2003, a new disease was observed in common bean (*Phaseolus vulgaris)* in Spain. Symptoms consisted of internervial yellowing in leaves, combined with chlorosis and necrosis, hook-shaped fruits, and severe stunting. This leaf spot disease is spreading in Southern Spain where it has quickly become a serious problem causing losses as high as 50%. About 100 ha of the affected areas have been inspected periodically and as much as 10% of the whole area has been analyzed. A total of 303 samples were taken from symptomatic plants from 2004 to 2007. Three different pathogens were associated with the symptoms: Bean yellow disorder virus (BnYDV) a *Bemisia tabaci*-transmitted crinivirus, and the bacteria *Erwinia persicina* Hao *et al.*, and *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Hedges) Dowson. The detection rates were 50.5%, 7% and 3.3%, respectively. Mixed BnYDV and *E. persicina* infections were observed in 8.25% of samples. Considering the economic losses that have resulted during the last three years and since *E. persicina* has been detected in animals and humans, there is a clear and urgent need to develop rapid techniques to analyze a large number of samples.

21.17 TOMATO BACTERIAL SPOT IN GEORGIA. **N. Giorgobiani, P. Eliashvili, Sh. Kharadze and G. Kvesitadze.** *Durmishidze Institute of Biochemistry and Biotechnology, David Agmasheneblis Kheivani 10 km,Tbilisi 0159, Georgia. Email: gkvesitadze@yahoo.com*

Tomato is an important vegetable for Georgia. In this country of 69,000 km^2 the average annual production in private and state farms is 125,000 tons of high quality tomato. Over the past centuries bacterial plant diseases have caused serious damage, resulting in economic loss and human suffering. Among the diseases of tomato the most deleterious is the black bacterial spot. Spread of the disease was studied in different climatic zones of Georgia. It was found that all plant organs including fruits are exposed to disease at different stages of plant growth and development. Spread of the disease by regions varies from 15-20% to 40-60%. Several hundred bacteria were isolated from diseased plants and their pathogenicity was studied. In total 57 pathogenic strains causing tomato bacterial spot were isolated. Bacterial spot of tomato is caused by *Xanthomonas campestris* p.v. *vesicatoria* (Doidge) Dye. Several pathological changes in biochemical characteristics of diseased plants were revealed. Search for biological control methods based on bacteriophage is in progress.

21.18 ETHIOLOGY OF DECLINE OF LOQUAT (*ERIOBOTRYA JAPONICA*) IN EASTERN SPAIN. E. González-Domínguez, A. Pérez-Sierra, L.A. Álvarez, P. Abad-Campos, J. Armengol and J. García-Jiménez. *Instituto Agroforestal Mediterráneo (IAM), Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Email: aperesi@eaf.upv.es*

Spain is the first producer of loquat [*Eriobotrya japonica* (Thunb.) Lindl.] in the Mediterranean area and the second in the world. The province of Alicante (eastern Spain) is the main producer; in 2006, 1269 ha were cultivated with a yield of 21815 t. In recent years an increasing problem has been the decline and death of established and newly planted trees. A survey of 32 loquat orchards was carried out from 2004 to 2007. Diseased trees showed leaf chlorosis, defoliation, branch dieback, basal cankers and root rot. Occasionally, gum exudations were observed in the trunk. Eventually, affected trees died. Isolations were made from roots, cankers and soil. *Armillaria mellea*, *Rosellinia necatrix*, *Phytophthora nicotianae*, *P. citrophthora*, *P. cambivora*, *P. cactorum*, and *P. cryptogea* were identified by morphological and molecular techniques, and their pathogenicity to *E. japonica* was studied. The incidence and distribution of these pathogens showed that the main species associated to the decline was *A. mellea* (40.6% of the orchards surveyed) followed by *R. necatrix* (34.4%) and *Phytophthora* spp. (18.7 %). In general, each orchard presented infections caused by only one pathogen. Combined infections were detected occasionally. These results indicate that the decline of loquat trees in Spain is caused by a complex of soil-borne pathogens.

21.19 MOLECULAR CHARACTERIZATION AND PHYLOGENIES OF ITALIAN ISOLATES OF *CITRUS EXOCORTIS VIROID*. M. Guardo, G. Sorrentino, T. Marletta and A. Caruso. *CRA - Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acireale (CT), Italy. Email: maria.guardo@entecra.it*

The nucleotide sequences of six *Citrus exocortis viroid* (CEVd) isolates found in different source plants and geographical locations in Italy were estimated by sscp and sequence analysis. Three isolates were collected from lemon trees of the historical citrus collection in the 'Medici villas of Boboli' in Florence, one from Procida lemon in Procida island (NA), one from 'Castagnaro' Bergamot in Reggio Calabria province, and one isolated from a 'Monachello' lemon in Catania province. Variability was mainly found in the V and P domains. Nucleotide diversity estimated from nucleotide distances ranged from 0.005 to 0.042, and these low values suggest a negative selective pressure. In particular the Sicilian isolates were very similar to an isolate from the 'Medici villas of Boboli' collection, with nucleotide distance of 0.005. This may be due to viroid transmission by infected tools or to a similar selective pressure. Instead the Procida Lemon isolate showed the highest distance value of 0.042, due probably to its isolated location.

21.20 GRAPEVINE TRUNK DISEASES: A METHOD TO INVESTIGATE RELATIONSHIPS BETWEEN INTERNAL WOOD DECAY AND FOLIAR SYMPTOMS. L. Guérin-Dubrana, J.P. Goutouly, J. Piot and N. Maher. *U.M.R Santé Végétale INRA/ENITA de Bordeaux IFR 103, ISVV, 1 Cours du Général de Gaulle CS40201, 33175 Gradignan, France. Email: l-guerin@enitab.fr*

The symptomatology of grapevine trunk diseases (esca and *Eutypa* dieback) tends to be quite complex: erratic foliar symptoms and internal wood discolorations are the result of fungi developing in the vascular tissues. To better understand the spatio-temporal dynamics of esca and associated diseases, we need tools to estimate the real incidence of the disease taking into account both external and internal symptoms. The objectives of this study were to develop a method to quantify internal wood necrosis in vines, and to analyse relationships between the type of foliar symptom and the degree of wood alteration. Cordons, trunks, and rootstocks of 140 vines (cv. Cabernet Sauvignon) sampled from 11 Bordeaux vineyards, were systematically cross-sectioned into pieces 6 cm long. The sections were analysed by image processing to measure the surface area of each necrosis type. Multivariate statistical methods were used to define development stage categories of internal necrosis. We found a strong correlation between disease levels in the trunks and branches but no continuity of necrosis between the rootstock and the scion. As expected, most vines expressing '*Eutypa* dieback' foliar symptoms showed central and dominant wedge-shaped necrotic lesions. The acute form of esca most frequently showed the most severe necrosis, with the presence of white rot, and damaged sap tissues. Furthermore, vines expressing the chronic form of esca could only be distinguished from symptomless vines by analysing necrosis in the branches, with necrotis surfaces and damaged sap tissues being more prominent.

21.21 POWDERY MILDEW CONTROL ON ALMOND IN CALIFORNIA. B. Holtz, T. Martin-Duvall and J. Adaskaveg. *University of California, 328 Madera Ave. Madera, CA 93637, USA. Email: baholtz@ucdavis.edu*

Two species of powdery mildew have been reported on almond, the apple powdery mildew fungus *Podosphaera leucotricha* and the peach powdery mildew fungus *Sphaerotheca pannosa*. We believe the apple powdery mildew fungus is responsible for causing disease like symptoms on almond similar to symptoms associated with peach rusty spot, but both organisms may be present. Fungicide applications were timed according to fruit development of the Carmel variety from petal fall to 60 days after full bloom in a completely randomized design with five replications. Applications were made approximately four and six weeks after petal fall. All treatments were evaluated for powdery mildew incidence by randomly collecting 25 nuts per plot and determining the number of nuts infected with powdery mildew. Laredo exhibited the least incidence of powdery mildew followed by Topsin tank-mixed with Microthiol, Gem, and Pristine that were all significantly less than the control. Scala had less mildew than the control but the difference was not significant. The nuts were shaken from the Laredo treatment and non-treated control trees and weighed. Sub-samples were collected, dried, and hulled in order to examine the effect of powdery mildew on yield and nut quality, and 200 nuts per sub-sample were shelled and weighed and percent shrivel recorded. Two applications of Laredo resulted in a 21.6 percent increase in yield and 35% less almond shrivel when compared to the non-treated control.

39.1 SEED TRANSMISSION OF *PHOMA GLOMERATA*, CAUSAL AGENT OF CROWN ROT DISEASE OF FENNEL IN SOUTHERN ITALY. E. Lahoz, R. Caiazzo, A. Fanigliulo, A. Carella, F. Porrone and A. Crescenzi. *CRA-CAT, Unità di Ricerca per le Colture alternative al Tabacco, Via P. Vitiello 108, 84018 Scafati, Italy. Email: ernesto.lahoz@entecra.it*

In the autumn of 2005 and 2006 millions of fennel seedlings with serious symptoms of damping off and root necrosis were ob-

served in greenhouse nurseries in Basilicata, Calabria, Campania and Apulia regions, southern Italy. Here we describe confirmation of the causal agent of this disease, applying Koch's postulates. The fungus species was identified by means of the internal transcribed spacers and the 5.8 rDNA gene (ITS1-5.8-ITS2). The amplified DNA was sequenced and compared with those present in GenBank (NCBI). The fungus isolated after morphological and molecular characterization was ascribed to the species *Phoma glomerata* (Corda) Wollenweber & Hochapfel, and this appears to be the first report of *P. glomerata* as agent of crown rot of fennel. Isolation was attempted from seeds treated for 1 min in 1% sodium hypochlorite, and yielded from 1 to 20% of colonies of *P. glomerata*, after 1-5 days. The application of coating did not influence the ability of the fungus to infect plants. Relationships between isolates recovered from plants and seeds were investigated by means of PCR amplification with random and specific primers of the internal transcribed spacers and the 5.8 rDNA gene (ITS1-5.8-ITS2), and will be discussed. High similarity confirmed that infections originated from seeds.

39.2 FIRST OBSERVATIONS ON THE ROLE OF RAIN IN THE PENETRATION OF *EUTYPA LATA* INTO PRUNING WOUNDS. **P. Larignon.** *Institut Français de la Vigne et du Vin, Domaine de Donadille, 30230 Rodilhan, France. Email: philippe.larignon@itvfrance.com*

The choice of methods for evaluating fungicides able to protect pruning wounds against *Eutypa lata* depends on a good knowledge of the first stages of infection under natural conditions. The objective of this study was to obtain information on the role of rain in the penetration of ascospores of *E. lata* into the vessels after pruning. In natural conditions, pruning wounds subjected to rain in the presence of wood pieces carrying perithecia of *E. lata* showed that the spores were not distributed homogeneously over the entire length of the wound. They were rather present in woody tissues located between 6th and the 15th millimetre (58% of isolations). They were seldom present more deeply than 20th millimetre. Experiments with artificial inoculation using a suspension of ascospores showed that the spores were preferentially located in the first five millimetres when they were inoculated in a dry period whereas under rain, they were distributed more deeply in the wound and homogeneously through the first twenty millimetres. This study demonstrated the role of water in migration of the spores into wounds and subjacent tissues. Evaluations of the efficiency of products for protection of pruning wounds against *E. lata* will have to take account of this important factor.

39.3 PROPAGATION OF PIONEER FUNGI ASSOCIATED WITH ESCA DISEASE BY VEGETATIVE MATERIAL IN FRENCH GRAPEVINE NURSERIES. **P. Larignon, M. Coarer, K. Girardon, F. Berud and O. Jacquet.** *Institut Français de la Vigne et du Vin, Domaine de Donadille, 30230 Rodilhan, France. Email: philippe.larignon@itvfrance.com*

Esca disease of grapevines is caused by a white rot in the wood, which is also correlated with various foliar symptoms (mild form, apoplexy). The white rot results from the action of several microrganisms: pioneer fungi leading to the formation of brown necrosis in a central position [*Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium aleophilum* (*Pal*)] or in sectorial position (*Eutypa lata*), which is then colonized by *Fomitiporia mediterranea*. The organisms responsible for the foliar symptoms are not known, as Koch's postulates are incomplete. Since no control measures are known in the vineyard, measures must be taken in nurseries to manage this syndrome. To achieve this goal, we studied the life cycle of fungi associated with esca disease in nurseries, then tested some control measures aimed at obtaining healthy plants. Only, *Pch* and *Pal* occurred in the woody tissues of canes (grafts, rootstocks), and at their surface. They were also isolated from plants after planting. Their isolation in higher percentage suggested contaminations during the grapevine propagation process. This possibility was examined during two steps (callusing, planting). *Pch* contaminated the plants during callusing of the wounds located at the base of grafted cuttings. *Pal* would contaminate the aerial part during planting. Among the different treatments tested, only hot water treatment (45 min, 50°C) showed good efficiency against *Pch*. Only by combining different measures associated with HWT will it be possible to achieve our goal.

39.4 RED WINE CHARACTERISTICS AFFECTED BY GRAPEVINE VIRUSES. **F.J. Legorburu, E. Recio, E. López, J. Baigorri, M. Larreina, L. Caminero, F. Cibriain and F. Aguirrezábal.** *NEIKER-Tecnalia, Apartado 46, Vitoria-Gasteiz, Basque Country, Spain. Email: jlegorburu@neiker.net*

The effect of grapevine viruses on the yield and composition of fruit is well stablished. Some wine parametres, like alcohol level or titratable acidity, can be measured in, or reliably predicted from, fruit or must. Other red wine components are extracted from from grape skins and seeds by the alcohol produced during fermentation. Anthocyanins and tannins, responsible for red colour and long-term stability, respectively, are among them, and measuring these in the fruit is not standarized and cannot be done in the winery in real time. As a result of a previous survey on grapevine viruses, plants infected by either *Grapevine fanleaf virus* (GFLV, *Nepovirus, Comoviridae*) or *Grapevine leafroll-associated virus 3* (GLRaV-3) were noted in several vineyards of the red variety Tempranillo in the Rioja wine region, in northern Spain. Over three years, separate microvinifications were done from infected and uninfected plants. Although this approach introduces more variables in the system, increasing the experimental error, it more reliably reflects the extraction of polyphenols during wine making. Even though the effect of the viruses was much smaller than the differences among vineyards, GLRaV-3 was found to decrease final alcohol contend by half a degree and seriously diminish colour intensity. Unexpectedly, no increase in acidity was detected. GFLV-infected plants often produced more concentrated wine than healthy ones, but this effect did not compensate for the overall yield loss due to the virus.

39.5* THE VIRUS WORLD OF FIG MOSAIC DISEASE. **G.P. Martelli, D. Boscia, M.A. Castellano, M. Conti, A. De Stradis, M. Digiaro, T. Elbeaino, G. Gattoni and A. Minafra.** *Department of Plant Protection and Applied Microbiology, University of Bari, Via Amendola 165/A, 70126 Bari, Italy. Email: martelli@agr.uniba.it*

Fig mosaic disease (FMD) is a cosmopolitan disease of fig (*Ficus carica*) characterized by various patterns of discolouration (mosaic, chlorotic mottling and blotching, vein banding, ringspots, line patterns) and malformation (twisting, puckering, rosetting) of the leaves. Infected plants have reduced vigour and may bear small mottled fruits. FMD has a viral aetiology but its causal agents are still under scrutiny. Up to 1971, enveloped round to ovoid bodies 90-200 nm in diameter (double-membrane bodies or DMBs), were the only anomalous intracellular struc-

tures consistently associated with FMD. DMBs are likely the particles of what can tentatively be referred to as fig mosaic virus (FMV). In 1971, an isometric virus [*Sowbane mosaic virus* (SoMV)] was reported from Italy, then viruses with filamentous particles from Japan (unidentified possibile carlavirus), Herzegovina (unnamed potyvirus) and Spain (unassigned unidentified virus). A turning point came in 2006-2007 when a putative closterovirus [Fig leaf mottle-associated virus 1 (FLMaV-1)], a putative ampelovirus [Fig leaf mottle-associated virus 2 (FLMaV-2)] and a partially characterized flexivirus, were reported first from Italy, then from different European, Mediterranean, and North and South American countries. FMD is perpetuated by vegetative propagation and is graft- but not seed-transmitted. However, FMV (i.e. the DMBs) is transmitted by the eriophyid mite *Aceria ficus* and indications are that FLMaV-2 is vectored by pseudococcid mealybugs. The contemporary presence of multiple viruses in symptomatic plants from widely separated geographical areas suggests that FMD is a complex disorder and that its putative agents have travelled with infected propagative material, so as to achieve a worldwide distribution.

39.6 GRAPEVINE DISEASES IN GEORGIA. **G. Mepharishvili, E. Megrelidze, Z. Sikharulidze and L. Gorgiladze.** *Ministry of Education and Science, Institute of Plant Immunity, Kobuleti 6200, Georgia. Email: gmeparishvili@bk.ru*

Georgia is rich in its diversity of native Georgian grapevine varieties and is a classical wine-making country. In 2004-2006 the incidence and severity of vine diseases were monitored in four viticulture zones of Georgia: Kakheti, Kartli, Imereti, and the humid subtropics. The following diseases were detected: anthracnose (*Elsinoe ampelina*), downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*), white rot (*Coniella diplodiella*), grey mould (*Botrytis cinerea*), and *Phomopsis* cane and leaf spot (*Phomopsis viticola*). The major and economically important diseases are downy mildew and anthracnose; these diseases occurred in all the investigated zones and regions. Nearly all varieties, whether commercial (Rkashtiteli, Saperavi, Aladasturi, Odjaleshi, Tcholikauri), aboriginal (Kamuri, Khikhivi, Kabashi) or introduced (Isabella shavi, Isabella tetri, Isabella tchiteli, Veluri amerikuli) were affected by downy mildew and anthracnose. Incidence of downy mildew and anthracnose was between 60-80%, but anthracnose was distributed more widely than downy mildew in western Georgia. Because of early infection and favorable conditions during growing seasons very high incidence and severity of anthracnose were found in 2004, and it caused losses which reached 80%. Where pesticides were used, severity of both diseases decreased but in Georgia the level of pesticides usage is low.

39.7 NEW DISEASES OF AROMATIC CROPS RECENTLY OBSERVED IN ITALY. **A. Minuto, P. Pensa, M.L. Gullino and A. Garibaldi.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: andrea.minuto@unito.it*

Aromatic plants (*Rosmarinus officinalis*, *Salvia* spp., *Origanum* spp.; *Mentha* spp., *Thymus* spp, *Ocimum basilicum*, *Diplotaxis* spp., *Taraxacum officinale*) are cash crops grown in northern Italy for culinary and ornamental use. *Diplotaxis tenuifolia* was reported as host of *Peronospora parasitica* and *Sclerotinia sclerotiorum* respectively in 2002 and 2003. Moreover during 2002-2003 *S. sclerotiorum* was reported to infect *Thymus* × *citriodorus* cv. Silver Queen, *R. officinalis* 'Prostratus' potted plants and *Salvia officinalis* grown in containers. In 2006 *S. sclerotiorum* was observed on *Taraxacum officinale* and *Origanum vulgare* and severe infections of *Botrytis cinerea* were reported on *S. officinalis*, *Mentha* × *piperita, Origanum majorana*, and *Melissa officinalis*. During the period 2001-2004, in Switzerland, Italy and France, severe outbreaks caused by *Peronospora* sp. on basil in open fields and under protection were observed. This disease, never reported before in the Mediterranean area, is now rapidly spreading in non-EU countries; infected seed and market globalisation may be key factors for this new epidemic. Other observed outbreaks might be due to several factors: the intensive cultivation of aromatic plants in northern Italy with more than 50 and 60 million potted plants respectively in 2003 and 2005 only in the Albenga area; the reduced availability of registered fungicides; climatic conditions characterized by mild autumns and, particularly in 2006/07, also a mild winter. In addition the need to increase the number of crop cycles per year might cause increased use of N-fertilizer, raising host susceptibility to fungal diseases.

39.8 ACTIVITY OF MEPTYLDINOCAP AGAINST POWDERY MILDEW OF GRAPEVINE. **A. Minuto, G. Gilardi, M.L. Gullino, A. Garibaldi, A.E. Hufnagl and L. Bacci.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: minuto.andrea@tiscali.it*

During 2005, 2006 and 2007 experimental trials were carried out in Liguria (northern Italy) to investigate the activity of meptyldinocap (DE-126 GF-1478) against powdery mildew (*Uncinula necator*) on grapevine. In 2005, six treatments of meptyldinocap at 140 and 210 g active ingredient (a.i.) /ha gave results similar to dinocap applied in the same manner. Treatments with myclobutanil (56.25 g of a.i./ha) and spiroxamine (394 g of a.i./ha), compared with meptyldinocap applications, significantly reduced the percentage of infected bunches, but did not differ in percentage of infected berries. In 2006, when meptyldinocap, dinocap, myclobutanil, or spiroxamine were applied before flowering (6 sprays) or after fruit setting (3 sprays), reduced efficacy was observed with the second type of application. Finally, in 2007 two sprays carried out with a 5-day interval with all fungicides (meptyldinocap, dinocap, myclobutanil, spiroxamine) at three disease severity thresholds (1-5%, 20-30%, 50-60% of infected grape bunches) were effective particularly when sprays were carried out at the lower disease threshold. Our trials demonstrate the possibility to replace dinocap with meptyldinocap without negative consequences in term of disease control. On the basis of the results obtained in 2006 and 2007, further investigations aimed at defining the most effective strategy for meptyldinocap application are needed.

39.9 RESULTS OF DIFFERENT STRATEGIES OF CHEMICAL AND BIOLOGICAL CONTROL OF GREY MOULD OF GRAPEVINE IN NORTH-WESTERN ITALY. **M. Monchiero, G. Gilardi, I. Luongo, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: m.monchiero@alice.it*

Control of grey mould (*Botrytis cinerea*) of grapevine can be achieved by using a range of chemical and biological measures. In five trials carried out from 2005 to 2007 in Piedmont (north-western Italy), boscalid and fenhexamid provided good control of *B. cinerea* when used at phenological stage B (touching of berries), followed by pyrimethanil or cyprodinil+fludioxonil at phenological stage C (colour change). The control was comparable to that obtained by using pyrimethanil at stages B and C or in

alternation with the mixture cyprodinil+fludioxonil. Less interesting results were obtained by using fenhexamid at stage B and iprodione at stage C. In the presence of medium-high grey mould incidence, there was significant reduction of the percentage of damaged berries and bunches, on vines treated with fenhexamid at stage B and iprodione at stage C, provided by three applications of trifloxystrobin, to prevent powdery mildew, in July. The biological agents *Trichoderma harzianum*, *Metschnikowia pulcherrima* and *Bacillus subtilis* gave partial control, especially when used in combination with chemicals.

39.10 EPIDEMIOLOGY OF *MONILIA* DISEASE OF QUINCE IN SOUTHERN SPAIN. J. Moral, D. Cabello, M.J. Benítez, M. Lovera, O. Arquero and A. Trapero. *Department of Agronomy, University of Córdoba, Campus de Rabanales, 14071 Córdoba, Spain. Email: trapero@uco.es*

Monilia disease of quince (*Cydonia oblonga)* caused by *Monilinia linhartiana* (anamorph *Monilia cydoniae*) is a serious concern for quince production in southern Spain. Disease epidemics have been characterized during 2004–07 in four commercial orchards in the Subbetica region of southern Spain. Two major infection periods were identified coinciding with shooting and flowering. These infection periods were associated with two different disease syndromes: a) leaf blotch and shoot blight, and b) mummification of young fruits. Foliar infection was caused by airborne ascospores produced on mummified fruits that overwintered on the soil surface, while flower infection was due to conidia produced on leaf lesions. Infected leaves showing lesions covered with conidia had a very characteristic odour, probably related with the transmission of conidia by insects. Disease incidence greatly varied amongst orchards and years and was correlated with density of mummified fruits on soil. Formation of apothecia on mummified fruits on the soil and discharge of ascospores into the air were related with the frequency and amount of rain during the budding and shooting periods. Based on these results, disease control measures have to be directed to reduce inoculum on soil and to protect the trees with fungicides applied at the beginning of the budding stage of the crop.

39.11 SPREAD OF *PHYTOPHTHORA* SPECIES THROUGH ORNAMENTAL PLANT TRADE. E. Moralejo, A. Pérez-Sierra, L.A. Álvarez, L. Belbahri and E. Descals. *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n 46022 Valencia, Spain. Email: vieaemr@uib.es*

The genus *Phytophthora* comprises plant pathogens causing significant economic losses in agriculture, horticulture and forestry. Recent studies on population genetics indicate that some new *Phytophthora* species have probably been introduced or have emerged and/or spread via the horticultural trade or forest plantations. Plant trade has been recognised as a principal pathway for the introduction of invasive plants and exotic pathogens. However, few studies have been focussed on screening the species diversity within a taxonomic group accompanying this movement of plants. A *Phytophthora* survey was conducted from 2001 to 2006 in nurseries and garden centres in eastern Spain and the Balearic Islands. A total of 125 *Phytophthora* isolates were obtained from 37 different host species ranging from herbs to trees and grouped into 17 putative species. Seven of these species were common in ornamental plants around the world, five were rare in Europe and five were unknown to science prior to the survey.

39.12 MAJOR SEED-BORNE FUNGAL DISEASES OF ONION (*ALLIUM CEPA*). N. Özer. *Department of Plant Protection, Faculty of Agriculture, Namık Kemal University, Tekirda, Turkey. Email: nurayozer@hotmail.com*

Aspergillus niger, Botrytis aclada and *Fusarium oxysporum* f.sp. *cepae* are important seed-borne fungi of onion (*Allium cepa* L.) as causal agents of black mould, neck rot and basal rot diseases, respectively. These pathogens can be transmitted from infected seeds to seedlings, sets or bulbs. Pectolytic enzymes and isoenzymes of these pathogens contribute to virulence during onion seed or bulb colonization. They eventually kill the entire plants through degradation of the tissues. No onion cultivar with high resistance to *A. niger* has been produced. Resistance to *B. aclada* was determined by using transplants and bulbs. Numerous onion lines and cultivars were found to be resistant to *F. oxysporum* f. sp. *cepae* under different experimental conditions. The mechanism of resistance of onion to these pathogens was examined. Chemical treatments are generally suggested for their control. In recent years, alternative compounds or treatments to pesticides have been evaluated.

39.13 RECENT ADVANCES ON THE CONTROL OF OLIVE DISEASES BY COPPER COMPOUNDS. L.F. Roca, J. Moral, J.R. Viruega, A. Avila, R. Oliveira and A. Trapero. *Depto. Agronomía, Universidad de Córdoba, Campus de Rabanales, C4, 14071 Córdoba, Spain. Email: trapero@uco.es*

Copper compounds are effective in controlling major foliar and fruit diseases of olive, mainly leaf spot or peacock spot caused by *Spilocaea oleagina*, anthracnose incited by *Colletotrichum* spp., and cercosporiose due to *Pseudocercospora cladosporioides*. However, their efficacy depends a great deal on the specific disease, time of application, and persistence on olive leaves and fruits. The commercial formulations of copper fungicides markedly influence their persistence, but the type of copper salt has no influence on persistence. The negative effects of copper fungicides have been and are being evaluated in olive orchards, although no phytotoxic effects have been observed on leaves and flowers. Some copper fungicides may induce systemic acquired resistance (SAR) in olive trees and experiments are being carried out to study this phenomenon and its use in an integrated disease management system.

39.14 MOLECULAR IDENTIFICATION OF *FUSICLADIUM ERIOBOTRYAE* THE FUNGAL PATHOGEN ASSOCIATED WITH MEDITERRANEAN LOQUAT SCAB. P. Sánchez-Torres, R. Hinarejos and J.J. Tuset. *IVIA, Centre of Plant Protection and Biotechnology, Lab of Micology, Ctra Moncada-Naquera km 4,5 Moncada, 46113 Valencia, Spain. Email: palomas@ivia.es*

Different *Fusicladium eriobotryae* strains have been isolated from loquat with scab symptoms. Mediterranean loquat scab symptoms are generally very evident and serious as they appear on both sides of leaves and fruit in the form of green or olive-brown spots. The lesions are usually circular and as they increase in size they becomes olive-coloured and velvety due to the production of asexual spores. In this work, loquat plants were infected using two different isolates, which differed in virulence. Both isolates of *F. eriobotryae* were compared to other closely related fungi such as V*enturia inaequalis, V. pyrina, Spilocaea pomi, S. eriobotryae* and *Fusicladiun carpophilum*, and symptoms and infection processes were studied. The results confirm that *F. eriobotryae* (Fe-S) is relatively virulent in cultivars found in the Mediterranean region and

therefore could be useful for testing resistant/tolerant varieties using this *in vivo* system. All the different strains were characterised to establish their interrelationships, using rDNA together with RFLP and RAPD techniques. We were able to specifically identify *F. eriobotryae*. This work provides the first molecular insight into the fungal pathogen causing loquat scab and offers a good method for *F. eriobotryae* identification.

39.15 SUSCEPTIBILITY OF WINE AND TABLE GRAPE CULTIVARS TO POWDERY MILDEW. **A. Santomauro, C. Dongiovanni, C. Giampaolo, M. Di Carolo and P. Pollastro.** *Dipartimento di Protezione delle Piante e Microbiologia applicata, Università degli Studi di Bari, Via Amendola, 165/A 70126 Bari, Italy. Email: a.santomauro@agr.uniba.it*

Sixty-three grapevine cultivars commonly grown in Italy were rated for susceptibility to powdery mildew under conditions of natural infection. The trial was carried out in Apulia (southern Italy), in a vineyard ("tendone" training system) where several grapevine cultivars are grown. A fully randomized design with 4 replicated plots of 4 vines was adopted. Symptom severity was assessed by observing all bunches in each plot. An empirical scale with 8 classes of infection was used to calculate the percentage of infected bunches, disease severity and McKinney's index. On 26 June, symptoms were observed on all bunches of several cultivars, and a broad variation in susceptibility levels was observed. In particular, the percentage of infected bunches ranged from 44% (cv. Inzolia and Centennial Seedless) to 100% (cvs Bianco d'Alessano, Fiano, Cabernet Sauvignon, Malvasia Bianca, Chardonnay and Uva di Troia), with values of McKinney's index ranging from 7% to 95%. Other cultivars, such as Bombino Nero, Primus, Negroamaro, Italia and Regina dei Vigneti showed intermediate infection values. The results partly confirm those previously obtained in Apulia. The level of susceptibility can be influenced by several factors, including the phenological growth stage of vines and the occurrence of conditions conducive to infection. The information obtained could be useful for the choice of the cultivar at the moment of planting new vineyards, especially in organic farming.

39.16 APPENDAGE-BEARING COELOMYCETES ON GRAPEVINES IN AUSTRALIA. **V. Sergeeva, N.G. Nair, M. Priest and R. Spooner-Hart.** *Centre for Plant and Food Science, University of Western Sydney, Locked Bag, 1797, South Penrith, DC, NSW 1797, Australia. Email: v.sergeeva@uws.edu.au*

The occurrence of various fungi such as *Botryosphaeria, Eutypa lata, Phaeomoniella and Phaeoacremonium* associated with infection of canes and trunks of grapevines (*Vitis vinifera* L.) in Australia has been reported. However, little is known about incidence and distribution, or role in pathogenesis of various appendage-bearing Coelomycetes on grapevines. We recognized five morphologically distinct taxa of appendaged Coelomycetes occurring on grapevines in eastern Australia. Morphological features of the fungi and symptoms caused by them are described: *Pestalotiopsis uvicola* (Spegazzini) Bissett with its mostly concolorous cells and rugose cell walls has been isolated from bleached canes, internal wood rot, leaf spots, flower rachises and berries. *P. menezesiana* (Bres. & Torr.) Bisset, distinguished from *P. uvicola* by its opaque upper cells and darkened septum has been isolated from bleached canes and internal wood rot. *Seimatosporium hysterioides* (Fuckel) Brockman was isolated from dead stems and from cankered canes. *Truncatella angustata* (Pers.: Link) Hughes has been isolated from dormant canes and roots. *Sporocadus rhodendri* (Schw.) Morelet was isolated from symptomless canes. Infection studies showed that grapevine berries were infected by *P. uvicola* more readily at later stages of berry development than at the earlier stages. The delay in infection of berries at the pea size stage of growth may indicate that the fungus undergoes a quiescent phase after flower infection. Several appendaged Coelomycetes are associated with grapevines in Australia and their role in pathogenesis is worth further investigation.

39.17* DIAGNOSIS AND DETECTION OF FUNGI OCCURRING ON OLIVES IN AUSTRALIA. **V. Sergeeva, R. Spooner-Hart and N.G. Nair.** *Centre for Plant and Food Science, University of Western Sydney, Locked Bag, 1797, South Penrith, DC, NSW 1797, Australia. Email: v.sergeeva@uws.edu.au*

Several fungi, some of pathogenic importance, were observed on olives from different olive-growing regions of Australia during the period 2002-2007. Samples of olive leaves, flowers and berries were received from olive groves in New South Wales, Victoria, Queensland and South Australia. Berries and leaves from different cultivars, Manzanillo, Nevadillo, FS-17, Correggiola, Picual, Barnea, Frantoio were examined. Conventional methods of detection such as incubation of surface-sterilised and unsterilized tissues in moist chambers, and growth on common culture media (potato dextrose agar) were used. Fungi in nine different genera were isolated from these samples. These were *Colletotrichum, Botryosphaeria, Pseudocercospora, Fusicladium, Coleophoma, Phoma, Phomopsis Pestalotiopsis and Alternaria.* 30 isolates of *Colletotrichum* were isolated from berries. It appears therefore that *Colletotrichum* is the predominant pathogen of olive berries. These species caused different types of symptom on the berries. Three different species of *Colletotrichum* were isolated from olive berries. These species were *C. acutatum, C. gloeosporioides* and *C. orbiculare.* Few isolates of *C. acutatum* were obtained from olive leaves. Of the fungi isolated, *Pseudocercospora cladosporiodes, Fusicladium oleagineum, Coleophoma olea, Phoma* sp. and *Colletotrichum orbiculare* were new records on olive berries in Australia. Three genera, *Phoma, Phomopsis and Colletotrichum* were new records on olive leaves in Australia. Fungal pathogens taint olive oil. For instance, it has been reported that at over 30% of infection of the olives by *C. gloeosporiodes,* the oil could not be considered "extra virgin" because of a high increase in free acidity and peroxide number.

39.18 FUNGAL AND OOMYCETE PATHOGENS ASSOCIATED WITH DECLINE AND DEATH OF STRAWBERRIES IN INFERTILE, SANDY SOILS OF WESTERN AUSTRALIA. **I. Tvede, D. Phillips, K. Sivasithamparam and M.J. Barbetti.** *School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA, 6009, Australia. Email: tvedei01@student.uwa.edu.au*

Western Australian strawberry production accounts for 90% of Australia's strawberry exports, and is worth over $1.1 million a year to the state's economy. Over the past three growing seasons, the major WA strawberry growing region, Perth metropolitan area, has suffered losses from root and crown diseases of up to 1 million plants per year. The infertile, sandy soils of the region, along with the cultural practices used, including continuous cropping, together have resulted in an environment conducive to root and crown rots. Three distinct periods of plant deterioration in commercial fields have been recorded, including immediately after planting in late autumn, mid-winter and in spring. The most

significant of these periods is in spring, and it often extends and worsens into the summer months. Throughout these periods, symptoms of general necrosis of feeder roots, distinct root lesions, early root death, internal crown discolouration, wilting and death of outer leaves, stunting and/or death of plants can be found. A range of different fungal and oomycete pathogens have been isolated from plants with these symptoms. Pathogenicity tests indicate that various fungi/oomycetes, singly and in combination, are associated with the symptoms observed in the field.

39.19 MYCELIAL COMPATIBILITY AND INTER-SIMPLE SEQUENCE REPEATS (ISSR) ANALYSES REVEAL A CLONAL STRUCTURE AMONG STRAINS OF *ROSELLINIA NECATRIX* ISOLATED FROM *CYPERUS ESCULENTUS*. **A. Vicent, M. León, M. Berbegal, P. Abad-Campos, J. García-Jiménez and J. Armengol.** *Instituto Agroforestal Mediterráneo (IAM), Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Email:jarmengo@eaf.upv.es*

Tuber rot of tiger nut (*Cyperus esculentus* L.) caused by *Rosellinia necatrix* was first described in 1998 in Valencia province (eastern Spain) and has become an important disease of this crop which is used to produce tiger nut milk ("horchata"). Twenty-four strains of *R. necatrix* obtained from *C. esculentus* and seventeen from other hosts were evaluated for mycelial compatibility in 2% malt extract agar. A representative group of these strains was further characterized by inter-simple sequence repeats (ISSR) analysis. All strains from *C. esculentus* belonged to a single mycelial compatibility group (MCG) irrespective of their geographical origin or year of isolation and were incompatible with the strains from other hosts. Four ISSR primers: $(CA)_7$, $(AG)_7$, $(ACA)_5$ and $(GT)_7$ provided good and polymorphic banding patterns and were selected to analyze the genetic variability of *R. necatrix* strains. Cluster analysis revealed the existence of genetic diversity in *R. necatrix* from different hosts but all strains from *C. esculentus* were grouped into a single cluster. These results indicate that the population structure of *R. necatrix* affecting *C. esculentus* is clonal, suggesting that this pathogen was introduced recently and spread throughout the tiger nut production areas by infected tubers which are used to propagate this crop.

39.20 IDENTIFICATION OF *BOTRYOSPHAERIA* SPP. CAUSING BUNCH ROT IN GRAPEVINES. **N. Wunderlich, C.C. Steel, G.J. Ash and S. Savocchia.** *National Wine and Grape Industry Centre, School of Wine and Food Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. Email: nwunderlich@csu.edu.au*

Species of the fungus *Botryosphaeria* are responsible for cankers in the wood and bunch rots of grapevines. Pathogenicity tests have been conducted on grapevine wood for several species, but it is unknown which of these infect bunches. *Botryosphaeria* spp. have been isolated from bunches in Australian vineyards, but again little is known about the infection pathway into bunches. This project aims to identify *Botryosphaeria* spp. isolated from grapevines at different phenological stages (dormant buds, flowers, pea-sized berries, berries at veraison and berries at harvest) and from the wood. Samples will be collected over 3 seasons from *Vitis vinifera* cv. Chardonnay and Shiraz from two vineyards in the Lower Hunter Valley in south-eastern Australia. Pathogenicity tests will be conducted on berries using the species isolated from the survey. Species variation within a plant will be analysed and compared to the species found in diseased wood of the same plant. To date, *Botryosphaeria* spp. have been isolated from 6% (3% from Chardonnay and 3% from Shiraz) of bud samples in one vineyard, and 17% (12% from Chardonnay and 5% from Shiraz) in a second vineyard. *Botryosphaeria* spp. have also been isolated from approximately 50% of the wood samples collected from these vineyards. The results from this study will contribute to understanding the epidemiology of *Botryosphaeria* bunch rot and will lead to better management strategies for this disease.

39.21 MOLECULAR VARIABILITY OF *MOROCCAN WATERMELON MOSAIC VIRUS* IN THE AFRICAN CONTINENT. **S. Yakoubi, C. Desbiez, H. Fakhfakh, C. Wipf-Scheibel, M. Marrakchi and H. Lecoq.** *Laboratoire de Génétique Moléculaire, Immunologie et Biotechnologie, Faculté des Sciences de Tunis, Campus Universitaire, El Manar 2092, Tunis, Tunisia. Email: yakoubisoumaya@yahoo.fr*

During a survey carried out in October 2005, cucurbit leaf samples showing virus-like symptoms were collected from the major cucurbit-growing areas. DAS-ELISA tests showed the presence of *Moroccan watermelon mosaic virus* (MWMV, Potyvirus), detected for the first time in Tunisia, in 62% of symptomatic samples collected from Cap Bon (northern Tunisia). In contrast, none of the samples collected from Bizerte (northern Tunisia) and Monastir (Sahel of Tunisia) were found infected by MWMV. The localization of MWMV in Cap Bon could be due either to a recent introduction in this area or to specific ecological conditions in this region such as the presence of an efficient reservoir or specific cultural practices not found elsewhere in Tunisia. Sequence analysis on parts of the CP gene of MWMV isolates from different parts of the world revealed an interesting geographic structure of MWMV variability, with three different clusters: one cluster including isolates from the Mediterranean region, a second including isolates from Western and Central Africa, and a third one including isolates from southern Africa. Genetic distances between isolates correlated with geographic distances, suggesting a limited long-distance dispersal of the virus. Isolates from countries in the Mediterranean region where MWMV has recently emerged (France, Spain and Portugal) have highly conserved sequences suggesting that they may have a common and recent origin. MWMV from Sudan, the most divergent variant, appears to be at the fringe of the MWMV species.

DISEASES OF ORNAMENTALS AND TURFGRASSES

24.1 ANTHRACNOSE CAUSED BY *COLLETOTRICHUM ACUTATUM* ON EVERGREEN AZALEA (*RHODODENDRON AZALEA*) CULTIVARS: SUSCEPTIBILITY TRIALS. **D. Bertetti, M.L. Gullino and A. Garibaldi.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: domenico.bertetti@unito.it*

In several nurseries located in the Verbano-Cusio-Ossola province (northern Italy) anthracnose causing leaf spots and defoliation was observed on evergreen azaleas. *Colletotrichum acutatum* was isolated from infected leaves and stems and was identified as the causal agent of this disease. Two trials were carried out to evaluate the susceptibility of 70 evergreen azalea cultivars grown in nurseries in the Lake Maggiore area. Three plants of each cultivar tested were inoculated with a conidial suspension of the pathogen, and then kept in a high humidity chamber, inside the greenhouse, for 3 days. After symptom appearance, the number of leaf spots was counted on 15 leaves per inoculated plant,

chosen at random. For each susceptible cultivar the average number of spots/leaf was calculated. The experiment was repeated twice. Sixty of the cultivars failed to develop disease symptoms and were considered resistant to *C. acutatum*. Cultivars showing symptoms were classed as follows: moderately susceptible (MS = average number of spots/leaf 0.1 to 5), susceptible (S = average number of spots/leaf 5.1 to 20), highly susceptible (HS = average number of spots/leaf higher than 20). 'Conversation Piece', 'Eikan' and 'Martha Hitchcock' showed moderate susceptibility. 'Addy Wery', 'Fior di Pesco Cavadini', 'Geisha Orangerot', 'Kermesina', 'Orion', 'Palestrina' and 'Snow' developed severe symptoms. 'Snow' was susceptible in the first trial and highly susceptible in the second.

24.2 POWDERY MILDEWS RECENTLY OBSERVED IN ITALY ON ORNAMENTAL PLANTS. D. Bertetti, M.L. Gullino and A. Garibaldi. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: domenico.bertetti@unito.it*

We report the results of a survey, over the last six years, of powdery mildews on ornamental plants cultivated in nurseries, and in public and private gardens located in the regions of Piemonte and Liguria in Italy. Several powdery mildews were found on new hosts. Symptoms on new hosts were described, and characteristics of conidia and conidiophores were observed in the light microscope (dimensions, shape, presence of fibrosin bodies etc.). If the perfect stage was present, chasmothecia, asci and ascospores were also described. If possible, identification of the causal agent of the disease was followed by ITS analysis to confirm the classification. Symptoms were reproduced on new plants by experimental inoculation. Several varieties of the same host species were inoculated to test their susceptibility to the pathogen. The following causal agents of powdery mildew are reported on the following new hosts: *Oidium* sp. on *Akebia quinata* and *Papaver nudicaule*, *Oidium* sp. subgen. *pseudoidium* on *Salvia scabra*, *Lonicera caprifolium*, *Mandevilla splendens*, *Berberis thunbergii* var. *atropurpurea* and *Wisteria sinensis*, *Oidium* sp. subgen. *fibroidium* on *Euryops pectinatus*, *Oidium* sp. subgen. *octagoidium* on *Acer negundo*, *Oidiopsis* sp. on *Asclepias curassavica, Erysiphe azaleae* on *Rhododendron japonicum × R. molle, Erysiphe aquilegiae* var. *aquilegiae on Aquilegia flabellata, Erysiphe orontii* on *Veronica spicata* and *Antirrhinum majus, Erysiphe biocellata* on *Verbena × hybrida, Golovinomyces orontii* on *Petunia × hybrida* and *Lamium galeobdolon, Leveillula clavata* on *Euphorbia pulcherrima, Podosphaera fusca* on *Coreopsis lanceolata, Podosphaera leucotricha* on *Photinia × fraserii, Podosphaera aphanis* var. *aphanis* on *Potentilla fruticosa, Podosphaera spiraeae* on *Spiraea japonica.*

24.3 *FUSARIUM* AND *VERTICILLIUM* WILTS NOTED ON ORNAMENTAL PLANTS FOR THE FIRST TIME IN ITALY. D. Bertetti, G. Gilardi, M.L. Gullino and A. Garibaldi. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: domenico.bertetti@unito.it*

Several *Fusarium* and *Verticillium* wilts were recently noted, for the first time in Italy, on ornamental plants in nurseries and private gardens located in Piemonte and Liguria regions. Symptoms were observed on crops grown in greenhouses and in the open, for the production of cut flowers and pot plants. The causal agents of the diseases were isolated and identified, experimentally reproducing the disease symptoms. Trials were made to test the susceptibility of the most widespread varieties of the new hosts. *Fusarium oxysporum* was observed on *Lewisia cotyledon* and *F. oxysporum* f. sp. *chrysanthemi* was found on African daisy (*Osteospermum* spp.). Wilt of lisianthus (*Eustoma grandiflorum*) caused by *F. oxysporum* f. sp. *eustomae* is causing severe attacks in many commercial farms producing cut flowers. Trials to test the most susceptible cultivars of lisianthus were followed by evaluation of the effect of temperature and pathogen isolate on disease development under controlled conditions. Wilt caused by *Verticillium dahliae* was found on African daisy, and the same pathogen was isolated from Washington lupin (*Lupinus polyphyllus*).

24.4* RECOVERY OF *PHYTHOPHTHORA* SPECIES FROM POTTED ORNAMENTALS IN COMMERCIAL NURSERIES IN ITALY. S.O. Cacciola, A. Pane, P. Martini, G.E. Agosteo, F. Raudino and G. Magnano di San Lio. *Department of Scienze e Tecnologie Fitosanitarie, University of Catania, Via S. Sofia 100, 95123 Catania, Italy. Email: apane@unict.it*

During surveys of ornamental retail nurseries in Italy, *Phytophthora* species were very frequently recovered from roots of potted plants. Isolates obtained with BNPRAH and PARPNH selective media were identified using morphological criteria, electrophoretic patterns of mycelial proteins and isozymes and sequencing of the internal transcribed spacer (ITS) region of rDNA. The species recovered most frequently from the roots and rhizosphere soil were *P. nicotianae*, *P. palmivora*, *P. cryptogea* and *P. citricola*. Other less common species were *P. asparagi*, *P. cactorum*, *P. cinnamomi*, *P. drechsleri*, *P. gonapodyides*, *P. hedraiandra*, *P. inundata*, *P. niederhauseri*, *P. tentaculata* and unidentified *Phytophthora* spp. included in ITS clades 1 and 6. *Phytophthora* isolates were also obtained from symptomless plants, confirming the risk of spreading exotic and endemic pathogens through nursery stocks.

24.5 EXPLOITING BIORESOURCES IN MANAGING *FUSARIUM* WILT OF CARNATION, A SERIOUS THREAT TO COMMERCIAL PRODUCTION. S. Chandel and R. Kumar. *Department of Mycology and Plant Pathology, Dr. Y.S.P. University of Horticulture and Forestry, Nauni, Solan (H.P)-173230, India. Email: schandelmpp@rediffmail.com*

Fusarium wilt (*Fusarium oxysporum* f.sp. *dianthi*) is rapidly becoming a limiting factor to commercial production of carnation (*Dianthus caryophyllus* L.) under protective conditions. The disease is soilborne in nature, hence difficult to manage. Excessive use of chemicals is causing eco-environmental pollution and health hazards. To overcome this situation bioresources provide a better alternative in manipulating the rhizosphere by adding beneficial microorganisms to the soil. The present study was focused on the use of organic amendments, plant extracts, and neem formulations in mitigating losses caused by *Fusarium*. Nine organic amendments were tried but only two, neem cake and pine needles, gave above 70% disease control whereas 54-57% disease suppression was recorded in treatments of Mustard and Cotton seed cakes if applied 25 days prior to planting of carnation cuttings. Extract of garlic (*Allium sativum*) was superior to other plant extracts by giving minimum mycelial growth (22.25 mm) with maximum inhibition (75.28%). Marigold (*Tagetes erecta*), tulsi (*Ocimum sanctum*) and Neem (*Azadirachta indica*) were equally good in restricting the growth of fungus at different concentration levels, with higher concentrations being more effective. Achook (1.0%) and Nimbicidine (0.3%) among commercial neem formulations gave lower incidence using the root dip method than soil drench application. Integration of best neem formulation with efficacious plant extracts

yielded greater wilt reduction in some combinations: Achook+ *Azadirachta indica* (67%), Achook+*Allium sativum* (65.79%) and Achook+*Tagetes erecta* (64.48%).

24.6* DETECTION AND IDENTIFICATION OF POTYVIRUSES OF BULBOUS ORNAMENTALS IN NEW ZEALAND. **S.J. Cowell, M.N. Pearson, T. Wei, A.G. Blouin, D. Cohen and G.R.G. Clover.** *School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand. Email: scow013@ec.auckland.ac.nz*

A number of bulbous flower species are important ornamental horticultural crops for New Zealand, but knowledge of which viruses are present in these crops is incomplete. To ensure that phytosanitary regulations are appropriate, the New Zealand Ministry of Agriculture and Forestry (MAF) initiated a targeted survey of viruses of the most significant bulbous flower crops in 2005. The *Potyvirus* genus, the largest group of plant viruses, contains some of the most important viral pathogens of bulbous ornamentals and other important crop species. Consequently, the aim of this research was to add to the body of knowledge required by MAF by focusing on the detection and identification of potyviruses of bulbous ornamentals in New Zealand. *Bean yellow mosaic virus*, *Freesia mosaic virus* (FreMV), *Hippeastrum mosaic virus* (HiMV), *Iris severe mosaic virus*, *Lily mottle virus*, *Hyacinth mosaic virus* (HyaMV), *Nerine virus Y*, *Nerine yellow stripe virus* (NeYSV), *Ornithogalum mosaic virus* and *Ornithogalum virus 2* were isolated from bulbous flower samples. FreMV, HiMV, HyaMV and NeYSV are new potyvirus records for New Zealand. In addition, there were a number of new host records for some viruses. Some presumed potyvirus isolates were of uncertain identity, and may prove to be new strains or species of *Potyvirus*. This information will better enable the development of suitable phytosanitary regulations for bulbous ornamental importation, protocols for disease management, and also allow negotiation of market access for bulb exports.

24.7 GENETIC DIVERSITY OF BELGIAN *PHYTOPHTHORA RAMORUM* ISOLATES. **I. De Dobbelaere, A. Vercauteren, K. Heungens and M. Maes.** *Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96 bus 2, Belgium. Email: kurt.heungens@ilvo.vlaanderen.be*

Phytophthora ramorum is a relatively recently described quarantine species that causes extensive mortality of selected oak trees on the west coast of the USA, the so-called "sudden oak death". Only a few forest or park trees have been found infected by *P. ramorum* in Europe, where the pathogen is mainly present in nurseries, especially on *Rhododendron*, *Viburnum*, and *Camellia*. It is suspected that *P. ramorum* was recently introduced in Europe and therefore possesses only a limited amount of genetic diversity. To verify this hypothesis and to develop molecular markers that would help to study the dispersion of the pathogen, we have screened 80 *P. ramorum* isolates with AFLP and SSLP. The isolates originated from Flanders and were mainly selected based on differences in isolation year and location. Using the AFLP method with five primer combinations, 13 polymorphic fragments were identified. These markers identified only 8 isolates that differed from the main genotype by one to three polymorphisms. For SSLP, 132 candidate polymorphic microsattelites were prescreened using 10 isolates belonging to different EU genotypes. Finally, six primer pairs were selected and used for screening the 80 isolates. These revealed 10 isolates that differed from the main genotype, two of which were also genetically distinct based on the AFLP analysis. The overall level of genetic diversity within these isolates of *P. ramorum* would indeed appear to be limited, indicating recent dispersion of the pathogen. Several new molecular markers were found, but these mainly identified genotypes that differed from the main EU lineage by single mutation events.

24.8* *PHYTOPHTHORA* DIVERSITY IN UK GARDENS. **G. Denton, J. Denton, I. Waghorn and B. Henricot.** *Plant Pathology Department, The Royal Horticultural Society, Wisley, Woking, Surrey, GU23 6QB, UK. Email: geoffdenton@rhs.org.uk*

Phytophthora is a common cause of death of a range of herbaceous and woody plants in gardens. Over the last 10 years the Plant Pathology department at the Royal Horticultural Society has diagnosed over 1000 enquiries with *Phytophthora* from more than 185 genera. The most common hosts affected are *Taxus*, *Rubus*, *Rhododendron*, *Prunus* and *Viburnum* with *Taxus* accounting for over 20% of the cases. Confirmation of the pathogen has traditionally been via apple baiting and then water float for sporangial production. Alternative diagnostic methods of a commercial lateral flow device and a nested PCR, based on the ITS region, are being compared to traditional baiting for their efficacy with soil and/or plant samples. Initial findings indicate that the lateral flow and nested PCR systems detect *Phytophthora* at higher frequency from symptomatic plants compared to apple baiting. Through baiting we recovered *P. cactorum, P. cinnamomi, P. citricola, P. citrophthora*, *P. cryptogea*, *P. gonapodyides* and *P. niederhauserii*. With the nested PCR we detected a wider range of *Phytophthora* species and additionally identified *P. alni, P. cambivora, P. hibernalis, P. megasperma, P. porri, P. quercina* and *P. syringae*. Sequence results also identified *Pythium* species from dying plants in association with *Phytophthora* or alone, indicating their possible involvement in root death. Species commonly identified are *Pythium attrantheridium*, *Py. heterothallicum*, *Py. intermedium, Py irregulare* and *Py. sylvaticum*. Future work will investigate their role in causing diseases on ornamentals.

24.9 INCIDENCE AND DIVESITY OF CAULIMOVIRUSES ASSOCIATED WITH DAHLIA MOSAIC IN *DAHLIA* SPP. **K.B. Druffel, S. Eid, D. Saar and H.R. Pappu.** *Department of Plant Pathology, Washington State University, P.O. Box 646430, Pullman, WA 99164, USA. Email: kdruffel@mail.wsu.edu*

Mosaic disease of dahlia (*Dahlia variabilis*) is a serious disease affecting dahlia in several countries. In addition to a caulimovirus (DMV-Portland) reported previously, we have isolated and characterized a caulimovirus (DMV-D10) associated with dahlia mosaic. Recent surveys of dahlia from different parts of the US showed that DMV-D10 was the most prevalent virus in dahlia. The double stranded DNA genome of D10 was ca. 7.0 kb in size and shared many of the features of the members of the genus *Caulimovirus.* However, the virus differed from known caulimoviruses in that the aphid transmission factor was absent and the coat protein was smaller than its counterpart in known *Caulimovirus* species. Sequence identity of the deduced protein sequences with the known caulimoviruses ranged from 32-70% at amino acid level. In addition to DMV-D10 and -Portland, we have recently characterized another caulimovirus (DMV-Holland) from dahlia. Based on sequence similarity with the DMV-D10 and -Portland, DMV-Denmark was found to be a distinct caulimovirus species. A survey of dahlias from the US and Europe for the three caulimoviruses showed that D10 was most

widely prevalent followed by DMV-Holland and DMV-Portland. Southern hybridization analysis showed that the viral genome is integrated into the host genome. Several wild species of dahlia are being evaluated for the presence of these three caulimoviruses. Production of DMV-free stock and the virus management tactics for dahlia mosaic should take into account the diversity of caulimoviruses extant in dahlia.

24.10 MIXED VIRUS INFECTION – CAUSE OF DAPHNE DIEBACK IN THE CZECH REPUBLIC? **J. Fránová, K. Petrzik, D.-E. Lesemann, M. Navrátil, R. Karešová, M. Šimková and J. Nebesářová.** *Department of Plant Virology, IPMB, BC ASCR, v.v.i., Branišovská 31, 370 05 České Budějovice, Czech Republic. Email: jana@umbr.cas.cz*

Leaf mosaic symptoms of *Daphne mezereum* plants have been observed with increasing frequency in the Czech Republic. Some plants also showed leaf necrosis, precocious yellowing/reddening with branch defoliation and flowering twice or three times a year. Most of the shrubs with severe symptoms died. Shrubs with leaf mosaic contained flexuous filamentous virions (696×13 nm) and cylindrical inclusions typical of the subdivision III of Edwardson's classification for inclusions induced by members of the family *Potyviridae*. Daphne mosaic virus (DapMV) was identified, characterised thoroughly, and based on the nucleotide sequence of the coat protein gene, was proposed as a new species of the genus *Potyvirus*. Double infection of DapMV and bacilliform virions was observed in samples from daphne shrubs, which revealed simultaneously mosaic symptoms, precocious leaf reddening, defoliation, and repeated flowering with subsequent decline and dieback. Extensive aggregations of bullet-shaped particles (166-370×65 nm and 169-233×68-78 nm) were present in the nucleus or perinuclear space of diseased root and leaf tissues, respectively; suggesting that the virus could belong to the genus *Nucleorhabdovirus*. Unexpectedly, bacilliform virions which, in thin sections, measured 162-215 nm by 62-75 nm were also detected in symptomless daphne plants. The presence of mixed virus infection could be the cause of *D. mezereum* dieback in the Czech Republic. The work is funded by the GA ASCR No. 1QS500510558 and AV0Z50510513.

24.11 INFECTION SOURCE OF PETAL BLIGHT OF CHRYSANTHEMUM GROWN IN GLASSHOUSES. **T. Furukawa and K. Kishi.** *Div. Biological Sciences, Graduate School of Science and Engineering, Tokyo Metropolitan University, Hachioji, Tokyo, 192-0397, Japan. Email: furukawa-toshiko@tmu.ac.jp*

In Japan, chrysanthemum is cultivated in fields for cut flowers, and in glasshouses to produce special plants, the flowers of which are very large, about 20 cm in diameter, and should have no staining or necrosis on the petals and leaves. They are mainly grown for competition. In the glasshouses, petal blight by *Itersonilia perplexans* Derx has occurred sporadically in many places for decades. The symptoms, light brown spots, suddenly appear on many flowers simultaneously in a glasshouse, although no disease was observed the previous night. If once the disease occurs, it occurs every year, however hard the glasshouse is disinfected. As the occurrence of this disease is not observed on field-grown chrysanthemum, we thought the fungi overwinter in glasshouses. We surveyed the candidates; composts, soils in the pots, timber for the workbench and so on. But the overwintering place of the spores was not identified. Next we studied the plants growing near the glasshouses. *I. perplexans* was isolated only from the flowers belonging to Asteraceae, and it caused the same symptom on chrysanthemum flower within a day after inoculation. Moreover, the disease never occurs when Asteraceae plants do not exist around glasshouses. The infection source of the disease is, therefore, strongly suggested to be some Asteraceae plants that bloom at the same time as or just before chrysanthemum blooms.

24.12 STUDIES ON IDENTIFICATION AND BIOLOGICAL CHARACTERISTICS OF THE CAUSAL ORGANISM OF TREE PEONY ROOT ROT IN ANHUI, CHINA. **M. Guo, Z. Gao, Y. Pan and T. Wang.** *College of Plant Protection, Anhui Agricultural University, Hefei 230036, P.R. China. Email: gzm@ahau.edu.cn*

Tree peony is an ornamental and medical plant in China, and root rot of this plant is an important disease causing serious loss in recent years in Anhui. The causal organism was identified and its biological characteristics were studied. The results showed that fungi could produce both microconidia and macroconidia, which grew on vase-shaped conidiophores. The microconidia were ovoid with no or one septum. The macroconidia were sickle- or spindle-shaped with 3 to 5 septa and the top cell shorter and blunt. The size was about 20.15-37.21×3.98-5.27 µm. Chlamydospores were nearly spherical, borne singly or paired on the mycelium. Based on the above, the pathogen was identified as *Fusarium solani* Sacc., according to Booth's *Fusarium* classification system. The fungi could grow at 5-40°C, and the optimum temperature for the mycelial growth was 25-30°C. The lethal temperature for conidia was 52°C for 10 minutes. The range of pH for mycelial growth was 4.1 to 12.5, with the optimum being 5.5-7.0. The optimum pH for conidium germination was between 4.0 and 7.0. Aerial hyphae and spores were produced best in darkness. The fungus could use many carbohydrate and nitrogen sources, but the most suitable carbon sources were glucose, maltose and mannose. For conidial production, the optimum carbon source and optimum nitrogen source were fructose and urea, respectively. Peptone was the optimum nitrogen source for mycelial growth. The optimal media for mycelial growth were PSA, starch, PDA and eggplant broth medium.

24.13 STUDIES ON THE CONTROL OF *CYLINDROCLADIUM BUXICOLA* USING FUNGICIDES AND HOST RESISTANCE. **B. Henricot, G. Denton and E. Wedgwood.** *Plant Pathology Department, The Royal Horticultural Society, Wisley, Woking, Surrey, GU23 6QB, UK. Email: beatricehenricot@rhs.org.uk*

Cylindrocladium buxicola is a fungal pathogen that causes a severe leaf and twig blight on *Buxus* spp. Laboratory experiments were carried out to study the *in vitro* effect of 10 fungicides on mycelial growth and conidial germination of the fungus. The results showed that carbendazim, prochloraz and kresoxim-methyl completely inhibited mycelial growth. Kresoxim-methyl had the lowest EC50 value and was the most effective at inhibiting conidial germination. Field experiments on *B. sempervirens* 'Suffruticosa' are being carried out to test the protective and curative roles of the most effective fungicides, alone or in combination. Based on the results of low levels of leaf drop, leaf spotting and stem streaking throughout the trial, the preventative treatments Bravo 500 (chlorothalonil) and Opponent (epoxiconazole, kresoxim-methyl and pyraclostrobin) were the most effective. The curative treatments Octave (prochloraz) and Opponent were the best at stopping further disease development. Pathogenicity assays showed that the host range of the fungus was not limited to the genus *Buxus* as *Sarcococca* was also susceptible. None of the 10

box species and cultivars tested were immune to the disease although *B. balearica* as well as *Sarcococca* showed significantly lower levels of infection as measured by the expression of leaf symptoms and the number of conidia produced on host tissue. Microscopic observation showed that disease development was very rapid and aggressive on *B. sempervirens* 'Suffruticosa' where it was able to survive at least 5 years on decomposing fallen leaves.

24.14 *PHYTOPHTHORA NIEDERHAUSERII* IN GREEHOUSE POT PLANTS IN NORWAY. **M.L. Herrero, A.M. de Cock, S. Klemsdal and B. Tope.** *Norwegian Institute for Agricultural and Enviromental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås. Norway. Email: maria.herrero@bioforsk.no*

In 2006, a survey of root diseases in greenhouse pot plants was started in Norway. During this survey an unknown *Phytophthora* sp. was isolated several times. The ITS rDNA regions of these isolates were sequenced and compared to the GenBank database. The sequences of the isolates matched the sequence of *Phythophthora niederhauserii*. The diseased plants came from 5 different greenhouse sites. Isolates were obtained from ivy (*Hedera helix*), begonia hybrids (*Begonia* × *hiemalis* and *Begonia* × *cheimantha*), gloxinia (*Sinningia speciosa*) and kalanchoë (*Kalanchoë blossfeldiana*). Symptoms on begonia, gloxinia and ivy included root and stem necrosis, with the necrosis advancing to the leaves via the petioles. With ivy and gloxinia, wilting of the whole plant was observed. In kalanchoë only discoloration of roots and reduced plant growth was seen. Koch's postulates have been completed for ivy and gloxinia. The pathogen caused aggressive root rot in 22 different ivy cultivars.

24.15 STEM ROT OF *CAMELLIA JAPONICA* CAUSED BY A CORTICIOID FUNGUS. **T. Kubayashi and N. Maekawa.** *Nagasaki Agricultural and Forestry Experiment Station, Isahaya 854-0063, Japan. Email: kubayashi@pref.nagasaki.lg.jp*

We conducted a survey on stem rot of *Camellia japonica* in the Goto Islands, Nagasaki, Japan. A fungus causing the stem rot often induced diebacks and stem breakage of *C. japonica,* and the infected parts of sapwood showed necrosis and discoloration. The fungus associated with the infection was macroscopically characterized as having resupinate, effused and white basidiocarps. These features indicate the fungus is a corticioid species belonging to Basidiomycota, but it has not yet been identified because of immaturity. Mating tests revealed that an isolate from the stem rot were identical with that from the immature basidiocarp tissue. Furthermore, both isolates, when inoculated, induced stem rot on *C. japonica*. We will identify the causal fungus based on microscopic features of its mature basidiocarps and on DNA sequences.

24.16 CONTROL OF TURFGRASS DISEASES WITH PHOSPHONATE FUNGICIDES. **P. Landschoot, P. Cook and M. Schlossberg.** *Dept. Crop and Soil Sciences, 116 ASI Bldg., Pennsylvania State University, University Park, PA, 16802, USA. Email: pjl1@psu.edu*

Phosphonate fungicides are used to control *Pythium* and anthracnose diseases on golf courses in the United States. Limited information is available concerning efficacy differences between phosphite and fosetyl-Al products. This study was designed to determine the influence of different formulations of phosphite and fosetyl-Al, applied at equivalent rates of phosphorous acid, on control of *Pythium* blight (*Pythium aphanidermatum*) and anthracnose basal rot (*Colletotrichum cereale*) on cool-season turfgrasses. Two phosphite fungicides, two fosetyl-Al fungicides, a solution of reagent-grade potassium phosphite, mefanoxam (*Pythium* trials), and thiophanate methyl (anthracnose trials) were compared in field trials in University Park, PA. All fungicide treatments provided good control (>89%) of *Pythium* blight on creeping bentgrass and perennial ryegrass; and no differences in control were detected among phosphonate fungicides at anytime during the test. None of the phosphonate fungicides completely controlled anthracnose; but the Chipco Signature formulation of fosetyl-Al and the solution of reagent grade potassium phosphite treatments had significantly less disease than the untreated control. Chipco Signature performed better than Aliette (the other fosetyl-Al fungicide), indicating that the Chipco Signature formulation may be enhancing disease control. The fact that the reagent grade potassium phosphite showed significantly less disease than the control indicates that phosphite compounds may have some benefit in suppressing anthracnose under certain conditions.

24.17 CONTROL OF FUSARIUM WILT OF CARNATION BY PRE-INOCULATION WITH FUNGAL ANTAGOSNISTS. **C.J. López-Herrera, A.M. Prados-Ligero, D. Ruano-Rosa, M.J. Basallote-Ureba and J.M. Melero-Vara.** *IAS-CSIC, Apdo. 4084, 14080 Córdoba, Spain. Email: lherrera@cica.es*

Nine monosporic isolates of *Trichoderma* spp. were selected by dual and cellophane cultures with 5 monosporic isolates of *Fusarium oxysporum* f.sp. *dianthi* (Fod).They were tested for antagonism on rooted carnation cuttings of the susceptible cv. Annelies, which were inoculated 15 days later with three isolates of Fod. Of 67 monosporic isolates of *F. oxysporum* obtained from soil and carnation plants growing in a naturally infested greenhouse, nine isolates were found as possible antagonists of three Fod isolates in artificial co-inoculation of cuttings of carnation cv. Exotica. In a greenhouse naturally infested by Fod two biological control treatments with the above-mentioned fungal genera were compared with methyl bromide (MB) treatment. One of those consisted of a mixture of wheat seed colonised by the 4 best isolates of *Trichoderma*, added to the soil at planting (10 g.plant^{-1}) and at 8 and 3 months thereafter until 19 months after planting. For the second treatment, a mixed conidial suspension of two selected isolates of non-pathogenic *Fusarium* (NPF) was applied as a root dip of carnation cuttings of the two carnation cvs. studied (Master and Fancy Schubert). Regardless of the cultivar used, disease onset was ca. 200 days after planting for MB whereas it was delayed 100 days and the areas under disease progress curves in the NPF and *Trichoderma* treatments were reduced to 1/3 and 1/5 that of the MB treatment.

24.18 NUCLEOTIDE SEQUENCES AND DISTRIBUTION OF *CHRYSANTHEMUM STUNT VIROID* IN JAPAN. **Y. Matsushita.** *Research Team for Growth and Flowering, National Institute of Floricultural Science, Tsukuba, Japan. Email: yousuken@affrc.go.jp*

Stunting caused by *Chrysanthemum stunt viroid* (CSVd) is one of the most damaging diseases of cultivated chrysanthemum (*Chrysanthemum morifolium* Ramat. = *Dendranthema grandiflorum* Kitam.), the most important cut flower in Japan. We assayed for CSVd in cultivated chrysanthemum collected from 10 prefectures in Japan and 8 wild species (*Chrysanthemum* spp.) cultivat-

ed at the National Institute of Floricultural Science (NIFS) and determined complete nucleotide sequences of CSVd isolates infecting the plants. CSVd was detected in 80 of 89 samples of cultivated chrysanthemum, and samples from all prefectures were infected. Since all 8 wild species had CSVd in RT-PCR results, they were recognized as hosts of CSVd, even though no stunt symptoms were observed. Five sequence variants were distinguished among the 21 isolates based on differences in the nucleotide sequences. Mutations were common in the P (pathogenicity) domain. Variant 5 from *C. morifolium*, and variant 4 from *C. yoshinaganthum* had different nucleotide sequences from those reported previously. Variant 1 was most frequently detected from samples in 6 prefectures and is assumed to be the predominant CSVd variant distributed in Japan.

24.19 EPIDEMIOLOGY OF *PHYTOPHTHORA RAMORUM* ON *RHODODENDRON*. V. McDonald and N.J. Grünwald. *Horticultural Crops Research Laboratory, USDA ARS, Corvallis, OR, USA. Email: grunwaln@science.oregonstate.edu*

Phytophthora ramorum is the causal agent of sudden oak death in forests and ramorum shoot dieback and leaf blight on ornamental hosts. The known population of *P. ramorum* worldwide consists of three distinct clonal lineages. The objective of this study was to determine if there exist clear differences in pathogenic fitness in the three clonal lineages of *P. ramorum* on *Rhododendron*. We evaluated differences in pathogenic fitness using three isolates randomly selected from within each clonal lineage. Fitness of each isolate on each host was determined by measuring the fitness components of lesion area, sporulation capacity, incubation period and the area under the lesion expansion curve. Effect of cultivar was evaluated using a susceptible cultivar and a moderately resistant cultivar of *Rhododendron*. In all three trials there was a significant cultivar effect for most fitness components. In contrast, there was no effect of clonal lineage on most fitness components. In general, we observed low levels of sporulation (< 300 sporangia per cm^2 lesion) and short incubation periods (1.8-2.7 days for 50% infection) relative to other foliar *Phytophthora* diseases.

24.20 NEW *FUSARIUM* WILTS OF ORNAMENTAL CROPS IN ITALY. A. Minuto, M.L. Gullino and A. Garibaldi. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: angelo.garibaldi@unito.it*

Through commerce, new diseases may suddenly appear and be rapidly spread by infected propagative material. During the last 10 years several *Fusarium* wilts have been observed in Liguria, northern Italy. *Fusarium oxysporum* was detected in summer 1997 on potted plants of Paris daisy (*Argyranthemum frutescens*, cv. Camilla). In summer 1999, a wilt caused by *F. oxysporum* on potted *Hebe* sp. (cvs Paula, Linda and Heidi) was observed. In summer 2002, gerbera plants (*Gerbera jamesonii* cv. Kaiki) with wilt symptoms were reported in a soilless crop system at Albenga (Savona). A similar wilt in soil crops was also observed during the summer of 2002 and 2003 in Imperia province, on cvs Red Bull, Anedin, Gud finger, Basic and Cirill. The same wilt was observed in soilless gerbera (cv. Jaska) in 2004 in Spain, and in 2007 in gerbera plants, either hydroponic or soil-grown, in Brazil (cvs Basic, Xena and Olimpia). During autumn 2002, wilted plants of African daisy (*Osteospermum* sp.), grown in Albenga, were found infected by *F. oxysporum*. *Fusarium* strains isolate in Italy from Paris daisy, African daisy and gerbera belong to forma specialis *chrysanthemi*, confirming the risks of intensive cultivation and propagation of different Asteraceae (Compositae) plants in the same greenhouses. Finally, during the spring of 2004, a new *Fusarium* wilt was observed on bitterroot (*Lewisia cotyledon*), in Piedmont.

24.21 RESISTANCE OF GERBERA AND AFRICAN DAISY TO *FUSARIUM OXYSPORUM* f.sp. *CHRYSANTHEMI*. A. Minuto, P. Pensa, D. Bertetti, M.L. Gullino and A. Garibaldi. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: domenico.bertetti@unito.it*

Gerbera grown for cut flowers, was reported as host of *Fusarium oxysporum* in 2002 in Italy and in 2007 in Spain. The *Fusarium* wilt agent of gerbera belongs to the *forma specialis chrysanthemi*. *Argyranthemum frutescens* (Paris daisy) and *Osteospermum* sp. (African daisy) are also hosts of *F. oxysporum* f.sp. *chrysanthemi*. Since cultivars appear to differ in susceptibility, experimental trials were carried out to evaluate the resistance/susceptibility of available cultivars of gerbera to the fungus. The pathogenicity of two fungal isolates obtained from infected gerbera (*Gerbera jamesonii*) and chrysanthemum (*Chrysanthemum morifolium*) was tested on 57, 55 and 53 gerbera cultivars in 2004, 2005 and 2006 respectively. The results showed that in these years 47, 65 and 75% of the cultivars were highly resistant to the chrysanthemum isolate of *F. chrysanthemi* and 48, 56 and 72% were highly resistant to the gerbera isolate. Similar trials were carried out during the period 2004-2005 on 20 cultivars of African daisy: in both years around 50% of cultivars tested were resistant to each of the two *F. chrysanthemi* isolates, while only 30% were resistant to *F. chrysanthemi* obtained from *Osteospermum* sp. Because some differences in cultivar susceptibility were observed, the presence of physiological races among the isolates of *F. chrysanthemi* is suggested.

24.22 REDUCED SENSITIVITY TO FUNGICIDES OF *PYTHIUM* SPP. PATHOGENIC ON TURFGRASS IN ITALY. M. Mocioni, P. Titone, J. Liu, A. Garibaldi and M.L. Gullino. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: mmocion@tin.it*

Pythium blight, incited by different species of *Pythium*, is one of the most severe diseases of turfgrass in Italy. In high-maintenance stands such as golf courses, control strategies rely on use of chemicals on a preventive basis, in order to avoid damage to large areas of turf, due to the remarkable speed of disease progress. In recent years, fungicides have failed to work effectively on some Italian golf courses. The sensitivity of 21 fungal isolates obtained in 9 different areas in Northern and Central Italy was tested against metalaxyl, propamocarb, fosetyl Al and azoxystrobin. Minimal inhibitory concentration (MIC) and concentration inhibiting 50% of growth (EC_{50}) were determined for each isolate. Most of the isolates were resistant to all the fungicides tested, with MIC values > 300 µg/ml. Some resistant isolates showed a high degree of virulence when inoculated on potted *Agrostis stolonifera* L. The *Pythium* species, determined by molecular analysis, were classified as *P. aphanidermatum* (Edson) Fitsp., *P. myriotylum* Drechs. and *P. torulosum* Coker & F. Patterson. *P. aphanidermatum* showed an optimum growing temperature of 30°C *in vitro*, while the other isolates grew rapidly at 20-25°C. This is the first report of resistance to fungicides in *Pythium* spp. on turfgrass in Italy.

24.23 EFFECTS OF *CHELIDONIUM MAJUS* PLANT EXTRACT ON FUNGAL MORPHOLOGY OF *BOTRYTIS CINEREA*. **M. Parvu, O. Rosca-Casian, C. Craciun, L. Barbu-Tudoran, L. Vlase and M. Tamas.** *42 Republicii Street, 400015 Cluj-Napoca, Romania. Email: mparvucluj@yahoo.com*

Chelidonium majus plant extract was obtained from the above ground plant organs, and was analysed for chelidonine and berberine content. It was tested, in different concentrations, for its effects on germination and growth of conidia and sclerotia of *Botrytis cinerea* isolated from rose flowers and grown on nutritive medium. The effects of the extract on *B. cinerea* morphology were studied using scanning and transmission electron microscopy. At the minimum fungicidal concentration (250 µg/ml), the extract induced clear ultrastructural changes of the cell wall, plasma membrane, cytoplasmic content and organelles, and caused loss of conidial and sclerotial germinability. The cytological effects of the extract on *B. cinerea* conidial and sclerotial morphology may explain its potent anti-*Botrytis* properties.

24.24 BLIGHT DISEASE OF *IXORA* IN KONKAN, MAHARASHTRA (INDIA). **S.A. Patil, P.A. Fugro and V.B. Mehta.** *Dr.B.S. Konkan Krishi Vidyapeeth, Dapoli 415 712, M.S., India. Email: sfugro@yahoo.co.in*

For the first time, *Ixora signapurensis* ornamental plants commonly grown in Konkan (India) were found severely infected with blight, with heavy defoliation of leaves and blossom drop. A pathogenic fungus was isolated and pathogenicity was confirmed on test plants. Fungal colonies on potato dextrose agar medium (PDA) were cottony, white, floccose and wrinkled with profuse growth. Sporulation was observed late. The maximum fungal growth was obtained on Richard's medium followed by Coon's agar and PDA. Mycelium was hyaline, septate, slender, branched (2.3-3.0 µm in width). Conidia produced from whorls of phialides were cylindrical, bullet shaped and unicelled with light coloured black wall and measuring 6.2×2.2 µm. Sporodochia were sessile, found in large masses, olivaceous green to dark in colour. Conidial germination occurred at room temperature (27±1 °C). Citric acid and urea were the best sources of carbon and nitrogen, respectively for growth and sporulation at pH 6.0 to 7.5. The pathogenic fungus was identified as *Myrothecium roridum* Tode ex Fries. This appears to be the first record of *Myrothecium roridum* causing blight of *Ixora*. A host-range study indicated that *Mussaendra*, *Gardenia*, *Diffenachia*, *Aglonema*, *Acalypa*, *Peperomia*, *Pilea* and egg plant are hosts of *Myrothecium roridum* in this region. *In vitro* evaluation of bioagents showed that *Trichoderma harzianum* was most effective in suppressing the growth of *Myrothecium roridum*.

24.25 CHEMICAL ALTERNATIVES TO METHYL BROMIDE (MB) FOR CONTROL OF *FUSARIUM* WILT AND *MELOIDOGYNE* ROOT KNOT DISEASES OF CARNATION. **A.M. Prados-Ligero, M. Talavera, M.D. Vela, M.J. Basallote-Ureba, C.J. López-Herrera and J.M. Melero-Vara.** *IAS-CSIC, Apdo. 4084, 14080 Córdoba, Spain. Email: cs9mevaj@uco.es*

Fusarium oxysporum f. sp. *dianthi* (Fod) and *Meloidogyne* spp. are the most important pathogens of carnation crops in southern Spain. During 2005-2008 two experiments were carried out in greenhouses infested by both or the former pathogen, respectively planted with cvs. Master and Picaro, or Master. The efficacy of soil application of different chemicals in the control of these diseases was evaluated. Because the higher infestation level by *Meloidogyne*, disease incidence (DI) was higher in the susceptible 'Picaro' than in 'Master' (susceptible to Fod). After 19 months, the treatments 1,3-D+CP, DMDS, DMDS+CP reached DI<15% in the latter, being respectively 50, 80, and 90% in 'Picaro'. Consequently, cumulative carnation yields over the untreated control were respectively increased by 99, 83 and 83% in 'Master', whereas the increases were 278, 323 and 213% in 'Picaro'. In the greenhouse infested only by Fod and cropped to 'Master', DI was ca. 100% in the untreated control by the end of summer 2007 whereas it was less than 2% in plots treated with MB or 1,3-D+CP, with yields 2.4 times those of untreated control; and in the DMDS+CP and Na azide treated plots, the DIs were respectively 12 and 16%, and the yields 2.3 and 2.1 as compared to untreated controls.

24.26 INFLUENCE OF GREENHOUSE ENVIRONMENT ON ATMOSPHERIC *ERYSIPHE CICHORACEARUM* CONIDIA AND DISEASE ON *GERBERA JAMESONII*. **L.E. Sconyers and M.K. Hausbeck.** *Department of Plant Pathology, Michigan State University, East Lansing, MI 48824-1311, USA. Email: hausbec1@msu.edu*

Gerbera daisies (*Gerbera jamesonii*) are grown in the United States as cut flowers in outdoor nurseries in the southern states or as potted plants in greenhouses in the north. Gerbera daisies are typically grown at high densities, creating environmental conditions that are optimal for disease development. Signs of powdery mildew include white, talcum-like colonies on leaf, stem, or flower surfaces. Under optimal environmental conditions, powdery mildew causes leaf blighting and plant death. Atmospheric concentrations of *Erysiphe cichoracearum* conidia and environmental conditions were monitored in two greenhouses containing powdery-mildew infected gerbera. Concentrations of airborne conidia were monitored at both locations using 7-day volumetric spore samplers. Conidia counts were converted to number of conidia/m^3/hour. Temperatures in both greenhouses were maintained at 20 to 22ºC and artificial lighting provided a 14-hour photoperiod. Powdery mildew incidence (percentage of all plants infected) and severity (percentage of foliage infected) were assessed every 14 days in both greenhouses. Atmospheric conidial concentrations were greatest in the first greenhouse during 0800 to 1600 hours, with a peak (150 conidia/m^3/hour) occurring at 1500 hours. Peak conidial concentrations in the second greenhouse occurred at 0800 (135 conidia/m^3/hour) and 1500 (185 conidia/m^3/hour) hours. Large conidial release events were often associated with fluctuations in relative humidity and watering activity. Conidial releases were significantly reduced when temperatures exceeded 30ºC. Information from this study will be used for developing a disease management strategy for gerbera powdery mildew and timing fungicide applications.

24.27 RUST DISEASES ON ORNAMENTAL PLANTS IN THAILAND. **S. Seemadua, B. Udomsak, Y. Chiemchaisri and P. Athipunyakom.** *Plant Protection Research and Development Office, Department of Agriculture, Ministry of Agriculture & Cooperatives, Chatuchak, Bangkok, 10900, Thailand. Email: seemadua@yahoo.com*

A rust survey was conducted on ornamental plants from various locations in Thailand during October 2004 to September 2006. Morphological characteristics of various spore types were observed under compound and scanning electron microscopes. Rust fungi were encountered on *Plumeria acuminata* Ait., *Canna*

indica L., *Chrysanthemum morifolium* Ramat., *Justicia fragilis* Wall., *Justicia* sp. and *Dianthus caryophyllus* L.. Three genera and four species of rust fungi were identified as *Coleosporium plumeriae, Puccinia thaliae, P. horiana* P. Henn., *P. thwaitesii* and *Uromyces dianthi* (Pers.) Niessl. Dried specimens were maintained in the Thai Plant Pathology Herbarium, Department of Agriculture, Bangkok, Thailand.

24.28 EFFECT OF DAYLENGTH ON FORMATION, RELEASE, AND GERMINATION OF POWDERY MILDEW CONIDIA IN ROSES AND SEVERITY OF THE DISEASE. A. Suthaparan, M.L. Herrero, R.I. Pettersen, S. Torre, A. Stensvand, D.M. Gadoury and H R. Gislerød. *Dept of Plant and Environmental Sciences, University of Life Sciences, P.O. Box 5003, 1432 Ås, Norway. Email: aruppillai.suthaparan@umb.no*

We studied the effect of daylength on formation, release, and germination of conidia of rose powdery mildew (*Podosphaera pannosa* var. *rosae*) and severity of the disease. Whole plants or detached leaves were exposed to daylengths of 0, 12, 18, or 24 h. Experiments took place in growth chambers with artificial light. Light intensity, light quality and RH were kept constant in each experiment. Prior to each experiment, plants or detached leaves were given uniform growing conditions and inoculum levels. The highest spore formation and germination were obtained at 18 h day length. Compared to 18 h day length (mean of 3 experiments), spore formation on detached leaves in Petri dishes was reduced to 61.8% under continuous light and germination was reduced to 72.3%. If whole plants were exposed to the four different light regimes over 7 days in wind tunnels and conidia were trapped continuously, 24, 12, and 0 h light regimes reduced the cumulative spore numbers to 22.1, 18.3, and 0.4% (mean of 3 experiments), respectively, compared to 18 h. Continuous light also strongly reduced disease severity compared to 18 h day length. Powdery mildew is the most severe fungal disease in greenhouse-grown roses worldwide, and our results suggest that increased day lengths may suppress the disease significantly compared to the 16 to 18 h days normally used in commercial production and thus ultimately reduce the need for fungicide applications.

24.29 *GAEUMANNOMYCES GRAMINIS* VAR. *AVENAE* ON TURFGRASS IN ITALY: IDENTIFICATION AND MANAGEMENT. P. Titone, M. Mocioni, J. Liu and M.L. Gullino. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: patrizia.titone@unito.it*

In spring 2005, several patches were observed on *Agrostis stolonifera* greens on a golf course in northern Italy. Patches ranged from 3 to more than 50 cm in diameter and showed plants having completely dead leaves and roots covered with dark, ectotrophic mycelium., Discoloration and brown vascular tissues were observed on affected roots. After placing sterilised root sections on potato dextrose agar (PDA) containing 25 ppm streptomycin sulphate, several colonies were isolated, showing grey aerial mycelium, hyphae curling towards the centre of colony and a maximum daily growth rate of 7–8 mm at 25°C. Pathogenicity trials on 5 week-old *Agrostis stolonifera* showed that 19 of 20 strains were virulent. Plants were removed and washed 10 weeks after inoculation. Plants inoculated with virulent strains grew shorter roots and leaves, compared to the controls; roots were covered with dark, ectotrophic mycelium. Sections of infected roots, when disinfested and placed on PDA-streptomycin sulphate, produced the same colony morphology and characteristics as those previously isolated. The fungus was identified as *G. graminis* var. *avenae*. In September 2005, a trial was carried out to manage the disease by using manganese sulphate (2.25 and 4.5 kg/ha), potassium sulphate (60 kg/ha), *Trichoderma* sp. (1×10^7 CFU/ml) and a compost mixed with sand (70/30 v/v). Treatments were carried out twice in spring 2006, once in autumn 2006 and twice in spring 2007. Manganese sulphate, potassium sulphate and *Trichoderma* sp. treatments were effective in reducing disease incidence.

24.30 STUDY ON HOST RANGE OF SOME *COLLETOTRICHUM* SPECIES ON SCROPHULARIACEOUS ORNAMENTALS. K. Tomioka, J. Nishikawa, J. Moriwaki, Y. Hirooka, M. Endo, T. Nagai, H. Sawada, T. Aoki and T. Sato. *Genebank, National Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan. Email: tomioka@affrc.go.jp*

Colletotrichum (teleomorph *Glomerella*) is known to be virulent to various plants, and causes serious fungal diseases generally called "anthracnose". We previously reported the following data on pathogenicity of *C. destructivum*, *C. gloeosporioides*, *C. acutatum*, *C. dematium* and *C. circinans* to scrophulariaceous ornamentals; 1) They were virulent to snapdragon; 2) There was no virulence of the former three *Colletotrichum* species to angelonia, gloxinia penstemon, torenia and alpine speedwell; 3) foxglove and nemesia were attacked by *C. destructivum* but not *C. acutatum* or *C. gloeosporioides*. To better understand the host range on scrophulariaceous ornamentals, we checked pathogenicity of the five *Colletotrichum* species to cloven-lip and slipperwort as well as that of *C. dematium* and *C. circinans* to angelonia, gloxinia, penstemon, torenia, alpine speedwell, foxglove and nemesia. The five *Colletotrichum* species were also found to be virulent to cloven-lip and slipperwort. All isolates used caused only petal blight on cloven-lip, the same as that on snapdragon. All isolates of *C. gloeosporioides*, *C. acutatum* and *C. circinans* caused only petal blight on slipperwort. The isolates of *C. dematium* caused blight of the whole plant but only petal blight on slipperwort. Some isolates of *C. destructivum* were avirulent to slipperwort, and others were virulent, causing whole-plant blight of slipperwort. The results obtained from inoculation to slipperwort indicate differentiation of pathogenicity in *C. dematium* and *C. destructivum*. In addition, no virulence was detected of *C. dematium* and *C. circinans* to angelonia, gloxinia, penstemon, torenia, alpine speedwell, foxglove and nemesia.

24.31 ANTHRACNOSE OF *POLYGONATUM FALCATUM*. K. Tomioka, J. Moriwaki, Y. Hirooka, M. Endo, T. Nagai, H. Sawada, T. Aoki and T. Sato. *Genebank, National Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan. Email: tomioka@affrc.go.jp*

Polygonatum falcatum Asa Gray, a monocot perennial in the family Liliaceae, is known as an ornamental in Japan. A severe fungal disease causing spotting, blight and leaf fall was found on potted plants grown in the open in Kagawa prefecture, Japan, in May 2001. Chlorotic to brown spots less than 1 mm in diameter initially appeared on leaves. The lesions gradually extended along leaf veins to become fusiform to streaky, and often coalesced. The foliar lesions resulted in early blight and defoliation. Plants with numerous lesions withered rapidly. A mitosporic fungus isolated repeatedly from the diseased plants was identified as *Colletotrichum dematium* (Persoon: Fries) Grove based on morphological and cultural characters. Sequencing of the rDNA-ITS1 re-

gions of isolates supported the identification. When leaves of healthy plants were inoculated with the isolates, the natural symptoms were reproduced while controls had no symptoms. The fungus could consistently be re-isolated from diseased leaves that had been inoculated, but not from healthy controls, thereby demonstrating that the isolates examined were pathogenic to *P. falcatum*. This disease has not previously been reported, and *P. falcatum* was judged to be a new host for the fungus. We therefore named it "anthracnose of *P. falcatum*" as a new disease, because the genus of the pathogen was *Colletotrichum*. One of the isolates and its DNA sequence obtained in this study have been deposited in the Genebank and the DDBJ under accession no. MAFF239500 and AB334523, respectively.

24.32* *FUSARIUM FOETENS*, A NEW AND SEVERE PATHOGEN ON *BEGONIA* SPP. IN NORWAY. **B. Toppe, M.L. Herrero and M.B. Brurberg.** *Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division. Høgskoleveien 7 N -1432 Ås Norway. Email: brita.toppe@bioforsk.no*

In 2002, *Fusarium foetens* was detected for the first time in Norway in *Begonia* × *hiemalis* Fotsch (Begonia Elatior hybrids). Later, in 2006, severe outbreaks appeared in *B.* × *hiemalis*, in the new host plant *Begonia* × *cheimantha* Everett (Lorraine begonia hybrids) and in the new begonia cultivar Betulia. Mortality of 50% or more was observed for all three hosts at different greenhouse sites. In research greenhouses, *B.* × *hiemalis*, *Begonia* × *cheimantha*, and 'Betulia' showed symptoms and finally wilting, 3 to 6 weeks after artificial inoculation. Symptoms of the disease were pale green foliage and dark water-soaked discoloration of stems and vascular tissue. At an advanced stage of the disease, stems, petioles and leaves collapsed and became covered with the fungal sporodochia. The presence of *F. foetens* was confirmed using real-time PCR based on the mitochondrial small subunit (mtSSU) rDNA on pure isolates or infected plant material. So far, *F. foetens* has not been detected in symptom-free planting material of begonia in Norway, but another species, *F. begoniae*, was found twice from healthy-looking cuttings of *B.* × *hiemalis* during a preliminary survey. In 2007, the disease was recommended for regulation as a quarantine pest in Europe (EPPO A2-list). The Norwegian Food Safety Authority handles *F. foetens* as a quarantine pest in nurseries that propagate cuttings for sale, but thus far it has not been detected in such production in Norway.

24.33 *PHALAENOPSIS* ORCHIDS SHOWING CHLOROTIC RINGS, A NEW DISEASE CAUSED BY *CARNATION MOTTLE VIRUS*. **Y.X. Zheng, C.C. Chen and F.J. Jan.** *Department of Plant Pathology, National Chung Hsing University, Taichung 402, ROC. Email: fjjan@nchu.edu.tw*

A virus culture, 92-orchid-1, isolated from a *Phalaenopsis* orchid bearing chlorotic ring symptoms was established in *Chenopodium quinoa*, and characterized serologically and biologically. The virus reacted with *Carnation mottle virus* (CarMV) antiserum in ELISA, and isometric particles measuring about 32 nm were observed. The particles of 92-orchid-1 gave a single protein band of 40 kDa in SDS-PAGE. The coat protein (CP) of 92-orchid-1 and CarMV-TW isolated from carnation in Taiwan reacted strongly with the antiserum against 92-orchid-1. *Nicotiana benthamiana* and *N. rustica* displayed different host reactions to 92-orchid-1 and CarMV-TW and are good indicator plants for the two isolates. To determine the taxonomic relationships of the virus, the conserved region of the polymerase gene (ORF 1RT) and the complete CP gene were cloned and sequenced. The sequence of the conserved region shared 97.5%, 65.1%, 60.0% and 62.2% nucleotide identities and 98.6%, 69.7%, 65.5% and 64.8% amino acid identities with those of CarMV, *Pelargonium flower break virus* (PFBV), *Saguaro cactus virus* (SgCV) and Angelonia flower break virus (AnFBV), respectively, indicating that 92-orchid-1 is a carmovirus related to CarMV. Sequence analyses of the CP gene amplified using primers specific for CarMV showed that 92-orchid-1 is an isolate of CarMV, and 92-orchid-1 is therefore designated as CarMV-Ph. To our knowledge, this is the first report of CarMV infecting *Phalaenopsis* orchids.

DISEASES OF SOILLESS CROPS

36.1* EFFECTIVENESS OF SOLUBLE SILICON AND BIOLOGICAL CONTROL AGENTS FOR MANAGEMENT OF *PYTHIUM* ROOT ROT OF HYDROPONICALLY-GROWN KALE (*BRASSICA OLERACEA* L. VAR. *ACEPHALA* DC.). **T. Jaenaksorn and H. Rachniyom.** *Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand. Email: kjtanimn@kmitl.ac.th*

The effect of 4 kinds of biological control agent (BCA) and 3 concentrations of 3 kinds of soluble silicon were tested on root rot disease severity and yield of kale (*Brassica oleracea* L. var. *acephala* DC.) grown by deep flow technique in the presence of *Pythium aphanidermatum*. Survival of BCAs and the pathogen in the system was also determined. Of the 4 BCAs tested, three were formulations (spore suspension, starter and wettable powder) of bioproducts of *Trichoderma harzianum* while the fourth was an indigenous *Trichoderma*. Sodium silicate ($Na_2Si_3O_7$), potassium silicate ($K_2Si_3O_7$) and Phyton (SiO_2) were the tested as soluble silicon. The result showed that all BCAs gave different levels of disease control and the spore suspension formulation, either from bioproduct or indigenous, was more effective than the other formulations. Silicon additive at the rate of 250 ppm significantly reduced disease severity. Moreover, the presence of BCA added as spore suspension and any tested soluble silicon at 250 ppm significantly reduced root rot severity compared to the other treatments. Similar results were obtained for crop yield. That is, significantly highest fresh kale and root weight were observed in the treatments including BCA spore suspension and 250 ppm soluble silicon. We conclude that using soluble silicon with BCAs may be a useful component of root rot disease management in hydroponic cultures. We also discuss the advantages and limitations of these measures.

36.2 USE OF POTASSIUM SILICATE TO CONTROL POWDERY MILDEW IN SOILLESS TOMATOES. **A. Minuto, P. Pensa, M.L. Gullino and A. Garibaldi.** *AGROINNOVA, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. Email: marialodovica.gullino@unito.it*

Worldwide, powdery mildew is a serious disease in tomato and can be caused by *Leveillula taurica* or *Oidium neolycopersici*. Both pathogens are reported in Europe and in Italy, but *O. neolycopersici* causes severe outbreaks particularly on protected crops. Among the non-conventional strategies effective to limit powdery mildew epidemics, application of potassium silicate, through the nutrient solution, is a promising technique on some crops. During 2005, 2006 and 2007 we investigated the effects against *O. neolycopersici* of potassium silicate (K_2SiO_3) application via nutrient

solution (100 ppm) delivered by sub-irrigation or drip irrigation. All trials were carried out under high disease pressure (artificial inoculation). The results confirmed the beneficial effects of potassium silicate: disease incidence and severity were significantly reduced in silicate-treated plots (10-40% of infected leaves in treated plants and 50-90% in control plants; 10-15% of leaf surface infected in treated plants and 40-50% in control plants). Previous results showed the positive effects of silicates applied by nutrient solution in controlling cucurbit powdery mildews; our data confirmed the effects against tomato powdery mildew. Silicates reduce the incidence of powdery mildew probably by inducing higher mechanical resistance in plant tissues, and also higher production of phenolics. Recently it has been shown that Si treatment had no effect on the metabolism of unstressed plants, suggesting a non-essential role for the element. Nevertheless Si may stimulate a more efficient response to pathogen stress.

36.3 MICRORGANISMS ISOLATED FROM USED SOILLESS CROP SUBSTRATES CAN CONTROL *FUSARIUM OXYSPORUM* F. SP. *RADICIS-LYCOPERSICI* ON TOMATO. A. Minuto, F. Clematis, M.L. Gullino and A. Garibaldi. *AGROINNOVA, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. Email: angelo garibaldi @unito.it*

Soilless tomatoes are seriously damaged by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL). Since natural substrate suppressiveness of recycled media (rockwool, perlite and perlite-peat mix) is known to be effective in reducing FORL infections, several potential biocontrol agents were isolated from suppressive media and tested for control of tomato crown and rot root. One hundred and ten single spore/cell isolates belonging to fluorescent bacteria, *Trichoderma* spp. and *Fusarium* spp. were tested adopting a technique already used to verify the suppressiveness of soilless substrate samples. Five fluorescent bacterial isolates (FC6B, FC7B, FC8B, FC9B and FC24B), 5 strains of *Fusarium* (FC11F, FC14F, FC20F, FC28F, FC29F) and 1 *Trichoderma* (FC37F) showed significant control of FORL incidence on tomato seedlings. All the bacteria were effective when applied simultaneously with the pathogen, but the strain of *Trichoderma* was significantly effective only when applied before pathogen inoculation. On the contrary, *Fusarium* strains appeared effective irrespective of time of application. All trials were repeated at least twice and a commercial biocontrol agent (Mycopstop) served as control. Our data confirm the importance of the resident microflora in the suppression of FORL in recycled substrates for soilless tomatoes. Further trials are needed to verify the efficacy of selected biocontrol agents under semi-commercial field conditions.

36.4 EFFECT OF ELECTRIC CONDUCTIVITY OF NUTRIENT SOLUTION ON SEVERITY OF *BOTRYTIS CINEREA* ON SOILLESS TOMATOES. A. Minuto, M.L. Gullino and A. Garibaldi. *AGROINNOVA, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. Email: angelo.garibaldi@unito.it*

Soilless crops may enable the adoption of innovative control methods aimed at changing host susceptibility by modifying the availability of water and nutrients. Varying the electric conductivity (EC) of nutrient solution (NS) may theoretically vary the susceptibility of the host to *B. cinerea*, by affecting water availability in the soilless system. During 2005, 2006 and 2007 we investigated the effects of NS concentration on *B. cinerea* susceptibility in soilless tomatoes, simulating a drip and sub-irrigation system for NS delivery. Three EC levels (1.8 mS/cm; 3.0 mS/cm; 4.7 mS/cm) were obtained by adding 0.87 and 1.74 g/l of NaCl to the NS commonly used for soilless tomatoes. *B. cinerea* was periodically inoculated by spraying with a water suspension of conidia (1×10^4 CFU/ml). Before inoculation, wounds were made by removing secondary buds and basal leaves. We then counted the number of infected wounds and measured the size of infected areas (length of stem or petiole infected by *B. cinerea*). The results confirmed the positive effects of higher EC in reducing susceptibility to *B. cinerea* infection. The percentage of infected wounds dropped from 40.0, 10.0 and 9.0% to 12.0, 5.0 and 1.0 % respectively in tomatoes irrigated with standard or NaCl-treated NS during the 1st, 2nd and 3rd trial. In these trials the of length of pruned stem and petioles was 5.3, 5.7 and 4.4 cm and 2.6, 3.7 and 0.7 cm respectively in tomatoes irrigated with standard or NaCl-treated NS.

36.5 NON-CHEMICAL DISEASE CONTROL OF *FUSARIUM* CROWN ROOT ROT IN SOILLESS TOMATO CROPS. A. Minuto, F. Clematis, M.L. Gullino and A. Garibaldi. *AGROINNOVA, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. Email: marialodovica.gullino@unito.it*

Soilless tomatoes are seriously damaged by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL). The aim of this study was to evaluate the natural substrate suppressiveness of used media (rockwool, perlite and perlite-peat mix) against FORL infections. New and used substrates, sampled from closed soilless systems, were either disinfected or not, either artificially inoculated with *F. oxysporum* f. sp. *radicis-lycopersici* or not and, finally, sown with tomato seeds cv Cuore di Bue. The effects of disinfected/non-disinfected and used/new substrates on FORL incidence were assessed by evaluating the symptoms of crown-rot on the root-shoot transition zone of the seedlings. Non-disinfected and inoculated used rockwool, perlite and perlite-peat mix significantly reduced FORL incidence when compared with non-disinfected and inoculated new substrate. Disinfected and inoculated used rockwool and perlite-peat mix did not suppress FORL, similarly to new and inoculated substrates. These results are in accordance with other research that, on the cucumber-*Pythium* host-pathogen complex in a closed rockwool soilless system, demonstrated the key role of resident microflora in suppressing the root rot disease. Recycled perlite also suppressed FORL incidence and severity when sterilized before inoculation with the pathogen. In reused perlite the mechanism of suppressiveness seems to be mediated not only by the resident microflora, as observed on recycled rockwool substrate, but also by chemical characteristics. In conclusion, tomato plants grown in used rockwool, perlite and, perlite-peat mix after at least 6 months of crop cycle, may exploit natural suppressiveness to effectively control FORL infections.

36.6 PYTHIUM ROOT ROT IN SOILLESS GROWN LETTUCE. EFFECTIVENESS OF CHLORINATION AND EFFECTS ON LETTUCE QUALITY AND THE NUTRIENT SOLUTION. H.E. Palmucci, Z. Premuzic, M. Nakama, S. Wolcan, J. Tamborenea and N. Donofrio. *Facultad de Agronomía Universidad de Buenos Aires (FAUBA) Av. San Martín 4453, Capital Federal (1416), Argentina. Email: palmucci@agro.uba.ar*

Chlorination constitutes a control alternative, within the chemical disinfection methods for waterborne pathogens. Previous experiment was performed applying sodium hypochlorite (0.55, 5.5 and 11 ppm chlorine) to lettuce (*Lactuca sativa*) 'Mantecosa' grown in a closed, soilless system to determine the optimum dose without affecting production and quality or causing phytotoxicity.

The production (fresh weight and dry matter) and some commercial and nutritional quality factors (phytotoxicity, vitamin C, nitrates) were analyzed and related to the contents of sodium and chlorides in plants and to the chemical changes: pH, electrical conductivity (EC) and chlorides, in the nutrient solution. Another trial was developed using 0.55 ppm with the aim to study the effectiveness of control of three different isolates of *Pythium* spp. The treatments were: T0: inoculated plants + nutrient solution; T1: nutrient solution with chlorine; T2: inoculated plants + nutrient solution with chlorine. The inoculum consisted of adding 9 g of colonized potato dextrose agar (PDA) of each *Pythium* isolate per pot. The effects caused by different chlorine doses were evaluated as aerial and root fresh weights and percentage of dry matter. The chemical changes in the nutrient solution: EC, pH and chloride concentration were measured weekly. All experimental designs were completely randomized. ANOVA was performed on the data and significant differences were further examined via post-hoc comparison test LSD ($p \leq 0.05$) using SPSS software. We concluded that the addition of 0.55 ppm chlorine was effective to control *Pythium* spp in lettuce grown in a closed soilless system.

36.7* SPOILAGE AND ROT OF MUNG BEAN SPROUTS BY SOME FUNGI. **T. Sato, M. Aoki, T. Aoki, K. Tomioka, H. Sawada, Y. Hirooka, M. Endo and T. Nagai.** *Genebank, National Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan. Email: s1043@affrc.go.jp*

Mung bean (*Vigna radiata*) sprouts are a common raw food in eastern Asia including Japan. Since they are produced in darkness under warm and humid conditions, the white cotyledons and hypocotyls are easily attacked not only by plant pathogens but also saprophytic microorganisms during production. *Erwinia carotovora, Pseudomonas fluorescens, Fusarium solani, Macrophomina phaseolina, Rhizoctonia solani* and *Colletotrichum* sp. have already been reported to spoil sprouts (Aoki *et al.*, 1986). In the present study, we also found other fungi spoiling the sprouts. *Alternaria alternata, Apiospora montagnei, Fusarium oxysporum, Geotrichum candidum, Absidia corymbifera, Rhizopus oryzae, Cercospora kikuchii, Colletotrichum gloeosporioides, Colletotrichum truncatum, Fusarium equiseti, Gibberella fujikuroi and Phoma* sp. were isolated from spoiled sprouts in several sprout production factories located in Tokyo. The natural symptoms were reproduced by inoculation with the relevant fungal isolates, and the inoculated fungi were consistently re-isolated from the spoiled sprouts, demonstrating that the fungi were the causal agents. Diagnostic symptoms are as follows; 1) *Alternaria alternata*, *Apiospora montagnei* and *Fusarium oxysporum* cause hypocotyl rot. 2) *Geotrichum candidum* causes hypocotyl rot accompanied by a distinctive odor. 3) *Absidia corymbifera* and *Rhizopus oryzae* cause characteristic entangling as well as rot of hypocotyls. 4) Six other fungi, generally known as seed-borne pathogens, cause brown rot of cotyledons and hypocotyls. Field sanitation during mung bean cultivation and complete washing of the raw seed before sprout production are necessary to control the spoilage.

36.8* SPOILAGE OF SOYBEAN SPROUTS BY *GIBBERELLA ZEAE* AND *PHOMA MEDICAGINIS*. **T. Sato, M. Aoki, T. Aoki, K. Tomioka, H. Sawada, Y. Hirooka, M. Endo and T. Nagai.** *Genebank, National Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan. Email: s1043@affrc.go.jp*

Soybean (*Glycine max*) sprouts are a common raw food in eastern Asia including Japan. Since they are produced in darkness under warm and humid conditions, seed-borne pathogens easily spoil the achlorophyllous cotyledons and hypocotyls during production. We found rotten soybean sprouts covered with white mold in a factory for sprout production located in Tokyo. A fungus isolated repeatedly from the spoiled sprouts formed reddish colonies on PDA to produce conidia. The fungus also formed similar conidia on SNA, and produced perithecia on SNA with slips of sterilized filter paper under a black light. Conidia were falcate with obvious foot cells, pointed at the apex, (3–) 5–7 septate and 44–70 × 4–5 µm in size. Perithecia contained numbers of asci each having eight 4-celled ascospores. The fungus was identified as *Gibberella zeae* (anamorph: *Fusarium graminearum*). We also found in other factories another fungus causing brown spots on cotyledons before hypocotyl elongation. The fungus formed pycnidia on PDA to produce pinkish conidial masses. Conidia were phialidic, hyaline, aseptate, ellipsoid to cylindrical and 3.4–10.5 × 1.7–4.4 µm in size. The fungus was identified as *Phoma medicaginis.* The spoilage was shown to be caused by *Gibberella zeae* and *Phoma medicaginis* through inoculation tests with the respective fungal isolates. Field sanitation during cultivation of soybean and complete washing as well as heat sterilization of the raw seed materials before sprout production are effective for spoilage control.

FASTIDIOUS BACTERIA

23.1* VECTOR TRANSMISSION OF *XYLELLA FASTIDIOSA* TO PLANTS. **R. Almeida, N. Killiny and M. Daugherty.** *Dept. Environmental Science, Policy and Management, University of California, Berkeley, USA. Email: rodrigo@nature.berkeley.edu*

Xylella fastidiosa is a xylem-limited plant pathogenic bacterium transmitted by sharpshooter leafhopper vectors. In grape, *X. fastidiosa* causes Pierce's disease, and in this model system we are studying the *X. fastidiosa*-vector molecular interactions and conducting transmission assays to model this system mathematically. In vitro tests have shown that proteins are associated with *X. fastidiosa* attachment to polysaccharides and that hemagglutinin-like proteins are important for cell adhesion to sugars. Transmission tests with knockout mutants of several adhesins support our in vitro observations. We have also determined that the number of infective insects on plants is associated with transmission rates, but there is no relationship between the number of *X. fastidiosa* cells carried by vectors and transmission efficiency. Furthermore, symptom development and bacterial populations within stems are associated with the number of insects on plants. Our results appear to be the first to dissect the *X. fastidiosa*-vector interface. We are also modeling *X. fastidiosa* transmission using the variables described above to determine the relative contribution of different biological factors to transmission efficiency.

23.2* COMBINED USE OF AN SNP-BASED ASSAY AND MULTILOCUS SSR MARKERS FOR DETECTING AND DIFFERENTIATING *XYLELLA FASTIDIOSA* SUBSP. *PAUCA* GENOTYPES AND ISOLATES FROM CITRUS AND COFFEE. **B.B. Landa, J.R.S. Lopes, M. Montes-Borrego, L. Zambolim, and R.M. Jiménez-Díaz.** *Instituto de Agricultura Sostenible, CSIC, Apdo. 4084, 14080 Córdoba, Spain. Email: blanca.landa@ias.csic.es*

Xylella fastidiosa is a xylem-limited, nutritionally fastidious bacterium comprised of subspecies that cause severe plant diseases in several host plants. Strains of *X. fastidiosa* subsp. *pauca* (*Xfp*) cause leaf scorch in coffee (CLS) and variegated chlorosis in citrus (CVC). In this study, we identified two genotypes of *Xfp*

based on a single nucleotide polymorphism (SNP) found in the *gyrB* sequence of 90 isolates from 26 citrus and 64 coffee trees showing CVC or CLS symptoms, respectively. From this sequence, a primer was designed that exactly adjoins to stop just at the 5' end of the SNP, which when used in a SNaPshot protocol differentiates *Xfp* genotypes according to the host source of strains. The robustness of the SNaPshot protocol for predicting *Xfp* host source was confirmed in a blind test using DNA from 18 isolates, and subsequent sequencing of the *gyrB* gene. We also validated the usefulness of the SNAPShot technique using DNA extracted from infected plants and insect vectors. Results showed that coffee plants may be naturally infected with both genotypes as indicated by phylogenetic analysis of *gyrB* sequences. The combined use of the SNaPshot protocol and a published PCR assay based on multilocus simple sequence repeat (SSR) markers indicated that the two genotypes and distinct isolates of *Xfp* infect citrus and coffee in Brazil, and that genotypic diversity exists within an orchard or an infected plant. The combined use of both molecular assays can greatly facilitate epidemiological studies of CVC and CLS diseases.

23.3* INTERACTION OF A BIOLOGICAL CONTROL STRAIN AND A PATHOGENIC STRAIN OF *XYLELLA FASTIDIOSA* IN GRAPEVINE. R. Oliver, W. Owens and D.L. Hopkins. *University of Florida, MREC, 2725 Binion Road, Apopka, FL 32703, USA. Email: dhop@ufl.edu*

Pierce's disease (PD) of grapevine, caused by *Xylella fastidiosa* subsp. *piercei*, limits the grape industry in the southeastern U.S. In Florida, inoculation of 'Cabernet Sauvignon' with a benign strain (EB92-1) of *X. fastidiosa* subsp. *piercei* provided control of Pierce's disease for more than 10 seasons in the vineyard; all untreated vines died within 4 years. To gain understanding of the mechanism of biological control by the benign strain, colonization and movement of the pathogenic strain in 'Carignane' grapevine protected by the biocontrol strain was determined in greenhouse tests using the enhanced cyan fluorescent protein gene and the enhanced yellow fluorescent protein gene as markers to distinguish the two strains *in planta*. The biocontrol strain colonized the grapevines at a level that was at least 100-fold lower than pathogen populations associated with disease development. The biocontrol strain reduced systemic colonization by the pathogen by 10 to 100 fold, compared to plants inoculated with pathogen only. Systemic movement of the pathogen in the plant was limited by the biocontrol strain. The effects on pathogen populations were observed in parts of the plant that were beyond those colonized by the biocontrol strain. The biocontrol strain appears to prevent PD development by limiting the pathogen population below the threshold for symptom production. Since the control occurs in sections not populated by the biocontrol strain, the mechanism is probably systemic induced resistance.

Genomics and proteomics

10.1 COMPARATIVE PROTEOMICS AND DIFFERENTIAL EXPRESSION TO IDENTIFY *ZEA MAYS* PROTEINS ASSOCIATED WITH RESISTANCE TO *ASPERGILLUS FLAVUS*. L. Balzano, J. Alezones, C. Bernal, I. Galindo and N. Diez. *Laboratorio de Genética y Polimorfismo Genético, Instituto de Biotecnología. Fundación Instituto de Estudios Avanzados IDEA, Valle de Sartenejas, Venezuela. Email: leobalzano@gmail.com*

The phytopathogenic fungus *Aspergillus flavus* is one of the agents that most affect maize production and the possibility of aflatoxin generation after infection. Previous studies with other fungi have only analyzed the differential expression response of embryonic tissue. In this study we included the endosperm tissue to complete the analysis of changes in protein pattern in seeds. We extracted protein from the seeds of 11 *Zea mays* lines previously identified as resistant or susceptible to *A. flavus*, using an *in vitro* infection methodology of maize seeds and extraction buffers with different pH values. We determined the quantity of protein in extracts by the Bradford QuickStart® method and obtained one-dimensional protein profiles using 12% SDS-PAGE stained with Coomasie blue. We then obtained two-dimensional patterns using a non-linear 3–10 pH frame and silver-stained 12% SDS-PAGE. Analysis of the different culture lines permitted the detection of multiple protein-expression variations principally related to catabolism-activation pathway proteins and antioxidant enzymatic proteins. This work establishes a correlation between these differentially expressed proteins and the plant resistance or susceptibility to *A. flavus*.

10.2 cDNA AFLP ANALYSIS OF PEACH FRUIT SUSCEPTIBILITY TO *MONILINIA LAXA*. E. Baraldi, P. Zubini and P. Bertolini. *Biotechnology lab., DIPROVAL (Dept. of Agrifood Protection and Improvement)-CRIOF, University of Bologna, Bologna, Italy. Email: elena.baraldi@unibo.it*

Monilinia brown rot is one of the most important diseases of peach (*Prunus persica*) fruit. During fruit growth, susceptibility to *Monilinia* rot strongly decreases over two weeks, corresponding to pit hardening (S2 phase) and accumulation of aromatic compounds. Susceptibility increases again thereafter. To investigate the molecular bases of this temporary loss of susceptibility to *Monilinia*, large-scale gene expression analysis was undertaken to compare susceptible fruits (S fruits; end S1 growth phase) with the two week-older resistant fruits (R fruits; full S2 phase). The mRNA was isolated from the peel of S and R fruits, both after three hours inoculation with *M. laxa* (SI and RI fruits) or mock inoculation (SH and RH fruits). The RNA was retrotranscribed into cDNA and used for cDNA AFLP analysis. More than 100 sequences were differentially expressed in S and R fruits, and more than 340 in H and I fruits. The most interesting sequences were quantified by Real-Time PCR and their putative role in the temporary loss of susceptibility of peach fruit to *Monilinia* rot was investigated using the recombinant expressed proteins in in vitro systems.

10.3* PATTERNS IN POPULATION DIVERSITY REFLECT GLOBAL MOVEMENT OF THE RED BAND NEEDLE BLIGHT PATHOGEN, *DOTHISTROMA SEPTOSPORUM*. I. Barnes, M.J. Wingfield and B.D. Wingfield. *Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa. Email: Brenda.wingfield@fabi.up.ac.za*

The population diversity of a world-wide collection of *Dothistroma septosporum* was studied utilising 15 polymorphic microsatellite markers specifically developed to study this pathogen. Isolates from northern hemisphere countries, including Austria, Poland, Hungary and Bhutan exhibited high gene diversities. This result is consistent with the view that the pathogen is endemic to the areas sampled or that it has been present for an extended period, on native trees including *Pinus nigra* and *P. wallichiana*. Both mating types of the pathogen were also present in

isolates from these northern hemisphere countries. In contrast, *D. septosporum* populations in southern hemisphere countries are typical of an introduced pathogen. The South American populations from Chile and Ecuador were clonal, exhibiting identical alleles and allele frequencies. The New Zealand and Australian populations were also clonal, however, they contained alleles that were unique to these countries, suggesting that the origin of these populations is in an area that has thus far not been sampled. The hypothesis that *D. septosporum* was introduced into Australia from New Zealand via trade winds from the Tasman sea is supported by results of this study. Isolates representing all four southern hemisphere populations contained only one mating type gene, emphasising the clonal reproduction of these populations. Populations of *D. septosporum* from non-native *P. radiata* in both Kenya and South Africa showed high gene diversity and the presence of both mating types. These results indicate that there have been multiple introductions of the pathogen into the African countries considered in this study.

10.4 CLONING OF LACCASE GENE FRAGMENTS AND DETECTION OF LACCASE ACTIVITY IN *SETOSPHAERIA TURCICA*. **Z.Y. Cao and J.G. Dong.** *Mycotoxin Laboratory, Agricultural University of Hebei, Baoding, P.R. China. Email: caozhiyan@hebau.edu.cn*

Melanin is a brown to black pigment produced by some fungi, plants and animals. It is known that DHN melanin is a virulence factor in *Setosphaeria turcica.* Molecular genetic studies have identified four genes (*PKS*, *4HNR*, *3HNR*, *SCD*) involved in the DHN melanin biosynthetic pathway of *S. turcica*. The synthesis pathway often involves a laccase for the catalization of the last reaction step. In our research, three PCR-products were obtained from *S. turcica* by designing three pairs of degenerate primers according to the conserved domain of other fungal laccases. The fragments were named LacI, LacII and LacIII, respectively and their sizes were 931bp, 500bp and 1141bp. Both LacI and LacIII share 65% identity with the laccase of *Phaeosphaeria spartinicola*. LacII showed similarity of 46% with the laccase of *Hortaea acidophila*. This study shows that there are at least three laccase genes in *S. turcica*. The laccase activity was detected by monitoring the increase of absorbance (420nm) with a spectrophotometer following addition of ABTS (2,2'-azino-bis(3-ethyl-2,3-dihydro-1,3-benzthiazole-6-sulfonate) as the common substrate. To stimulate laccase activity, copper concentration in Fries medium was varied between zero and 2mM by addition of copper sulfate. The result showed that laccase activity could be induced by addition of 5-1000 µM copper with an optimum between 50 and 500 µM. In order to illustrate the role of laccase in *S. turcica*, further studies will focus on making laccase mutants and analyzing the role of laccase in the DHN melanin biosynthesis pathway.

10.5 PROTEOMIC ANALYSIS OF *PERONOSPORA VICIAE* PRE-INVASION STRUCTURES. **J.L. Chuisseu Wandji, J. Harrison, H. Macdonald and P.T.N. Spencer-Phillips.** *Centre for Research in Plant Sciences, University of the West of England, Coldharbour Lane, Bristol BS16 1QY, UK. Email: Josiane.Chuisseuwandji@uwe.ac.uk*

The oomycete *Peronospora viciae* causes downy mildew of pea (*Pisum sativum*) resulting in up to 55% losses in yield, with breakdown of host resistance and an increase in pathogen resistance to fungicides recently observed. Proteomics provides a novel method for analysis of host-pathogen interactions and has the potential to identify key marker and target proteins for diagnosis and control. We have used two-dimensional gel electrophoresis (2-D) and mass spectrometry to analyse proteins extracted from three pre-invasion developmental stages: 1) conidia from sporulating infections; 2) germinating conidia; 3) conidia with germ tubes and appressoria. An enhanced protein extraction method has been developed which results in over 700 protein spots visible on Coomassie blue-stained gels. MALDI-TOF peptide mass fingerprints and Q-TOF analysis of amino acid sequences of the 32 most abundant proteins gave 9 with no match to oomycete or plant proteins, 12 appearing to be of plant origin and 11 matching oomycete proteins. The latter included those involved in protein and amino acid synthesis, protein binding, transport and folding, metabolic and energy pathways, and cellular organisation. Changes in the abundance of more than ten proteins during development of infection structures have been observed post-germination, and our latest data will be presented.

10.6 EXPRESSION PROFILING SOYBEAN RESPONSES TO PATHOGENS AND HERBICIDES. **S.J. Clough, J. Zhu, B. Calla, O. Radwan and M. Li.** *USDA-ARS / University of Illinois, Urbana, IL, USA. Email: sjclough@uiuc.edu*

Soybean plants are attacked by a wide variety of pathogens requiring effective means of defense. Gene activation is a major defense mechanism to reduce the degree of damage caused by pathogens as it leads to the rapid, regulated production of toxins and enzymes, in addition to structural reinforcements. Defense responses often include down regulation of genes associated with photosynthesis. To compare gene expression responses to pathogens and herbicides that inhibit photosynthesis, we used soybean cDNA microarrays to measure differential gene expression in soybean in response to three different pathogens and two photosynthesis-inhibiting herbicides. The plant-pathogen interactions that were analyzed in this study included *Pseudomonas syringae* compatible and incompatible interactions in leaves, *Sclerotinia sclerotiorum* infection in susceptible and partially resistant stem tissue, and the effect of *Fusarium virguliforme* toxin in leaf tissue. The herbicides used to examine gene expression responses to the chemical inhibition of photosynthesis included atrazine and bentazon. Statistical analysis of expression data from approximately 38,000 spotted gene representatives identified hundreds of genes that were significantly activated or repressed during these interactions. Summaries of common and treatment specific expression will be presented.

10.7 QTL MAPPING OF *ASCOCHYTA* BLIGHT RESISTANCE IN INTERSPECIFIC PROGENIES FROM CHICKPEA AND *CICER RETICULATUM*. **N. Danehloueipour, M.N. Nelson, G. Yan, H.J. Clarke and K.H.M. Siddique.** *School of Plant Biology (MO84), Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, WA 6009, Australia. Email: ndaneh@plants.uwa.edu.au*

The objectives of this study were to identify regions of the chickpea genome controlling *Ascochyta* blight resistance, days to flowering and plant growth habit. An interspecific F_2 mapping population, derived from a cross between chickpea accession ICC 3996 (resistant to *Ascochyta* blight, early flowering, and semi-erect plant growth habit) and *Cicer reticulatum* accession ILWC 184 (susceptible to *Ascochyta* blight, late flowering, and prostrate habit), was used to construct a genetic map. F_2 plants were cloned through stem cuttings. Clones were screened for *As-*

cochyta blight resistance in controlled environment conditions using a 1–9 scale. Three quantitative trait loci (QTLs) were found in this population that explained approximately 49% of the variation for resistance. Two of these QTLs were located on linkage group (LG) 4 and explained 21.1% and 4.9% of the phenotypic variation. The other QTL, located on LG3, explained 22.7% of the phenotypic variation for resistance. Linkage group 3 had two major QTLs for days to flowering (explaining 90.2% of phenotypic variation) and a major single QTL for plant growth habit (explaining 95.2% of phenotypic variation). In this population, there was a negative correlation between resistance and days to flowering (r=-0.22, *P<0.001*) and a positive correlation between days to flowering and plant growth habit (r=0.36, P<0.001). Furthermore, we discuss how the markers flanking QTLs identified in this study could be used for efficient introgression of new allelic diversity into cultivated chickpea from a related wild species.

10.8 IDENTIFICATION AND EVOLUTION OF SPECIFIC EFFECTORS IN THE *MELAMPSORA*–POPLAR INTERACTION. **N. Feau, D.L. Joly, P. Tanguay and R.C. Hamelin.** *Service canadien des forêts, Ressources naturelles Canada, Centre de Foresterie des Laurentides, 1055 du PEPS, P.O. Box 3800, Québec G1V4C7, QC, Canada. Email: nicolas.feau@rncan.gc.ca*

According to recognition and coevolution models, fungal effectors implicated in host-pathogen interaction are predicted to exhibit a number of common structural and functional features as well as significant sequence variation. Fungal genetic determinants involved in host adaptation are expected to rapidly diverge between closely related species and may be restricted to certain lineages. We designed a multifaceted approach based on comparative genomics and sequence data trimming and analyses for the identification of effectors implicated in host-adaptation. Poplar leaf tissues infected with four closely related *Melampsora* rust species were sampled at different stages and used to generate a collection of expressed sequence tags (14,541 clones sequenced; 4,867 ESTs). Bioinformatics and comparative analyses were used to extract: 1), extracellularly expressed secreted proteins, 2) determinants that are unique to biotrophic fungi and to each *Melampsora* species, and, 3) the presence of positive selection using intra- and interspecific comparative genomics within the *Melampsora* genera. Ratios of non-synonymous/synonymous nucleotide-substitutions between homologous sequences from the different libraries revealed the signature of positive selection within five genes that were predicted to be extracellular effectors. One of these candidate genes was highly expressed in compatible vs incompatible *M. larici-populina/P. deltoides* × *P. trichocarpa* interactions based on SAGE and reverse-transcriptase quantitative PCR analyses.

10.9 ANALYSIS OF *BOTRYTIS CINEREA* SECRETOME. F.J. Fernández-Acero, T. Colby, A. Harzen, U. Wieneke, C. Garrido, M. Carbú, I. Vallejo, J. Schmidt and J.M. Cantoral. *Laboratory of Microbiology, Marine and Environmental Sciences Faculty, University of Cádiz, Pol. Río San Pedro s/n, Puerto Real, 11510, Cádiz, Spain. Email: jesusmanuel.cantoral@uca.es*

The ascomycete *Botrytis cinerea* is a phytopathogenic fungus infecting a number of crops including tomatos, grapes and strawberries, and causing significant yield losses in these crops in Andalusia (Spain) each year. In the last few years, *B. cinerea* has been adopted as an important model system in molecular phytopathology. Several approaches have been applied to this fungus to unravel its mechanisms of infection. These studies have revealed the complexity and wide variety of infection strategies used by *B. cinerea* that are mediated by a complex set of genes/proteins called pathogenicity factors. Many of these factors have been described in phytopathogenic fungi as proteins excreted to the media (secretome). The study of this differential subproteome can contribute to a comprehensive view of the role of these factors and may reveal new foci for the fight against the pathogen. In this work a proteomic approach based on 2-DE has been developed to establish the proteins secreted to culture media supplemented with various plant elicitors. Proteins were obtained from the culture media by an improved method based on DOC-TCA precipitation and phenol extraction. From the 2-DE analyses, a relevant set of proteins has been identified including known virulence factors such as pectin methyl esterases and proteases. Identification of other enzymes implicated in the infection mechanism indicate that this proteome-mining is a promising strategy for discovering new pathogenicity factors.

10.10 PROTEOME ANALYSIS OF THE IMPACT OF TRICHOTHECENES AND *FUSARIUM GRAMINEARUM* IN WHEAT *FUSARIUM* HEAD BLIGHT. N.A. Foroud, A. Laroche, B.E. Ellis and F. Eudes. *Agriculture and Agri-Food Canada, Lethbridge Research Centre, Alberta T1J 4B1, Canada. Email: foroudn@agr.gc.ca*

Fusarium Head Blight (FHB) is a devastating disease of wheat caused by *Fusarium graminearum* and related species. Two major forms of FHB resistance include resistance to initial infection (Type 1) and resistance to disease spread (Type 2). *F. graminearum* produces trichothecene mycotoxins that inhibit eukaryotic protein synthesis and may play a role in virulence and disease spread. A trichothecene non-producing mutant (GZT40, wild-type GZT3639) is capable of establishing infection in susceptible wheat genotypes but is less virulent than the wild-type and is incapable of spreading from floret to floret. Thus, wheat genotypes that do not normally exhibit Type 1 or 2 resistances effectively display these forms of resistances when trichothecenes are not being expressed by the pathogen. In the current study, two-dimensional polyacrylamide gel electrophoresis was performed on spikelets of infected heads from three wheat genotypes, one susceptible cultivar (Superb) and two resistant double haploid lines (DH1 and DH2). DH1 and DH2 were generated from crossing and backcrossing the resistant cultivars CIMMYT11 and CM82036, respectively, with 'Superb'. DH1 and DH2 each share 75% genetic identity with their susceptible parent ('Superb'), and respectively exhibit good Type 1 and Type 2 resistance. Five inoculums, GZT3639, GZT40, GZT40 supplemented with deoxynivalenol (DON; the major trichothecene of GZT3639), DON, and water, were used to point-inoculate the central florets during anthesis. Spikelets above and below the inoculation point were collected for proteome analysis in order to compare both the systemic changes and the constitutive differences in the proteomes of susceptible and resistant genotypes.

10.11 PROTEOME AND TRANSCRIPTOME ANALYSIS OF THE IMPACT OF TRICHOTHECENES AND *FUSARIUM GRAMINEARUM* IN BARLEY. N.A. Foroud, J. Geddes, B. Selinger, A. Laroche, and F. Eudes. *Agriculture and Agri-Food Canada, Lethbridge Research Centre, Alberta T1J 4B1, Canada. Email: foroudn@agr.gc.ca*

Two-dimensional polyacrylamide gel electrophoresis was per-

formed on proteins from infected heads of six barley genotypes: one susceptible cultivar (Stander), two intermediate resistant cultivars (CDC bold and Chevron) and three resistant genotypes (CI4196, Harbin and Svansota). Five inoculums, GZT3639, GZT40 (a trichothecene non-producing mutant of wild-type GZT3639), GZT40 supplemented with deoxynivalenol (DON), DON, and water, were used to point-inoculate every second spikelet at anthesis. Spikelets were collected for proteome analysis in order to compare both systemic changes and constitutive differences in the proteomes of susceptible and resistant genotypes. Spikelets from Chevron and Stander where also harvested for mRNA extraction and transcriptome analysis using the barley Affymetrix chip. Thirty-three acidic protein spots were differentially expressed 3 days post-inoculation. Proteins responsive to FHB included those associated with oxidative burst and oxidative stress response, and defence response (t-test, $P<0.05$). An increase in abundance of PR-1, PR-3 or PR-5 was associated with the resistant genotypes, as well as the intermediate resistant genotypes. The microarray investigation revealed a complex cellular network in the barley cells in response to the fungus, the mycotoxin, and the subsequent interaction between them. Both Chevron and Stander appeared to up-regulate gene transcripts associated with the jasmonic acid pathway; and showed up-regulation of many gene transcripts coding for PR-proteins (e.g. PR-10, PR-13, and LTPs), but differed in their responses to specific treatments and their induction timing. At least three distinct response patterns are reported from these 6 barley genotypes.

10.12* IDENTIFICATION THROUGH COMPARATIVE PROTEOMICS OF PROTEINS ASSOCIATED WITH RESISTANCE OF RICE (*ORYZA SATIVA)* TO *PYRICULARIA GRISEA*. **M.A. Franco, Y. Jayaro, A. Gonzalez, C. Ramis, N. Diez and I. Galindo Castro.** *Laboratorio de Genómica y Proteómica. Centro de Biotecnología. Fundación IDEA. Carretera Baruta, Hoyo de la Puerta Sarteneja, Caracas, Venezuela. Email:marialefranco@gmail.com*

Rice is one of the most important crops; its most significant economic features are crop yield, nutritional value, culinary quality and pathogen resistance. Rice blast, caused by the fungus *Pyricularia grisea*, costs Venezuelan farmers more than 30% of crop production every year. The use of resistant cultivars by pyramiding resistance genes through classical or molecular marker-assisted breeding has proved to be the most economic and effective method to control the disease. Plants respond to pathogen attack by activating multiple defence mechanisms to protect themselves from infection. Some wild resistant plants have been identified. In the field, some isolines have been identified that offer natural resistance to a great number of fungal isolates, but the mechanisms are not known by which the plant-pathogen interaction allows the plant to recognize the presence of the pathogen outside its cells and respond successfully before the attack. The objective of this work was to compare the protein expression profiles through time of rice plants susceptible or resistant to *P. grisea*, using two-dimensional gel analysis to identify the proteins expressed differentially. Proteome maps were obtained from the susceptible and resistant samples, and comparing them demonstrated the important differences between the total proteins of each material.

10.13 *PEPINO MOSAIC VIRUS* IN CANADA; PHYLOGENY OF COMPLETE SEQUENCES AND EFFECTS OF VIRUS INFECTION ON FRUIT YIELD. **C.J. French, A. Bunckle, G. Ferguson, C. Dubeau, M. Bouthillier and M.G. Bernardy.** *Agriculture & Agri-Food Canada, Pacific Agri-Food Research Centre, Highway 97, Summerland, B.C. VOH 1ZO, Canada. Email: frenchc@agr.gc.ca*

Pepino mosaic potexvirus (PepMV) was first reported in Ontario, Canada, in 2000. Bright yellow mosaics have been observed but more typical symptoms on tomato in Ontario are a mild leaf mosaic, leaf bubbling and necrotic spots. We have now completely sequenced many tomato isolates from Ontario greenhouses and conducted phylogenetic analyses including published virus sequences. The Ontario isolates were very similar to European tomato isolates and to each other (approximately 99% overall nucleotide homology). However, representative isolates from Ontario were distinct from two previously reported tomato isolates (CH-1 and CH-2; average nucleotide identity 82% and 78%, respectively). In controlled greenhouse trials, infection of tomato by an isolate from Ontario had little effect on fruit yield whereas infection with an isolate from Southern Europe reduced fruit size and overall yield. The effects of virus infection on fruit yield varied between growing seasons.

10.14 TELOMERIC FINGERPRINTING OF *COLLETOTRICHUM ACUTATUM* ISOLATES CAUSING ANTHRACNOSE IN STRAWBERRY. **C. Garrido, M. Carbú, F.J. Fernández-Acero, I. Vallejo and J.M. Cantoral.** *Laboratory of Microbiology, Marine and Environmental Sciences Faculty, University of Cádiz, Puerto Real, 11510 Cádiz, Spain. Email: jesusmanuel.cantoral@uca.es*

Colletotrichum acutatum is a major worldwide plant pathogen infecting a broad range of host plants. It causes anthracnose on a number of economically important woody and herbaceous crops, including ornamentals, fruits, conifers and forage plants. *Fragaria ananassa*, cultivated strawberry, is particularly susceptible to anthracnose. In the present study, an extensive sample of *C. acutatum* isolates, closely or distantly related, collected from different strawberry cultivars, were analysed to study the molecular polymorphism among the isolates, as well as to estimate their chromosome number. Genomic DNA of forty nine *C. acutatum* isolates was digested to completion with four restriction enzymes (*Bam*HI, *Eco*RI, *Hind*III and *Pst*I) in independent experiments. DNA was transferred to Hybond-N membrane and then hybridised by Southern-blot using a telomeric DNA probe. The telomeric fingerprint patterns were analysed using Fingerprinting II software (Bio-Rad). Results indicated that the minimun number of estimated chromosomes ranged between six and nine, and established the level of molecular polymorphism among *C. acutatum* isolates.

10.15 CLONING AND EXPRESSION OF THE G PROTEIN β SUBUNIT (STGB) GENE FROM *SETOSPHAERIA TURCICA*. **Z.M. Hao and J.G. Dong.** *Mycotoxin Laboratory, Agricultural University of Hebei, Baoding, P.R. China. Email: hzm_0322@163.com*

Filamentous fungi recognize and respond to signals from the environment and hosts by altering their growth and development. Signal pathways mediated by G protein play an important role in fungal development. For plant pathogens, such as *Magnaporthe grisea*, Gβ subunit genes are known to regulate virulence and development. The ortholog in *Cryptococcus neoformans* is required for mating but dispensable for virulence, emphasizing that signaling pathways are put to different uses, depending on the fungal species. We obtained a homologous fragment of Gβ gene from *Setosphaeria turcica*, which was named Stgb and is similar to homologus regions of other fungal Gβ genes. The 3' cDNA end of

Stgb contained a 3'UTR (145 bp) but no typical poly(A) tailing signal. Because of its 100% identity with Cgb-1 in *Cochliobolus heterostrophus*, we designed specific primers, which corresponded to the ORF of Cgb-1. As a result, we obtained the Stgb ORF, which was 1056 bp long and encoded 351 amino acid residues. The predicted protein sequence shared a high identity with Gβ protein from *C. heterostrophus* (100%), *Mycosphaerella graminicola* (96%), *Aspergillus nidulans* (92%), and *Coccidioides immitis* (91%). The sequence had been deposited in the GenBank/EBI Data Libraries under Accession No. EF407555. Stgb was expressed in the pET system. Results of SDS-PAGE and Western blotting showed that the recombinant protein, calculated molecular mass about 40 kDa, was expressed in *E. coli*. In conclusion, we have successfully cloned a Gβ subunit gene of *S. turcica* and gained a His-tag recombinant protein.

10.16 EFFICIENT VIRUS-INDUCED GENE SILENCING IN PLANTS USING A MODIFIED GEMINIVIRUS DNA 1 COMPONENT. C. Huang and X. Zhou. *Institute of Biotechnology, Zhejiang University, Hangzhou 310029, P.R. China. Email: zzhou@zju.edu.cn*

Virus-induced gene silencing (VIGS) is currently recognized as a powerful reverse genetics tool for application in functional genomics. DNA 1, a satellite-like single-stranded DNA molecule associated with begomoviruses (Family *Geminiviridae*), is known to replicate autonomously but requires the helper virus for its dissemination. We developed a VIGS vector based on the DNA 1 component of *Tobacco curly shoot virus* (TbCSV), a monopartite begomovirus, by inserting a multiple cloning site (MCS) between the Rep ORF and the A-rich region for subsequent insertion of DNA fragments of genes targeted for silencing. When a host gene (sulfur, *Su*) or transgene (green fluorescent protein, *GFP*) was inserted in the modified DNA 1 vector and co-agroinoculated with TbCSV, efficient silencing of the cognate gene was observed in *Nicotiana benthamiana* plants. More interestingly, we showed that this modified DNA 1 could effectively suppress *GFP* in transgenic *N. benthamiana* or endogenous *Su* in tobacco plants with *Tomato yellow leaf curl China virus* (TYLCCNV), another monopartite begomovirus that does not induce any viral symptoms. A gene-silencing system in *Nicotiana* spp., tomato (*Solanum lycopersicum*) and *Petunia hybrida* plants was then established using TYLCCNV and the modified DNA 1 vector. It can be used to silence genes involved in meristem and flower development. Previous studies have reported that DNA 1 is associated with both mono- and bipartite begomoviruses as well as curtoviruses. This vector system can therefore be applied for the study, analysis and discovery of gene function in a variety of economically important crop plants.

10.17 PROTEOMIC APPROACH FOR SEEKING THE PROTEINS RELATED TO DEVELOPMENT OF *FUSARIUM OXYSPORUM* IN THE HOST PLANT. M. Kawabe, T. Arie and Y. Arimoto. *Applied Biology for Plant Protection Research Unit, RIKEN Institute, Saitama, Japan. Email: mkawabe@riken.jp*

Fusarium oxysporum f. sp. *lycopersici* (FOL) causes soil-borne vascular wilt disease on tomato. Previously, it was reported that FOL could develop in stems but not in leaves of tomato. Tomato leaves do not contain glutamine but tomato stems contain a high concentration. This suggested that glutamine concentration might affect the development of FOL. In further experiments, it was reported that FOL grown on tomato leaves in the presence of exogenous glutamine could invade tomato and cucumber cotyledons through stomata, but FOL grown on tomato leaves without added glutamine could not. These results suggest that glutamine may affect the invasion of FOL into host plants and development of FOL in them. We analyzed the protein expression profiles between FOL grown on medium with and without glutamine by two-dimensional difference gel electrophoresis (2D-DIGE). We found differences in the expression of some proteins. The amino acid sequences of proteins which were up-regulated or down-regulated by the addition of glutamine were determined, as were the functions of some proteins which were up- or down-regulated. Here we present the putative functions of some of the proteins regulated by glutamine.

10.18 PTR TOXB-INDUCED CHANGES IN THE LEAF PROTEOME OF TOXIN-SENSITIVE WHEAT (*TRITICUM AESTIVUM* L.). Y.M. Kim, N.N.V. Kav and S.E. Strelkov. *4-10 Agriculture/Forestry Centre, University of Alberta, Edmonton, Alberta T6G 2P5, Canada. Email: ymkim@ualberta.ca*

The ascomycete fungus *Pyrenophora tritici-repentis* causes tan spot, an important foliar disease of wheat. The pathogen produces at least three host-specific toxins, including Ptr ToxB, a 6.6 kDa protein that induces chlorosis in sensitive wheat genotypes. To gain a better understanding of the role of this toxin in disease development, we examined proteome-level changes induced by hexahistidine-tagged Ptr ToxB in toxin-sensitive leaf tissue. Leaves of the wheat cv. Katepwa were infiltrated with toxin or water and changes in the proteome occurring at 12, 24 and 36 h after treatment (but prior to the development of chlorosis symptoms) were compared by two-dimensional gel electrophoresis. A total of 102 protein spots were identified as either increasing (66 spots) or decreasing (36) in intensity relative to water-treated controls. The identities of some of these proteins were established MS/MS, and included proteins involved in the stress/defense response, photosynthesis, and carbohydrate and nitrogen metabolism. Levels of antioxidant enzymes including ascorbate peroxidase were found to increase at 24 h, a finding consistent with previous research suggesting the involvement of reactive oxygen species (ROS) in Ptr ToxB-induced chlorosis. However, the quantity of germin-like protein 1, which has been proposed to have a function in pathogen defense of cereals, was found to decrease five-fold 36 h after treatment with the toxin. This would support the role of Ptr ToxB as a pathogenicity factor that confers susceptibility in wheat to toxin-producing isolates, and may suggest an inactivation of the host defense response.

10.19 PHOSPHITE-INDUCED CHANGES IN GENE EXPRESSION OF *PHYTOPHTHORA CINNAMOMI*. M. King, J.A. McComb, W. Reeve, G.E.StJ. Hardy and P.A. O'Brien. *Centre for Phytophthora Science and Management, School of Biological Science and Biotechnology, Murdoch University, Murdoch WA 6150, Australia. Email: M.King@murdoch.edu.au*

Phosphite, an analogue of phosphate, is widely used to control oomycete diseases of plants. It inhibits growth and zoospore production in species such as *Phytophthora cinnamomi* both *in vitro* and *in planta*. As an approach to understanding the mechanism of phosphite on *P. cinnamomi* we compared the transcriptomes of untreated and phosphite-treated mycelium using microarray analysis to identify genes that are differentially expressed. A cDNA library was constructed using RNA from phosphite-treated mycelium and used to construct an array containing

over 9,000 clones as probes. The array was hybridised with differentially labelled RNA from untreated and phosphite-treated mycelium. From the arrays seventy-two transcripts with altered patterns in gene expression (change ≥ 2 fold) were identified. Forty five clones represented genes that were down-regulated with changes in gene expression ranging from 2- to 3.5-fold. Thirty-two cDNA transcripts were up-regulated with changes in gene expression ranging from 2- to 16-fold. The identity of the most highly expressed clones was determined by sequencing and comparison with sequences in GenBank.

10.20 ANALYSIS OF GENE EXPRESSION OF *R. SOLANI* (AG-4) TO UNDERSTAND ITS VIRULENCE AND BIOLOGY. **D.K. Lakshman.** *USDA-ARS, Floral and Nursery Plants Research Unit, Beltsville, MD 20705, USA. Email: Dilip.Lakshman@ars.usda.gov*

The basidiomycete *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) is comprised of important plant pathogens, saprophytes and mycorrhizae and exists in several hyphal anastomosis groups (AGs) with distinct host specializations. AG-4 isolates cause diseases in a broad range of hosts, including ornamentals and turfgrasses. Knowledge of the molecular mechanism of *Rhizoctonia* pathogenicity and virulence is only rudimentary at present. It is important to develop a greater understanding of the interactions involved in pathogenicity, particularly to differentiate the genes involved in establishment of infection from those involved in maintaining an infection, as different approaches would be taken to prevent infection compared to controlling infection. A thorough knowledge in this area is also necessary to assist breeders in identifying genes that can be targeted in order to generate resistant varieties and to determine whether such resistance is likely to be strain-specific or broadly effective. In an effort to extend our knowledge of the molecular mechanism of virulence of this pathogen, we have developed two normalized EST libraries specific to a virulent isolate and a 3-O-methylglucose induced virulence-repressed isolate of *R. solani*, AG-4, isolate Rs23. Thus far, about 2000 EST clones have been sequenced and an overall analysis of the cDNA sequences is in progress. Subsequently, additional sequencing of the ESTs will be conducted and their differential gene expression will be analyzed to target pathogenicity associated genes of *R. solani*. Knowledge gained from gene expression of the AG-4 isolate will be utilized for conducting comparative genomic and proteomic studies of other AGs that infect ornamentals.

10.21 FUNCTIONAL AND GENETIC CHARACTERIZATION OF CALMODULIN FROM *SETOSPHAERIA TURCICA*. **Z.Y. Li and J.G. Dong.** *Mycotoxin Laboratory, Agricultural University of Hebei, Baoding, P.R. China. Email: leezhiyongds@126.com*

Calmodulin (CaM) modulates intracellular calcium signaling and acts on several metabolic pathways and on gene expression regulation in many phytopathogenic fungi. Therefore, the elucidation of the possible role played by the CaM gene will be helpful to better understand the mechanism of fungal invasion, expansion, and reproduction in host plants. The CaM antagonist trifluoperazine (TFP) can inhibit conidial germination and appressorium formation of *S. turcica.* The inhibition percentage of 10-30 μmol l^{-1} ranges from 56% to100%. DNA and cDNA fragments of the CaM gene were obtained by polymerase chain reaction (PCR) amplification from degenerate primer sets. The 3' and 5' cDNA sequences of CaM were obtained through the method of 3' RACE and 5' RACE. The *S. turcica* CaM gene was found to encode a putative protein of 149 amino acids, which shows a high degree of identity with CaM of other phytopathogenic fungi. The coding region of the gene is interrupted by four introns. To determine the start site of transcription of the *S. turcica* CaM gene, genome walking was performed. The promoter was analyzed using the Softberry software and the results showed that it contained several regulatory elements. Southern-blot results strongly suggested that CaM is represented by one copy in the genome of *S. turcica.* To establish what function the CaM gene plays in growth and pathogenesis of *S. turcica*, further studies will deal with making CaM mutants and analyzing the role of the CaM gene.

10.22 MITOCHONDRIAL GENOMICS IN *PHYTOPHTHORA* AND *PYTHIUM*; IMPLICATIONS FOR PHYLOGENETICS AND DEVELOPMENT OF MOLECULAR MARKERS. **F.N. Martin.** *1636 East Alisal St., Salinas, CA, USA. Email: fmartin@pw.ars.usda.gov*

Mitochondrial genomics can be useful for investigating the evolutionary processes contributing to mitochondrial sequence divergence among species and hence, provide insight to the phylogenetic relationships. While the mitochondrial genomes of the genera *Pythium* and *Phytophthora* encode a similar suite of genes, they differ from each other by the presence of an inverted repeat (IR) in *Pythium* that can represent approximately 75% of the genome size. While an IR is not usually found in *Phytophthora* genomes, a small IR was observed in *P. ramorum* and *P. hibernalis* (< 1.5 kb). In an effort to gain a better understanding of the evolutionary forces responsible for sequence divergence in genomes with and without an IR and to clarify the phylogenetic relationships within the individual genera the mitochondrial genomes of a number of *Pythium* and *Phytophthora* species were sequenced. Comparative genomics among species within a genus indicated that certain regions of the genome were more polymorphic than others. In *Pythium*, the small unique region and adjacent IR sequences were the most polymorphic while in *Phytophthora* genomic inversions and translocations were observed. While the IR in *Pythium* appeared to stabilize the genome from rearrangements, the rates of evolutionary divergence was more dependent on the specific gene and not its location in the IR. Sequencing multiple genomes of the same species (*P. ramorum*) identified intraspecific variation for identification of mitochondrial haplotypes. Gene order differences between the two genera have identified several regions suitable for the development of genus and species specific molecular markers.

10.23 CHARACTERIZATION ON TRANSCRIPTIONAL CIRCUITRY REGULATING PATHOGENICITY USING A PROTEOMICS APPROACH. **T. Mitchell, J-R Xu, H. Zhu, H. Rho, M. Gowda and R. Dean.** *The Ohio State University, Department of Plant Pathology, Columbus OH, USA. Email: tkmitche@yahoo.com*

Gene expression is controlled in part by a network of protein interactions that result in the activation or deactivation of transcription factors often through phosphorylation. These signalling networks continue to be fertile ground for generating insights into fungal biology and virulence, but full characterization of the downstream transcription factors they directly regulate is lacking. To unravel signalling networks used by the fungus *Magnaporthe grisea* to coordinate gene expression during growth, development and infection, a protein chip containing all predicted transcription factors for this fungus was created. Through a combined automated and manual annotation process, we have identified over 500 putative transcription factors in the genome and confirmed

the expression of > 90% using data from EST, RL-SAGE and MPSS studies. We cloned >80% of the transcription factors and expressed each in yeast to print the *M. grisea* transcription factor protein microarray. Protein arrays were used to assay kinase phosphorylation specificity and activity. Gene disruption and over-expression strategies were then used to map transcription factor binding motifs and identify genes regulated by selected transcription factors using a ChIP-chip hybridization strategy. We will present results from annotation, cloning, and phosphorylation studies as well as ChIP-chip studies.

10.24 PROTOPECTINASE ACTIVITY OF POLYGALACTURONASES FROM PATHOGENIC AND NONPATHOGENIC ISOLATES OF *GEOTRICHUM CADIDUM* GOVERNS THEIR PATHOGENICITY TO CITRUS FRUIT. **M. Nakamura, K. Nakamura and H. Iwai.** *Faculty of Agriculture, Kagoshima University, Kagoshima 890-0065, Japan. Email: masa@agri.kagoshima-u.ac.jp*

Geotrichum candidum is a yeast-like fungus that causes diseases in humans, animals and plants. As a phytopathogen, the fungus causes sour rot, which is an important cause of postharvest loss of citrus fruit and has been reported from many areas in the world where citrus is grown. *G. candidum* is divided into two types based on pathogenicity to citrus fruit. The citrus type is pathogenic, and the non-citrus type is nonpathogenic. Morphologically, both types are indistinguishable. Both fungi secrete polygalacturonase (PG) which is thought to be a factor in pathogenicity. Thus, to elucidate whether PGs are really involved in pathogenicity, S31PG1 and S63PG1, derived from citrus and non-citrus types respectively were expressed in the fission yeast *Schizosaccharomyces pombe* and their PG and protopectinase (PP) activities were measured. S31PG1 showed high PG and PP activities, and severely degraded lemon peel, whilst S63PG1 showed only PG activity but none of PP, and did not degrade lemon peel at all. These results indicate that the different PP activities of the PGs are a key to the pathogenicity of *G. candidum* to lemon fruit. Interestingly, comparison of both PG structures predicted by the homology modelling method showed that the regions which sandwich the catalytic site differ significantly in the two PG proteins, suggesting that the different structures of the regions might influence the affinity to pectic substance (protopectin) in lemon peel and thereby determine binding.

10.25 PROTEOMIC ANALYSIS OF *CARICA PAPAYA* L. ROOT PROTEINS AND THEIR INTERACTION WITH ROOT-ROT PATHOGEN, *PHYTOPHTHORA PALMIVORA*. **M.D. Paidi, Q.Li, P. Moore and Y.J. Zhu.** *Hawaii Agriculture Research Center, 99-193 Aiea Heights Drive, Aiea, HI 96701, USA. Email: jzhu@harc-hspa.com*

The objective of this project is to evaluate the potential of applying proteomic approaches to discover the basis for susceptibility of Hawaiian papaya cultivars to the root-rot pathogen *Phytophthora palmivora*. We selected two cultivars, Kamiya as the most resistance cultivar and SunUp as a susceptible one, for a comparative proteomic analysis. Soluble root proteins were extracted from 3-month-old plants using phenol-SDS extraction buffer. Approximate 200 mg of protein was resolved by two-dimensional electrophoresis (2-DE), followed by staining in Coomassie Blue solution. Spots in 2D gels were analyzed using PDQuest 8.0 software (Biorad). Over 300 protein spots were compared for differences in abundance between SunUp and Kamiya and significantly over-expressed or down-regulated protein spots were identified from each cultivar. These selected protein spots were extracted and analyzed with nano 2D-LC-MS/MS and further evaluated for their responses to P. palmivora in a time course during the post infection. Over-expression of defense-related proteins in salicylate acid (SA)- and jasmonate acid (JA)-dependent pathways were found exclusively in Kamiya following inoculation, but absent in SunUp. RT-PCR assays for molecular markers of SA- and JA-dependent pathways were also carried out to confirm results of proteomic analysis. These differences in defense-related proteins could infer a different mechanism of host interacting with pathogen in a tolerant cultivar. This study contributes toward the identification and characterization of proteins that are differentially expressed between the resistant cultivar and susceptible cultivars to provide a systematic approach to elucidate the mechanisms of phytophthora resistance.

10.26 THE ADVANTAGES OF ASCOSPORE PRODUCTION BY *FUSARIUM GRAMINEARUM*. **M. Pasquali, K. Seong and H.C. Kistler.** *Department of Plant Pathology, University of Minnesota and USDA ARS Cereal Disease Laboratory, St. Paul, USA. Email: matias.pasquali@gmail.com*

Ascospores of *Fusarium graminearum* are the primary inoculum source for *Fusarium* head blight disease of wheat and barley. Produced within perithecia, ascospores are known to be slightly less pathogenic, but more frequently found in aerobiological surveys, than conidia. To identify the precise biological and epidemiological role of ascospores in the *F. graminearum*–wheat pathosystem, a genomic approach has been adopted. A full genome microarray study has been carried out comparing gene expression during germination and drought stress response of both conidia and ascospores. Moreover, the biological consequences of drought stress has been compared for the two spore types. *Ascospores* and conidia differ in viability and infectivity after drought stress: ascospores maintain their ability to infect while conidia became significantly less able to cause disease on wheat. As inferred from gene expression data, ascospores are able to maintain basic cell metabolism and function while conidia more rapidly lose their ability to survive, probably by way of programmed cell death. This may be caused by the relative lack of nutritional reserves in conidia or by the inability of these cells to sense the changes in the environment resulting in precipitous depletion of energy sources within the cell. Thus environmental adaptability of ascospores might be a crucial factor for their epidemiological success in primary infection processes.

10.27 GENOME-WIDE IDENTIFICATION OF GENES CONTROLLING COLONY GROWTH OF *MAGNAPORTHE ORYZAE*. **Y.L. Peng.** *The State Key Laboratory for Agrobiotechnology and Department of Plant Pathology, China Agricultural University, Beijing 100094, P.R. China. Email: pengyl@cau.edu.cn*

Magnaporthe oryzae infects host plants by a process involving conidiation, appressorium formation, penetration and invasive growth of infection hyphae. Understanding of molecular mechanisms regulating pathogenesis will contribute to design of novel approaches for disease control. We suppose that many genes controlling colony growth are also necessary for pathogenicity, and we have thus started genome-wide identification of genes required for colony growth. In order to identify the genes, an insertion mutagenesis library was generated with a field isolate, P131. The library contained about 68,000 independent transformants.

After screening the library, about 600 mutants showing slow colony growth were isolated. Genetic co-segregation analysis was carried out on all the mutants and showed that the phenotype change in less half of the mutants co-segregated with the selection marker of hygromycin resistance, suggesting that not all the mutations were caused by the insertion. TAIL PCR was used to isolate the sequences flanking the insertion sites of co-segregation mutants. So far, the sequences from about 200 mutants have been determined, and through gene complementation and targeted gene disruption, more than 100 genes have been identified that control colony growth. It was also found that many of the colony growth-controlling genes are also required for conidiation, appressorium formation, penetration and invasive growth. A detailed report will be given on the genetic network controlling colony growth of *M. oryzae.*

10.28* BIOLOGICAL IMPACT OF *GRAPEVINE FANLEAF VIRUS* RNA-1 GENETIC VARIABILITY. **M. Pompe-Novak, U. Čepin, I. Gutiérrez-Aguirre and M. Ravnikar.** *Department of Biotechnology and Systems Biology, National Institute of Biology (NIB), Večna Pot 111, 1000 Ljubljana, Slovenia. Email: marusa.pompe.novak@nib.si*

Grapevine fanleaf virus (GFLV) is the causal agent of grapevine fanleaf degeneration disease, one of the most important viral diseases of grapevine, resulting in a progressive decline of infected vines, yield loss and poor fruit quality in all wine-producing areas in the world. The virus is naturally spread by the nematode vector *Xiphinema index* and through the use of infected planting material. GFLV is known to exist as a mixture of variants. Concern over the biological and economical impact of the different GFLV variants has lately activated studies on the biological diversity of the virus, mostly restricted to the RNA2 of the viral genome. In our study, the genetic variability of GFLV RNA1 was assessed by immunocapture (IC) - reverse transcription (RT) - polymerase chain reaction (PCR) - restriction length fragment polymorphism (RFLP), followed by cloning and sequencing. Nucleic and amino acid diversities were tested. The possibility of mixed infection and recombination was examined, as was the association between symptomatology and genetic variability. The study provided new insights into the biological diversity of GFLV.

10.29 FROM APOPLAST PROTEOMICS TO GENE IDENTIFICATION IN HEXAPLOID WHEAT BY ANALYZING THE HOST RESPONSE IN LEAF RUST-RESISTANT LR1 AND LR9 LINES. **V. Pós, É. Hunyadi-Gulyás, M. Cserháti, K. Manninger, K. Medzihradszky, J. Györgyey and N. Lukács.** *Dept. of Plant Physiology and Plant Biochemistry, Corvinus University of Budapest, Budapest, Hungary. Email: veronika.pos@uni-corvinus.hu*

We characterized changes in the apoplast protein pattern in response to leaf rust infection in three near-isogenic wheat lines of 'Thatcher' differing in resistance to *Puccinia recondita* f. sp. *tritici* pathotype 43722. Intercellular fluid was obtained from vacuum-infiltrated leaves; SDS-PAGE-separated proteins were identified by MALDI-TOF and LC-MS-MS. We found that endo-1,3-beta-D-glucanase and chitinase (type I or II) enzymes and proteins belonging to the PR 1 family were induced earlier and more intensely in the resistant genotypes Lr1 and Lr9 than in the susceptible control line. Colorimetric assays confirmed the increased activity and differing induction kinetics; the pH optimum of enzyme activities was at the slightly acidic pH characteristic of the apoplast. These antifungal proteins are known participants in the antifungal defence of numerous resistant and sensitive plants, but since they may also be significant for seedling resistance when expressed differentially, we performed promoter analysis using the fully sequenced rice genome. The corresponding most homologous genes were identified in rice, and the promoter regions of these isoenzymes were compared to reveal potential common regulatory motifs. Several putative motifs, which may be involved in the differential control of gene expression, were identified in the promoter region of the most sequence-similar isoenzymes. PCR analysis using primers derived from protein sequences further supported the proteomic results, but also showed the refinement required for gene identification by reverse genetics in a hexaploid plant. (Supported by GVOP-3.1.1.-2004-05-0163/3.0).

10.30 MAPPING OF QUANTITATIVE TRAIT LOCI CONFERRING FIELD RESISTANCE TO BLAST IN IRANIAN RICE (*ORYZA SATIVA* L.) POPULATIONS. **H. Sabouri, A. Rezai, M. Kavousi, M. Katouzi and A. Moumani.** *Gorgan University of Agricultural Sciences & Natural Resources, Golestan, Gorgan, Gonbad, Iran. Email: sabouriho@yahoo.com*

In order to study the inheritance of blast field resistance in Iranian rice, an experiment was conducted at the Rice Research Institute of Iran. In this study, we performed a linkage mapping of quantitative trait loci (QTLs) for blast field resistance using F_2 plants/F_3 progenies derived from crosses between the cvs. Tarommahalli (susceptible) and Khazar (resistant). 192 plants of the F_2 population were used to construct a linkage map. A total of 74 markers which covered the whole rice genome and revealed polymorphisms between the parents, were used to determine SSR genotypes of each plant in the F_2 population. We mapped seven putative QTLs on chromosome 1, 3 (two QTLs), 4, 5 and 9 (two QTLs). These QTLs explained 11, 17, 19, 15, 12, 12 and 20% of the total phenotype variation in F_3 lines, respectively. Alleles of 'Khazar' increased the level of resistance in qBFRI-3a, qBFRI-3b, qBFRI-4 and qBFRI-9a QTLs whereas alleles of 'Tarommahalli' decreased the level of resistance in qBFRI-1, qBFRI-5 and qBFRI-9a QTLs. The high level of resistance to blast in 'Khazar' is mainly explained by qBFRI-3b and qBFRI-9b. Application of newly found QTLs for breeding rice with blast field resistance is discussed.

10.31 EXPRESSION OF *SOYBEAN CHLOROTIC MOTTLE VIRUS* REVERSE TRANSCRIPTASE IN THE YEAST *PICHIA PASTORIS.* **M. Saito, M. Suzuki, Y. Takemoto, M. Ugaki and T. Hibi.** *Laboratory of Bioresource Technology, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, 202 Bioscience Building, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan. Email: ugaki@k.u-tokyo.ac.jp*

Soybean chlorotic mottle virus (SbCMV) is the type species of the genus *Soymovirus*, a distant relative of the genus *Caulimovirus* that includes the well-characterized *Cauliflower mosaic virus* (CaMV). Viruses in both genera replicate through transcription of a double-stranded (ds) genomic DNA into an RNA intermediate followed by reverse transcription of the RNA into dsDNA. The virus-encoded reverse transcriptase (RT) is essential for their life cycles. The nucleotide sequence of the 8.2-kb SbCMV genome has been determined, and the RT may be encoded in ORF V, but its characterization has never been attempted. The RT of CaMV was reportedly expressed both in *E. coli* or the yeast *Saccharomyces cerevisiae*, but was active only when expressed and correctly processed in yeast. To analyze the properties of SbCMV

RT, we expressed it in the yeast *Pichia pastoris*, because expression can be induced with methanol. ORF V was modified by optimizing its codons according to the codon usage of *P. pastoris* and was tagged with six histidine residues and a myc epitope. Expression was performed in MMH medium with induction using 0.5% methanol. After 48hr of induction, cells were collected and lysed in the extraction buffer using glass beads. Expressed SbCMV RT was purified using an Ni-NTA resin column, and was detected by Western blotting using an anti-myc-HRP antibody. Further characterization is in progress.

10.32 TRANSCRIPTOME ANALYSIS OF PGPR-MEDIATED DISEASE RESISTANCE AND GROWTH PROMOTION IN RICE. **K. Saveetha, L. Karthiba, D. Saravanakumar, M. Raveendran, T. Raguchander, P. Balasubramanian and R. Samiyappan.** *Department of Plant Pathology, Centre for Plant Protection Studies, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, India. Email: savee_patho2003@yahoo.co.in*

Sheath blight disease in rice caused by *Rhizoctonia solani* is one of the most devastating diseases of rice, causing significant yield loss worldwide. Till now, no major resistance genes to sheath blight have been discovered in cultivated rice. In an attempt to find effective biological means of controlling this disease, *Pseudomonas fluorescens* strain TDK-1 (Thadiankudisai) was found to exhibit a higher degree of suppression of sheath blight and was also found to promote growth in rice. However, the molecular mechanism behind this disease control and growth promotion is not well understood. We attempted to study the PGPR-induced physiological, biochemical and molecular changes in rice. Transcript profiling through DD-RT-PCR strategy was done in rice plants, challenged with sheath blight pathogen (*Rhizoctonia solani*) in the presence and absence of PGPR. Preliminary results showed that PGPR inoculation had significant effects on the expression of around 138 genes (95 upregulated and 43 down-regulated). Challenge by *R. solani* in PGPR-treated plants induced the expression of key defence-related genes like jasmonate O-methyl transferase, ACC deaminase, ubiquitin protein ligase, β-glucosidase, proteinase inhibitor protein, NBS-LRR disease resistance protein, endoglucanase and aspartic proteinase. The results indicated that rice transcripts modulated by PGPR strain TDK-1 play a major role in inducing systemic resistance against sheath blight. Further experiments are in progress to understand the exact role of PGPR in inducing disease resistance and growth promotion in rice.

10.33 GENOME AND TRANSCRIPTOME OF THE FOREST PATHOGEN *HETEROBASIDION ANNOSUM*. **J. Stenlid, M. Garbelotto, U. Kües, F. Martin, H. Solheim, M. Karlsson and Å. Olson.** *Dept Forest Mycology and Pathology Swedish University of Agricultural Sciences, Box 7026, Uppsala, Sweden. Email: jan.stenlid@mykopat.slu.se*

Heterobasidion annosum causes a devastating root rot in conifer plantations and natural forests throughout the northern hemisphere. In a collaboration with the Joint Genome Institute, the genome of *H. annosum* will be the first plant-pathogenic homobasidiomycete to be sequenced, allowing for new insights into plant-microbe interactions. Comparisons with plant pathogens with a gradient of taxonomic relatedness to *H. annosum* will help understanding the evolution of pathogenicity factors. The transcriptomes of *Heterobasidion* during pathogenic and saprotrophic growth have been studied using large-scale EST sequencing and macro-array analysis. Important changes in metabolism during pathogenic growth include upregulation of oxidative metabolism, transporter systems, hydrophobin and toxin production. This project will also gain insights into fungal evolutionary history and biology including development, non-self recognition, mating, white rot pathways, and secondary metabolism.

10.34 CHARACTERIZATION OF THE *PI40(T)* GENE FOR DURABLE RESISTANCE TO BLAST DISEASE IN *JAPONICA* RICE. **J.P. Suh, J.H. Roh, Y.C. Cho, S.S. Han, Y.G. Kim and K.K. Jena.** *National Institute of Crop Science, RDA, Suwon 441-857, Republic of Korea. Email: suhjp@rda.go.kr*

Rice blast caused by the fungus *Magnaporthe grisea* is one of the most destructive rice diseases. Durable resistance to multiple rice blast pathogen populations is an important goal for *japonica* rice breeding. A new source of resistance to Korean blast isolates was identified in an *indica* introgression line, IR65482-4-136-2-2 that has inherited the resistance gene from an EE genome wild *Oryza* species, *O. australiensis* (Acc. 100882). A major resistance gene designated *Pi40(t)* has been located on the short arm of chromosome 6. *Pi40(t)* was linked to a DNA marker, 9871.T7E2b in the 70 Kb chromosomal region using haplotyping and association analysis. *Pi40(t)* has conferred resistance to a number of blast Korean isolates as well as Philippine isolates suggesting broad-spectrum resistance. Four breeding lines possessing favorable agronomic traits as well as the *Pi40(t)* gene in a *japonica* genetic background were developed by marker-assisted backcrossing. These breeding lines showed resistance against 29 virulent Korean field isolates tested by the sequential planting method (SPM) in the greenhouse. The results were similar to the farmer's field data and suggested that *Pi40(t)* might give broad-spectrum durable blast resistance. We are studying the molecular characteristics of the breeding lines carrying *Pi40(t)* and the effect of introgressed chromosomal segments on durable resistance expression in *japonica* background has been determined.

10.35 COMPLETE NUCLEOTIDE SEQUENCE AND GENOME CHARACTERIZATION OF TWO BEET CRYPTIC VIRUSES BCV-1 AND -2. **A. Szegő, N. Enünlü, D. Veliceasa, S.D. Deshmukh, L. Szilák and N. Lukács.** *Corvinus University of Budapest, Department of Plant Physiology and Plant Biochemistry, Budapest, Hungary. Email: noemi.lukacs@uni-corvinus.hu*

Beet cryptic viruses belong to the *Partitiviridae* family and have small, segmented dsRNA genomes with segments of 1-2 kbp. Up to now three different beet cryptic viruses (BCV-1, -2, -3) have been identified. Our aim was to characterize the genomic dsRNA of BCV-1 and -2 by cDNA cloning and sequence analysis. The BCV-1 dsRNAs were 2008 bp and 1783 bp long; computer analysis of the sequences predicted ORFs encoding the putative RNA-dependent RNA-polymerase (RdRp, 616 aa, 72.5 kDa) on dsRNA1 and a putative coat protein (489 aa, 53.4 kDa) on dsRNA2. The putative RdRp of BCV-2 is encoded by a 1575 bp dsRNA, whose predicted protein consists of 475 aa (54.2 kDa). The other dsRNA (1598 bp) of BCV-2 encodes a predicted protein of 426 aa (49.6 kDa). The viral RdRps were identified on the basis of conserved motifs considered as markers of dsRNA viral RdRps; six such motifs were found in each putative RdRp sequence. Multiple RdRp alignments showed that BCV-1 and BCV-2 clearly belong to different groups of cryptic viruses. BCV-1 RdRp showed most homology to *Vicia cryptic virus*, *White clover cryptic virus 1* and to some fungal viruses in the Partitiviridae,

whereas BCV-2 resembled a group of cryptic viruses, which, to our present knowledge, occur only in plants. The untranslated regions of the two viruses were clearly different, whereas the 5'- and 3'-ends of both segments of each virus had strong homology. (Supported by OTKA T032393 and GVOP-3.1.1. - 2004-05-0163/3.0; Anita Szegő is a Ferenc Deák Scholarship fellow.)

10.36 HAPLOTYPE ANALYSIS OF *FUSARIUM* RESISTANCE QTL IN WHEAT FROM NORTH AMERICA AND EUROPE. **L. Tamburic-Ilincic D. Somers, A. Brule-Babel, G. Fedak and A. Schaafsma.** *University of Guelph, Ridgetown Campus, 120 Main St. E, Ridgetown, Ontario, N0P 2C0, Canada. Email: tamburi@ridgetownc.uoguelph.ca*

Fusarium head blight (FHB) is an important disease of wheat in all temperate regions of the world. *Fusarium graminearum* Schwabe is the predominant species that causes FHB and produces the mycotoxin deoxynivalenol (DON) in the grain. The most practical way to control FHB is through development of resistant cultivars. However, breeding for FHB resistance and low DON content in grain has been difficult because various types of resistance to FHB in wheat are quantitatively inherited. We developed a line of winter wheat germplasm named RCATL33 with combined FHB resistance from spring wheats 'Sumai 3' from China and 'Frontana' from Brazil. Genetic analysis indicated that RCATL33 contains Frontana alleles on chromosome 3A and Sumai 3 alleles on chromosome 3BS. Additional sources of FHB resistance have now been identified in winter wheats from North America and Europe. We have initiated studies to identify additional sources of resistance with which to bolster the known FHB resistance. Winter wheats from North America, France, Hungary, Czechoslovakia and the Netherlands were haplotyped with microsatellite markers linked to FHB resistance quantitative trait loci (QTLs) on chromosomes 2D, 3A, 3B, 5A and 6B. The diagnostic band sizes for markers associated with QTLs for FHB resistance in winter wheat from North America and Europe will be reported. Parents with unique FHB resistance QTLs have now been identified and strategies for pyramiding the various sources of resistance will be discussed.

10.37 LOCATION OF SEXUAL AND VEGETATIVE COMPATIBILITY LOCI ON AN AFLP-BASED GENETIC LINKAGE MAP OF *AMYLOSTEREUM AREOLATUM*. **M.A. van der Nest, B. Slippers, M. Wilken, K. van Zyl, J. Stenlid, M.J. Wingfield and B.D. Wingfield.** *Department of Genetics, FABI, University of Pretoria, Pretoria, 0002, South Africa. Email: brenda.wingfield@FABI.up.ac.za*

Amylostereum areolatum, together with its symbiont *Sirex noctilio,* seriously threatens pine forestry in the southern hemisphere. In this symbiosis, the fungus mainly reproduces asexually, with *A. areolatum* arthrospores being spread by the female wasp. However, sexual reproduction also occurs. *Amylostereum areolatum* has a tetrapolar heterothallic mating system where sexual compatibility is determined by two unlinked mating-type (*mat*) loci (A and B), each with a number of sub-loci. Only genetically distinct monokaryons with different allele specificities at both their *mat* loci are sexually compatible. This limitation is thought to play an important role in maintaining diversity for adaptation to changing environments. Asexual reproduction, is thought to keep the fungal population sufficiently homozygous for stability of the *Amylostereum-Sirex* mutualism. A phenomenon known as vegetative incompatibility may further ensure preservation of genotypes adapted for symbiosis. In the vegetative compatibility system, hyphal fusion is only permitted between genetically similar heterokaryons that share the same alleles at all of their heterokaryon incompatibility (*het*) loci. Our research focuses on the genetic mechanisms that control sexual and self recognition in *A. areolatum*. The aim of this study was to characterize the genes present at the *mat* and *het* loci and to locate their position on an AFLP genetic linkage map. Mating and vegetative incompatibility studies suggest that sexual recognition in *A. areolatum* is controlled by two multi-allelic *mat* loci. These studies showed that self recognition in this fungus is controlled by at least two multi-allelic *het* loci. Linkage mapping further demonstrated that these loci are unlinked.

10.38 IN SILICO EVALUATION OF MITOCHONDRIAL GENES AS DNA BARCODE FOR RUST FUNGI. **A. Vialle, N. Feau and R.C. Hamelin.** *Service canadien des forêts, Ressources naturelles Canada, Centre de Foresterie des Laurentides, 1055 du PEPS, P.O. Box 3800, Québec G1V4C7, QC, Canada. Email: nicolas.feau@rncan.gc.ca*

DNA barcoding promises a fast and easy bio-identification system using a 648-bp sequence of the mitochondrial gene encoding cytochrome c oxidase I (CO1-5'). The occurrence of introns and copies of CO1 in fungal mitochondrial genomes can compromise the utilization of this gene for the elaboration of a barcode in urediniales. We proposed an in silico evaluation of the potential of 14 mitochondrial genes, including CO1, commonly found in mitochondrial genomes of Basidiomycota as barcode. First, we inventoried all mitochondrial DNA data publicly available for this phylum, including three rust species, and designed mitochondrial genome recovery strategies. Fourteen mitochondrial genomes were characterized. Second, each gene retrieved was evaluated with a particular emphasis on: 1) intron position and size; 2) occurrence of copies of the gene in both nuclear and mitochondrial genomes and; 3) information provided in context of barcoding e.g. the compromise between the need for universal application across taxa and discrimination among taxa. The genes targeted showed high values of divergence (measured by K2P distances) indicating a good potential for the resolution of lower-level relationships. However, numerous introns, potentially interfering with PCR amplification of the target barcode sequence, occurred in CO1 as well as 6 other genes specifically in rusts. Considering these results and the optimal length of 600-bp recommended for a barcode, we propose three alternative genes to CO1 (ATP6, CO3 and NAD6), to elaborate a barcode for Basidiomycota. Experimental validation of these last genes is currently under investigation among the rust genera *Melampsora* and *Chrysomyxa*.

10.39 MICROARRAY ANALYSIS OF GENES REGULATED BY CAMP IN FUNGAL SYMBIONTS DURING INTERACTIONS WITH GRASSES. **C.R. Voisey, M.J. Christensen, A.K. Khan, R.D. Johnson, Z.A. Park, L.J. Johnson, J.P. Koolaard, J.M. Pratt, S. Rasmussen and G.T. Bryan.** *AgResearch Grasslands, Private Bag 11-008, Palmerston North 4442, New Zealand. Email: christine.voisey@agresearch.co.nz*

The fungal endophytes, *Epichloë* and *Neotyphodium*, form symbiotic associations with cool-season grasses. Growth of the endophytes within grass leaves and reproductive tissues is confined to the intercellular spaces and tightly regulated. The signalling mechanisms coordinating the interaction between host and symbiont remain largely unknown. Invasion of plant tissues by pathogenic fungi is usually dependent on processes regulated

(at least in part) by the ubiquitous signalling molecule, adenosine 3¢, 5¢-cyclic AMP (cAMP). To identify the key molecular mechanisms regulated by cAMP during colonisation of perennial ryegrass (*Lolium perenne*) by the fungal endophyte *Epichloë festucae*, we disrupted the fungal adenylate cyclase gene (*acyA*) which synthesises cAMP from ATP. Growth and morphology of cAMP mutants in culture were severely affected; however some mutants were still capable of forming symbiotic associations with grasses. Microscopic analysis of mutant cAMP endophytes within the host revealed that the tight regulation of hyphal growth normally observed in plants was disrupted and hyphae appeared hyperbranched. To undertake expression profiling of transcripts from ryegrass plants infected with fungal cAMP mutants we custom-designed a dual-species (endophyte/ryegrass) Affymetrix GeneChip® representing ESTs from the closely-related asexual species, *Neotyphodium lolii*. In total 8,540 genes were represented on the microarrays. Comparison of transcript changes between fungal cAMP mutants and wild type controls in association with ryegrass have been used to identify host and symbiont genes affected by disruption of the endophyte cAMP signalling network during interactions with grasses.

10.40 FUNGAL PLASMIDS IN RICE SHEATH BLIGHT FUNGAL PATHOGEN *RHIZOCTONIA SOLANI* AG-1 I A STRAIN GXE4. J. Xie, Y. Fu, D. Jiang and G. Li. *The key lab of plant pathology of Hubei Province, Huazhong Agricultural University, Wuhan, 430070, Hubei, P.R. China. Email: daohongjiang@mail.hzau.edu.cn*

Linear plasmids are extra-chromosomal DNA elements that widely exist in prokaryotes and eukaryotes, for example, linear plasmids were found in some fungi; usually, fungal plasmids do not affect virulence and other phenotypes significantly. *Rhizoctonia solani,* a ubiquitous inhabitant of soils in many parts of the world, harbors several types of linear plasmid. A strain named GXE4 of rice sheath blight fungal pathogen *R. solani* AG-1A was isolated from a typical lesion of sheath blight at Guilin, Guangxi Province, P R China. GXE4 grew on PDA plates slowly, with reduced sclerotium production, and formed abnormal colonies, and almost lost the ability to attack rice. A 3.6 kb nucleic acid segment was always co-extracted with the genomic DNA. This segment was gel-purified and treated with DNase I, RNase A and restriction endonucleases, and the results showed that it was not sensitive to RNase A, but sensitive to DNase I and some endonucleases, confirming that it was DNA. It could be digested by exonuclease III, suggesting that it did not have a protein or knob-shaped structure at the 3' terminus. The segment was subjected to sequencing analysis, and a 3.3 kb spliced sequence was obtained; however, from the sequence, no open reading frame (ORF) could be predicted with the DNAMAN program. So far as we know, the 3.6 kb DNA in strain GXE4 may represent a new type of fungal plasmid.

10.41 PROTEOMIC ANALYSIS OF EXTRACTS OF POTATO TUBERS INFECTED BY *PECTOBACTERIUM CAROTOVORUM* SSP. *ATROSEPTICUM*. R. Yahiaoui-Zaidi. *Université de Béjaia, Faculté des Sciences de la Nature et de la Vie, Route Targa-Ouzzemour, 06000 Béjaia, Algeria. Email: rachida_zaidi@yahoo.fr*

The study of the *Pectobacterum*–potato interaction by using proteomics initially required the development of tools which allowed a better separation of proteins of the potato tuber. Research on pathological markers of particular interest was carried out on protein extracts obtained from potato variety Bintje either healthy or infected with a strain of *Pectobacterium carotovorum* ssp. *atrosepticum*. The extracts were subjected to a two-dimensional electrophoresis (E-2D) to highlight differential proteins. Image analysis with the software "Melanie" made it possible to detect differential protein spots between the two extracts.

10.42 PROTEOME ANALYSIS OF INFECTION-SPECIFIC PROTEINS FORMED IN JAPANESE BIRCH PLANTLETS BY INFECTION OF *INONOTUS OBLIQUUS*. S. Yokota, Y. Takashima, F. Ishiguri, K. Iizuka and N. Yoshizawa. *Department of Forest Science, Utsunomiya University, Utsunomiya, Tochigi, Japan. Email: yokotas@cc.utsunomiya-u.ac.jp*

Inonotus obliquus is a fungus causing stem heart rot of birch trees. The present study aimed at identifying proteins produced specifically in plantlets of *Betula platyphylla* var. *japonica* No.8 by infection of *I. obliquus* strain IO-U1, 2 days after infection. Crude protein preparations were obtained from 3 groups of birch plantlets: those infected with the fungus (T), intact and without injury (C1), and with injury (C2). They were collected 2 days after the treatments, deep-frozen in liquid N2, powdered, and then extracted with a buffer to obtain crude protein preparations. The protein samples were then subjected to two-dimensional electrophoresis (2DE). The gels were stained with silver nitrate, and the gel images analyzed with a 2DE software. The infection-specific proteins were estimated by comparing their pIs and MWs with those in the data base. Comparison with the data base suggested the formation of proteins involved in oxidative burst (carbonic anhydrase, L-ascorbate peroxidase, glutathione peroxidase, glutathione *S*-transferase), lignin biosynthesis (caffeoyl-CoA O-methyltransferase), flavonoid biosynthesis (chalcone-flavonone isomerase), molecular chaperone (heat shock 60 kDa protein), hypersensitive reaction (hypersensitive-induced response protein, glyceraldehydes-3-phosphate dehydrogenase, 20S proteasome), and PR protein (PR10-1). In addition, glutathione *S*-transferase and heat shock 60 kDa protein were identified by MALDI-TOF-MS analysis. It is assumed, therefore, that oxidative burst mainly occurred in birch No.8 plantlets as a defence reaction against *I. obliquus* strain IO-U1 during the early stage of infection.

10.43 INSIGHTS INTO THE GENOME OF THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*. L. Zurletto, E. Deleury, J. Gouzy, P. Wincker, E. Danchin, B. Favery, M.N. Rosso, P. Castagnone-Sereno and P. Abad. *INRA-UNSA-CNRS, UMR1064-6192, Interactions Plantes-Microorganismes et Santé Végétale, 400 Route des Chappes, F-06903 Sophia - Antipolis, France. Email: rosso@sophia.inra.fr*

A high-quality draft genome sequence of the obligatory plant parasite root-knot nematode (RKN), *Meloidogyne incognita* has been completed by scientists from Genoscope, the Genopole Toulouse Midi-Pyrénées and INRA Sophia-Antipolis. Because *M. incognita* is an important parasite with a broad host-range, its biology has been intensively studied. It reproduces by mitotic parthenogenesis with a life cycle of 6 to 8 weeks; second-stage juveniles (J2) hatch from eggs in the soil and invade the root tissues towards the vascular cylinder using a secreted cocktail of enzymes that degrade the plant cell wall and assist the migration. *M. incognita* growth and reproduction depend on the successful establishment and maintenance of a specialized feeding site within the root. After the feeding site is induced, J2s moult into sedentary J3 and J4 juveniles and finally into females that lay eggs out-

side the root. We produced EST libraries from different developmental stages to facilitate the automatic annotation of the genome. A restricted international consortium, involved in the molecular analysis of plant – *M. incognita* interactions, is carrying out a joint effort to produce expert manual annotation, which will greatly benefit from the concomitant availability of other nematode genomes. The complete genome sequence of *M. incognita* offers a great opportunity to improve our understanding of the processes underlying nematode infection of plants, and also to elucidate evolutionary and functional relationships between different lifestyles in the nematode phylum such as mitotic versus meiotic RKNs, animal- versus plant-parasitic species, and parasitic versus free-living species.

GLOBAL SEED HEALTH: CONCERNS AND SOLUTIONS

48.1 DETECTION AND IDENTIFICATION OF SEED-BORNE FUNGI OF PEPPER (*CAPSICUM ANNUUM*).

M.H. Ali, A.M. Abdelmonem and M.R. Rasmy. *Plant Pathology Research Institute, ARC, Giza, Egypt. Email: dimamt@yahoo.com*

Sixteen pepper (*Capsicum annuum* L.) seed samples were collected from Alexandria markets and examined for the presence of seedborne fungi using the standard blotter and agar plate methods (ISTA, 1981). We found that *Alternaria alternata, Aspergillus* spp., *Cladosporium herbarum, Colletotrichum capsici, Curvularia lunata, Fusarium oxysporum, F. semitectum, F. solani, Penicillium* spp., *Rhizopus* sp., *Rhizoctonia solani* and *Stemphylium* sp. were the predominant fungi associated with pepper seeds. The standard blotter method was better than the agar plate method as it detected 11 fungi compared to 8 fungi. Pathogenicity tests revealed that some of the pepper seedborne fungi could produce damping-off and wilt on the pepper cultivars tested. Seed infection levels of pepper with *F. oxysporum* had a significant effect on wilt incidence. Culture filtrate either of *F. oxysporum* or *F. solani* significantly reduced pepper seed germination as compared to *F. semitectum*. By means of specific primers, the *F. oxysporum* was identified as f. sp. *capsici.*

48.2 DEVELOPMENT OF MOLECULAR DIAGNOSIS FOR DETECTION AND QUANTIFICATION OF *ALTERNARIA* SPP. IN TAIWAN.

L.C. Chen, Y.M. Chang, Y.S. Chen, J.C. Tu, C.H. Chiang and T.C. Fang. *Department of plant pathology, National Chung Hsing University 250, Kuo Kuang Rd., Taichung 402, ROC. Email: lcchen@dragon.nchu.edu.tw*

Alternaria species attack numerous crops because of their wide host ranges and persistent survival, and require further study. We collected samples of *Alternaria* spp. from Cruciferae, Solanaceae and the legume family in parts of Taiwan. We verified the presence of *A. tenuissima*, *A. alternata*, *A. longipes* and *A. brassicicola*. Using PCR technology, eleven random primers were used for random amplified polymorphic DNA (RAPD) analysis to find specific DNA markers of *A. brassicicola*. The primer OPA-09 amplified a distinct 830-bp fragment, subsequently cloned and used to design the *A. brassicicola*-specific primers Ab536R and Ab536F. The primers amplified a 536-bp fragment specific for *A. brassicicola*. This PCR test could detect 50 pg of *A. brassicicola* genomic DNA. We are collecting more samples from many host crops to probe into *Alternaria* spp. interspecific and intraspecific genetic variations.

48.3 USE OF OZONE TO CONTROL POSTHARVEST GREY MOLD OF TABLE GRAPES.

F. Ciccarese, T. Ziadi, A. Ambrico, A. Ciccarese, M. Sciacovelli and M. Gallo. *Department of Biology and Plant Pathology, University of Bari, Via G. Amendola 165/A, 70126 Bari, Italy. Email: fciccare@agr.uniba.it*

The main objective of postharvest disease control of table grapes is to preserve quality (appearance, texture, flavour, nutritive value and safety) in the face of attacks by phythopathogenic microrganisms. To reduce table grape losses during harvest and commercialization, ozone, classified as a safe substance "GRAS" by the U.S. Food and Drug Administration, is used. This work reports the results of a trial of ozonated water treatment on table grapes in cold and in ambient temperature storage. The trial was carried out in November in a covered vineyard of table grape 'Italia'. Bunches were treated with ozone or the bio-control agent *Aphanocladium album* isolate Mx-95, or sulphur dioxide. Bunches just after harvest were immersed in ozonated water at a concentration of 4 ppm for 3 minutes. A suspension of Mx-95 was applied with a nebulizer in the field, at a concentration of 1×10^7 CFU/ml three days before harvest. Sulphur dioxide was applied as a film upon the bunches in the package. The incidence of grape rot was assessed after various days of cold and/or ambient temperature storage. Treatments with ozonated water and Mx-95 gave satisfactory disease control. Sulphur dioxide gave the best results in rot control. We acknowledge the technical cooperation of "ECO.AGRO.SERVICE", chemical and environment analysis laboratory, Adelfia (Bari), Italy.

48.4 INCIDENCE OF *MACROPHOMINA PHASEOLINA* – A FUNGUS CAUSING ROOT ROT IN SEEDS OF CLUSTERBEAN, AND MANAGEMENT IN STORAGE BY TRADITIONAL SEED-TREATING MATERIALS AND OILS.

S.C. Jain and R.K. Jaiman. *Department of Plant Pathology, S K N College of Agriculture, Rajasthan Agricultural University, Bikaner, Jobner Jaipur, Rajasthan 303329, India. Email: scjskn@yahoo.com*

Incidence of *Macrophomina phaseolina* was as high as 9.75% in clusterbean, *Cymopsis tetragonoloba* (L.) Taub seed sample CB-5 collected from Jaipur district, out of twelve seed samples collected from farmers in the major clusterbean-growing districts of Rajasthan state. Of four traditional seed-treating materials and four oils used as pre-storage seed dressers and seed coaters respectively, neem leaf powder followed by turmeric powder (at 10 g/kg of seed) as seed dresser and neem oil followed by mustard oil (10ml /kg seed) as seed coater were found effective in reducing the incidence of *M. phaseolina* in clusterbean seed, with improved seed germination after up to six months of storage.

48.5 EVALUATION OF DRY HEAT TREATMENTS FOR ERADICATING *ACIDOVORAX AVENAE* SUBSP. *CITRULLI* FROM WATERMELON SEEDS.

J. Kim, J. Feng, X. Liu and J. Li. *Dept. of Plant Pathology, China Agricultural University, Beijing 100094, P.R. China. Email: lijq231@cau.edu.cn*

Acidovorax avenae subsp. *citrulli* (Aac), causes bacterial fruit blotch (BFB) of watermelon and other cucurbits. Losses associated with possible infected seeds have threatened the existence of the watermelon seed and transplant industries. The most effective control of BFB is exclusion of the bacterium from the field along with the use of pathogen-free seeds. Presently, dry heat treatment (DHT) has been employed for controlling seed-transmitted viruses or other pathogens. However, no reliable DHT for eradication

of Aac from watermelon seeds is available. We evaluated eight different DHT protocols for eradication of Aac from inoculated triploid watermelon seeds cv. Jingxin No.5 and No.6 using grow-out and Bio-PCR assays. The results showed that temperatures from 35 to 50 ºC cannot effectively eradicate the pathogen, and temperatures from 75 to 80 ºC followed cooling without buffer temperatures eradicated the pathogen but significantly decreased seed germination and vigor. The following protocol resulted in effective eradication of Aac without significant impact on germination and seedling vigor: 1) pre-treatment of 35 ºC for 24h followed by 50 ºC for 24 h; 2) treatment of 75 ºC for 72 h; and 3) post-treatment of 50 ºC for 24 h followed by 35 ºC for 24. This work using inoculated seeds suggests that this dry heat treatment protocol may be used in commercial production and marketing.

48.6 PATHOGENIC FUNGI ON WHEAT GRAIN IN SERBIA. **V. Krnjaja, J. Levic and S. Stankovic.** *Institute for Animal Husbandry, Belgrade-Zemun, 11080, Republic of Serbia. Email: VesnaKrnjaja.IZS@gmail.com*

Wheat is one of the most important crops in Serbia, grown on approximately 600,000 ha with average yield of 3,600 kg/ha. Wheat is mainly used to make bread and in human nutrition. For livestock nutrition wheat grain can be used as concentrated livestock feed, and the whole plant can be used as fodder. Considering the economic importance of wheat, primarily in human nutrition, but also in livestock nutrition, the microflora of the wheat grain harvested in 2007 in the vicinity of Belgrade in Serbia was investigated. Wheat grains (3,300) were examined in regard to presence of potentially toxigenic fungal species, especially of genus *Fusarium.* After surface disinfection in sodium hypochlorite, wheat grains were placed on 2% water agar, 10 grains per Petri dish, and incubated for 7 days at 26°C. According to methods by Ellis (1971), Burgess *et al*. (1994) and Watanabe *et al*. (1994), fungal genera were determined with special focus on species of *Fusarium*. The presence of seven fungal genera was established, *Acremoniella* (0.09%), *Acremonium* (0.06%), *Alternaria* (96%), *Dreschlera* (0.3%), *Fusarium* (3.5%), *Nigrospora* (0.03%) and *Penicillium* (0.03%). Within *Fusarium* eight species were identified, *F. graminearum* (63.5%), *F. oxysporum* (1.7%), *F. poae* (0.9%), *F. proliferatum* (5.2%), *F. semitectum* (2.6%), *F. sporotrichioides* (20.9%), *F. subglutinans* (3.5%) and *F. verticillioides* (1.7%). High presence of species *F. graminearum* and *F. sporotrichioides* indicated potential danger of presence of mycotoxins zearalenone and trichothecene.

48.7 RAPID DETECTION OF *PEPPER MILD MOTTLE VIRUS* IN SEEDS OF PEPPER (*CAPSICUM ANNUUM*) BY INDIRECT DOT-IMMUNOBINDING ASSAY. **X. Li.** *Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, P.R. China. Email: lxh1962@yahoo.com.cn*

Pepper mild mottle virus (PMMoV) in pepper seeds was detected by indirect dot-immunobinding assay (I-DIBA) on nitrocellulose membrances and by DAS-ELISA. We found that in PMMoV-positive pepper seed, the detection titre was 1:12800 (w/v) by I-DIBA, and 1:51200 (w/v) by DAS-ELISA. PMMoV in pepper leaf could be detected at maximum dilution of 1:3200 (w/v) by both I-DIBA and DAS-ELISA. Healthy pepper leaf did not give a positive reaction when diluted to 1:800 (w/v). Detection PMMoV in pepper seed by I-DIBA was repeatable and uniform. To clarify rates of PMMoV infection in pepper seeds, seeds of 17 commercial pepper varieties were tested by I-DIBA. In 12 of the varieties, PMMoV was seedborne with different rates from 1.25% to 100%.

48.8* CIMMYT SEED HEALTH LABORATORY: A GUARANTEE FOR THE SAFE EXCHANGE OF WHEAT AND MAIZE SEED AROUND THE WORLD. **M. Mezzalama, N. Valencia-Torres and N. Lozano-Ramirez.** *CIMMYT, Ap 6-641, 06600 México DF, Mexico. Email: m.mezzalama@cgiar.org*

Seed-borne pathogens can infect or kill plants and cause chemical changes in grain, degrading grain content or releasing mycotoxins, with potentially harmful effects on humans or livestock. To guarantee the safe movement of CIMMYT germplasm with partners in more than 100 countries worldwide, the CIMMYT Seed Health Laboratory (SHL) monitors and certifies the quality of Center germplasm products for the absence of pathogens and for seed viability, as well as guarding against the establishment or spread of exotic pests and diseases on imported seed. The SHL applies national and international standards for seed testing to guarantee quality and reliability; in 2006, the laboratory obtained full accreditation with the ISO/IEC 17025-2005 norm, *General requirements for the competence of testing and calibration laboratories.* In 2006 the SHL tested 5,000 maize, wheat, barley, and triticale samples for the presence of seed-borne fungal, bacterial, and viral diseases, including pathogens of quarantine importance such as *Tilletia indica, Fusarium verticilloides, Pantoea stewartii*, *Xanthomonas translucens* pv. *undulosa*, *Barley stripe mosaic virus*, *Wheat streak mosaic virus*, and *Maize dwarf virus*. During 2006, CIMMYT sent 368 shipments of small grain cereals (wheat, triticale, and barley) to more then 100 countries and 298 shipments of maize to more then 150 countries.

48.9 EVALUATION OF HEALTH AND GERMINATION OF BEAN (*PHASEOLUS VULGARIS*) SEEDS BEFORE AND AFTER PROCESSING. **M.H.D. Moraes, A. Tremocoldi, M.L.P. Ghizzi and J.O.M. Menten.** *USP/ESALQ, C.P. 09, Piracicaba, SP, 13418-900, Brazil. Email: mhdmorae@esalq.usp.br*

Bean seeds may carry important pathogens such as *Sclerotinia sclerotiorum, Phaeoisariopsis griseola* and *Xanthomonas axonopodis* pv. *phaseoli*, which may be carried internally or externally. Besides causing disease, some fungi may affect seed germination. Processing aims to improve the characteristics of a seed lot through the removal of impurities and of seeds that present undesirable characteristics. The seed characteristics such as size, shape and weight are taken into account. Some pathogens may affect seed development, making them smaller, wrinkled or deformed. Our purpose was to check the effect of processing on bean seed health and germination. The samples were characterized as follows: fields I and II (first and second stages), III (third stage), IV and V (fourth and fifth stages). In fields I, II and III white mold and angular spot were detected, while in fields IV and V angular spot and *Fusarium* wilt occurred. The health of samples was evaluated using 3 methods (blotter test, modified blotter test and semi-selective medium); for germination we used the roll paper method. The bacteria *X. axonopodis* pv. *phaseoli* was not found. *S. sclerotiorum* was detected in two samples and, after the processing, it was eliminated from one of those samples. *P. griseola* was detected in almost all samples but processing did not eliminate the fungus from the seeds. The results showed that processing had not eradicated the seed pathogens and had not improved the germination.

48.10* SEED HEALTH INITIATIVES AT ASIAN SEED HEALTH CENTRE. **H.S. Prakash, N.M. Carmen and J. Torp.** *DOS in Applied Botany and Biotechnology, University of Mysore, Manasagangotri, Mysore 570006, India. Email: hsp@appbot.unimysore.ac.in*

Seed health is of great concern to farmers, quarantine personnel, germplasm managers, seed producing agencies, certification agencies, phytosanitary authorities, quality control managers, seed traders, seed sector programmes and regulatory authorities. Seed health is a specialized discipline, and personnel managing the seed sector need specialized training in detection and diagnosis of seed-borne pathogens, especially in Asia and Africa. With this background, the Danish Seed Health Centre for Developing Countries (DSHC) has established two regional centres for Asia and Africa. The Asian Seed Health Centre (ASHC) at Mysore, India, has developed course modules such as short courses, modular courses, a proficiency certificate course and a Master in Philosophy course for seed health training. In addition ASHC has identified research areas like creation of a molecular database for seed-borne pathogens, molecular diagnostics, and biological control and management of mycotoxin problems. Seed health is an important non-tariff seed trade barrier, hence accurate diagnosis of seed-borne pathogens using serological and molecular techniques is of special significance. This demands creation of a molecular database and country-wide seed health status reports. The molecular techniques should be validated against conventional seed health tests. Biological seed treatment is gaining special attention in organic farming. The mycotoxins in seed could also be an important trade barrier; it is therefore intended to develop molecular markers to identify toxigenic fungal strains and to manage mycotoxin problems. In the coming years we hope to consolidate these efforts initiated by DSHC.

48.11 BUNT AND SMUT DISEASES OF WINTER WHEAT IN LATVIA. **I. Priekule.** *Latvian Plant Protection Research Centre, Lielvardes 36/38, Riga LV-1006, Latvia. Email: ilze.priekule@laapc.lv*

Bunts and smuts are harmful to winter wheat *Triticum aestivum* L. in Latvia. The most widespread from the beginning of 20th century is common bunt, *Tilletia caries* (DC.) Tul. Spread of the disease is increasing sporadically. The main causes are introduction of minimalized no-ploughing soil management, supplemented by wheat growing without crop rotation, use of infected seed material treated with fungicides of unsatisfactory quality, and favourable climatic conditions during the germination period. In 2006 a previously unknown bunt disease was observed on winter wheat. Identification was made using light microscopy. Morphologic characteristics were similar those reported for dwarf bunt (*T. controversa* Kühn.). Epifluorescence microscopy was used to confirm the identity as *T. controversa*. The introduction of this disease into Latvia can derive from import of seed material from European countries where dwarf bunt is widespread. After accession of Latvia to the European Union, some of the seed material control measures currently in force were cancelled. Dwarf bunt was a quarantine disease, and seed material import was controlled in Latvia till 2004, preventing the introduction of such diseases. In 2007 loose smut, *Ustilago tritici* (Pers.) Rostr., was observed on winter wheat (previously found rarely in spring wheat). The main reasons of disease appearance can be seed import, and use chiefly of surface-active fungicides for winter wheat seed treatment. In conclusion, global trade of cereal seed material without quarantine barriers can lead to introduction of new diseases, therefore it is necessary to have local control measures of imported lots and field monitoring to avoid new fungi spreading from primary sources.

48.12* ALTERNATIVE SEED TREATMENTS FOR ORGANIC LEGUME PRODUCTION. **F. Tinivella, L. Hirata, M.A. Celan, S. Wright, T. Amein, A. Schmitt, E. Koch, J. van der Wolf, S.P.C. Groot, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: federico.tinivella@unito.it*

European law provides that, in organic farming, organically produced seed should be used. Therefore new sanitation treatments need to be developed which do not use classical fungicides but still produce seed free from pathogens which can strongly affect yield of the crop. Greenhouse trials were carried out in order to test the efficacy of different seed treatments alternative to chemicals against *Colletotrichum lindemuthianum* causing anthracnose on bean and *Ascochyta pisi* causing *Ascochyta* blight on pea, respectively. Resistance inducers, commercially formulated micro-organisms, non-formulated selected strains of different micro-organisms (fungi, bacteria and yeasts) and plant extracts were applied as dry or liquid seed treatments on naturally infested seeds. Seedling emergence and disease incidence and/or severity were recorded. Possible suppression of *Ascochyta* blight in peas was masked by the high rate of infection (around 20%), so almost all seed treatments turned out to be ineffective in controlling infection, with the exception of treatments with thyme oil and a strain of *Clonostachys rosea*. *C. lindemuthianum* infection was successfully controlled by all resistance inducers. However, they also caused a significant reduction of plant emergence. Among the micro-organism formulations, *Bacillus subtilis*-based formulations provided the best protection to anthrachnose. Some bacterial strains, a disease-suppressive saprophytic strain of *Fusarium oxysporum* and the mustard powder-based product Tillecur™ proved to be effective against bean anthrachnose. Tillecur™ (Schaette AG, Bald Waldsee, Germany) and thyme oil are promising for application in integrated pest management and could possibly be used in organic farming.

48.13 EFFECTIVENESS OF SEED TREATMENT AGAINST SEED TRANSMISSION OF PLANT PATHOGENS WITH DIFFERENT ECOLOGICAL CHARACTERISTICS. **E.U. Toropova, V.A. Chulkina.** *Chair of Plant Protection Systems and Plant Epidemiology, Novosibirsk State Agricultural University, P.O. Box 253, Krasnoobsk, Novosibirsk region, Russia. Email: helento@ngs.ru*

In Siberia, plant pathogens causing more than 75 per cent of the analyzed 250 most frequent and harmful diseases are transmitted by seed and transplanting stock. Seed is a second transmission pathway for soilborne and leaf-stem pathogens and the main way for seedborne ones. Presowing seed treatment is used for control of pathogens from different epidemiological groups; however, the effectiveness of up-to-date seed treatment depends heavily on adaptation of plant pathogens to different kinds of ecological environment. Research shows that presowing seed treatment halts the life cycles of seedborne plant pathogens (smut, ergot) by interrupting the main transmission mechanism. As the main means of transmission for soilborne pathogens (common root rot, *Fusarium* wilt) is through soil, with seed transmission as additional, the dynamics of epidemics depend on the number of pathogens in soil, and seed treatment can only prevent the formation of new epidemic foci in non-infected soil. In the

case of leaf-stem infections (*Septoria* blight, rust, powdery mildew), seed treatment prevents the formation of primary epidemic foci around plants which grew from infected seeds. Systemic seed treatments lower plant susceptibility to leaf-stem infections and slow down the epidemic process.

48.14 SIMULTANEOUS DETECTION OF MULTIPLE PATHOGENS IN SEEDS USING MAGNETIC CAPTURE HYBRIDIZATION AND REAL TIME PCR. **R.R. Walcott, Y. Ha and K. Johnson.** *4315 Miller Plant Sciences, Athens GA, 30602, USA. Email: rwalcott@uga.edu*

To improve the detection of plant pathogens in seeds and thereby limit global dissemination, we evaluated magnetic capture hybridization and multiplex real-time PCR. Single-stranded hybridization capture probes targeting *Acidovorax avenae* subsp. *citrulli* and *Didymella bryoniae* DNA were covalently attached to magnetic particles and used to selectively concentrate template DNA from cucurbit seed extracts. Sequestered template DNAs were then simultaneously amplified by multiplex real-time PCR using pathogen-specific TaqMan PCR assays. After optimization, MCH multiplex real-time PCR displayed a detection threshold of 10 and 10^5 *A. avenae* subsp. *citrulli* and *D. bryoniae* CFU /ml, respectively which was ten times more sensitive than direct real-time PCR. MCH also improved the reliability of the detection of both pathogens. MCH multiplex real-time PCR consistently detected both target pathogens in artificially and naturally infested cucurbit (watermelon and melon) seedlots (n=5,000 seeds) containing as little as 1 seed infected with *A. avenae* subsp. *citrulli* and 1 seed infected with *D. bryoniae*. Using this technique large seed samples can be tested for multiple pathogens in less than 36 h which is a significant improvement over conventional seedling grow-out or blotter tests that require at least 14 days. Since this assay does not require analysts to be familiar with the unique morphological characteristics of the pathogens it can be readily deployed in private, and government seed health testing laboratories worldwide. Finally, the assay is applicable to all pathogen and seed types and its application for the detection of different combinations of phytopathogenic bacteria, viruses and fungi will be discussed.

HOST-PATHOGEN INTERACTIONS

37.1 CHANGES IN TOTAL PHENOLS AND RESISTANCE TO *SCLEROTINIA* STEM ROT IN RAPE OILSEED *BRASSICA NAPUS* CULTIVARS GROWN IN NORTHERN IRAN UNDER GREENHOUSE CONDITIONS. **A. Abasi Moghadam and M. Behrozin.** *Genetic Division and National Plant Gene bank of Iran, Seed and Plant improvement Institute, Mahdasht Ave., Karaj, Iran. Email: abasimoghadam@gmail.com*

Stem rot or white mould of rape caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is prevalent in northern Iran. During 2004-2005 canola cultivars grown in this area were tested with the local *Sclerotinia* isolates SS1Go, SS2SA and SS3CA. Complete randomized block design was used with three replications in two groups; one group was inoculated with three-day-old mycelial discs grown on PDA with the addition of a wound on the petiole; the other group was inoculated without wounding. The experiment was repeated three times. Results indicated significant different between treatments ($p < 0.01$) regarding host resistance and fungal virulence. Okapi and Talaieh were the most resistant cultivars and SS1Go was the most virulent isolate. Disease development was greater when inoculation included wounding. Total phenols (mg/g leaf) were measured on four spring cultivars (Hayola, RGS003, Sarigol and Quantum) and two winter cultivars (Okapi and Talaieh) following inoculation with isolate SS1Go with and without wounding, at seedling stage (SS) or stem-formation stage (SFS) using a complete randomized block design with three replications. Sampling was done at 24, 48, 72, 100 and 144 h after inoculation for SS plants, with an additional sampling at 192 h for SFS plants. There was a significant difference between treatments ($p < 0.01$) regarding total phenol changes in SS and SFS plants. Increase in total phenol had started 24 h after inoculation and continued till 72 h after inoculation. 'Okapi', then 'Talaieh', showed the greatest total phenol increase for SFS plants, whereas 'Talaieh', then 'Hayola', showed most increase for SS plants, inoculated with or without wounding.

37.2 FIRST REPORT OF *ARMILLARIA MELLEA* ON *QUERCUS* SPP. IN AZERBAIJAN. **D.N. Aghayeva and T.C. Harrington.** *Institute of Botany, Azerbaijan National Academy of Sciences, Patamdar 40, Baku AZ1073, Azerbaijan. Email: adilzara@hotmail.com*

Fruiting bodies of a species of *Armillaria* were found at the base of dead and dying oak trees (*Quercus robur* and *Quercus castaneifolia*) in October and November 2006 at several locations in the Great Caucasus region of Azerbaijan. Basidiomycetes (specimens MAH96D and MAH96Q, Central Herbarium, Institute of Botany) were also found on *Quercus* spp., *Castanea sativa*, and *Juglans regia* in 2004. Isolates from 27 basidiomycetes (Harrington *et al*., 1992) formed abundant rhizomorphs on malt extract agar. The identification was confirmed by rDNA sequences of the intergenic spacer region (IGS-1, accession numbers EF637084 and EF637085) and internal transcribed spacer region (ITS, EF637086, EF637087). Fragments of approximately 610 bp were amplified and sequenced from two isolates (including CBS 121534) with the IGS-1 primers LR12R and O1 (Coetzee *et al*., 2005). Fragments of approximately 800 bp were amplified and sequenced from eight isolates using the primers ITS1F and ITS4 (White *et al*., 1990). These sequences closely matched those of *A. mellea* isolates from Europe (AF163600, AF451822, AY163605, AF163599, AY848938 and AF163580). *A. mellea* from Asia, Europe, eastern North America and western North America form four distinct lineages based on ITS and IGS sequences (Coetzee *et al*. 2005). Phylogenetic analyses grouped the Azerbaijan isolates with isolates from Europe and Iran (*A. mellea* subsp. *mellea*) and separated them from from Asian (*A. mellea* subsp. *japonica*, Terashima *et al*., 2006) and North American isolates. This is the first confirmed report of an *Armillaria* species from Azerbaijan and the first identification of *A. mellea* sensu stricto.

37.3 SELECTION FOR EXTREME BASAL RESISTANCE OF BARLEY AGAINST POWDERY MILDEW. **R. Aghnoum and R.E. Niks.** *Wageningen University, Laboratory of Plant Breeding, P.O. Box 386, Wageningen, The Netherlands. Email: reza.aghnoum@wur.nl*

Powdery mildew caused by the biotrophic pathogen, *Blumeria graminis* f.sp *hordei,* is an important disease of barley worldwide. More than 100 race-specific resistance genes that mainly act by hypersensitivity response (HR) have been identified in barley and were deployed in breeding programs to combat the disease. Due to appearance and spread of virulent isolates of the pathogen, virtually all of these genes have become ineffective. As an alternative

approach for achieving race non-specific and durable resistance, genes for quantitative resistance may be accumulated to deploy this basal resistance. QTLs for basal resistance were mapped in four populations of barley: L94 × Vada, Nure × Tremois, Steptoe × Morex and Oregon Wolfe. From each mapping population one genotype with the lowest infection frequency was selected, and the most resistant segregants of the four populations were intercrossed in two rounds to accumulate QTLs for basal resistance. Progeny of the crosses between the lines of the different mapping populations showed transgressive segregation both towards resistance and susceptibility. The most resistant double cross F2 progeny only allowed about 15% successful haustorium formation. The race specificity of basal resistance after inoculation with races of the pathogen with different virulence spectra is being determined.

37.4 EVOLUTION OF TOXIN PRODUCTION AND PATHOGENICITY IN THE TOMATO PATHOTYPE OF *ALTERNARIA ALTERNATA*. **Y. Akagi, H. Akamatsu, H. Otani, M. Yamamoto, T. Tsuge and M. Kodama.** *Laboratory of Plant Pathology Tottori University, Tottori, Japan. Email: mk@muses.tottori-u.ac.jp*

The tomato pathotype of *A. alternata* (*A. alternata* f. sp. *lycopersici*) produces host-specific AAL-toxins and causes *Alternaria* stem canker. A 120 kb genomic region that contains the AAL-toxin biosynthetic (*ALT*) gene cluster in the tomato pathotype was sequenced and compared with corresponding sequences of the fumonisin biosynthetic (*FUM*) gene cluster in *Gibberella moniliformis*. The genomic region includes 19 putative ORFs and 12 of these showed similarity to the genes in the FUM gene cluster. The *ALT* gene cluster resides on a 1.0 Mb conditionally dispensable chromosome (CDC) found only in the pathogenic and AAL-toxin-producing strains of *A. alternata*, and homologues of the genes were not detected in non-pathogenic strains of *A. alternata*. Genomic sequences of *ALT1* and another PKS gene located outside of the *ALT* gene cluster, both of which reside on CDCs in the tomato pathotype strains were compared to tomato pathotype strains collected worldwide. This revealed that the sequences of the genes located on the CDCs, in the strains with different geographical origins, are identical. On the other hand, sequences of other genes located on the chromosomes other than CDCs are not identical in each strain, indicating that the origin of the CDCs might be different from other chromosomes in the tomato pathotype. We propose that the ability to produce AAL-toxins determining specific pathogenicity could be potentially spread among *A. alternata* strains by horizontal transfer of the CDCs, and thus provide a possible mechanism whereby new pathogens can arise in nature.

37.5 INFLUENCE OF ENVIRONMENTAL FACTORS AND RELATIVE WATER CONTENT ON THE SUSCEPTIBILITY OF CITRUS CULTIVARS TO *PHYTOPHTHORA CITROPHTHORA* AND *P. NICOTIANAE*. **L.A. Álvarez, D. Gramaje, P. Abad-Campos and J. García-Jiménez.** *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Email: luialber@eaf.upv.es*

Variability in the susceptibility of three *Citrus* cultivars: mandarin Clementine cv. 'Hernandina', Fortune hybrid and sweet orange cv. 'Lane-Late' to monthly inoculations of *P. citrophthora* and *P. nicotianae* (syn. *P. parasitica)* was evaluated from October 2004 to December 2006. Inoculations were made on branches in the field and excised branches *in vitro* with both pathogens. The areas of canker obtained were found to be correlated with environmental parameters and relative water content. In field assays, cultivars inoculated with *P. citrophthora* developed largest lesions during March-June (spring) and with *P. nicotianae* from June to August. However, canker areas on excised citrus branches inoculated with both pathogens were largest during March–May. There were no correlations between environmental parameters during the 27-month period and the extent of colonization by *P. citrophthora.* Nevertheless, a significant relationship was observed for mean relative humidity and mean ambient temperature with the length of lesions during October to May of each year. There was a strong positive correlation between mean maximum relative humidity and mean maximum temperatures with the size of cankers caused by *P. nicotianae.* Canker areas were significantly related to relative water content of Fortune and Lane-late cultivars in orchard assays. In excised branches, the lesions caused by both pathogens were not significantly related to any seasonal change in water content. Seasonal changes in susceptibility of citrus cultivars to *P. citrophthora* and *P. nicotianae* may facilitate timing of disease control measures to coincide with periods when disease development is greatest.

37.6 EFFECT OF SUPERNATANT OF *MAGNAPORTHE GRISEA* CONIDIAL SUSPENSIONS ON THE INFECTION TO RICE PLANTS. **S. Ando, S. Tanabe, H. Shigemori, K. Yamada, C. Akimoto-Tomiyama, Y. Nishizawa and E. Minami.** *National Institute of Agrobiological Sciences, Tsukuba, 305-8602, Japan. Email: sando@affrc.go.jp*

Rice blast disease caused by the hemibiotrophic fungus *Magnaporthe grisea* leads to serious damage worldwide for production of rice. In the rice – *M. grisea* interaction, there are several reports that *M. grisea* produces some factors, such as toxins, to enhance their infection. However, the chemical or biological features of these are not fully understood. We found infection-promoting activity in the supernatant of conidial suspensions (SCS) prepared from *M. grisea* grown on oatmeal agar medium. Washed conidia were resuspended in water or SCS and inoculated to excised rice leaf sheaths (cv. Nipponbare), and infection rates were scored by light microscopy at 40-48 h post-inoculation. Addition of SCS promoted the extension of infectious hyphae compared to water. The activity against Nipponbare (Pia) was found in the SCS from five virulence and three avirulence strains of *M. grisea*, and the effect was much more evident in compatible than incompatible interactions. Boiled SCS retained the activity, indicating that the factor is heat-stable. The factor was soluble in methanol but not in ethyl acetate. Chemical and biological characterization of the factor will be reported.

37.7 DIFFERENTIAL PATHOGENIC RESPONSE IN STRAWBERRY LEAVES AND PETIOLES BY *COLLETOTRICHUM ACUTATUM*. **F.T. Arroyo, J. Moreno, P. Daza, J. Torreblanca, B. de los Santos and F. Romero.** *IFAPA Centro Las Torres-Tomejil, Apdo Oficial, Spain. Email: franciscot.arroyo@juntadeandalucia.es*

The susceptibility of leaves and petioles from 'Camarosa' strawberry plants to *Colletotrichum acutatum* was tested using a severity index based on infection response. Symptoms developed on both leaves and petioles were characterised through 30 days. Small flecks or light brown spots were observed on leaves and they reached a size of 1 to 5 mm. However, symptoms caused on petioles were dark brown sunken lesions and they reached a size of 30-50 mm. Well-developed acervuli, which produced masses of orange-pink spores, were also observed at this stage on petioles.

According to infection response, leaves were evaluated as resistant to *C. acutatum* whereas petioles were evaluated as susceptible. The fungal infection process on these tissues was also studied using light and electron microscopy. Conidium development was similar on both leaves and petioles, and conidia began to germinate within 4 h after inoculation by forming a germ tube from the ends of the conidium. Also, globose or subglobose appressoria were formed from the germ tube tip or directly from the conidium. Secondary conidium production or microcyclic conidiation, produced from conidial and hyphal phialides, was observed on leaves but not on petioles. At ultrastructural level, differences between the infection and colonisation of leaves and petioles by *C. acutatum* were also noted. Necrosis of the epidermal and parenchyma cells caused by hyphae was extensive in petioles, but colonisation of the pathogen was limited to 2-5 epidermal cells in leaves.

37.8* SUPPRESSION OF DEFENCE-ELICITING MAMPS BY CALCIUM-BINDING EPS: A COMPONENT OF BACTERIAL VIRULENCE. S.N. Aslam, A. Molinaro, D. Chinchilla, T. Boller, G. Erbs, M-A. Newman and R.M. Cooper. *Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK. Email: bssrmc@bath.ac.uk*

Bacterial surface components are shed inadvertently during infection, and result in activation of myriad defences. These defence elicitors or MAMPs include flagellin, LPS and elongation factor (EF-Tu), and we have found that Gram-negative peptidoglycan (PGN) is also active. Some pathogens have evolved MAMPs to avoid recognition. We will describe examples with PGN and its more active constituent muropeptide dimer. Early plant responses involve generation of reactive oxygen species (ROSs), extracellular alkalinization, and deposition of wound callose. Most defence pathways require an initial influx of calcium ions from apoplast to symplast. Our data suggest that many bacteria chelate apolplastic Ca^{2+} and suppress this mode of signalling. Most extracellular polysaccharides (EPSs) such as alginate (*Pseudomonas syringae*), xanthan (*Xanthomonas campestris* pv. *campestris*) and amylovoran (*Erwinia amylovora*) are polyanionic and bind calcium. We have shown with aequorin-transformed *Arabidopsis* that pure EPSs from all the main genera of Gram-negative plant pathogens can suppress calcium flux associated with MAMP recognition. Specific binding of flg22 to FLS2 receptor on *Arabidopsis* cells is not blocked by EPSs. EPS knockout strains trigger calcium flux to the cytosol that is not induced by the respective WT strains. Defence gene expression, callose deposition, ROS and pH increase are elicited by MAMPs and by EPS-minus mutants, but are suppressed by EPS supplied in pure forms or produced from WT cells. The widespread production of these ion-binding polymers by diverse pathogens suggests that EPSs play a key role in establishing compatibility by suppressing MAMP recognition as well as providing protective functions from biotic and abiotic stresses.

37.9 THE DYNAMICS OF *RHYCHOSPORIUM SECALIS* IN WINTER AND SPRING BARLEY AND THE ROLE OF INOCULUM SOURCES. S.D. Atkins, B. Fraaije, J.A. Lucas and B.D.L. Fitt. *Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK. Email: simon.atkins@bbsrc.ac.uk*

Leaf blotch, caused by *Rhynchosporium secalis*, is an economically important disease of barley, resulting in annual yield losses of $ 5.2M. To limit epidemics, cultivar resistance and fungicides are used. However, the pathogen is variable and can render barley resistance ineffective and develop insensitivity to fungicides. To understand the dynamics of *R. secalis* and investigate potential inoculum sources, winter and spring barley trials were established at sites throughout the UK, using replicated plots of barley cultivars (two susceptible; two resistant), with half the plots sprayed with a conventional fungicide regime to control *Rhynchosporium*. The dynamics of disease development were investigated by visual assessment and quantitative PCR (qPCR). Burkard spores samplers were operated at two sites to measure concentrations of air-borne *R. secalis* particles. Grain samples, alternative hosts (grasses and other cereal crops) and soil were tested to determine their role in the spread of *R. secalis*. Results indicated that the pathogen can colonise plants extensively without developing visual symptoms throughout the growing season, and can infect the grain. Visual assessment of symptoms is therefore not a reliable method of scoring disease. Colonisation of winter barley was greater than colonisation of spring barley and was detected earlier (GS13). QPCR distinguished susceptible and resistant cultivars. Air-borne *R. secalis* particles were scarce. The presence of both mating types was established at all trial sites. Amounts of *R. secalis* DNA on harvested grain were greater than amounts on grain sown and was found on grain from plants with no visual symptoms.

37.10 DISSECTING MOLECULAR EVENTS THAT GOVERN THE COMPATIBLE INTERACTION BETWEEN *PHYTOPHTHORA PARASITICA* AND *ARABIDOPSIS THALIANA*. A. Attard and H. Keller. *UMR-Interactions Plantes-Microorganismes et Santé Végétale, INRA1064-CNRS6192, Université Nice-Sophia Antipolis, B.P. 167, 400 Route des Chappes, 06903 Sophia Antipolis Cedex, France. Email: agnes.attard@sophia.inra.fr*

Oomycetes of the genus *Phytophthora* are fungus-like plant pathogens that are devastating for crop plants. Due to their particular physiological characteristics, no treatments against diseases caused by these microorganisms are available. To develop such treatments, it appears essential to dissect the molecular mechanisms that underlie the interaction between *Phytophthora* species and host plants. Data are scarce because pathosystems involving *Phytophthora* have not been amenable to genomic approaches. To analyze the susceptibility mechanism in *Arabidopsis thaliana*, we developed a new pathosystem involving the model plant and *P. parasitica*. Like the majority of *Phytophthora* species, *P. parasitica* is soilborne, infecting a wide range of host plants. The new pathosystem was developed based on inoculating roots with *P. parasitica* zoospores, thus representing the most common, natural mode of infection for soilborne oomycetes. Cytological analyses showed that *P. parasitica* is responsible for massive invasion and complete destruction of *A. thaliana* roots. Zoospores reach the root tip surface, germinate and develop an appressorium-like structure that enables penetration of epidermal cells. Invasive growth occurs mainly between cells, and subsequently hyphae reach the aerial organs, which leads to plant killing within 18 days. The novel interaction model we present here offers considerable possibilities to investigate the genetic factors that govern root infection by pathogenic oomycetes. The results of whole-genome transcript profiling developed.

37.11 EARLY PRO- / ANTIOXIDATIVE INTERPLAY BETWEEN HOST AND PARASITE. A.A. Aver'yanov, T.D. Pasechnik, V.P. Lapikova, T.S. Romanova, L.M. Gaivoronskaya, O.S. Abramova, Vl.V. Kuznetsov and C.J. Baker. *Research Institute of Phytopathology, B. Vyazemy, Moscow region, 143050, Russia. Email: averyanov@post.ru*

The fungal pathosystems of rice blast (with *Magnaporthe grisea*) and of cucurbit scab (cucumber with *Cladosporium cucumerinum*) were studied with regard to events occurring in the infection droplet (including formation of reactive oxygen species and their scavenging by antioxidants) at one day post-inoculation, as these are criticial for the disease outcome. It was found that pure water alone, in long contact with leaves, might confer some resistance on the plant. The effect was coupled with enhanced superoxide production. In addition to the plant leaves, germinating spores of the parasite may contribute significantly to the overall production of O_2^- and H_2O_2. ROS of fungal origin may even suppress fungal development along with disease severity, as was observed in too dense or too dilute spore suspensions. Antioxidant activities, namely, those of catalase and superoxide dismutase were found in diffusates of both rice and *M. grisea* cells. Intense light stimulated the fungal secretion of H_2O_2-scavengers that may be an adaptation to photodamage. While antioxidant activity, regardless of its origin, in the infection droplet may favor compatibility, evidence has been obtained that plants seem to inactivate alien antioxidant enzymes. Therefore, the liquid phase of the infection droplet exhibits opposing pro-oxidant/antioxidant activities whose balance is likely one of the determinants of innate and acquired disease resistance.

37.12 NADPH OXIDASE-MEDIATED GENERATION OF REACTIVE OXYGEN SPECIES AND THEIR ANTIOXIDANTS DURING *COLLETOTRICHUM SUBLINEOLUM* INFECTION IN SORGHUM. **P. Basavaraju, N.P. Shetty, H.S. Shetty and H.J. Lyngs Jørgensen.** *University of Copenhagen, Faculty of Life Sciences, Department of Plant Biology, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark. Email: bpu@life.ku.dk*

Infection of sorghum by the hemibiotrophic pathogen *Colletotrichum sublineolum* (isolate CP2126) induced a superoxide burst 12 hours after inoculation (hai), followed by accumulation of H_2O_2 at 48 hai in the resistant cv. SC146 and the intermediately resistant cv. SC326 but not in the susceptible cv. BTx623. In BTx623, a late and massive H_2O_2 accumulation at 96 to 120 hai correlated with the necrotrophic stage of infection. In-gel assays for NADPH oxidase showed induction at 6 and 12 hai in SC146 but not in BTx623. Also immunoblots of the $gp91^{phox}$, $p67^{phox}$, $p47^{phox}$ and $p22^{phox}$ subunits of the NADPH oxidase complex indicated that they were involved in generation of ROS. Native PAGE analysis for superoxide dismutase showed four isoforms, two of which (SOD2 and SOD3) were CuZn-SODs, SOD1 was a Mn-SOD and SOD4 a Fe-SOD. In SC146, SOD2, SOD3 and SOD4 were induced after inoculation, whereas none of the SOD isoforms were induced in BTx623. Infiltration of diphenyleneiodonium to inhibit NADPH oxidase and catalase to scavenge H_2O_2 reduced H_2O_2 accumulation and increased infection in all the three cultivars. Activities of the important H_2O_2 scavengers catalase, ascorbate peroxidase and glutathione S-transferase were induced differently after infection in the three cultivars, whereas there was no apparent correlation with the pattern of H_2O_2 accumulation. This indicates that the antioxidants work in a concerted way with varying importance during the course of infection.

37.13 ASSESSMENT OF *ALTERNARIA DAUCI* HOST RANGE AND PRELIMINARY STUDIES OF FUNGAL PHYTOTOXIC COMPONENTS. **C. Boedo, P. Hudhomme, M. Briard, V. Le Clerc, T. Guillemette, P. Simoneau and P. Poupard.** *UMR PaVé No. 77, INRA/INH/Université d'Angers, UFR Sciences, 2 Bd Lavoisier, 49045 Angers, France. Email: cora.boedo@etud.univ-angers.fr*

Alternaria dauci is a necrotrophic pathogen responsible for *Alternaria* leaf blight (ALB) of carrot. *A. dauci* host range is not well defined: for some authors this fungus may have a narrow host range limited to some Apiaceae species, and for others, it may infect species from different botanic families. Little is known concerning the *A. dauci* pathogenicity determinants. The purposes of the present study were (1) to clarify the *A. dauci* host range and (2) to identify potential phytotoxic compounds in *A. dauci* culture filtrates. Two *A. dauci* strains were inoculated to ten Apiaceae species, six wild *Daucus* species and five species from other botanic families. Symptoms were scored using a disease rating scale. Isolations were made from plants with developed symptoms and *A. dauci* was diagnosed on the basis of conidium morphology and specific PCR. Symptoms due to *A. dauci* were observed for all plant species tested, except for maize and leek. Further experiments are necessary to better characterize the ALB symptoms. In order to identify potential phytotoxic compounds produced by the fungus, *A. dauci* crude culture filtrates were applied to detached carrot leaves. Brown necrotic spots surrounded by a chlorotic halo developed, strongly suggesting the synthesis of toxic compound(s) by *A. dauci*. Chemical characterization of the putative toxins is in progress.

37.14 RESISTANCE TO *PYRENOPEZIZA BRASSICAE* (LIGHT LEAF SPOT) IN *BRASSICA NAPUS* (WINTER OILSEED RAPE). **E.F. Boys, J.S. West, C.P. Werner, G.J. King, P.S. Dyer and B.D.L. Fitt.** *Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK. Email: emily.boys@bbsrc.ac.uk*

Light leaf spot (*Pyrenopeziza brassicae)* is one of the most important diseases of oilseed rape in the UK and northern Europe, with estimated UK yield losses ranging from € 9 million to € 32 million per season. It decreases leaf photosynthetic area and plant vigour, increases susceptibility to frost damage in winter and causes premature pod ripening and seed shedding in summer. Resistance to *P. brassicae* in current commercial cultivars is not thought to be major gene-mediated and is insufficient to control the disease without the use of fungicides. A doubled haploid population derived from a cross between a susceptible breeding line and a resistant breeding line thought to carry major gene-mediated resistance to *P. brassicae* was used in this work. An experiment was carried out in controlled environment conditions in which eight doubled haploid lines, their two parents and commercial cultivars Elan (the most resistant current commercial cultivar) and Hearty (susceptible) were inoculated with a mixed suspension of *P. brassicae* conidia, and symptom development was compared between lines/cultivars. Plants were then left to senesce, and inoculated leaves incubated to induce the production of apothecia (containing ascospores). This experiment confirmed that the doubled haploid population is segregating for resistance to *P. brassicae*. The fungus is able to undergo asexual sporulation on "susceptible" lines, but not on "resistant" lines. Sexual reproduction resulting in the production of ascospores, however, was possible on debris from both "susceptible" and "resistant" lines, whose leaf surfaces had been extensively inoculated artificially with conidia.

37.15 INVESTIGATING THE PHENOTYPE OF A MAJOR GENE-MEDIATED RESISTANCE TO *PYRENOPEZIZA BRASSICAE* IN *BRASSICA NAPUS*. **E.F. Boys, K.H. Reilly, J.S. West, C.P. Werner, P.S. Dyer and B.D.L. Fitt.** *Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK. Email: emily.boys@bbsrc.ac.uk*

Pyrenopeziza brassicae causes light leaf spot on winter oilseed

rape (*Brassica napus*), currently controlled through the use of fungicides and cultivars with quantitative resistance. 'Imola' (a breeding line from CPB Twyford) is thought to contain at least one major resistance gene against *P. brassicae*. Previous work has shown that *P. brassicae* is unable to undergo asexual sporulation on 'Imola' and a "black flecking" phenotype is instead observed after inoculation with *P. brassicae* conidia in controlled environment conditions. Unlike asexual sporulation, which is widespread across the leaf lamella in susceptible cultivars, the "black flecking" phenotype is only associated with the leaf vein tissue. An experiment was carried out in controlled environment conditions in which 'Imola', a susceptible breeding line and four commercial cultivars ('Elan', 'Winner', 'Navajo' and 'Hearty') were inoculated with a suspension of *P. brassicae* conidia, and the amount of *P. brassicae* DNA present in the leaf tissue was compared between lines/cultivars using quantitative PCR. In addition to whole leaf samples, samples of leaf vein tissue and leaf lamella tissue were also compared for each line/cultivar. This experiment showed that tissue from the resistant line, 'Imola', had significantly less *P. brassicae* DNA than tissue from the other cultivars (including 'Elan', currently the most resistant commercial cultivar) and that the amount of *P. brassicae* DNA increased with time although 'Imola' remained asymptomatic. The proportion of *P. brassicae* DNA present in samples of vein tissue compared to lamella tissue was greater in 'Imola' than in other cultivars.

37.16 EFFECT OF ESCA-ASSOCIATED FUNGI ON WHITE AND RED GRAPEVINE CULTIVARS. G. Bruno and L. Sparapano. *Department of Biology and Plant Pathology, University of Bari, Via Amendola, 165/A, 70126 Bari, Italy. Email: sparlor@agr.uniba.it*

Togninia minima (Tmi), *Phaeomoniella chlamydospora* (Pch) and/or *Fomitiporia mediterranea* (Fme) are three well known esca-associated fungi. We compared two cultivars of *Vitis vinifera*, 'Italia' and 'Sangiovese' differing in susceptibility to these fungi. Scytalone, isosclerone, pullulan, and enzymes (laccase, tannase, peroxidase) produced by the pathogens, and host defence compounds (phenolics) were used for the comparison. The fungal metabolites were found in bioactive concentrations in leaves, rachises and berries of esca-affected vines at various stages of growth. The findings suggest that at least a part of esca foliar and fruit symptoms are caused by toxic fungal metabolites originating in the infected tissues and translocated to the crown via the xylem stream. Absorption of weak solutions of the fungal metabolites by sound leaves and berries of both cvs. induced esca-like symptoms. The presence of laccase, tannase and phenoloxidase in infected tissues aided symptom development. The concentration of constitutive or inducible phenolics in both cvs. varied in relation to phenological phases. Values of almost all the parameters measured in this study indicated that grapevines infected with Tmi and Pch gave a different response than vines infected by Tmi, Pch and Fme. These results indicate that infection by all three fungi gives rise to a range of biochemical and physiological events leading to the full scale of esca symptoms.

37.17 ESCA-ASSOCIATED FUNGI AND GRAPEVINE RESPONSE: INDUCTION OF HOST DEFENCE SUBSTANCES AND THEIR DEGRADATION BY THE PATHOGENS. G. Bruno and L. Sparapano. *Department of Biology and Plant Pathology, University of Bari, via Amendola, 165/A, 70126 Bari, Italy. Email: gbruno@agr.uniba.it*

Esca of grapevine (*Vitis vinifera*) is a complex disease whose symptoms may arise from the combined effect of several pathogenic and physiological factors. After infection, grapevines synthesize several compounds such as phenolics, phytoalexins (e.g. viniferins) and glycolic acid. During vegetative growth, synthesis and accumulation of some phenolics were monitored in leaves of cv. 'Italia'. Leaves were collected from vines showing severe symptoms of brown wood-streaking associated with dual infection of *Togninia minima* (Tmi) and *Phaeomoniella chlamydospora* (Pch), or of brown wood-streaking plus white rot caused by Pch, Tmi and *Fomitiporia mediterranea* (Fme) together. Healthy vines were used as controls. Fungal infection caused an increase in total phenolics as determined by the Folin-Ciocalteu method. Concentration of some phenolics (benzaldehyde derivatives, benzoic acid derivatives, cinnamic acid derivatives, flavonols, flavonol-3-O-glycosides, flavan-3-ol derivatives) increased in esca-affected vines compared with healthy vines. Synthesis and localization of these phenolics appeared to be associated with resistance against esca-associated fungi. Assays on plates containing malt-agar or modified Czapek-agar or in a liquid Czapek medium amended with 0.01-1 mM of the phenolics mentioned above, showed only slight inhibition against Pch, Tmi and Fme cultures. However, the presence of several phenolics in the growth medium induced each fungus to produce extracellular phenol-degrading enzymes (laccase and peroxidase). In infected vines, esca-associated fungi were able to elicit defence reactions which led to production of antimicrobial phenolics. Conversely these substances are easily degraded by the enzymes produced by the pathogens.

37.18 RESISTANCE OF A COLLECTION OF RYE AND TRITICALE VARIETIES TO ERGOT (*CLAVICEPS PURPUREA*). B. Cagas, A. Lebeda, M. Ondrej and L. Odstrcilova. *Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71 Olomouc-Holice, Czech Republic. Email: ales.lebeda@upol.cz*

The reason for studying resistance of rye and triticale to ergot of rye [*Claviceps purpurea* (Fr.) Tul.] was a large number of sclerotia in harvested rye in the Czech Republic in recent years. Under greenhouse conditions varieties of rye (*Secale cereale* L.) and triticale (*Triticosecale* Wittm.) were artificially inoculated with conidial suspensions of ergot. The level of resistance of each variety was assessed by the rate of honeydew production and by the number and weight of sclerotia produced. The highest honeydew production was found in the varieties Seldo, Aventino and Albedo (2.88-2.32 drops/ear), and the lowest was in Picasso, Fernando and Pollino (0.05-0.68 drops/ear). In inoculated varieties of triticale no honeydew was observed. The highest number of sclerotia was produced in the rye varieties Albedo, Matador and Aventino (9.6-7.6 sclerotia/ear), the largest weight of sclerotia in the varieties Albedo, Aventino and Selgo (0.33-0.23 g/ear), whereas the smallest number of sclerotia was recorded in Fernando, Fugato and Picasso (2 sclerotia/ear), and the lowest weight Picasso, Fernando and Pollino (0.056-0.09 g sclerotia/ear). In all inoculated varieties of triticale, except Lupus and Sekundo, sclerotia were at a minimum number of 0.37-0.026 sclerotia/ear and their weight was 0.018-0.0019 g/ear. The experiments confirmed that rye varieties are more susceptible to ergot than triticale, and that large differences in resistance between rye varieties are genetically controlled.

37.19 EFFECTS OF NITROGEN AND CARBON STARVATION ON POLYGALACTURONASE GENE EXPRESSION IN *PYRENOCHAETA LYCOPERSICI*. R. Caiazzo, E. Lahoz, M.

Aragona, A. Infantino and A. Crescenzi. *CRA-CAT, Unità di Ricerca per le Colture alternative al Tabacco, Via P. Vitiello 108, 84018 Scafati, Italy. Email: rosa.caiazzo@entecra.it*

Cell wall degrading enzymes (CWDE), such as pectin methyl esterase, pectin liase, cellulase and polygalacturonase are secreted by fungi during the initial stages of pathogenesis. In particular, polygalacturonases (PGs) are involved in the pathogenesis of several host-pathogen interactions. The present study reports the effects of nutrient limitation on the expression of PG genes in order to understand whether starvation could mimic the conditions of the initial stage of pathogenesis of *P. lycopersici* on tomato. For this purpose cDNA, prepared from starved and replete fungal mycelia was amplified with primers PER-PEF, specific for PG. PG expression of *P. lycopersici* grown on complete medium was compared with cDNA obtained from fungi grown on media low in nitrogen or carbon. Differences in patterns of amplified cDNA were observed: two characteristic fragments were obtained when the fungus was grown in low nitrogen. In contrast, fungus grown in complete media and in presence of low carbon showed a similar pattern except for the presence of one fragment found only under carbon starvation. Each amplified fragment could represent a different isoform of the enzyme. The different fragments were sequenced and characterized in order to better understand which were the molecular forms expressed during interaction with the host. The results suggest that the low availability of carbon and nitrogen does mimic the initial growth conditions of *P. lycopersici* in tomato and, probably, the involvement of polygalacturonase regulation in pathogenesis.

37.20 *SUGARCANE MOSAIC VIRUS* INFECTION UP-REGULATES GENE EXPRESSION IN MAIZE. Y.Y. Cao, Y. Shi, T. Zhou, H.F. Li and Z.F. Fan. *Department of Plant Pathology and State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing 100094, P.R. China. Email: fanzf@cau.edu.cn*

Sugarcane mosaic virus Beijing isolate (SCMV-BJ) systemically infects maize and causes dwarf mosaic symptoms. To investigate the viral infection-induced up-regulated gene expression changes in maize at different time intervals post inoculation, a suppression subtractive hybridization cDNA library was constructed. A total of 454 cDNA clones had their expression up-regulated by SCMV-BJ infection, and 168 of these have been sequenced. Sequence analyses indicated that 58 sequences were redundant, and among the 108 viral infection-induced up-regulated cDNA clones, 7 were identical to known maize sequences, 30 were putative novel genes, and 71 contained regions that share 60%~90% identities to various sequences from the GenBank. The significance of these results will be discussed together with those from investigations on the molecular interactions between SCMV and maize genes to identify the genes of maize involved in development of this viral disease.

37.21 DIFFERENTIAL GENE EXPRESSION IN TOMATO INFECTED BY *TOMATO SPOTTED WILT VIRUS* IN PRESENCE OR ABSENCE OF ARBUSCULAR MYCORRHIZAL COLONIZATION. M. Catoni, L. Miozzi, V. Fiorilli, M. Novero, L. Lanfranco and G.P. Accotto. *CNR – Institute of Plant Virology, Strada delle Cacce 73, 10135, Torino, Italy. Email: m.catoni@ivv.cnr.it*

Tomato (*Solanum lycopersicon*) is a member of the Solanaceae, one of the most economically important plant groups and the most valuable in terms of vegetable crops. Tomato is used as a model solanaceous plant susceptible to a wide range of microrganisms. Among viruses, the thrips-vectored *Tomato spotted wilt virus* (TSWV, genus *Tospovirus*) is a major cause of crop loss in tomato and several other vegetables worldwide. Tomato can also establish symbiosis with arbuscular mycorrhizal fungi (AMF) such as *Glomus mosseae*, which are known to confer benefits on host plants in terms of mineral nutrition and protection against some kinds of stress. In this work, using microarray technology and a set of approximately 12,000 tomato genes represented in the TOM2 oligonucleotide chip (Cornell University, USA), we compared the genes differentially regulated during TSWV infection in tomato shoots and roots, in presence or absence of *G. mosseae* root colonization. Experiments were conducted with three biological replicates for each condition studied: mock inoculation, virus infection, AMF colonization, and virus infection of AMF-colonized plants. Although virus accumulation in leaves and roots seemed not to be influenced by fungal colonization, several genes showed differential expression during virus infection in the presence of *G. mosseae*. Microarray expression results were validated by real-time RT-PCR. These studies will bring better understanding on how mycorrhizas can influence metabolic pathways in virus-infected plants.

37.22 CELLULAR INTERACTIONS BETWEEN *MYCOSPHAERELLA FIJIENSIS* AND BANANA: IMAGING ROS PRODUCTION. M.J.B. Cavalcante, J. Escoute, M. Lartaud and J.L. Verdeil. *Histology and Plant Cell Imaging Laboratory, CIRAD TA A 96/02 Avenue Agropolis, 34398 Montpellier, France. Email: maria.barbosa@cirad.fr*

Most plants present natural defense strategies against biotic stresses, being regulated by two main mechanisms: Non-host resistance and gene-to-gene resistance. One of the faster activated defense reactions in the host plant tissues against the pathogen is the accumulation of reactive oxygen species (ROS) like H_2O_2, O_2 – and OH-. The oxidative explosion is a defense answer after pathogen recognition, inducing a hypersensitive reaction (HR) leading to a localized cell death, resulting in the limitation and blocking of pathogen development. Black Sigatoka resistant banana cultivar, 'Calcutta 4', and the susceptible one, 'Grande Naine', were in vitro infected by *Mycosphaerella fijiensis*. For these two cultivars, a comparative kinetics study of the interactions was done 2, 5, 7, 10 or 16 days after inoculation using DAB (3,3–diaminobenzidine) dye for imaging the production of H_2O_2 under the microscope. DAB reacts with H_2O_2 producing a dark brown pigment in the cells near the infection site, characterizing the hypersensitivity reaction. After 7 days of inoculation, the resistant banana cultivar 'Calcutta 4', presented accumulation of H_2O_2 inducing the hypersensitivity reaction that was increased with time until 16 days. Meanwhile, the susceptible one 'Grande Naine' did not present H_2O_2 accumulation either hypersensitivity reaction during time course. For the first time we report here the presence of H_2O_2 that could be related to banana resistant cultivar defense, however, the entire process remains to be elucidated.

37.23 DIVERSITY OF GENE EXPRESSION AND PRODUCT STRUCTURE IN *BOTRYTIS CINEREA*. E. Cettul, P. De Marta, D. Rekab and G. Firrao. *Dipartimento di Biologia Applicata alla Difesa delle Piante, Università di Udine, Via Scienze 208, 33100 Udine, Italy. Email: firrao@uniud.it*

The high genetic variability of natural populations of the ascomycete pathogen *Botrytis cinerea* (telomorph *Botryotinia fucke-*

liana) is a key to its success, enabling it to use diverse attack strategies and molecular weapons. In the model system *B. cinerea* - *Arabidopsis thaliana*, we identified several combinations of pathogen strain / host accession with different behaviors. We report time course monitoring of pathogen and host gene expression that shed light on the variability of signal perception and gene activation occurring during this interaction. We also found that *B cinerea* populations show high diversity in selected gene sequences, according to the role of the given gene product during the interaction with the host. Our sequence analysis of the complete set of endopolygalacturonase genes (*Bcpg1 – 6*) of 32 *B. cinerea* strains showed that genes with the same biochemical function are under different selection pressures. *Bcpg3*, *Bcpg4*, *Bcpg5*, and *Bcpg6* showed modest and mostly silent polymorphism, with minimal occurrence of mutants that could compromise enzymatic activity. In contrast, *Bcpg1* and *Bcpg2* were more variable, and a likelihood ratio test showed them to be under positive selection, suggesting that escaping host recognition is the driving selection force for these genes. Thus, different sequence divergence and selection are consistent with different roles played by the different gene products during the interaction of pathogen and host.

37.24 PRIMARY STUDIES ON SYMPTOM RECOVERY OF TOBACCO PLANTS INFECTED BY *CUCUMBER MOSAIC VIRUS* STRAIN M. M.S. Chen, Y.X. Ma, S.F. Zhu, H.Y. Chen, Z.X. Du and L.N. Chen. *Insitute of Animal and Plant Quarantine, Chinese Academy of Inspection and Quarantine, Beijing 100029, P.R. China. Email: zhushf@netchina.com.cn*

The M strain of *Cucumber mosaic virus* (CMV) can induce the phenomenon of symptom recovery on tobacco (*Nicotiana tobacum* cv. White Burley). The CMV-infected bottom leaves exhibit yellowing symptoms, while the middle leaves above the initially infected bottom leaves showed no symptoms, although the later newly formed upper leaves showed symptoms again. To investigate the mechanisms of this symptom recovery, the concentrations of the virus in the lowest virus-infected leaves, in the middle recovered ones, and in the upper ones with symptoms were analyzed by DAS-ELISA. The virus concentrations were calculated using a serial dilution of purified CMV particles as standard. The results showed that the virus concentration is positively related to symptom severity. The virus concentration in the recovered symptomless middle leaves was about 85 to 135 times less than that in the leaves with mosaic or yellow symptoms. Biological experiments and RT-PCR analysis showed that infectious virus did exist in the recovered leaves, but could not replicate to higher concentration and cause obvious symptoms. Our results indicated that there might be an efficient resistant mechanism in the symptom recovery leaves. Moreover, a full-length infectious clone of the M strain of CMV was constructed and the specific antibody against the CMV coat protein was prepared. Now the interaction mechanisms between the M strain of CMV and the tobacco host is being studied.

37.25 SEARCHING FOR PATHOGENICITY DETERMINANTS OF THE CHICKPEA BLIGHT PATHOGEN *ASCOCHYTA RABIEI*. W. Chen and D. White. *USDA-ARS, 303 Johnson Hall, Washington State University, Pullman, WA, USA. Email: w-chen@wsu.edu*

Understanding how pathogens cause plant diseases is a basic biological question that will allow us to explore novel methods of controlling plant diseases. Despite of the importance of *Ascochyta* blight of chickpea, little information about pathogenic mechanisms is available on the pathogen, *Ascochyta rabiei*. Insertional mutagenesis via *Agrobacterium*-mediated transformation was used to generate tagged transformants. After screening more than 800 transformants, eleven were identified to have significantly reduced virulence or lost pathogenicity. PCR and Southern hybridization were used to identify T-DNA integration. Inverse-PCR was used to localize the mutations to identify DNA regions adjacent to insertion sites. DNA sequences adjacent to insertion sites were used as queries in tBLASTx searches of the GenBank database and the genome database of *Stagnospora nodorum*. The translated DNA (576 bp) from transformant ArW8 shared 71% identity (91/128 aa) with the kinesin of *Cochlioblus heterostrophus*. Translated DNA from transformant ArW540 (440 bp) shared 66% identity (86/130 aa) with the transposase protein of the *S. nodorum* transposon molly. The remaining six sequences shared minimal or no sequence similarity with proteins in the databases. In addition, a DNA library of *A. rabiei* was constructed. Clones that contained the genes mutated in the transformants were identified from the DNA library and are being employed in complementation experiments to confirm the functions of the mutated genes. In addition, clones from the DNA library of *A. rabiei* that contained candidate genes like the polyketide synthase gene (*pks*1) and acyl-CoA ligase (*cps*1) gene were identified and will be used in targeted mutagenesis.

37.26 COMPARISON OF WHEAT DEFENCE GENES IN RACE-SPECIFIC AND NON-RACE-SPECIFIC RESISTANCES TO STRIPE RUST. X.M. Chen, T. Coram, M.L. Settles and M.N. Wang. *USDA-ARS, 361 Johnson Hall, Washington State University, Pullman, WA 99164-6430, USA. Email: xianming@wsu.edu*

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is an important wheat disease worldwide. Sustainable control of the disease relies on durable-type resistance and appropriate use of effective non-durable type resistance. All-stage (AS) resistance, which provides complete protection and is qualitatively inherited, is usually race-specific and not durable, while high-temperature adult-plant (HTAP) resistance, which is partial and quantitatively inherited, is non-race specific and durable. For understanding the molecular mechanisms of these two types of resistance, the wheat GeneChip was used to profile the transcriptional changes occurring in wheat lines with or without the *Yr5* gene for AS resistance and *Yr39* for HTAP resistance after inoculation with the stripe rust pathogen. The peak expressions of most *Yr5*-regulated genes occurred at 24 h while significance increase in expression level for most *Yr39*-regulated genes did not occur until 48 h after inoculation. A total of 64 transcripts were identified to be up-regulated by *Yr5* while 99 transcripts were induced during the *Yr39*-mediated HTAP resistance, indicating that HTAP resistance has a broader molecular basis than AS resistance. Of these genes, 14 were common in the two types of resistance and 11 of them were identified as hypersensitive-response genes, which might be related to the hypersensitive response observed in both types of resistance. Six of the *Yr39*-regulated genes but none of the *Yr5*-regulated genes were R genes. Race-specific AS resistance included more PR protein and hypersensitive response transcripts, while overall HTAP resistance involved a greater diversity of defence-related pathways.

37.27 IDENTIFICATION OF THE INTERACTION BETWEEN MAIZE FERREDOXIN-5 AND *SUGARCANE MOSAIC VIRUS*

HC-PRO. Y.Q. Cheng, J. Xu, Z. Liu, T. Zhou, Y. Shi, H.F. Li and Z. Fan. *Department of Plant Pathology, China Agricultural University, Beijing 100094, P.R. China. Email: chengyuqin@cau.edu.cn*

Identification of interactions between viral and host proteins is essential to elucidate the molecular mechanisms underlying the virus infection process in plants. The helper component-proteinase (HC-Pro) of potyviruses is a multifunctional protein playing an important role in symptom expression. To identify maize (*Zea mays*) proteins that interact with *Sugarcane mosaic virus* (SCMV) HC-Pro, we constructed a maize cDNA library in the yeast GAL4 activation domain expression vector, using RNAs extracted from seedlings of the maize inbred line Ye-107. The cDNA library was then screened using the full-length SCMV HC-Pro cistron fused with the GAL4 DNA binding domain. The plasmids of 42 positive clones were sequenced, and three clones shared an identical sequence of 665 bp encoding a predicted protein of 138aa. A BLAST query of the protein sequence revealed that it has a conserved domain fer2 and its sequence was most closely related to maize ferredoxin-5 (Fd-5, 94% sequence identity). Compared with maize Fd-5 which includes a transit peptide (1-38aa) and a mature chain (39-135aa), this protein has a putative 41-aa transit peptide; the mature polypeptide is of the same size but with 5 different amino acids. We also cloned the cDNA encoding the same ferredoxin by RT-PCR using RNA extracted from the leaves of another maize inbred line Zong-san 1. Taken together, we assign this protein as maize Fd-5. The interaction between Fd-5 and HC-Pro in living plant cells were confirmed by using bimolecular fluorescence complementation (BiFC) the reconstitution of YFP fluorescence.

37.28* A PATHOGEN-RESPONSIVE MENTHONE REDUCTASE PLAYS A CRUCIAL ROLE IN RESISTANCE AGAINST MICROBIAL PATHOGENS IN PLANTS. H.W. Choi, B.G. Lee, N.H. Kim, Y. Park, C.W. Lim, I.S. Hwang, D.S. Choi, D.S. Kim, H.K. Song and B.K. Hwang. *Laboratory of Molecular Plant Pathology, College of Life Sciences and Biotechnology, Korea University, Anam-dong, Sungbuk-ku, Seoul 136-713, Republic of Korea. Email: bkhwang@korea.ac.kr*

Plants elaborate a vast array of enzymes that synthesize defensive secondary metabolites in response to pathogen attack. Here, we isolated the pathogen-responsive *CaMNR1* (*Capsicum annuum* menthone: (+)-(3S)-neomenthol reductase) gene from pepper plants and found that the CaMNR1 protein belongs to a member of the short-chain dehydrogenase/reductase superfamily. Gas chromatography-mass spectrometry analysis revealed that purified CaMNR1 catalyzes a menthone reduction reaction with NADPH as a cofactor to produce neomenthol. The reaction has a pH optimum of 7.5 and K_M values of 35.1 and 21.7 *m*M for menthone and NADPH, respectively, with k_{cat} values of 0.039 and 0.053 s^{-1}, respectively. CaMNR1 also possesses a significant catalytic activity for neomenthol oxidation. The *CaMNR1* gene is strongly induced in pepper plants by infection with the avirulent strain Bv5-4a of *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), as well as by treatment with salicylic acid, ethylene and abscisic acid. We examined the cellular function of *CaMNR1* by using the virus-induced gene silencing technique in pepper plants and by overexpressing *CaMNR1* in *Arabidopsis*. *CaMNR1*-silenced pepper plants were highly susceptible to *Xcv* infection and expressed lowered levels of defense-related genes such as *CaBPR1*, *CaPR10*, and *CaDEF1*. Transgenic *Arabidopsis* overexpressing *CaMNR1* exhibited enhanced resistance to infection by the hemi-biotrophic pathogen *Pseudomonas syringae* pv. *tomato* DC3000 and the biotrophic pathogen *Hyaloperonospora parasitica* isolate Noco2, accompanied by induction of the defense-related *PR1* and *PDF1.2* genes. Together, these results indicate that the novel menthone reductase gene *CaMNR1* positively regulates defense responses of pepper plants to pathogen invasion.

37.29 IDENTIFICATION OF PUTATIVE PATHOGENICITY FACTORS IN *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM*. J.R. Conroy, K.C. Goulter, P.M. Schenk and E.A.B. Aitken. *School of Integrative Biology, University of Queensland, St Lucia, QLD, Australia. Email j.conroy1@uq.edu.au*

The identification of fungal pathogenicity genes in the past has focussed on aerial phytopathogens. More recently, additional pathogenicity genes, or genes that contribute to pathogenicity, have been identified in soilborne fungi with *Fusarium oxysporum* being used as a model for understanding these pathosystems. We aim to identify fungal sequences that are preferentially expressed during the early infection stages of *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) on cotton. *Fusarium* wilt disease is potentially devastating to the Australian cotton industry. Currently there is no resistant germplasm, and management of the disease relies upon farm hygiene, crop rotation, and the use of tolerant cultivars. Identifying putative pathogenicity genes in Fov would lead to a greater understanding of this pathosystem, and in the future may facilitate the development of novel control strategies for the industry. Genes differentially expressed during the Fov–cotton interaction have been previously investigated by constructing cDNA libraries and performing microarray analyses. In comparison, we are observing the cotton–Fov interaction transcriptome during the early stages of infection using cDNA-AFLPs (amplified fragment length polymorphisms). Further analysis of differentially regulated fungal sequences includes BLAST analyses against the *F. oxysporum* genome database, and observing gene expression during early infection using real-time quantitative PCR. We are also investigating potential toxic proteins in Fov via the culture filtrate method. Fov produces a range of proteins in liquid culture, and by employing stem cutting and calli bioassays we are able to ascertain protein toxicity through crude and purified fractions.

37.30 SOME ASPECTS OF GENETIC CONTROL OF SOYBEAN'S REACTION TO *FUSARIUM* INFECTION. L. Coretchi, A. Cornescu and N. Gheorghita. *Institute of Genetics and Plant Physiology, Academy of Sciences of Moldova Republic, Chisinau, Moldova. Email: lcoretschi@mail.ru*

Our aim was the elucidation of some aspects of the genetic control of soybean reaction to *Fusarium* infection in a backcross hybrid system. The reaction of soybean to *Fusarium* infection was established for parental genotypes and hybrid populations F1, F2, BC1 BC2, which were obtained by hybridization of the genetic form with varieties differing in level of resistance to *Fusarium* infection: Bucuria, Alina, Kizelniska, and Ki237. Using different mathematical models, the genetic control of resistance to *Fusarium* was elucidated analyzing the intra-allelic (dominance) and inter-allelic (epistatic) gene interaction between homozygote (aa), between heterozygote (dd) and between homozygote and heterozygote loci (ad). The dominance level and heritability coefficients were calculated using the Simmonds and Allard formulas. The results showed that the resistance level of the F_1 genotypes was higher than the parental genotypes, and in the F_2 generation, significant lowering of resistance was not observed. This suggests that additive genes favourable for this character have been accumulated. Analysis of backcross values demonstrated the depend-

ence on genitor values of the respective backcross. The results elucidated the all detected genetic actions implications in the genetic control of soybean reaction to *Fusarium* infection. The variances of the analyzed genetic effects had higher values for the *d* and *dd* effects. The dominance level was partial. The heritability coefficients (broad sense) showed medium values for most of the hybrid combinations. The highest value (0.78) was found for K003 × Kizelniska combinations.

37.31 CHARACTERISATION OF CELLULAR AND MOLECULAR PLANT RESISTANCE MECHANISMS TO *PHYTOPHTHORA CINNAMOMI*. **J.A. Cullum and D.M. Cahill.** *Deakin University, School of Life and Environmental Sciences, Pigdons Road, Waurn Ponds, Victoria 3217, Australia. Email: janealis@deakin.edu.au*

The oomycete *Phytophthora cinnamomi* Rands is a soil-borne plant pathogen of worldwide economic and environmental significance. With a host range greater than 1000 species, it causes devastating losses in agricultural and natural systems by colonising the root system of a susceptible host. In 2000, it was identified as a key threat by the Australian federal government. Field observations have confirmed that some plant species continue to thrive or actively recolonise diseased areas, but very little is known about the mechanisms that underpin resistance. Australian native plants identified as field-resistant were inoculated and examined for the production of cellular resistance markers such as lignin, callose, suberin and reactive oxygen species using histochemistry. In resistant species such as the monocot *Lomandra filiformis*, production of lignin and callose was consistently up-regulated over a period of 7 days. Each of these markers was inhibited to further determine their specific role in defence against the pathogen. A *Zea mays – P. cinnamomi* pathosystem was also optimised in this study as a monocot model to facilitate molecular analysis. Similar cellular responses to those of resistant native plants have been found in this pathosystem, and the induction of resistance pathways is being examined through expression analysis of defence-related genes. Insights gleaned from the study of these interactions will assist in developing effective control methods to combat the pathogen.

37.32 RESISTANCE TO *ARMILLARIA MELLEA* IN *GENISTA MONOSPERMA*: ROLE OF ROOT ISOFLAVONOIDS. **P. Curir, F. Galeotti, M. Dolci and C. Pasini.** *C.R.A.-Unità di Ricerca per la Floricoltura e le Specie Ornamentali, C.so Inglesi 508, 18038 Sanremo, Italy. Email: p.curir@istflori.it*

Genista monosperma Lam. is an important ornamental shrub, grown in the western areas of the Liguria region as a cut flower. Its main fungal parasite is *Armillaria mellea*, a basidiomycete that causes a severe root disease. Several *G. monosperma* cvs are utilized in floriculture and, among them, different levels of constitutive resistance towards the pathogen have been observed, ranging from moderate tolerance (cv Seborghina) to high resistance (cv Merello). The root isoflavonoid contents of these two cvs, plus the susceptible ones 'Alma' and 'Rabassina', were studied. Qualitative and quantitative differences were found. In particular, the isoflavones genistein (4',5,7-trihydroxyisoflavone) and biochanin A (5,7-dihydroxy-4'-methoxyisoflavone) were detected in all four cvs but the ratios and concentrations of these compounds depended on the cv, and the highest isoflavone concentrations were found in the more resistanct cvs. This relationship is probably not a mere association between a molecular marker and a resistant genotype, but may represent a plant defensive response. *In vitro* assays performed with Shaw and Roth medium supplemented with the two isoflavones showed that these molecules are toxic to *A. mellea*. The results obtained suggest that root isoflavones may play a defensive role in *G. monosperma*.

37.33 COORDINATED DEFENCE RESPONSES ELICITED AGAINST *PHYTOPHTHORA*. **R. Daniel and D.I. Guest.** *Faculty of Agriculture, Food & Natural Resources, The University of Sydney, NSW 2006, Australia. Email: d.guest@usyd.edu.au*

Disease resistance in plants results from the timely signalling and expression of complex defences; when these signals fail or are suppressed, plants succumb to disease. *Phytophthora* species have globally-significant cultural, ecological and economic impacts, and can be effectively managed using phosphonate. Phosphonate targets the plant–pathogen interaction but we have yet to discover the particular components of the signalling network on which it acts. We have shown that phosphonate restricts pathogen development by enhancing defence responses in *Arabidopsis thaliana* following challenge by *Phytophthora palmivora*. Within 3 hours of inoculation, *P. palmivora* is arrested within the first three cell layers in phosphonate-treated seedlings. Extracellular release of superoxides occurs 6-7 hours after inoculation, immediately preceding hypersensitive cell death. Phosphonate treatment also enhances the accumulation of soluble phenolics including the phytoalexin camalexin and the deposition of lignin. Our studies indicate that application of phosphonate to *A. thaliana* induces a similar host defence response to that observed in other incompatible host-pathogen interactions.

37.34 THE E3 UBIQUITIN LIGASE SFO1 MEDIATES DISEASE RESPONSES AGAINST SEVERAL NECROTROPHS. **B.M.A. De Coninck, S.L. Delauré, W. Van Hemelrijck, M.F.C. De Bolle, M. Wogulis, J. Callis and B.P.A. Cammue.** *Centre of Microbial and Plant Genetics, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium. Email: bruno.cammue@biw.kuleuven.be*

The *Arabidopsis* esa1 mutant displays enhanced susceptibility towards several necrotrophic fungi including *Botrytis cinerea*, a broad spectrum pathogen, and the non-host pathogen *Fusarium oxysporum* f. sp. *cubense* (Foc), the causal agent of Panama disease on banana. Super-mutagenesis by activation tagging resulted in the isolation of an esa1sfo1 mutant. Compared to esa1 this mutant was even more susceptible to the above pathogens. Subsequently we showed that SFO1 can act independently of ESA1 since down-regulation and overexpression of SFO1 in Col0 plants resulted in higher susceptibility against Foc and increased tolerance towards *B. cinerea*, respectively. The SFO1 gene encodes a RING-type E3 ubiquitin ligase, belonging to the ATL-family. Functional characterization indeed proved that the SFO1 protein has ubiquitin ligase activity *in vitro*, indicating that the enzyme can ubiquitinate target proteins for subsequent degradation by the 26S proteasome. Since SFO1 acts as a positive regulator of the defence response it can be postulated that the target(s) function as negative regulator(s) of the disease response. From a Y2H screen at least two potential targets of SFO1 were identified. First, we will test whether both substrates can be ubiquitinated by SFO1 *in vitro*. Second, their potential role in diseases responses will be examined by analyzing plants with overexpression or downregulation of the corresponding genes. These results will be discussed.

37.35 FUNCTIONAL ANALYSIS OF REACTIVE OXYGEN-RELATED GENES IN THE WHEAT PATHOGEN *MYCOSPHAERELLA GRAMINICOLA*. **S. Deller, J. Keon, J. Antoniw, K. Hammond-Kosack and J. Rudd.** *Centre for Sustainable Pest and Disease Management, Department of Plant Pathology and Microbiology, Rothamsted Research, Harpenden, Hertfordshire, UK. Email: sian.deller@bbsrc.ac.uk*

Mycosphaerella graminicola (anamorph *Septoria tritici*) is a fungal pathogen of wheat leaves causing chlorotic/necrotic lesions that reduce the photosynthetically active leaf area. In the early stages of infection the fungus grows in the intercellular spaces without causing visible symptoms. In the later infection stages fungal biomass increases, hyphal nutrition becomes necrotrophic and localised host-programmed cell death occurs (1). Reactive oxygen species (ROS)-specific stains have shown that hydrogen peroxide and superoxide are present in infected leaves, and that levels increase with the appearance of disease symptoms (1, 3) and are closely associated with the fungal asexual fruiting bodies. Expression profiling of *M. graminicola* during infection of susceptible wheat genotypes has shown a number of ROS-associated genes that show greatly increased expression as symptoms become visible (1, 2, 3). Candidate genes have been selected for functional analysis based either upon the literature or their transcriptional up-regulation in planta during symptom development. Sequencing of the *M. graminicola* genome has enabled deletion of genes through targeted *Agrobacterium*-mediated transformation. Results will be presented describing the functional characterisation of fungal genes involved in the production of ROS or the oxidative stress response. 1. Keon *et al.* 2007, MPMI, 20, 178-193; 2. Keon *et al.* 2005, Fungal Genet. Biol. 42, 376-389; 3. Keon *et al.* 2005, Mol. Pl. Pathol. 6, 527-540. This project receives financial support from Syngenta and the Biotechnology and Biosciences Research Council (BBSRC) of the UK. Rothamsted Research receives grant-aided support from the BBSRC.

37.36 CLUBROOT RESISTANCE IN CAULIFLOWER ASSOCIATED WITH DEPOSITION OF CALLOSE AND A DECREASE IN ROOT ABSCISIC ACID IN THE HOST UPON INFECTION. **E.C. Donald, B. Czerniakowski and I.J. Porter.** *Department of Primary Industries Victoria, Private Bag 15 Ferntree Gully Delivery Centre, Victoria 3156, Australia. Email: caroline.donald@dpi.vic.gov.au*

Fluorescent staining with aniline blue (0.5% aniline blue in 1.15 M dipotassium hydrogen orthophosphate for 12 h) was used to study the deposition of α 1-3 glucan (callose) in roots of clubroot-resistant and susceptible lines of *Brassica oleracea* var *botrytis* (cauliflower) following inoculation with *Plasmodiophora brassicae*. Increased deposition of callose occurred in the resistant host. Two types of reaction were observed. The first type was a 'local' reaction in which small deposits were usually, but not exclusively, observed at the base of infected root hairs. This reaction occurred in 14% of resistant and only 1% of inoculated susceptible root hairs. The second type was a 'general' reaction in which the whole root hair and/or epidermal cell would fluoresce. This reaction occurred in 17% of resistant and only 3% of inoculated susceptible root hairs. Changes in root abscisic acid concentration following inoculation were studied using an indirect ELISA. The largest differences between resistant and susceptible varieties were observed 4 days after inoculation. At this time the concentration of ABA increased by 36 % following inoculation of the susceptible host and decreased by 35 % following inoculation of the resistant host. By 11 days after inoculation these differences were lower, although the trends (increased ABA in the susceptible host and decreased ABA in the resistant host) remained the same. These results suggest that resistance to clubroot in the line of *B. oleracea* var *botrytis* studied was characterised by deposition of α 1-3 glucan (callose) and a decrease in abscisic acid production following infection.

37.37 ATTEMPTS TO DEVELOP TOOLS TO SCREEN COCOA FOR *CACAO SWOLLEN SHOOT VIRUS* RESISTANCE OR TOLERANCE. **H. Dzahini-Obiatey and R.T.V. Fox.** *University of Reading, School of Biological Sciences, Whiteknights, Reading, Berkshire, RG6 6AS, UK. Email: h.k.dzahiniobiatey@reading.ac.uk*

Lack of rapid screening tools for *Cacao swollen shoot* virus (CSSV) is hampering breeding for resistance against the virus, which causes severe destruction to cocoa in West Africa. We recently reported the encasement of viral particles by a dense matrix of phenolic substances, the presence of nucleic acid-rich inclusion bodies and apoptosis, as structural and functional changes that accompany CSSV infection. These changes were found in only two of all the plant tissues examined. We now provide further evidence to confirm that one of the tissues, cotyledons of an infected plant are indeed rich in CSSV DNA. The virus was detected in the cotyledons just 3 days after inoculation using 3 pairs of primers in PCR assays – this is an improvement on previous detection times. The implication of these findings in breeding for CSSV-resistant cocoa is discussed.

37.38 A GEMINIVIRUS DNA β SATELLITE TARGETS THE MIRNA PATHWAY TO PRODUCE AN ABNORMAL PHENOTYPE IN PLANTS. **O. Eini, J.W. Randles and M.A. Rezaian.** *School of Agriculture, Food and Wine, Waite Campus, University of Adelaide, Glen Osmond, South Australia. Email: John.Randles@adelaide.edu.au*

DNA β is a satellite molecule associated with some monopartite begomoviruses in the family *Geminiviridae*. It co-infects with the helper viruses to induce severe symptoms in some host plants, causing economically important diseases. The βC1 protein encoded by DNA β is a pathogenicity determinant and has been reported to suppress post-transcriptional gene silencing (PTGS). We report here that βC1encoded by the DNA β associated with cotton leaf curl disease is also a suppressor of PTGS. Expression of this βC1 in *Arabidopsis* plants produced severe developmental abnormalities which resembled those produced by the mutation of key genes in the miRNA pathway. Expression analysis of genes known to be targeted by the miRNA pathway, and the key genes in the miRNA pathway, showed that βC1 enhances the expression level of some miRNA target genes such as MYB33, which is correlated with abnormal leaf shape in plants. Furthermore, the level of AGO1, a key component of the RNA-induced silencing complex (RISC), but not DCL1, was increased in these transgenic plants. We conclude that βC1 produces abnormal phenotypes in plants possibly by targeting the RNA-induced silencing machinery through interacting with AGO1 or other key factors of the miRNA pathway.

37.39 ELECTRON MICROSCOPY STUDY ON THE INTERACTION BETWEEN *MACROPHOMINA PHASEOLINA* AND SESAME SEEDS. **D.A. El-Wakil and A.M. Mahdy.** *Plant Pathology Research Institute, Agriculture Research Centre, Giza, Egypt. Email: deiaaelwakil@yahoo.com*

The direct impact of seed-borne fungi on seed is considerable. Many fungi are serious parasites of immature and maturing seeds and reduce seed yield both quantitatively and qualitatively. Other fungi, including saprophytes and very weak parasites, may lower seed quality by causing discoloration which may seriously depreciate the commercial value of seeds, particularly of grain when graded for consumption. Studies using scanning electron microscopy (SEM) confirmed the importance of the seed coat, and of seed cells as infection sites, as well as location of the mycelium of the fungus investigated, *Macrophomina phaseolina*. Using SEM we studied the colonization, infection and fungal establishment on different parts of sesame seeds. Colonization of seed tissues by *M. phaseolina* was also detected. Pycnidia of different shapes were observed.

37.40 INNATE IMMUNITY: PLANT RECOGNITION OF BACTERIAL MAMPs. **G. Erbs, T.T. Jensen, J.M. Dow, A. Molinaro, M. Parrilli, R.M. Cooper and M.A. Newman.** *University of Copenhagen, Faculty of Life Sciences, Dept of Plant Biology, Frederiksberg C, Denmark. Email: ger@life.ku.dk*

Innate immune defences of plants include a set of basal responses that can be triggered by the perception of general elicitors that have been termed Microbe Associated Molecular Patterns (MAMPs). Examples of MAMPs include lipopolysaccharides (LPS) from Gram-negative bacteria, peptidoglycans (PGN) from both Gram-positive and Gram-negative bacteria, flagellin, fungal cell wall glucans and chitin. Recognition of MAMPs in both insects and mammals leads to activation of defences and is often mediated by LRR (Leucine Rich Repeat) proteins such as Toll in *Drosophila* and the Toll-like receptors (TLRs) in mammals. LRR proteins also serve as receptors for MAMPs in plants; examples include the flagellin receptor FLS22 and elongation factor Ef-Tu receptor EFR. It is now established that LPS and PGN have myriad effects in plants including elicitation of plant defence responses, induction of the oxidative burst, nitric oxide synthesis, and phosphorylation of mitogen-activated protein (MAP) kinase. However little is known about the perception of LPS and PGN by plants or the associated signal transduction pathways that trigger the plant immune responses. Progress towards identification of plant receptors in *Arabidopsis* for LPS and PGN purified from the black rot pathogen *Xanthomonas campestris* will be presented.

37.41 PATHOGENICITY OF DIFFERENT ISOLATES OF TWO SPECIES OF *FUSARIUM* ON SIX CULTIVARS OF BEAN. **M. Faraji and S.M. Okhovvat.** *Department of Plant Protection, College of Agriculture, University of Tehran, Karadj, Iran. Email: Mfaraji1976@yahoo.com*

Bean plants with yellowing and wilting symptoms were surveyed in different areas of two provinces of Iran. Of 20 isolates of *Fusarium* collected, seven (A1 Gogan, A2 Bilverdi, A3 Savojbolagh, A4 Karadj, A5 Khomein, A6, Ramjin, and A7 Varamin) were found to be pathogenic on all bean cultivars tested in this study. According to their macroscopic and microscopic appearance on appropriate media such as PDA, CLA and PSA, A7 was identified as *Fusarium solani* and six other isolates were identified as *F. oxysporum*. The roots of bean seedlings were soaked in suspensions of the 7 isolates for 5 minutes (10^6 spores/ml.), then transplanted into sterilized soil in 4 pots (as replicates). For the control (A8), the roots were soaked in distilled water. The results showed that the mean percentage of necrotic roots and crowns of isolates A1, A2, A3, A5, A6 and A7 was 20.31% (group a); for isolate A4, it was 43.52% (group b), and for isolate A8 it was 2.77% (group c). A4 was more infectious than the others, rapidly causing wilting, leaf yellowing and decreased growth, but A5 with 25.32% of necrotic roots and crowns, was more pathogenic. Red bean cultivar Goli was more tolerant (with 10.02%) than Red bean cultivar Naz (16.29%) and the others (up to 25.15%).

37.42 PROTEOMICS AND REVERSE GENETICS TO UNDERSTAND GRAPEVINE RESPONSES TO *BOTRYTIS CINEREA*. **S. Ferrari, N. Bertazzon, E. Angelini, M. Borgo and B. Mattei.** *CRA Centro di Ricerca per la Viticoltura, Conegliano (TV), Italy. Email: michele.borgo@entecra.it*

Susceptibility of grapevine towards *Botrytis cinerea*, the causal agent of grey mould, shows great genetic variation. Investigation of the response of different cultivars after infection might identify a correlation between plant basal or induced resistance and the levels of specific proteins. Large-scale analytical techniques may allow the identification of polypeptides more abundant in resistant genotypes or whose levels increase after induction of resistance by infection or elicitor treatment. Protein profiles in grapevine cultivars with different susceptibility to *B. cinerea* were obtained by 2D-gel analysis. The corresponding genes will eventually be identified comparing the protein sequences obtained by mass spectrometry and the genetic sequences deposited in public databases. To confirm the role in defence of the genes thus identified, it is necessary to use reverse genetics. In contrast to other plant species, routine high-throughput methods to study gene function in grapevine are currently not available. For this reason we explored the feasibility of transient expression of double-strand RNA (dsRNA) through infiltration of grapevine leaves with *Agrobacterium tumefaciens*, with the aim of silencing specific genes by RNA interference (RNAi). Transient expression in grape leaves was initially validated using a 35S-GUS reporter construct. Constructs to silence known grapevine defence genes, encoding a polygalacturonase inhibitor, a stilbene synthase and a WRKY transcription factor were tested. This approach may unveil the function of grapevine genes without recourse to generating stable transgenic plants.

37.43 C AND N ALLOCATION AND DIFFERENTIAL GENE EXPRESSION IN EUROPEAN BEECH INFECTED WITH *PHYTOPHTHORA CITRICOLA*. **F. Fleischmann, R. Schäufele, M. Olbrich, H. Schnyder, D. Ernst and W. Osswald.** *Section Phytopathology of Woody Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany. Email: fleischmann@wzw.tum.de*

We investigated changes in allocation patterns of C and N metabolites in *Fagus sylvatica* saplings after root infection with *Phytophthora citricola* and correlated these results with differential gene expression data. For this, we performed a steady state labeling experiment with the stable isotopes ^{13}C and ^{15}N under ambient and elevated CO_2 regimes during the early stage of infection. During the experiment we measured gas-exchange of beech mesocosms including the isotopic signature of CO_2. The C and N allocation of saplings was measured from biomass samples taken eight times within a two week period after infection. Differential gene expression analyses were performed in leaves and roots using a cDNA micro-array for beech. *P. citricola* infection reduced dark respiration rates of beech mesocosms, while net assimilation rates remained unchanged under elevated CO_2 and were even increased under ambient CO_2. We found a significant increase in dark respiration of "old" carbon pools after *P. citricola* infection, while respi-

ration of "new" assimilated carbon remained unchanged. Local and systemic effects of *P. citricola* infection on C and N allocation were found. Partitioning of "new" carbon was significantly increased in leaves and reduced in roots, whereas the opposite was found for the partitioning of "new" nitrogen. The physiological data were compared with differential gene expression analyses of leaves and roots. The first results showed that the reduction of nitrogen partitioning in leaves was correlated with down-regulation of genes of primary amino acid metabolism as well as of specific amino acid transport pathways in beech leaves.

37.44 FUNGUS-WAX INTERACTIONS OF APPLE SOOTY BLOTCH AND FLYSPECK FUNGI, AND THEIR DIVERSITY IN SLOVENIA AND GERMANY. **J. Frank, B. Oertel, M. Žerjav, A. Munda and H.-J. Schroers.** *Hacquetova 17, 1000 Ljubljana, Slovenia. Email: jana.frank@kis.si*

We studied sooty blotch and flyspeck (SBFS) fungi and disease symptoms caused by these fungi on apples. Results from preliminary identifications based on morphological characters and sequences of the internal transcribed spacer regions of the ribosomal DNA show that more than 15 fungal species of various genera of the Capnodiales (*Cladosporium*, *Pseudocercosporella*, *Dissoconium*) and others (*Peltaster*, *Zygophiala*) belong to the SBFS complex. The diversity overlaps with that reported for other geographical regions. In slide culture systems, representatives of the taxa were inoculated on wax, which we obtained from fresh apples by chloroform extraction and amended with traces of minerals, ammonium nitrate and yeast extract. Remarkably, the colony phenotype of most taxa grown on wax in slide cultures was reminiscent of that encountered on apples naturally infected by the same species. In slide cultures, strong hyphal development was observed in *Pseudocercosporella* and in an unidentified *Cladosporium* species, moderate in *Zygophiala* and poor in *Peltaster fructicola*; dark pigmented cell-walls were encountered in all species; sporulation was seen in 1 of 2 *Cladosporium* species, 2 of 3 *Pseudocercosporella* species but absent in *Peltaster*; stroma only was observed in *Zygophiala* and hyphae only in *Cladosporium* and *Peltaster*; others formed both hyphae and stroma. From these results, we hypothesize that some of these SBFS fungi can utilize carbon from the apple wax. In order to understand fungus–wax interactions on a molecular level, gene expression studies are in preparation that will allow characterization of genes coding for wax decomposing proteins.

37.45 *STAGONOSPORA NODORUM* UTILIZES A SOPHISTICATED INVERSE GENE-FOR-GENE SYSTEM INVOLVING PROTEINACEOUS HOST-SELECTIVE TOXINS INTERACTING WITH DOMINANT WHEAT SENSITIVITY GENES. **T.L. Friesen, J.D. Faris, Z.H. Liu, P.S. Solomon and R.P. Oliver.** *USDA-ARS Cereal Crops Research Unit, Northern Crop Science Lab, 1307 18th Street North, Fargo ND, 58105, USA. Email: timothy.friesen@ars.usda.gov*

The *Stagonospora nodorum*-wheat pathosystem involves a complex of proteinaceous host-selective toxins that interact either directly or indirectly with host sensitivity/susceptibility gene products in an inverse gene-for-gene manner. Compatible interactions among these gene products are highly important in disease development. ToxA, a host-selective toxin originally identified in *Pyrenophora tritici-repentis*, moved from *S. nodorum* to *P. tritici-repentis* by a recent lateral gene transfer event. SnToxA, as well as SnTox1, SnTox2, and SnTox3, have each been shown to be highly important in disease development in the presence of the corresponding dominant wheat sensitivity genes, *Tsn1, Snn1, Snn2,* and *Snn3,* respectively. Preliminary data using a worldwide collection of isolates and multiple wheat mapping populations indicates that at least 10 additional host selective toxin-host sensitivity gene interactions are present. These wheat sensitivity genes have been identified on 11 different chromosomes on the A, B, and D genomes of wheat. This pathosystem may be an excellent model for other necrotrophic pathogens that utilize host-selective toxins to cause disease.

37.47 ALTERNARIC ACID FROM *ALTERNARIA SOLANI* IN EARLY BLIGHT DISEASE STIMULATES PHOSPHORYLATION ACTIVITY OF A CALCIUM-DEPENDENT PROTEIN KINASE (CDPK) FROM POTATO. **N. Furuichi, K. Yokokawa and H. Oikawa.** *Graduate School of Science and Technology and Center for Transdisciplinary Research, Niigata University, 2-8050 Ikarashi, Niigata 950-2181, Japan. Email: nfuru@agr.niigata-u.ac.jp*

We studied the effect of alternaric acid (AA), a host-specific toxin (HST) produced by *Alternaria solani*, on a putative plasma membrane and cytosolic kinase SdCDPK2 of potato and on hypersensitive cell death (HR) of host cells. Here we demonstrate that AA in the presence of Ca^{2+} and Mg^{2+} stimulates *in vitro* phosphorylation of His-tagged SdCDPK2, a Ca^{2+}-dependent protein kinase from potato cv. Rishiri. Ca^{2+} and Mg^{2+} played an important role in the interaction between AA and SdCDPK2. These results suggest that AA may regulate SdCDPK2 kinase during the infection process in a compatible interaction between host and *A. solani*, leading to the inhibition of HR, as does a suppressor of HR in the host cells. We suggest that AA is a primary determinant by which *A. solani* may stimulate CDPK activity in the host, suppressing HR. Key words: Alternaric acid, *Alternaria solani*, calcium-dependent protein kinase, host-selective toxin, hypersensitive cell death.

37.48 CYTOKININ IS A CRUCIAL PATHOGENIC FACTOR FOR CLUBROOT DEVELOPMENT IN *ARABIDOPSIS THALIANA*. **N. Galfe, A.A. Berger, M. Riefler and J. Siemens.** *Department of Biology, Molecular Biotechnology, Technical University Dresden, 01062 Dresden, Germany. Email: johannes.siemens@tu-dresden.de*

Arabidopsis thaliana is a host of the obligate biotrophic root parasite *Plasmodiophora brassicae*, the cause of clubroot. Local changes in cytokinin homeostasis by up-regulation of cytokinin receptor and down regulation of cytokinin-oxidases has been shown to be linked to pathogenesis (Siemens *et al.* 2006, Mol. Plant Microbe Int. 19: 480-494). Three sensor histidine kinases, AHK2, AHK3, and CRE1/AHK4 of *A. thaliana* have been shown to be cytokinin receptors, which revealed partially redundant functions (Riefler *et al.* 2006, Plant Cell 18: 40-54). The interaction with *P. brassicae* of loss-of-function mutants of all three receptors has been analysed. Single mutants showed wildtype clubs, whereas development of clubroot in roots of the double mutant AHK3/AHK4 was hampered. These double mutants showed reduced gall size. Histological analysis revealed an inhibition of pathogen development. Thirty days after infection, the root of the double mutant AHK3/AHK4 was colonised by vegetative secondary plasmodia of the pathogen but no mature spores could be detected in contrast to wildtype plants. In order to get further insights into the mechanism of this apparent blocking and the dependence of pathogen development on cytokinin-mediated signal transduction of host cells, whole genome expression was analysed comparing mutants as well as compatible and incompatible interactions.

37.49 TWO NOVEL N-LIKE GENES ENCODING FUNCTIONALLY COMPETENT DOMAINS TO INDUCE HYPERSENSITIVE RESPONSE. J-S. Gao, N. Sasaki, H. Kanegae, K. Konagaya, K. Takizawa, N. Hayashi, Y. Okano, M. Kasahara, Y. Matsushita and H. Nyunoya. *Tokyo University of Agriculture and Technology, Gene Research Center, Fuchu-shi, Tokyo 183-8509, Japan. Email: nyunoya@cc.tuat.ac.jp*

The tobacco N gene recognizes the helicase domain (p50) of *Tobacco mosaic virus* (TMV) replicase as an elicitor and mediates a hypersensitive response (HR). We obtained two cDNA clones encoding novel N-like (NL) proteins NL-C26 and NL-B69 from *Nicotiana tabacum* cv. Samsun NN. NL-C26 and NL-B69 had a Toll-interleukin-1 receptor/nucleotide-binding site/leucine-rich repeat (TIR-NBS-LRR) structure and showed 78 and 73% identities to N, respectively. The NL-C26 and NL-B69 genes were also expressed in *N. tabacum* cv. Samsun nn, which lacks the N gene. Unlike N, NL-C26 and NL-B69, when coexpressed with p50, failed to induce HR at the sites of agroinfiltration in Samsun nn leaves. However, the elicitor-dependent HR in cv. Samsun nn was induced efficiently by chimeric N proteins with the continuous TIR-NBS domains of NL-C26 and NL-B69. On the other hand, the efficiency of HR induction varied significantly among chimeric N proteins with either of the TIR and NBS domains of the NL proteins. In contrast, chimeras carrying the LRR domains of the NL proteins did not induce HR. Thus, the TIR-NBS domains of NL-C26 and NL-B69 could functionally adapt to the LRR domain of N, which may determine the specificity for the elicitor. We speculate that the NL genes are potential HR-inducing resistance genes for undetermined pathogens other than TMV.

37.50 HISTOLOGICAL AND TRANSCRIPTIONAL ANALYSES OF HOST- AND NON-HOST INTERACTIONS BETWEEN WHEAT AND BARLEY AND THE SMUT PATHOGENS *TILLETIA TRITICI* AND *USTILAGO HORDEI*. D.A. Gaudet, C. Penniket, F. Leggett, Z.X. Lu, M. Frick, B. Puchalski, G. Bakkeren and A. Laroche. *Agriculture and Agri-Foods Canada Research Center, P.O. Box 3000, Lethbridge, Alberta, Canada. Email: gaudetd@agr.gc.ca*

Transcription profiling during host- and non-host interactions can provide insights into genes and regulatory pathways important in imparting disease resistance. We have employed confocal and fluorescent microscopy to study wheat and barley inoculated with virulent and avirulent races of *Tilletia tritici* and *Ustilago hordei* in non-host and host compatible and incompatible interactions. Histological studies on the various interactions in barley and wheat demonstrated that non-host interactions occurred more rapidly than those of the incompatible host interactions but both were morphologically very similar and were restricted to the cells immediately adjacent to the invading hyphae, and very distinct from the compatible interaction. Using Affymetrix microarrays and real-time PCR, we compared defense-related gene expression of non-host incompatible with host-incompatible and compatible interactions and identified genes specific to non-host and host interactions. Results will be discussed in relation to the type of interaction and the differential regulation of transcripts involved in different signaling and metabolic pathways.

37.51 CHARACTERIZATION OF THE HYPERSENSITIVE REACTION CAUSED BY A C-STRAIN OF *XANTHOMONAS AXONOPODIS* IN DUNCAN GRAPEFRUIT (*CITRUS PARADISI*). A.M. Gochez and B.I. Canteros. *EEA INTA Bella Vista, CC 5, 3432 Bella Vista, Ctes., Argentina. Email: agochez@correo.inta.gov.ar*

Citrus canker is caused by several groups of strains of *Xanthomonas axonopodis* (Xac). The C-type canker, caused by C-group strains has been reported only in Brazil infecting Key lime (*Citrus aurantifolia*). The B-type canker, caused by B-group strains occurred on lemon (*Citrus limon*) in Argentina from 1929 to 1991. The A-type canker, caused by A-group strains is the most widespread and aggressive. The C-group strains cause necrosis similar to the hypersensitive reaction (HR) in grapefruit (*Citrus paradisi*) leaves. We compared data from C and B strains on Duncan grapefruit (DG) and Key lime (KL). Two-week-old leaves were infiltrated and electrolyte leakage and population growth determined at different times. Inoculum of 10^8 bacterial cells per ml of the C strain gave a conductivity of 154.3 µhom in DG after 72 hours and the same values in KL after 8 days whereas the B strain gave 13.5 in DG and 48.3 in KL after 72 hours. Inoculum of 10^4 bacterial cells per ml of C strain grew only to 8.4 cel./cm^2 after 2 weeks in DG and to 1.1×10^3 cel./cm^2 in KL developing 1.1 lesions/cm^2 after 3 weeks. In contrast the B strain grew to 2.5×10^5 in DG and 2.4×10^6 cel./cm^2 in KL after 7 days giving 21.9 and 46.2 les/cm^2 in DG y KL in 3 weeks. These results are typical of an HR single-gene resistant reaction of C-strain in grapefruit and pathogenic reactions of C in KL and of B in DG and KL. Work is underway to clone the avr gene causing this HR to understand single-gene resistance in citrus.

37.52 EFFECTOR GENE EVOLUTION IN *PHYTOPHTHORA RAMORUM*, CAUSAL AGENT OF SUDDEN OAK DEATH AND *RAMORUM* BLIGHT. E.M. Goss, C.M. Press and N.J. Grünwald. *Horticultural Crops Research Laboratory, USDA ARS, Corvallis, OR, USA. Email: grunwaln@science.oregonstate.edu*

Pathogen effectors can serve a virulence function on behalf of the pathogen or trigger a rapid defense response in resistant hosts. Sequencing of the *Phytophthora ramorum* genome and subsequent analysis identified a diverse superfamily of approximately 350 genes that are homologous to the four known effectors in plant pathogenic oomycetes and share with them two protein motifs (RxLR and dEER). While as a whole the genes in this superfamily exhibit modest sequence similarity, small groups of closely related genes can be identified. We have investigated the molecular evolution of two such groups of genes. Microarray data suggests that several of these genes are expressed in isolate Pr-102 and one group is part of a larger family that includes eight genes known to function as effectors in *Phytophthora infestans*. We sequenced the full coding region and flanking noncoding regions of each gene in the three clonal lineages of *P. ramorum* and cloned to obtain haplotypes when necessary. We also obtained orthologous sequence from sister taxa *Phytophthora lateralis*, *Phytophthora foliorum* and *Phytophthora hibernalis* allowing for examination of the evolution of these genes across species. There is evidence that since divergence from the most recent common ancestor of *P. ramorum* and *P. lateralis*, genes have been gained by duplication or have lost function. Within *P. ramorum*, genes at different loci show varying levels of polymorphism, suggesting different evolutionary pressures.

37.53 A SEARCH FOR RESISTANCE-RELATED METABOLITES IN PLANT ROOTS CHALLENGED BY *PHYTOPHTHORA CINNAMOMI*. T.K. Gunning, X. Conlan, N. Barnett and D.M. Cahill. *Deakin University, Waurnponds Campus, Victoria, Australia. Email: tkg@deakin.edu.au*

The soil-borne plant pathogen, *Phytophthora cinnamomi* Rands is the causal agent of widespread disease in many Australian native vegetation communities and is recognised as a major threat to Australia's biodiversity. Approaches to combating disease include induction and enhancement of natural resistance. Enhancement of resistance in susceptible species requires a detailed understanding of the host and pathogen interaction and associated defence strategies utilized by resistant species. Plants were chosen for analysis from sites in southeastern Australia, including The Great Otway National Park, where it is estimated that over 70% of the 620 heathland species are susceptible. Comparative floristic studies between un-infested and diseased sites confirmed a loss of plant diversity and a change in species composition after pathogen invasion. Seed was collected from a range of plants and grown and inoculated under controlled conditions. A number of potentially resistant species were identified and as previous studies had shown an association between production of secondary metabolites and resistance, a non-targeted liquid chromatography – mass spectrometry (LC-MS) analysis of root metabolites was performed. The metabolic profiles of un-inoculated and inoculated plants from several species were compared, and compounds associated with a resistance response identified. The opportunity now arises to elucidate underlying biosynthetic pathways, from gene to metabolite, and for the identified compounds to be used to induce or enhance resistance in susceptible species.

37.54 RICE TRANSCRIPTION FACTOR *WRKY89* IS INVOLVED IN RESPONSES TO BIOTIC AND ABIOTIC STRESSES IN RICE PLANTS. **Z. Guo, J. Hao, and X. Chen.** *State Key Laboratory of Agrobiotechnology, Department of Plant Pathology, China Agricultural University, Beijing 100094, P.R. China. Email: guozj@cau.edu.cn*

WRKY proteins are a large family of transcriptional regulators involved in a variety of biological processes in plants. Here we report functional characterization of a rice *WRKY* gene, *OsWRKY89*. RNA gel blot analysis indicated that *OsWRKY89* was strongly induced by treatments of methyl jasmonate and by UV-B radiation. Transient expression analysis of the OsWRKY89-eGFP reporter in onion epidermal cells revealed that OsWRKY89 was targeted to nuclei. Transcriptional activity assays of OsWRKY89 and its mutants fused with a GAL4 DNA-binding domain indicated that the 67 C-terminal amino acids were required for transcriptional activation and that the leucine zipper region at the N-terminus enhanced transcriptional activity. Overexpression of *OsWRKY89* led to growth retardation at an early stage and reduction of internode length. Scanning electron microscopy revealed an increase in wax deposition on leaf surfaces of the OsWRKY89 overexpression lines and a decrease in wax loading in the RNAi-mediated OsWRKY89 suppression lines. Moreover, extractable and cell-wall-bound phenolic compounds were decreased in the overexpressor lines, but SA levels were increased. Lignin staining showed an increase in lignification in culms of overexpressor lines. Interestingly, overexpression of *OsWRKY89* enhanced resistance to the rice blast fungus and white-back plant hopper as well as tolerance to UV-B irradiation. These results suggest that *OsWRKY89* plays an important role in response to biotic and abiotic stresses.

37.55 PNCCP OCCURS IN *PHYTOPHTHORA* LARGE PERIPHERAL VESICLES IN ZOOSPORES BUT IS SELECTIVELY SECRETED DURING ENCYSTMENT. **A.R. Hardham, L.M. Blackman and D. Skalamera.** *Plant Cell Biology Group, Research School of Biological Sciences, Australian National University, Canberra, ACT, Australia. Email: adrienne.hardham@anu.edu.au*

The initiation of host-plant infection by *Phytophthora* spores involves the regulated secretion of adhesives and protective mucilages from ventral and dorsal vesicles in the spore cortex. During this phase of development, a third category of spore cortical vesicle, the so-called large peripheral vesicles, are not exocytosed but become randomly distributed throughout the spore cytoplasm and are thought to serve as protein storage vacuoles. In recent research, we have identified another protein resident in the large peripheral vesicles. PnCcp is a 12 kDa protein that contains a single complement control protein module and has homologues in *P. infestans, P. sojae* and *P. ramorum. PnCcp* is a single-copy gene expressed at low levels in mycelia and germinated cysts but significantly up-regulated in sporulating hyphae and zoospores. Immunolabelling showed that PnCcp colocalizes with PnLpv, a high molecular weight glycoprotein that occurs in the large peripheral vesicles in zoospores. However, during encystment, PnCcp is selectively secreted as the large peripheral vesicles move away from the plasma membrane. In mycelia, the two proteins do not always co-localize, suggesting that the large peripheral vesicles may contain components that are synthesized separately during vesicle biogenesis.

37.56 HOST ADAPTATION AND ACQUISITION OF PATHOGENICITY OF *CITRUS EXOCORTIS VIROID*. **T. Hataya, T. Kawamura and T. Yonemoto.** *Laboratory of Pathogen-Plant Interactions, Research Group of Plant Breeding Science, Division of Applied Bioscience, Research Faculty of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kita-ku, Sapporo, 060-8589 Japan. Email: hataya@res.agr.hokudai.ac.jp*

To study the relationship between host adaptation of *Citrus exocortis viroid* (CEVd) and the pathogenicity of the resulting sequence variants, we analyzed progeny sequences of an isolate CEVd-H propagated in seven plant species; Etrog citron, tomato, eggplant, potato, *Physalis floridana*, cucumber, and *Gynura aurantiaca*. Major mutations were found at six positions in 370-372 nucleotide sequences. The two mutations found at nt 70/71 and 313/314 were closely correlated with the host plant. The dominant sequences were $GG_{70/71}$-$AU_{313/314}$ (To-type) in tomato but $G_{70/71}$-$GA_{313/314}$ (EC-type) in Etrog citron, eggplant, *Physalis floridana*, and cucumber plants. Analysis of progeny sequences derived from an infectious linear dimeric RNA having $GG_{70/71}$-$GU_{313/314}$ showed the host adaptation by mutations to $GG_{70/71}$-$AU_{313/314}$ (To-type) for tomato and $G_{70/71}$-$GA_{313/314}$ (EC-type) for Etrog citron. In addition, analysis of progeny sequences derived from infectious linear dimeric RNAs of To-type, EC-type, and *in vitro* mutants revealed that (1) mix-inoculation with To-type and EC-type resulted in the selection of To-type in tomato and EC-type in Etrog citron; (2) inoculation with EC-type to tomato resulted in mutations gradually to To-type in the following processes: $G_{70/71}$-$GA_{313/314}$ (EC-type) → $GG_{70/71}$-$GA_{313/314}$ → $GG_{70/71}$-$GU_{313/314}$ → $GG_{70/71}$-$AU_{313/314}$ (To-type); (3) the pathogenicity to tomato was stronger, in order, as follows: $G_{70/71}$-$GA_{313/314}$ (EC-type) < $GG_{70/71}$-$GA_{313/314}$ < $GG_{70/71}$-$GU_{313/314}$ < $GG_{70/71}$-$AU_{313/314}$ (To-type); (4) the duplication of 'G' at nts 70/71 was responsible for virulence to tomato. In conclusion, the adaptation of CEVd to tomato is closely correlated with the pathogenicity; that is, CEVd in tomato evolves in the direction of acquisition of strong virulence to this host.

37.57 INVOLVEMENT OF P10 OF *GRAPEVINE VIRUS A* IN SUPPRESSION OF RNA GENE SILENCING AND IN PATHOGENICITY. S. Haviv, S. Stukalov and M. Mawassi. *The S. Tolkowsky Laboratory, Plant Pathology Department, The Virology Unit, Agricultural Research Organization, the Volcani Center, Bet Dagan 50250, Israel. Email: mawassi@volcani.agri.gov.il*

GVA is a member of the genus *Vitivirus,* family *Flexiviridae*. It has a filamentous particle about 800 nm long, and is considered to be phloem-associated. The virus has a single-stranded RNA genome of about 7.4 kb, which consists of five open reading frames. ORF1 encodes a 194-kDa polypeptide with conserved motifs of replication-related proteins. ORF2 encodes a protein of 19 kDa, with unknown function. ORF3 encodes a 31-kDa movement protein. ORF4 encodes the coat protein. ORF5 encodes a small protein of 10 kDa that contains two distinct domains: a basic, arginine-rich motif (ARM) and a zinc-finger domain, and interacts with nucleic acids. Recently, it was suggested that the GVA p10 possesses weak activity as suppressor of RNA silencing. In our work we found that ORF 5 affects symptom appearance on *Nicotiana benthamiana* plants. Mutational analyses suggested that the ARM sequence of p10 is essential for GVA infection in plants, and symptoms are affected by the 8th residue in p10. Moreover, we have developed an assay whereby RNA silencing in leaves was induced by agroinfiltration of a mini-GVA-GFP replicon that possessed genes encoding viral RNA replicase and GFP fused to ORF2 product. The assay proved to be very sensitive for analyses of various RNAi suppressors. Using this system we found that p10 together with additional GVA-coded protein(s) or RNA(s) are involved in enhancement of RNA silencing suppression by the replicating virus.

37.58 STUDY OF THE BIOLOGICAL PROPERTIES OF BNYVV P25 PROTEIN: LOOKING FOR DOMINANT NEGATIVE MUTANTS TO CONTROL RHIZOMANIA. K. Hleibieh, E. Klein, A. Schirmer, L. Covelli and D. Gilmer. *Institut de Biologie Moléculaire des Plantes, 12 Rue du Général Zimmer, 67084 Strasbourg Cedex, France. Email: kamal.hleibieh@ibmp-ulp.u-strasbg.fr*

Rhizomania is a viral disease present in most of sugar beet-growing areas worldwide. The agent responsible, *Beet necrotic yellow vein virus* (BNYVV), has 4 or 5 plus-stranded RNAs. The encoded p25 protein has several effects and functions; it is responsible for symptom expression and influences viral pathogenicity, inducing root proliferation and decreasing sugar yields; it is a nucleo-cytoplasmic shuttling protein, possessing a nuclear localization signal and a nuclear export signal; it is able to activate transcription in a yeast one-hybrid system. p25 can interact with itself and is phoshorylated, as it is immunoprecipitated with phospho-ser and phospho-thr antibodies; p25 immunodetection after 2D separation reveals the presence of multiple spots. Site-specific mutagenesis has been performed on putative p25 phosphorylation sites to produce respectively, alanine mutants impaired in phosphorylation or aspartic acid mutants which mimic phosphorylated residues. p25 phosphorylation mutants have been tested for their capacities to modulate symptom expression, subcellular localization, transcription activation, and multimerization. We showed that phosphorylation of p25 modulates its activities. p25's cellular partners have been selected by yeast two-hybrid screening. p25 mutants will be tested for their interaction with cellular candidates. The goal of these studies is to select mutated proteins that are able to impair wild-type p25 functions. Such mutants will serve to obtain transgenic plants that will be further challenged with BNYVV.

37.59 MOLECULAR CHARACTERIZATION OF A POSSIBLE APOPTOSIS-RELATED CALCIUM-BINDING PROTEIN ASALG-2 IN OAT. T.X. Hoat, K. Uchihashi, H. Nakayashiki, Y. Tosa, S. Mayama. *Laboratory of Plant Pathology, Graduate School of Agricultural Science, Kobe University, Rokkodai-cho, Nada-ku, Kobe 657-8501, Japan. Email: mayama@port.kobe-u.ac.jp*

In victorin-sensitive oat lines, victorin, the host-selective toxin produced by the fungus *Cochliobolus victoriae*, induces programmed cell death (PCD) with characteristic features of animal apoptosis, such as mitochondrial permeability transition, chromatin condensation, nuclear DNA laddering and rRNA/mRNA degradation. In this study, we characterized the calcium-binding protein AsALG-2, which might have a role in victorin-induced PCD. *AsALG-2* is significantly homologous to the apoptosis-linked gene *ALG-2* identified in mammalian cells. Northern blots revealed that accumulation of *AsALG-2* transcripts increased during victorin-induced PCD but not during necrotic cell death. Salicylic acid, chitosan and chitin strongly activated the expression of general defence response genes such as *PR-10,* but neither induced cell death nor the accumulation of *AsALG-2* mRNA. Pharmacological studies indicated that victorin-induced DNA laddering and *AsALG-2* expression were regulated through similar pathways. The calcium channel blocker, nifedipine moderately inhibited accumulation of *AsALG-2* mRNA during cell death. Trifluoperazine (calmodulin antagonist) and K252a (serine-threonine kinase inhibitor) reduced victorin-induced phytoalexin accumulation but did not prevent victorin-induced DNA laddering or accumulation of *AsALG-2* mRNA. Taken together, our investigations suggest that *AsALG-2* could be involved in the signaling pathway of victorin-induced PCD in oat cells.

37.60 *ARABIDOPSIS* SIGNALLING COMPOUNDS INVOLVED IN COMPATIBLE PLANT-OOMYCETE INTERACTIONS. S. Hok, V. Allasia, E. Andrio, A. Attard and H. Keller. *UMR-Interactions Plantes-Microorganismes et Santé Végétale, INRA1064-CNRS6192-Université Nice-Sophia Antipolis, B.P. 167, 400 Route des Chappes, 06903 Sophia Antipolis Cedex, France. Email: sophie.hok@sophia.inra.fr*

Oomycetes are extremely devastating pathogens that provoke mildew diseases on a wide spectrum of crop plants. These microorganisms were long considered as true fungi, but they are more closely related to brown algae. Due to particularities concerning lifestyle, morphology, and physiology, pesticide treatments and resistance breeding approaches are basically inefficient against mildew pathogens. To follow up the growing urge for the development of efficient control methods, it is essential to increase our knowledge on the molecular bases that govern disease development. In this context, our aim is to identify plant functions that are manipulated by oomycete pathogens during the infection process. An analysis of the *Arabidopsis thaliana* transcriptome during the compatible interaction with the obligate biotroph, *Hyaloperonospora parasitica*, revealed a subset of activated genes that are involved in signal perception and transduction by plant cells. The functional analysis of two genes coding for a leucine-rich repeat receptor-like kinase (LRR-RLK) and a basic leucine zipper (bZIP)-like transcription factor were initiated. Knock-out mutants for both genes were analyzed, and we obtained overexpressors and transgenic lines harbouring promoter-reporter gene fusions, as well as lines expressing the proteins with fluorescent tags. The principal findings on the role of the two genes, for normal plant development and for the establishment of the compatible plant-oomycete interaction, will be presented.

37.61 STUDY OF GRAPEVINE YELLOWS AND INDUCED RESISTANCE TO THE DISEASE THROUGH GENE-EXPRESSION PROFILING. M. Hren, P. Ermacora, A. Rotter, N. Terrier, M. Ravnikar and K. Gruden. *Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia. Email: kristina.gruden@nib.si*

Flavescence dorée (FD) and bois noir (BN) phytoplasmas are the main causes of grapevine yellows. Phytoplasmas are intracellular bacteria, living inside the plant phloem. Consequently, direct treatment of plants against the pathogen with agrochemicals is ineffective. Factors determining the pathogenesis of both phytoplasmas as well as factors determining resistance of grapevines are poorly or not at all understood. The aim of this study was to monitor the gene-expression response of grapevines to phytoplasma infection. Relative expression of sucrose synthase (Susy), alcohol dehydrogenase I (Adh1) and heat shock protein 70 (Hsp70) was first monitored with real-time PCR in healthy, infected and recovered plants of cultivars Chardonnay, Barbera and Prosecco (123 samples altogether) to see if the field situation influences the response to infection. Ssh and Adh1 were significantly more expressed (t-test, $p<0.05$) in symptomatic plants of 'Chardonnay' (infected with BN). Similar results were obtained in symptomatic plants of 'Barbera' and 'Prosecco' (infected with FD). The expression of Hsp70 showed the same trend but the difference was not statistically significant. Based on similarity of these expression results, samples were pooled and genome-wide expression profiling of healthy 'Chardonnay' versus infected 'Chardonnay' was performed using DNA microarrays. The Mapman visualization tool was adapted for analysis of grapevine gene-expression data. Here significant differences in metabolism of photosynthesis, carbohydrates and in expression of genes related to biotic and abiotic stress were detected. Interestingly, changes in gene expression seemed to occur throughout the infected plants, despite uneven distribution of the pathogen in the plant.

37.62 ROLE OF THE PEPPER LIPOXYGENASE GENE *CALOX1* FOR PLANT DEFENSE AGAINST MICROBIAL PATHOGENS. I.S. Hwang, S.C. Lee, H.W. Choi, Y.J. Kim, D.S. Choi, D.S. Kim, N.H. Kim and B.K. Hwang. *Laboratory of Molecular Plant Pathology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Korea. Email: goonacruise@korea.ac.kr*

Lipoxygenases (LOXs) catalyze the hydroperoxidation of polyunsaturated fatty acids in plant cells. The products of LOX pathways may function physiologically as growth regulators for plant development and antimicrobial compounds for plant defense responses to pathogen invasion. The pepper lipoxygenase *CaLOX1* gene has been isolated and functionally characterized from pepper leaves infected with *Xanthomonas campestris* pv. *vesicatoria* (Xcv). This gene was differentially induced in leaves in compatible and incompatible interactions during *Xcv* infection, as well as after exposure to abiotic elicitors. Reverse-genetic approaches based on loss-of-function using virus-induced gene silencing (VIGS) and T-DNA insertion mutants or gain-of-function using transgene expression were applied to define the functions of *CaLOX1* in the response of plants to necrotrophic and biotrophic pathogens. *CaLOX1*-silenced pepper plants were susceptible to Xcv infection. *CaMV35S*::*CaLOX1* overexpression in transgenic *Arabidopsis* plants conferred enhanced resistance to *Pseudomonas syringae* pv. *tomato* and *Hyaloperonospora parasitica* infection, accompanied by cell death, reactive oxygen species accumulation and defense-related gene induction. By constrast, T-DNA insertion mutant plants of an *Arabidopsis AtLOX1* ortholog exhibited enhanced susceptibility to these diseases. Together, these results suggest that the pepper lipoxygenase *CaLOX1* gene is involved in resistance to bacterial and oomycete infection in pepper and *Arabidopsis*.

37.63 PENETRATION BEHAVIOR OF *ELSINOE FAWCETTII* CAUSING SCAB DISEASE ON CITRUS. J.W. Hyun, H.J. Kim, S.W. Ko, H.M. Kwon and Y.C. Jeun. *Citrus Experiment Station, National Institute of Subtropical Agriculture, R.D.A. Jeju 697-943, Korea. Email: jwhyun@rda.go.kr*

Penetration behavior of *Elsinoe fawcettii* on citrus was investigated by light and fluorescence microscopy and scanning electron microscopy. The conidia germinated and produced germ tubes from one or both ends of the conidia from 1 day after deposition on the citrus leaf surface. Globose appressoria were formed at the tip of germ tubes and mycelial swelling on germ tubes also occurred on the leaf surface, where no surface cracks or stomata were found. The fungal germ tubes were more often seen to penetrate the leaf surface directly by forming appressoria or mycelial swellings, rather than entering the leaf through stomata. The leaf surfaces underneath appressoria or swollen germ tubes were inflated and seemed degraded 3 day after inoculation, indicating possibility of direct penetration of the fungus by enzymatic degradation of the cuticle layers and relation of hormone in pathogenesis. It seemed that phenolic compounds accumulated around the infection site. The lesions were produced by artificial inoculation and natural infection on upper surfaces completely lacking stomata, or confined to midribs or major veins as well as on lower leaf surfaces which carried numerous stomata.

37.64 DISSECTING THE INFECTION MACHINERY OF *STAGONOSPORA NODORUM* USING DNA MICROARRAYS. S.V.S. Ipcho, J.K. Hane, P.S. Solomon and R.P. Oliver. *Australian Centre for Necrotrophic Fungal Pathogens, DHS, Murdoch University, South Street, Murdoch WA 6150, Australia. Email: 19987374@student.murdoch.edu.au*

The fungus *Stagonospora* (*Septoria*) *nodorum* [teleomorph *Phaeosphaeria* (*Leptosphaeria*) *nodorum*] is an important pathogen of cereals, causing both leaf and glume blotch. The genome sequence of this fungus is composed of 16116 genes, of which 10762 genes were predicted with a high level of confidence. RNA was extracted from lesions corresponding to early proliferation, vegetative state and sporulation on wheat leaves, and from mycelia of the fungus grown on minimal media reflecting vegetative state and sporulation. Based on the annotation of the genome sequence, a whole genome microarray was designed. Microarray experiments were undertaken using Cy3-labelled cDNA derived from the mentioned samples (Nimblegen Inc.). This robust methodological approach provided the expression profile of the pathogen during the key stages of plant infection and *in vitro* growth. These expression profiles were analysed and a subset of differentially expressed genes identified. Potential pathogenicity genes occurring as single copy in the genome and with interesting expression profiles were chosen for further study using a reverse genetics approach.

37.65* THE TRANSCRIPTION FACTOR ATAF1/HVNAC6 REGULATES PLANT DEFENCES AND ABIOTIC STRESS ADAPTATION. M.K. Jensen, M.F. Lyngkjær, P. Hagedorn and

D.B. Collinge. *Department of Plant Biology, Faculty of Life Sciences, University of Copenhagen, DK-1871 Frederiksberg C, Denmark. Email: dbc@life.ku.dk*

The plant-specific NAC transcription factors are involved in regulating abiotic and biotic stress responses as well as plant developmental processes. We have used a functional genomics approach in barley to unravel the functions of NAC transcription factors upregulated in powdery mildew (Bgh) infected epidermal cells. We identified a NAC protein, termed HvNAC6 (named after the rice NAC protein OsNAC6). Transient RNAi of *HvNAC6* renders barley epidermal cells significantly more susceptible to attack by the compatible Bgh isolate A6 compared to control cells. In the reciprocal approach, we studied transient overexpression, and found that *HvNAC6* overexpression leads to significantly more penetration-resistant cells compared to control transformed cells and two other HvNACs. Furthermore, a T-DNA mutation in the Arabidopsis *HvNAC6* homologue, *ATAF1* gene resulted in a significantly increased penetration rate compared to wild-type Col-0 plants, when inoculated by the non-host pathogen Bgh. ATAF1 has been described recently as a repressor of stress-sensitive genes, improving drought tolerance. To learn more about the downstream targets of *HvNAC6*, we used large-scale transcriptional profiles of *ataf1* and Col-0 plants +/- Bgh attack using the Affymetrix microarray. This study provided insight into the complex interplay of regulatory networks involved with perception and response to abiotic and biotic stresses in plants. M. K. Jensen *et al*. 2007, Plant. Mol. Biol. 65, 137-150; D. B. Collinge *et al*. Eur. J. Plant Path. *in press.*

37.66 GENETIC AND EPIGENETIC HERITABLE EFFECTS OF PATHOGEN STRESS ON PLANTS. P. Kathiria, A. Boyko, E. van Klei and I. Kovalchuk. *Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada. Email: igor.kovalchuk@uleth.ca*

Among higher eukaryotes, plants are the only organisms capable of fast acclimation and adaptation processes (1,2). These processes rely on the ability to reprogram the gene expression pattern according to changing environmental conditions (3,4). Here we present data showing that exposure of *Nicotiana tabacum or Arabidopsis thaliana* plants to viral (*Odontoglossum ringspot virus* and *TMV*) and bacterial (*P. syringae*) infection resulted in dramatic changes in the response of the progeny to the same and different stresses. Specifically, the progeny (S1) of these plants showed higher spontaneous recombination frequency, changes in global genome methylation and higher tolerance to the same pathogen and even to abiotic stress such as methyl methane sulfonate (MMS). The subsequent plant generation(s) exhibited a different pattern. Changes persisted when the first generation was grown in the presence of pathogen stress (S2), and changes substantially decreased, albeit not to the control levels, when they were grown in pathogen-free conditions (S1-Ct1). We hypothesize that the stressed plants inherit memory of stress, and that exposure to stress has to be persistent in order to maintain the same level of adaptive response. 1. Chinnusamy *et al.* 2004, J. Exp. Bot. 55, 225-36; 2. Turunen & Latola 2005, Environ. Pollut. 137, 390-403; Boyko *et al.* 2007, Nucleic Acids Res. 35, 1714-25.

37.67 RESPONSE OF *BRASSICA JUNCEA* GENOTYPES TO RACES OF *ALBUGO CANDIDA* IN WESTERN AUSTRALIA. P. Kaur, M.J. Barbetti and K. Sivasithamparam. *School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia Crawley, WA 6009, Australia. Email: kaurp02@student.uwa.edu.au*

During the past eight years, canola (*Brassica napus)* has become an integral component of the cropping systems of the Western Australian grainbelt. In Australia, a small proportion of the oilseed *Brassica* area (about 3000 ha) is sown to *B. juncea* mainly for use as condiments. Presently, canola-quality *B. juncea* is being developed to extend Brassica oilseed production to the lower rainfall areas of the southern Australian wheatbelt, as it is better adapted than canola to warmer and drier areas. Unfortunately, the varieties of *B. juncea* available in Australia appear highly susceptible to white rust. Seventeen races of *A. candida* have been reported from various cruciferous species worldwide. Of these, so far, 14 are on *Brassica* spp. while 6 among them are known to attack *B. juncea*. *B. juncea* cultivars previously reported as being resistant to white rust race 2 (now designated as race 2A) were subsequently found to be susceptible to a more virulent form of this race in Canada (now designated as race 2V) including newly developed canola-quality *B. juncea* which is widely sown across the Canadian prairies. Hence, studies were undertaken to find the response of *B. juncea* genotypes towards different races of *A. candida* occurring in Western Australia. These race delineations from different grainbelt regions are expected to help in selecting appropriate genotype resistance(s) for sowing in relation to the prevalent races. The information will also facilitate development of quarantine measures to prevent movement of infested seed to areas free of certain races.

37.68 DEFENSIVE ROLE OF A SMALL HEAT-SHOCK PROTEIN IN *NICOTIANA* PLANTS AGAINST *RALSTONIA SOLANACEARUM*. A. Kiba, M. Maimbo, K. Ohnishi, H. Yoshioka and Y. Hikichi. *B-200 Monobe, Nankoku, Kochi, 783-8502, Japan. Email: akiba@cc.kochi-u.ac.jp*

In tobacco, *Ralstonia solanacearum* OE1-1 (RsOE1-1) is pathogenic, whereas *R. solanacearum* 8107 (Rs8107) is non-pathogenic and induces the hypersensitive response (HR). To elucidate the molecular mechanisms of plant–*R. solanacearum* interactions, we used differential display to isolate a cDNA fragment, A6, regulated in tobacco when inoculated with RsOE1-1. The deduced amino acid sequence predicted from full length A6 cDNA showed similarity to small heat shock proteins from *Arabidopsis thaliana* (hypothetical protein), *Medicago truncatula* and *Cucumis melo*; we therefore designated A6 as Ntshsp17 (*Nicotiana tabacum* small heat shock protein 17). Recombinant Ntshsp17 overproduced in *Escherichia coli* exhibited molecular chaperone function. Expression of Ntshsp17 was increased in tobacco leaves inoculated with both RsOE1-1 and Rs8107. Expression was also induced by heat treatment, and by treatment with aminocyclopropane carboxylic acid, H_2O_2, methyl jasmonate and salicylic acid. Ntshsp17 expression was induced by inoculation with a hypersensitive response-and-pathogenicity (hrp) gene mutant of Rs8107 that does not induce the HR, but not by *Agrobacterium*-mediated transient expression of INF1, an HR elicitor. Nbshsp17 is an Ntshsp17 ortholog in *N. benthamiana*); in Nbshsp 17-silenced plants, expression of EREBP, PR-1a and PR-4 genes was compromised, but the expression of EF1-Éø was scarcely affected. Appearance of the HR was not affected in the silenced plants. In the silenced plants, growth of Rs8107 was accelerated. Bacterial growth and wilt symptoms elicited by RsOE1-1 were also accelerated in the silenced plants. These results indicate that this small heat shock protein may have a role in the HR-independent defences in *Nicotiana* plants.

37.69 STUDYING PATHOGENICITY OF *FUSARIUM* SPP. **M.I. Kiseleva, E.D. Kovalenko and N.S. Zhemchuzhina.** *All-Russia Research Institute of Phytopathology, Moscow, Russia. Email: kiseleva@vniif.rosmail.com*

Fusarium fungi are the most harmful disease agents of cereals (root rot, head blight, snow mould) and are a serious problem in Russia. Root rot is caused by a complex of *Fusarium* species: *F. culmorum, F. oxysporum, F. avenaceum, F. heterosporum, F. nivale* and *F. sporotrichioides*. Our aim was to compare the pathogenicity of *Fusarium* root rot of barley and wheat. The pathogenicity of *Fusarium* strains was determined by measuring the seed infection resulting from inoculating spore suspensions. The main parameters of pathogenicity were seed germination, root length and coleoptile length. *F. nivale* and *F. sporotrichioides*, which essentialy inhibited seed germination up to 12-70% and seedling growth (coleoptile length) up to 25-52%, were the most pathogenic species. The species of *Fusarium* were characterized by intraspecific variability of pathogenicity. Strains belonging to the same species had different levels (from low to high) of pathogenicity.

37.70* MODIFICATION OF A MAMP RECEPTOR, CEBIP, WITH AN R GENE PRODUCT LEADS TO FUNGAL DISEASE RESISTANCE. **K. Kishimoto, E. Nakajima, H. Kurano, H. Kaku, N. Shibuya, E. Minami and Y. Nishizawa.** *National Institute of Agrobiological Sciences, Tsukuba, 305-8602, Japan. Email: cucumber@affrc.go.jp*

A high-affinity binding protein for chitin oligosaccharide in the rice plasma membrane (CEBiP) was identified as a receptor for chitin elicitor, a representative microbe-associated molecular pattern (MAMP). CEBiP has two extracellular LysM motifs and a transmembrane domain (TM) (Kaku *et al.*, 2006). Here, we report the contribution of CEBiP in the basal disease resistance of rice plants against rice blast fungus, *Magnaporthe grisea,* and that manipulation of response to the MAMP enhances disease resistance. Knockdown of CEBiP by RNAi in rice plants resulted in the reduction of the basal resistance against a strain of *M. grisea* with lowered infectivity, suggesting that chitin elicitor recognition contributes at least partly to the basal resistance. To enforce HR cell death upon sensing the chitin elicitor, we produced transgenic rice lines expressing four types of chimeric genes (CRXa 1-4) constituting CEBiP and Xa21, an R gene of rice against *Xanthomonas oryzae* pv. *oryzae*. Cell lines expressing either CRXa1 or CRXa3, which contain the whole extra-cellular portion of CEBiP, TM, and the whole intra-cellular portion of Xa21, induced HR cell death accompanied by increased production of hydrogen peroxide and nitric oxide after treatment with chitin elicitor. Cell death induced by chitin elicitor was also observed in leaves expressing CRXa1 and CRXa3. These rice plants showed enhanced resistance to *M. grisea*, strongly suggesting that chitin elicitor is released during infection of *M. grisea*, and that manipulation of MAMP signaling by modifying MAMP receptors would be a novel strategy in molecular breeding for disease resistance.

37.71 CHANGES IN DISTRIBUTION OF RICE BLAST RACES IN JAPAN FROM 1976 TO 2001. **S. Koizumi.** *National Agricultural Research Center for Tohoku Region, Yotsuya, Daisen, Akita, 014-01 Japan. Email: skoizumi@affrc.go.jp*

Distribution of rice blast races in Japan and how this has changed over time were analyzed using the monitoring data of Yamada *et al.* (1979) and others on blast races, using nine Japanese differentials, and samples from all over the country in 1976, 1980, 1994 and 2001. The numbers of different races were 23, 22, 23 and 15 among 2,245, 2,376, 1,526 and 1,050 blast isolates collected in the respective four years. In 1979 and 1980 the most predominant race was 003 (virulent to resistance gene *Pia*) with 58% isolation frequencies (IFs), followed by races 007 (15%), 033 (11 and 9%), 001 (4 and 7%) and 103 (6 and 3%). In 1999 and 2001 the most prevalent race was changed from 003 to 007 (virulent to gene *Pii*) with 51 and 58% Ifs, followed by races 001 (23 and 17%), 003 (13 and 8%), 005 (4 and 8%), 037 (2 and 5%) and 033 (2 and 1%). Prevalence of blast races was affected by frequencies of corresponding complete resistance genes with gene-for-gene relationship to them in cultivated rice cvs., and statistically significant positive correlations were observed between IFs of blast races virulent to a resistance gene and frequencies of the gene in cultivated rice cvs. for each of *Pia, Pii, Pik, Pita/Pita-2* and *Piz.* Cluster analysis using a data set of IFs of blast races virulent to *Pia, Pii, Piz* and *Pita* in 1976, 1980, 1994 and 2001 divided the 37 prefectures with full data sets into five groups and three ungrouped prefectures.

37.72 PARAMETERS OF PARTIAL RESISTANCE IN WHEAT CULTIVARS TO *STAGONOSPORA NODORUM* BERK. **T. Kolomiets, O. Skatenok, I.V. Kudaikina and H. Bockelman.** *All-Russia Research Institute of Phytopathology, 143050, Moscow region, Bolshie Vyazemi, Russia. Email: nsgchb@vniif.ars-grin.gov*

Dynamics of Septoria development on more than 2000 wheats from a world germlasm collection was studied in an infectious nursery. On the basis of evaluations of Septoria intensity on wheats in the field during vegetation, the area under the disease progress curve (AUDPC) and resistance index (IR) were calculated. For studying partial resistance parameters (latent period, size of infectious spots) wheat cultivars with high and average indexes of resistance to Septoria were selected. Twenty-seven wheat cultivars with low indexes were studied for partial resistance parameters to *Stagonospora nodorum*. Evaluation of leaf pieces for resistance to 4 strains of the pathogen were carried out. The response of wheat cultivars to infection of *S. nodorum* pathotypes were different. The latent periods for cultivars Voronezhskaya 10, Lyra 98, Legenda and Owens were longer than for the control cultivar Priokskaya 1. Small infectious spots were found on cultivars Warigal*Dagger, Cltr 278222, Cltr 604225 and Enita, while latent periods were identical with a control cultivar. The cultivars with long latent period and small infectious spots were selected. These were 6 cultivars: Anderson, PI 278222 (USA), RAC 610, RAC 569, K-20 (Republic of South Africa) and SWS "A" N80 (Sweden).

37.73 ANALYSIS OF SYSTEMIC NECROSIS ON *NICOTIANA BENTHAMIANA* INDUCED BY *PLANTAGO ASIATICA MOSAIC VIRUS* INFECTION. **K. Komatsu, M. Hashimoto, J. Ozeki, M. Aoyama, Y. Yamaji and S. Namba.** *Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan. Email: akomatsu@mail.ecc.u-tokyo.ac.jp*

Like other plant pathogens, plant viruses induce various symptoms in their host plants, resulting in acute reduction in growth or yield. These symptoms include mosaic, distortion, yellowing, or dwarfing, and systemic necrosis. The symptoms are the dynamic manifestation of the systemic spread of changes in host metabolic pathways from a normal phase to an unusual phase triggered by interactions between the plant and the pathogen.

However, the mechanisms underlying symptom development are unresolved. To understand the mechanisms of systemic necrosis, we developed the pathosystem involving infection of *Nicotiana benthamiana* with *Plantago asiatica mosaic virus* (PlAMV; *Potexvirus*, *Flexiviridae*). We obtained two sub-isolates (Li1 and Li6) from a lily isolate of PlAMV (PlAMV-Li). Although the nucleotide sequences of Li1 and Li6 were highly conserved with over 99% complete nucleotide sequence identity, they showed different pathogenicity in *N. benthamiana*. Li1 caused systemic necrosis, whereas Li6 infected the plant symptomlessly. Inoculation tests with chimeric and point-mutated viruses revealed that amino acid 1154 of the RNA-dependent RNA polymerase (RdRp) contributes to the systemic necrosis. The accumulation level of the mutant viruses (Li1-1154Y and Li6-1154C), in which RdRp amino acid 1154 was exchanged to the wild type codon in Li1 and Li6, were almost equal. We also investigated whether the systemic necrosis induced by Li1 and Li6-1154C is associated with plant defence responses such as those observed in the hypersensitive response (HR).

37.74 SEARCHING FOR AGENTS IN RECYCLED IRRIGATION WATER THAT STIMULATE PLANT INFECTION BY *PHYTOPHTHORA* SPECIES. P. Kong and C.X. Hong. *Virginia Polytechnic Institute and State University, Virginia Beach, Virginia, 23455-3363, USA. Email: pkong@vt.edu*

The genus *Phytophthora* includes a group of destructive pathogens attacking a huge number of agriculturally and ornamentally important plants. We have been investigating the biology and management of *Phytophthora* species in recycling irrigation systems for 9 years. We have found that zoospore inoculum prepared with pond water results in markedly severer disease compared to inoculum prepared with tap or well water. This paper reports on a long search for infection-stimulating agents in pond water. Water quality analyses did not reveal significant differences in the contents and amount of common ions or compounds among pond, well, and tap water. The only exceptions were total Kjehldahl nitrogen (TKN) and total phosphorus which were higher in water from an irrigation reservoir than in that from other sources. We then looked into the involvement of other microbes in pond water that may have contributed to disease development. Water of interest was filtered through 0.2-µm pore size filters, exposed to Ultraviolet light and/or autoclaved. Treated water was then used as diluent to prepare inoculum and evaluated through a bioassay with leaf disks of *Rhododendron* sp. None of the sterilization treatments reduced the stimulating effect of pond water. These results suggest metabolites from some microbes may be involved in plant-infection enhancement. We also isolated some bacterial species from pond water and assessed their stimulating effect using the same bioassay. Some bacterial cell- and protein-free supernatants were as effective as pond water in stimulating zoosporic infection. Identification of infection-stimulating molecules in these supernatants is underway.

37.75 EVALUATION OF SPRING WHEATS FOR RESISTANCE TO LEAF RUST. E.D. Kovalenko, M.I. Kiseleva, A.A. Shcherbik and H. Bockelman. *All-Russia Research Institute of Phytopathology, Moscow, Russia. Email: kovalenko@vniif.rosmail.com*

The wheat leaf rust fungus (*Puccinia triticina)* is endemic and harmful in Russia, and success of breeding for resistance to leaf rust depends on the initial material. Selection of sources of spring wheat resistance to leaf rust was the aim of our research. More than 1200 spring wheat cultivars received from Germplasm Resources Information Network (USA) were evaluated for resistance to local leaf rust populations in the ARRIP infectious nursery. Wheats belonged to two genetic types: *Triticum aestivum* (hexaploid, 42 chromosomes) and *T. turgidum* subsp. *durum* (28 chromosomes). The spring wheat collection was represented by cultivars from North and South America, Europe, Asia, Africa and Australia. Seedlings of the same cultivars were evaluated for resistance to leaf rust in a greenhouse. According to data from the field nursery during vegetation, and greenhouse tests, the spring wheat cultivars were conditionally divided by leaf rust resistance into four groups: resistant (*T. aestivum* – 19.7%, *T. turgidum* subsp. *durum* – 67.6%); adult plant resistant (*T. aestivum* – 22.6%, *T. turgidum* subsp. *durum* – 22.7%); partially resistant (*T. aestivum* – 18.4%, *T. turgidum* subsp. *durum* – 5.5%); susceptible (*T. aestivum* – 39.1%, *T. turgidum* subsp. *durum* – 4.2%). Wheats from the different regions of the world differed in leaf rust resistance, that of *T. turgidum* subsp. *durum* being higher than that of *T. aestivum*. Many *T. turgidum* subsp. *durum* cultivars from the USA, Canada and Mexico were resistant to the pathogen.

37.76 PRODUCTION OF HIGH MOLECULAR WEIGHT PHYTOTOXIN(S) BY GERMINATING SPORES OF *MAGNAPORTHE GRISEA*. Y. Kumura, N. Kawakami, M. Ueno, J. Kihara and S. Arase. *Faculty of Life and Environmental Science, Shimane University, Matsue 690-8504, Japan. Email: makotou@life.shimane-u.ac.jp*

Production of phytotoxin(s) by germinating spores of *Magnaporthe grisea* was investigated. Detached rice leaves (cv. Asahi) were treated with *M. grisea* spore cultures germinated for 0, 3, 6, 12 or 24 h, and then kept under light or in the dark for 48 h. Under light, leaf necrosis was significantly induced by treatment with 3- or 6-h cultures, which correspond to an initial stage of germ tube formation, but not in the dark. Leaf necrosis was also induced in leaves of barley, crabgrass or finger millet. On the other hand, 12- or 24-h cultures, which correspond to the stage of mature appressorium formation, did not induce leaf necrosis, regardless of the light conditions. Leaf necrosis-inducing activity was lost by treatment with KIO_4 and α-mannosidase, but not by treatment with proteinase K, β-galactosidase, and β-glucosidase. The molecular weight of active compound(s) was estimated to be over 10 kDa. When 3- or 6-h cultures were applied to detached rice leaves, expression of resistance-related genes was observed under light. These results suggested that *M. grisea* produced high molecular weight phytotoxin(s), which act as an elicitor under light, at an initial stage of spore germination.

37.77 CELL WALL MUTANTS OF *BOTRYTIS CINEREA* AND THEIR INTERACTION WITH PLANT DEFENCE MECHANISMS. C. Kunz, P. Malfatti and M-C. Soulie. *UMR 217 INRA/Université ParisVI/AgroParisTech, Laboratoire Interactions Plantes-Pathogènes, 16 Rue Claude Bernard, 75231 Paris Cedex 05, France. Email: kunz@ccr.jussieu.fr*

Epidemics caused by the fungus *Botrytis cinerea* can be severe and economically damaging to many agricultural and horticultural crops. Chitin is an essential constituent of fungal cell walls, and chitin biosynthesis could be a suitable target for fungicides. Seven chitin synthase genes (Bcchs) have been identified in *B. cinerea*. We used Bcch mutants to study the specific role of chitin synthases in *B. cinerea* biology. So far, four mutants disrupted in Bcchs

classes I, IIIa, IV and VII were obtained and characterized. The four mutants showed different aggressiveness phenotypes on *Vitis vinifera* and *Arabidopsis thaliana*: Bcchs IV displayed a slight delay in infection whereas Bcchs1 and Bcchs 3a showed a reduction of aggressiveness of 25 and 70% respectively. The mutation of Bcchs VII was lethal, which makes chitin synthase VII an interesting fungicide target. Interestingly, Bcchs3 restored its aggressiveness (80%) on the *A. thaliana* pad3 defence mutant, which lacks the phytoalexin camalexin. The altered cell wall structure in Bcchs3 might cause an increased plant defence induction and/or the mutant imay be more sensitive to camalexin. We tested the effect of camalexin on growth of wild-type strain Bd90 and Bcchs3 *in vitro*, and first results indicated an increased sensitivity of Bcchs3 towards camalexin. In order to know if this chitin synthase mutant also induces higher levels of camalexin in infected leaves, we are currently investigating the amount of phytoalexin present in *A. thaliana* leaves infected with Bd90 or Bcchs3. We will also analyse if higher camalexin levels correlate with increased pad3 expression.

37.78 VARIATION OF HOST-PATHOGEN INTERACTIONS IN A WILD PLANT PATHOSYSTEM: A CASE STUDY *LACTUCA SERRIOLA – BREMIA LACTUCAE.* **A. Lebeda, I. Petrželová and Z. Maryška.** *Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71 Olomouc-Holice, Czech Republic. Email: ales.lebeda@upol.cz*

Research on the wild-plant pathosystem, *Lactuca serriola* (prickly lettuce) - *Bremia lactucae* (lettuce downy mildew), was conducted in the Czech Republic (CZ) in 1995-2006. The occurrence of *B. lactucae* on naturally growing *L. serriola* and other Asteraceae was recorded. *L. serriola* was the most common naturally growing host species of *B. lactucae.* Variation in resistance to *B. lactucae* was studied by using ten isolates (NL and BL races with known virulence patterns) on a metapopulation level, i.e. 250 *L. serriola* samples representing 16 populations from the CZ. The research revealed broad variation in host resistance among populations and also intrapopulation variability; altogether 45 resistance phenotypes were recorded, including completely resistant and fully susceptible; however, the most frequent were race-specific reaction patterns. Structural and temporal changes in virulence variation of *B. lactucae* populations on *L. serriola* were studied; 313 isolates of *B. lactucae* originating from the CZ were examined for the presence of 32 virulence factors (v-factors), and 93 different virulence phenotypes (v-phenotypes) were recorded. A study of v-factor frequency in *B. lactucae* populations on *L. serriola* showed that common v-factors in the pathogen population match some of the race-specific resistance *Dm* genes/R-factors originating from *L. serriola.* The highest frequency was recorded for v-factors v7, v11, v15-17, and v24-30. In the wild pathosystem, v-factors complementary to R-factors from *L. serriola* predominated, but in the crop pathosystem (*Lactuca sativa – Bremia lactucae*) v-factors matching R-factors from *L. sativa* were most common.

37.79 EXPLORING RESISTANCE IN WHEAT TO *FUSARIUM* EAR BLIGHT DISEASE. **S. Lee and K.E. Hammond-Kosack.** *Centre for Sustainable Pest and Disease Management, Rothamsted Research, Harpenden, Hertfordshire. AL5 2JQ, UK. Email: sarah.lee@bbsrc.ac.uk*

Fusarium ear blight (FEB), caused principally by the fungal pathogens *Fusarium graminearum* and *Fusarium culmorum,* is a devastating disease of wheat. The problems caused by ear infection are two fold: firstly, shrivelling of grain causes a reduction in yield and quality and secondly, the accumulation in the grain of *Fusarium* trichothecene mycotoxins, primarily deoxynivalenol (DON) and its acetylated derivates 3-ADON and 15-ADON and nivalenol (NIV), results in a reduction in quality and is a concern for food safety. Control of the disease is difficult and use of resistant cultivars is now considered to be the best control option. In this project, hexaploid wheat genotypes from around the world were screened in field trials over two years for resistance to FEB. Harvested grain from the trial was analysed using gas chromatography-mass spectrometry to quantify the DON mycotoxin present. Genotypes which showed reduced disease symptoms and/or mycotoxin accumulation are now being analysed further under controlled environment conditions. The infection biology of the more resistant genotypes is being investigated in two ways. Firstly, measurement of a DON breakdown product DON-3-glucoside will establish whether the mycotoxin is being broken down in planta. Secondly, using transgenic isolates of *Fusarium graminearum* producing the reporter protein β-glucuronidase (GUS), the route of infection and DON mycotoxin production is being further explored.

37.80 THE USE OF SUPPRESSION SUBTRACTIVE HYBRIDISATION (SSH) AND CDNA MICROARRAYS TO IDENTIFY DIFFERENTIALLY EXPRESSED CDNAS FROM *PLASMOPARA*-INFECTED GRAPEVINES. **G. Legay, A. Slaughter, J.M. Neuhaus and B. Mauch-Mani.** *Department of Molecular and Cellular Biology, University of Neuchatel, 2009 Neuchatel, Switzerland. Email: guillaume.legay@unine.ch*

Grapevine (*Vitis* species) is the world's largest and economic fruit crop because of its multiple uses including producing juice, fresh and dried fruit, distilled liquor and wine. Unfortunately, *V. vinifera* is susceptible to a wide number of diseases. Among these, the most threatening is downy mildew caused by the oomycete *Plasmopara viticola* which was introduced into Europe during the 19th century. Disease control is mainly achieved by regular application of various fungicides. This practice not only has detrimental consequences on the environment but also promotes resistant pathogen strains. For these reasons alternative ways of combating downy mildew are seriously required. Genomic approaches are likely to have particular value for grape improvement as they have the potential to identify transcriptional, biochemical and genetic pathways that contribute to disease resistance (e.g. specific resistance genes and downstream transcriptional pathways). Many processes are still unknown such as which defence mechanisms are delayed or repressed in grapevine varieties susceptible to downy mildew. To isolate differentially expressed genes involved in the defence against *Plasmopara*, a forward and reverse suppression subtractive hybridisation (SSH) library was constructed on the susceptible variety 'Chasselas'. A total of 2700 clones from this library were spotted on microarray slides representing both up- and down- regulated genes involved in *P. viticola* infection. This method provides a powerful tool for transcriptome analysis of biotic stresses in grapevine. Results from the microarray analysis will be discussed.

37.81 QUORUM SENSING REGULATES A TYPE I SECRETION SYSTEM IN *PSEUDOMONAS CORRUGATA*, CAUSAL AGENT OF TOMATO PITH NECROSIS. **G. Licciardello, I. Bertani, L. Steindler, P. Bella, V. Venturi and V. Catara.** *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. Email: vcatara@unict.it*

A PcoI-PcoR quorum sensing (QS) system was recently identified in *Pseudomonas corrugata* (Pc), causal agent of tomato pith necrosis. QS derivative mutants were affected in virulence on tomato and *in vitro* antagonistic activity against a number of fungi and bacteria. A 5611 bp operon encoding a tripartite resistance-nodulation-cell division (RND) transporter system was identified in the downstream region of the *pcoI* gene. It encodes an outer membrane protein, a periplasmic membrane fusion protein, and an RND-type cytoplasmic membrane protein. This transporter system is predicted to be involved in the export of drugs, cation or secondary metabolites. The RND type transporter system showed the highest amino acid homology with the PseABC efflux system of *P. syringae* pv. *syringae* B728a (Pss) involved in secretion of syringopeptins and syringomycin. Analysis of the operon promoter region revealed the presence of a *lux* box-like element centred 75 bp upstream from the putative translational start site. The presence of this putative regulatory element suggests that this transporter system is subject to QS regulation. The promoter region of the homologue of the *pseA* of Pss was cloned into the promoter probe vector pMP220 and conjugated into Pc, Wt strain and in *pcoI* and *pcoR* QS mutants. The beta-galactosidase activity was determined, and the transcription in Wt was 10 and 3 fold higher than in the *pcoR* and the *pcoI* mutants, respectively. This efflux system is hypothesized to have a role in secretion of secondary metabolites involved in pathogenesis and/or antimicrobial activity.

37.82 AUTOPHAGY VITALIZES APPRESSORIUM MORPHOGENESIS IN *MAGNAPORTHE ORYZAE*. X.-H. Liu, J.-P. Lu, T.-B. Liu and F.-C. Lin. *Biotechnology Institute, Zhejiang University, Hangzhou 310029, P.R. China. Email: fuchenglin@zju.edu.cn*

One of the most widespread and devastating plant diseases is rice blast which causes significant crop losses throughout China, South East Asia and South America, representing a loss of 157 million tons of rice per annum in the staple food crop of one half of the world's population. Major epidemics covering vast areas occur regularly, causing severe food shortages to entire nations and leading to multiple social crises. The pathogenic fungus, *Magnaporthe oryzae*, has emerged as a model system for dissecting fungal-plant interactions. Considerable progress has been made in identifying gene functions necessary for the regulation of pre- and post-penetration events in the development and pathogenicity of the fungus such as appressorium formation and invasive growth of the pathogen. However, the detailed molecular mechanisms of these developmental stages, especially appressorium morphogenesis, are still relatively poorly understood. We have isolated about 9 autophagic genes, which are highly conserved among other eukaryotes, including humans and plants. Disruption of these genes influenced the ability to survive starvation, and affected conidiation, conidial germination, lipid turnover, and appressorium turgor generation. As a result, these null mutants lose the ability to penetrate and infect the host plants. By reintroducing the autophagic genes to their null mutants, all the defects were restored. Taken together, clarification of the functions and network of these autophagy genes will lead to better understanding of the role of autophagic genes in this pathogen-host system.

37.83 PATHOGEN-DEFENCE-RELATED *ARABIDOPSIS* GENE DIFFERENTIALLY EXPRESSED IN RESPONSE TO *PHYTOPHTHORA INFESTANS*. Y.C. Liu, J.H. Xin, Q.Y. Weng and J.G. Dong. *Mycotoxin Laboratory, Agricultural University of Hebei, Baoding 071001, P.R. China. Email: liuyingchao@hebau.edu.cn*

Potato late blight, caused by the oomycete pathogen *Phytophthora infestans*, is one of the world's most devastating plant diseases. Late blight was responsible for the European potato famine in the 19th century, which caused the starvation deaths of more than one million people in Ireland alone. Control of late blight in China and other developed countries relies extensively on fungicide application. Cultivar resistance is one of the most ecomonic and effective approaches to the problem. We previously showed that the *Arabidopsis* ecotype Col is highly resistant to strain 17-B-1 of *P. infestans*. In this paper, genes of *Arabidopsis* differentially expressed after inoculation with *P. infestans* were studied using Differential Display Reverse Transcription-PCR (DDRT-PCR). One hundred and eight differentially expressed cDNA fragments were obtained. Among them 57 were found to hybridize to cDNA from inoculated and uninoculated plants. Four of the fragments were cloned and sequenced. Homologies indicate that one fragment was derived from a gene which encodes a member of the 1-aminocyclopropane-1-carboxylate synthase group, and another fragment was derived from gene At5g32405.1 the function of which is unknown.

37.84 PROGRESS TOWARD MAP-BASED CLONING OF AVRHAR FROM *PYRENOPHORA TERES* F. *TERES*. Z.H. Liu, J.D. Faris, M.C. Edwards and T.L. Friesen. *USDA-ARS Cereal Crops Research Unit, Northern Crop Science Lab, 1307 18th Street North, Fargo ND, 58105, USA. Email: timothy.friesen@ars.usda.gov*

The fungus *Pyrenophora teres* is a worldwide and economically important pathogen of barley, causing barley net blotch. A gene-for-gene relationship has been characterized in this host-pathogen system and four avirulence/virulence genes have been identified and mapped in the fungus. Here, our goal is to isolate *AvrHar*, the first *Avr* gene identified and mapped in *P. teres*, using a map-based cloning approach. Chromosome walking was initiated from an AFLP marker 2.2 cM from *AvrHar* using a 178-progeny fungal mapping population. Genotypic and phenotypic analysis has led to the construction of a 330 kb contig, which includes one fosmid and three BACs, within the *AvrHar* genomic region. We have completely sequenced 300 kb of the contig, which consists of a large amount of repetitive DNA and 42 predicted genes. A PCR based marker located about 160 kb from the original AFLP marker in the direction of the *AvrHar* candidate gene region has been shown to co-segregate with *AvrHar*. A candidate gene region consisting of 120 kb has been sequenced with only two predicted genes. We are presently verifying whether additional chromosome walking steps are necessary in order to cross the target gene. Interestingly, some of the repeat sequence has similarity to that surrounding *AvrLm1*, an *Avr* gene in *Leptosphaeria maculans*. The gene-coding region was shown to have high co-linearity to chromosome 2 of *Pyrenophora tritici-repentis*, a related pathogen that causes tan spot of wheat.

37.85 RELATIVE LEVELS OF *BEET NECROTIC YELLOW VEIN VIRUS* IN SUSCEPTIBLE AND RESISTANT GENOTYPES IN FIELD-GROWN SUGAR BEETS. S.B. Mahmoudi, R. Amiri, S. Darabi and M. Kakouinejad. *Sugar Beet Seed Institute, P.O. Box 31585-4114, Karaj, Iran. Email: mahmodi@sbsi.ir*

In order to study the trend of BNYVV concentration during seasonal growth, virus concentration was measured using DAS-ELISA in six genotypes including D-h and L-KWS (as resistant

commercial varieties), Z-83 (carries a 50% resistance gene), F2-93 (as F2 population with a 75% resistance gene), BC1-261-99 (population with 25% resistance gene) and Shirin (susceptible) and at four harvesting dates (2, 3, 4 months after planting and at harvest). The second and third harvesting dates had minimum and maximum ELISA values, respectively. A highly significant cultivar-harvest date interaction occurred. Absorbance readings for the first harvest clearly discriminated differences in cultivar reactions more than in subsequent harvests. So, early season virus multiplication (two months after planting) can be used to differentiate resistant and susceptible genotypes.

37.86 THE STUDY OF BIOCHEMICAL FEATURES OF PATHOGEN-DEPENDENT ENZYMES (ATPASE, PEROXIDASE) AND CONTROL OVER THEIR ACTIVITY UNDER INFECTION OF POTATO WITH *FUSARIUM SOLANI*. **A.M. Manadilova, A.Sh. Utarbayeva, O.A. Sapko and R.M. Kunaeva.** *Aitkhozhin Institute of Molecular Biology and Biochemistry, Dosmukhamedov St.86, Almaty, Kazakhstan. Email: Man_Alija@mail.ru*

This research was aimed at studying the activity and some physical-chemical characteristics of Ca^{2+}- ATPase and Peroxidase (POD) of potato varieties differing in resistance to the fungus *Fusarium solani*. An increase of Ca^{2+}-ATPase activity occurs in the first hours of infection with the fungus in resistant potato varieties, whereas no change is seen with non-resistant varieties. Infection leads changes in physical-chemical enzyme parameters (increase of K_m and V_{max}, change of incubation medium pH in resistant potato varieties). Data were obtained on the inhibitory effect of verapamil on the synthesis of phytoalexins (PA) and Ca^{2+}-ATPase activity. These data indicate the connection between activation of Ca^{2+} channels and PA synthesis, which participate protective reactions in potato. POD is an antioxidant enzyme which is important in protective responses. The effects of protein and non-protein fractions of culture filtrate and mycelium on tuber disks were studied. Culture filtrate proteins stimulated rapid induction of soluble and especially cell-wall bound forms of POD, both in tolerant and sensitivity varieties. The non-protein isolates also, but not significantly, induced soluble PODs. Induction of cell-wall bound PODs depended on plant sensitivity. In tolerant varieties there was a greater increase of enzyme activity than in sensitive ones. The influence of metabolites in culture filtrate and mycelium on POD activity in suspension cells was studied.

37.87 INTROGRESSION OF BARLEY GENES FOR PARTIAL RESISTANCE TO LEAF RUST INTO A GENETIC BACKGROUND SUSCEPTIBLE TO NON-HOST CEREAL RUST SPECIES. **T.C. Marcel, A. Lorriaux, F.Y. Kuok San, H. Jafary and R.E. Niks.** *Department of Plant Breeding, Wageningen University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands. Email: thierry.marcel@wur.nl*

Partial resistance is characterised by a reduced rate of epidemic development despite a susceptible infection type. Partial resistance represents the proportion of quantitative resistance that contributes to the basal defense of the plant against the intruding pathogen. Such a defense system prevents the formation of fungal haustoria and is due to genes located on so-called quantitative trait loci (QTL). Evidence suggests that basal resistance of barley to leaf rust (*Puccinia hordei)* is a weak form of non-host resistance, resulting from the partial success of the rust fungus to deal effectively with the defence that plant species mount against maladapted microbial intruders. A detailed chromosome map of barley was constructed and used as a platform to compare the genetic positions of QTLs across different mapping populations. Each mapping population segregated for a different set of QTLs. Five QTLs, selected for the size of their effect in barley seedlings, are being introgressed into SusPtrit background. Barley line SusPtrit was developed, through crosses and selection, by accumulating genes for high susceptibility to different heterologous rust taxa. The introgression of barley genes for partial resistance to leaf rust into the SusPtrit genetic background will permit to determine whether the same genes also have an effect against non-host rust species. Concurrently, high-resolution maps of the five target QTL regions are being constructed using synteny with rice and recent transcript maps of barley. This will allow determining the precise position of the genes underlying QTLs and to identify candidate genes explaining those QTLs.

37.88 IDENTIFICATION OF (POLY-) PHENOLIC SUBSTANCES IN SORGHUM (*SORGHUM VULGARE*) DURING INTERACTION WITH *SCLEROTIUM ROLFSII*. **S. Maurya, R. Singh, D.P. Singh, H.B. Singh, J.S. Srivastava and U.P. Singh.** *Assistant Professor, J.M.V., Ajitmal, Auraiya (Affiliated to Kanpur University), Auraiya, India. Email: maurya_sd@rediffmail.com*

Indentification of individual phenolic acids of sorghum (*Sorghum vulgare*) after interaction with *Sclerotium rolfsii* using high performance liquid chromatography (HPLC) showed presence of phenolics namely tannic, gallic, ferulic, chlorogenic and cinnamic acids in varying amounts. A maximum amount of ferulic acid (166.6 µg/g fresh wt) was present in the collar of inoculated plants 72 h after inoculation, with less in leaves and roots, the levels gradually decreasing with time. Similarly, chlorogenic acid was found present after 48 h, and cinnamic acid was detected within 72 h of inoculation. Reddish-brown pigmentation at the collar region of inoculated plants was also observed along with the high content of tannic acid. Among other phenolics, presence of gallic acid was recorded consistently, and maximum accumulation (139.3 µg/g fresh wt) was noted at the zone of interaction (collar region) 72 h post-inoculation. In contrast, maximum lignin deposition was observed in the collar region after 96 h. Induction of phenolic acids in sorghum along with lignin deposition and red pigmentation at collar region are considered as key biomarkers in the non-host-pathogen interaction of the *S. vulgare-S. rolfsii* pathosystem.

37.89 *FUSARIUM* SPECIES ISOLATED FROM SUGARCANE AND THEIR EFFECT ON DEVELOPMENT OF THE STALK BORER *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE). **S.A. McFarlane and R.S. Rutherford.** *SA Sugarcane Research Institute, P/Bag X02, Mount Edgecombe, 4300, South Africa. Email: sharon.mcfarlane@sugar.org.za*

Certain *Fusarium* species can affect the development and fecundity of *Eldana saccharina* Walker, an important pest of maize and sugarcane in parts of Africa. This was evident in studies conducted in West Africa where stalk damage in maize infected with *Fusarium verticillioides* Sacc. (Nirenberg) was significantly greater due to increased larval numbers and improved larval growth rates. There was also evidence that moth oviposition was positively affected by epiphytic and endophytic symptomless colonisation by *F. verticillioides*. A similar study was initiated in South Africa using *Fusarium* species isolated from sugarcane stalks with and without *E. saccharina* damage. A total of 223 isolates were obtained, representing 14 cultivars and 11 distinct geo-

graphic locations. Of these, 117 were isolated directly from borings, while 65 were isolated from surface-sterilised undamaged cane and were considered to be endophytic. The remaining 41 were isolated from cane showing symptoms of pokkah boeng, a disease affecting maize and sugarcane caused by *F. verticillioides* and *F. subglutinans*. Attenuated isolates were incorporated into a diet formulation used to rear *E. saccharina*. Some isolates resulted in improved survival and reduced time taken to pupation: these were considered to be beneficial to the development of *E. saccharina*. Other isolates appeared to be antagonistic, resulting in reduced survival and increased time to pupation. In olfactory choice assays, significant differences in the numbers of larvae attracted to maize kernels inoculated with different *Fusarium* isolates were noted. Molecular methods were used to group and identify the most beneficial and antagonistic *Fusarium* isolates.

37.90* THE MADS-BOX TRANSCRIPTION FACTOR SIG1 IS DOWNSTREAM OF MPS1 MAP KINASE AND IS REQUIRED FOR INFECTIOUS GROWTH IN *MAGNAPORTHE GRISEA*. **R. Mehrabi and J.-R. Xu.** *Seed and Plant Improvement Institute, Agricultural Research and Education Organization, P.O. Box 31585-4119, Karaj, Iran. Email: rahim.mehrabi@gmail.com*

Through a survey of the *Magnaporthe grisea* genome we found two genes encoding proteins belonging to the family of MADS-box transcription factors, which are homologs of *Saccharomyces cerevisiae MCM1* and *RLM1.* In this study we demonstrate that the homolog of *RLM1* in *M. grisea*, which we designated as *SIG1* plays an essential role for plant infection. Interestingly, the *sig1* mutant was able to form appressoria, penetrate the plant cells and form the primary infectious hyphae but was unable to extend the typical bulbous infectious hyphae and, therefore, failed to invade the host cells. More significantly, when the plant defence responses were repressed prior to inoculation, the *sig1* mutant could invade the host cells in a manner similar to the wild-type strain suggesting that the *sig1* mutant may be defective in overcoming the plant defense responses. We show that the Sig1 protein is expressed in and localized to the nucleus in conidia and particularly in appressoria and infectious hyphae, but not in vegetative hyphae. Furthermore, our data indicate that the evolutionarily conserved MADS-box domain is essential for transcriptional activation of Sig1 because the MADS-box deletion mutant was identical to the *sig1* mutant. In yeast-two-hybrid assays Sig1 strongly interacted with our previously identified MAPK, Mps1 suggesting that Sig1 is the downstream target of Mps1 MAPK. Overexpression of Sig1 in the Mps1 background failed to rescue the defective phenotype of the *mps1* mutant suggesting that transcriptional activation of Sig1 depends on the activity of Mps1 MAPK. To our knowledge no identical mutant with such a phenotype has been identified among plant pathogenic fungi.

37.91 THE EFFECT OF *SPHAEROPSIS SAPINEA* ON CONIFER DECLINE IN SERBIA AND MONTENEGRO. **T. Milijašević.** *University of Belgrade, Faculty of Forestry, Kneza Višeslava 1, Belgrade, Serbia. Email: Tafil@eunet.yu*

Sphaeropsis sapinea is widely distributed in Serbia and Montenegro in the continental and Mediterranean parts of these countries. It was researched intensively during late 80s and 90s. It is recorded on *Pinus nigra*, *P. sylvestris*, *P. halepensis*, *P. jeffreyi*, *P. peuce*, *P. pinaster*, *P. ponderosa*, *P. pinea*, *P. mugo*, *P. heldreichii*, *Abies concolor*, *Cedrus atlantica*, *Chamaecyparis lawsoniana*, *Cupressus sempervirens*, *Juniperus virginiana* and *Thuja occidentalis*. Most of the damage occurred in Austrian and Allepo pine plantations and also in urban areas. *Pinus heldreichii*, a Tertiary relic and Balkan subendemic is a new plant host in these countries. The pathogen was identified on individual trees near Pećka Patrijaršija and the Ostrog Monastery. On pines *S. sapinea* can affect almost all parts of the trees. The most common symptoms are shoot blight, bud wilt, stem canker, branch dieback, and necrosis and stunting of the seed cones. The critical period of infection is from mid April till mid May. It infects buds before they open in spring and also in the summer, in the year of their formation, young shoots through the bark and young needles. Changing of color of the infected needles can be seen at the begining of June, while in the middle of June they become yellow-brown. Pycnidia of *S. sapinea* are observed on young shoots and needles, pollen and seed cones, buds, current year and second year seed cones, and in the bark of older branches.

37.92 *BOTRYOSPHAERIA DOTHIDEA* – AGENT OF CANKER AND TREE DEATH IN SERBIA. **T. Milijašević and D. Karadžić.** *University of Belgrade, Faculty of Forestry*, *Belgrade, Serbia. Email: Tafil@eunet.yu*

The first report of *Botryosphaeria dothidea* (Mong ex Fr.) Cesat & Notaris in Serbia was on *Sequoiadendron giganteum* (giant sequoia) near Belgrade (Avala) in the early 90s. Later on, it was recorded on *Populus nigra* (clone I-214), *Quercus cerris*, *Q. petraea*, *Cedrus atlantica*, *Prunus laurocerasus* and *Viscum album*. The symptoms of the disease depend on plant species, but also on the part attacked. However, the greatest damage has occurred on *S. giganteum*. The shoots and thinner branches are usually killed, and cankers form on the thicker branches and on stems. Abundant resin is exuded where the cankers form. The fungus has two development stages. The pycnidial stage belongs to genus *Dothiorella* sp. and in Serbia it occurs throughout the year. The teleomorph is found more rarely, on the dead bark which is also colonized by other fungi. Control measures are very difficult. It is recommended that during the pruning of branches in tree rows, the cuts should be treated with a fungicide or with cambisane. As for suppressive control measures, our research shows that benomyl and copper fungicides produce good results.

37.93 RESPONSE OF RESISTANT AND SUSCEPTIBLE TOMATO PLANTS TO INFECTION WITH *RALSTONIA SOLANACEARUM*. **A. Milling and C. Allen.** *Dept Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706, USA. Email: cza@plantpath.wisc.edu*

Bacterial wilt of tomato and potato is caused by diverse strains of *Ralstonia solanacearum*, including strains adapted to tropical or temperate conditions. Resistance is the only practical way to control bacterial wilt, but wilt resistance is horizontal, multigenic and complex. Nothing is known about the mechanisms by which natural hosts like tomato defend themselves against *R. solanacearum*. To address these deficiencies, we examined the gene expression profiles of resistant and susceptible tomato plants infected with either a Race 1 tropical strain or a Race 3 biovar 2 temperate strain of *R. solanacearum*. Northern blot and qPCR experiments revealed that in response to *R. solanacearum* infections, tomatoes upregulated genes in the ethylene defense signal transduction pathway, such as PR1b. The salicylic acid pathway (indicated by expression of acidic glucanase gene GluA) was moderately upregulated, but we did not detect significant upregulation of the jasmonic acid pathway (indicated by expres-

sion of proteinase inhibitor gene PinII). Overall, the resistant breeding line Hawaii 7996 launched a much stronger defense than susceptible cultivar Bonny Best in response to infection with a tropical Race 1 strain. In contrast, the temperate Race 3 biovar 2 strain appeared to calm defense responses in both tomato lines, possibly facilitating the latent infections that make Race 3 strains especially dangerous. Interestingly, extrapolysaccharide (EPS)-deficient mutants of the pathogen triggered stronger host defense responses, suggesting that EPS normally functions to cloak *R. solanacearum* from host recognition and defense.

37.94 THE PATHOSYSTEM *LEPTOSPHAERIA MACULANS* AND CRUCIFEROUS CROPS (*BRASSICA* SPP.) IN CENTRAL MEXICO. O. Moreno-Rico, D.E. Manzano-Flores, G. Séguin-Swartz and J.J. Luna-Ruiz. *Departamento de Microbiología, Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Av. Universidad 940, Cd. Universitaria, C.P., 20100, Aguascalientes, Ags., Mexico. Email: omoreno@correo.uaa.mx*

We report the main results of investigations on the pathosystem between *Phoma lingam* (Tode ex Fr.) Desm [(teleomorph *Leptosphaeria maculans* (Desm.) Ces & De Not] and cruciferous cultivated crops of Central-Mexico. Based on typical sympthoms of diseased plants and morphometric observations of 100 picnidia and picniodiospores, we identified the anamorph *P. lingam* as responsible for blackleg disease in cauliflower, broccoli, and cabbage. Infection, development, and dispersion of *P. lingam* was favoured by an average temperature of 18 ºC, 122 mm of rainfall, and 66% relative humidity. These conditions occur from June through september in this region. Under these conditions, the pathogen has caused up to 90% production losses in cauliflower and romanesco (hybrid between cauliflower and broccoli). However, broccoli and cabbage are tolerant to blackleg in Mexico. In order to know if *P. lingam* belongs to the aggressive group, studies were conducted, including *in vitro* observations, pathogenicity tests using 14 cauliflower and 4 canola cultivars, caryotype electrophoresis analysis (CHEF), and nucleic acid hybridization using the repetitive element LMR1. The results with 10 isolates collected in two states (Aguascalientes and Zacatecas) indicated that they belong to the aggressive variant GP2 of *P. lingam*. We induced ascocarp formation by mixing cauliflower crop residues from the above two states of Central, Mexico. Based on morphometric observations conducted on 100 fruit bodies (ascoma, asci, and ascospores), the pathogen was identified as *L. maculans*.

37.95 HISTOPATHOLOGY OF *COLLETOTRICHUM* SPP. CAUSING OLIVE ANTHRACNOSE. C. Mota-Capitão, P. Talhinhas, V. Várzea, H. Oliveira and M.C. Silva. *Instituto de Investigação Científica Tropical, Centro de Investigação das Ferrugens do Cafeeiro, Quinta do Marquês, 2784-505 Oeiras, Portugal. Email: mariaceudasilva@gmail.com*

Olive anthracnose is a common and frequently severe disease, in most cases affecting fruits during maturation, causing premature fruit drop and reduced olive oil quality. Its incidence and severity has been increasing in the last few years in Portugal. *Colletotrichum acutatum* is the main pathogen associated with olive, but *C. gloeosporioides* also occurs, both species having the potential to infect other crops. Epidemiologic studies have demonstrated the asymptomatic presence of these fungi in different organs of the olive tree, such as branches and leaves, suggesting an important role as inoculum sources. Depending on the host, these two *Colletotrichum* species follow different penetration processes and colonization strategies. In this work, histopathologic studies show that conidia can germinate on the fruit surface (at diverse maturity stages) and produce melanised appressoria, followed by the direct penetration of the cuticle by means of a penetration peg. The period when only penetration pegs were observed corresponded to the absence of symptoms. At fruit maturity there was rapid hyphal growth in necrotic fruit cells, with subsequent development of lesions, formation of acervuli and cuticle disruption.

37.96 EXPRESSION OF DEFENCE-RELATED GENES AGAINST *FUSARIUM OXYSPORUM* F.SP. *CUBENSE* IN *MUSA* CULTIVARS. C.L. Munro, N. van den Berg, A.A. Myburg and A. Viljoen. *Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7600, South Africa. Email: altus@sun.ac.za*

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *cubense* (Foc), is one of the most damaging diseases of banana. Options available for the control of this disease are limited, with resistant cultivars the only apparent sustainable means whereby the disease can be managed. Little is known about the molecular processes underlying resistance responses, metabolic pathways and downstream signalling of the banana-Foc interaction. Several defence-related genes have previously been identified as being up-regulated in a tolerant Cavendish banana variety (GCTCV 218) upon infection with Foc (Van den Berg *et. al.,* 2007). Genes were also up-regulated in Lady Finger and Cavendish plants following Foc challenge after priming with non-pathogenic *F. oxysporum* (Forsyth, 2006). The aim of this study was to determine whether these genes played a significant role in the defence response of a range of banana cultivars resistant (Calcutta IV, Rose), tolerant (FHIA 17) and susceptible (Williams) to Foc race 4. Field trial data confirmed the susceptibility status of the respective cultivars, with neither Calcutta IV nor Rose showing any internal symptoms. Expression data from quantitative reverse-transcription real-time (qRT)-PCR results indicated that several of the genes investigated are potentially key genes in the defence response of the tolerant and/or resistant banana cultivars to Foc. Genes that showed up-regulation or constitutively higher levels in the tolerant and resistant cultivars include *POX*, *PR-3*, *lectin*, *PIR7A*, *PAE* and *catalase*. The continued pursuit of new defence-related genes is a research priority to better understand resistance in bananas to the *Fusarium* wilt pathogen.

37.97 GENE EXPRESSION ANALYSIS OF THE MECHANISM OF RESISTANCE TO SHEATH BLIGHT FUNGUS (*RHIZOCTONIA SOLANI*) ON SELECTED RICE LINES USING SEMI-QUANTITATIVE RT-PCR. J. Mutuku, J. Nakamura, S. Agarie and A. Nose. *Honjo-Machi, Saga 840-8502, Japan. Email: 06551104@edu.cc.saga-u.ac.jp*

The fungus *Rhizoctonia solani* Kuhn, is the causal agent of sheath blight disease in rice. To investigate metabolic changes associated with sheath blight, we selected a resistant line (2F18-7-32) and a susceptible line (2F21-21-29). Biochemical analysis revealed that lignin accumulated in the leaf sheath, and phenylalanine ammonia-lyase increased in the resistant line while sugar biosynthesis differentially changed with infection between the resistant and the susceptible lines. Expression levels of 59 enzyme genes were analyzed by semi-quantitative RT-PCR. Sampling was done at 1, 2 and 4 days post inoculation (dpi). The results

showed that the fructose-1,6-bisphosphate adolase gene was induced at a high level after infection in both resistant and susceptible lines while the fructose-1,6-bisphosphatase gene was induced at a low level. In the resistant line, there was a marked variation in the expression level at 1 dpi and 4 dpi, suggesting that the metabolic pathways shifted to favor the synthesis of fructose-1.6-biophosphate (FBP). Expression of genes in the shikimate pathway was induced in both resistant and susceptible lines at 1 dpi. Genes in the citric acid cycle showed differential expression, which was strongly induced in the resistant line but was suppressed in the susceptible line. In the resistant line, the Tal gene and genes responsible for the citric acid cycle and shikimate pathway were strongly induced at 4 dpi. These results suggested that synthesis and distribution of FBP, the activity of shikimate pathway and citric acid cycle seemed to be strongly involved in the sheath blight disease resistance.

37.98 INFECTION OF TOMATO LEAVES WITH A PATHOGENESIS-DEFICIENT STRAIN OF *BOTRYTIS CINEREA*. **M. Nakajima, R. Shinda, M. Maniwa and K. Akutsu.** *Laboratory of Plant Pathology, College of Agriculture, Ibaraki University, Ibaraki 300-0393, Japan. Email: mnakaji@mx.ibaraki.ac.jp*

We isolated an avirulent monoascosporic strain of *Botrytis cinerea* that had lost pathogenicity in several host plants, including tomato. Conidial germination, appressorium formation and infection hypha formation were investigated using onion epidermis. Conidia of the avirulent strain germinated well, and appressoria and infection hyphae were formed at 16 h and 20 h after inoculation, respectively. These behaviors were similar to those of the wild-type strain. The avirulent strain was arrested by a hypersensitive-like necrosis of tomato leaf tissue. In addition, autofluorescent compounds accumulated in infected epidermal cells. Because reactive oxygen species (ROS) production is one of the earliest defence responses in plant-pathogen interaction, we examined hydrogen peroxide (H_2O_2) accumulation at the inoculation site using DAB staining. Microscopic observation confirmed DAB staining in epidermal cells penetrated by the avirulent strain. When inoculation droplets containing conidia were supplemented with an H_2O_2 scavenger (ascorbic acid) or an NADPH oxidase inhibitor (diphenylene iodonium) this reaction was not seen in epidermal cells and hyphae were observed in mesophyll tissue.

37.99 *PHYTOPHTHORA* ROOT AND CROWN ROT ON FRUIT TREES IN BULGARIA. **M.B. Nakova.** *Department of Phytopathology, Agricultural University Plovdiv, 12 Mendeleev St., Plovdiv 4000, Bulgaria. Email: mnakova@au-plovdiv.bg*

Since the year 2000 symptoms of a new, unknown disease causing death of single trees or groups of trees have been found on apple, cherry and almond in Bulgaria. Laboratory analyses of the pathogenic fungi isolated proved that they belong to the genus *Phytophthora*. The prevailing species was *P. cactorum*. In vitro tests were done on utilization of different C, P, N, and S sources by *P. cactorum* and *P. citrophthora*. Physiological changes such as carbon/sugar, protein and dry matter content were studied, as well as N, P, and K amounts in infected and healthy cherry trees. Changes in amino acid contents and breeding activity were studied in apple, cherry, almond, peach and quince rootstocks.

37.100 INFECTION BIOLOGY OF *FUSARIUM VIRGULIFORME*, A CAUSAL AGENT OF SOYBEAN SUDDEN DEATH SYNDROME. **S.S. Navi and X.B. Yang.** *Department of Plant Pathology, 351 Bessey Hall, College of Agriculture and Life Sciences, Iowa State University, Ames, Iowa 50011, USA. Email: ssnavi@iastate.edu*

Soybean sudden death syndrome (SDS), caused by *Fusarium virguliforme*, is a root disease that results in severe foliar symptoms during the reproductive stage. For nearly three decades, SDS has been recognized as a major disease of soybean, *Glycine max* (L.) Merr., in the southern United States. The objective of our study was to understand the process of infection and colonization by the fungus leading to foliar symptom expression. To demonstrate the biology of infection, we used an effective and quantifiable seedling inoculation technique in which germinated seeds in a Petri dish were spray-inoculated with conidial suspension before being transplanted. Plants that had foliar symptoms showed taproots and basal stems discoloured both externally and internally, while plants with no foliar symptoms had only superficial discoloration. Microtome sectioning of taproots of plants with foliar symptoms revealed the presence of fungal structures in both xylem and phloem tissues, while plants without foliar symptoms revealed fungal structures only in phloem tissue. A SEM study showed a higher penetration frequency of the fungus near the root cap zone where few or no root hairs of the radicle were found. These results indicate that fungal penetration into the xylem tissue plays a role in foliar symptom expression.

37.101 FUNCTIONS OF THE 2B PROTEIN OF *PEANUT STUNT VIRUS* IN VIRAL REPLICATION, MOVEMENT AND PATHOGENICITY. **O. Netsu, K. Hiratsuka, S. Kuwata, T. Hibi, M. Ugaki and M. Suzuki.** *Laboratory of Bioresource Technology, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, 202 Bioscience Building, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan. Email: msuzuki@k.u-tokyo.ac.jp*

Peanut stunt virus (PSV) is a member of the genus *Cucumovirus*. The 3'-terminal part of PSV RNA 2 putatively encodes the 2b protein (2bP), whose gene overlaps 2a gene as in other cucumoviruses. We constructed two PSV mutants, which express an N-terminal eight amino acid truncated 2bP or no 2bP, designated mM1S2 or mM1S2M9, respectively. *Nicotiana tabacum* protoplasts and *N. benthamiana* plants were inoculated with *in vitro* transcribed RNA 2 derivatives and RNA 1 and 3 from corresponding cDNA clones of a PSV strain J2. Both 2bP mutants replicated as well as wild-type PSV in the protoplasts, suggesting that 2bP is not essential for viral replication. Both mutants moved systemically in inoculated *N. benthamiana* plants, showing that 2bP is not essential for viral movement. However, the plants infected by the 2bP mutants did not express any symptoms, and viral RNA accumulation of the 2bP mutants was significantly lower than that of wild-type PSV in the inoculated and the upper leaves. This means that PSV 2bP may be responsible at least for viral local movement and may influence systemic movement and pathogenicity. Notably, the viral movement or RNA accumulation of mM1S2 mutant was lower and higher than wild type and mM1S2M9 mutant, respectively, suggesting the important role of the N-terminal amino acids of 2bP. Furthermore, PSV 2bP was shown to act as a viral silencing suppressor, by transient expression assay with GFP reporter genes using *Agrobacterium*.

37.102 REACTION OF SOME AFRICAN RICE GERMPLASM TO *SAROCLADIUM ATTENUATUM* UNDER FIELD CONDITIONS. G.N. Ngala and M.O. Adeniji. *Bamenda University of Science and Technology, P.O Box 5135 Bamenda Nkwen, Mezam Division, N.W Province, Cameroon. Email: gnngala@yahoo.com*

Rice reminds most people of the Asian civilization. It is also African. Tropical *Oryza glaberrima* (TOG) was domesticated 3000 years ago in West Africa. Rice is in great demand today as a staple food in Africa, but production levels are very low. Africa suffers from rice diseases and pests unique to her, but only a few people work on these. Research should therefore address these problems since the potential for increased yield is good. The dirty panicle disease causing grain sterility and poor milling is one of the serious problems on both African and Asian rice, and *Sarocladium attenuatum* is the major pathogen. It was therefore of interest to screen TOG germplasm from different African countries for resistance to this fungus. At the 50% flowering stage, panicles of the test and control plants were inoculated by spraying with the pathogen (3.75×10^8 conidia/ml) and sterile distilled water respectively from a De Vilbiss No 15 sprayer. Disease symptoms developed within 6 to 12 days and were rated 4 times on a scale 1 to 5. Based on an established standard curve and the resistance levels defined by it, 21.4% of the TOG varieties gave a resistant reaction, 60.7% moderately resistant and 17.9% susceptible. The resistant and moderately resistant (82.1%) present a promising source of germplasm for improving resistance to the dirty panicle syndrome. These TOG germplasms should therefore be maintained and their production and genetic diversity increased.

37.103 SILENCING SUPPRESSION BY *CUCUMBER GREEN MOTTLE MOSAIC VIRUS* 129K PROTEIN: A COMPARISON BETWEEN SEVERE AND ATTENUATED STRAINS. M. Nishiguchi, H. Chen and N. Yamaoka. *Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama 790-8566, Japan. Email: mnishigu@agr.ehime-u.ac.jp*

Cucumber green mottle mosaic virus (CGMMV) is a tobamovirus infecting cucurbit plants. An attenuated strain of CGMMV, SH33b, has been used to protect muskmelon plants against wild severe strains in Japan. Silencing suppression was investigated by inoculation of GFP-silenced *Nicotiana benthamiana* with severe and attenuated strains, SH and SH33b, respectively. As a result, GFP expression was suppressed in SH-infected plants but not in SH33b-infected ones, suggesting that CGMMV SH genome encodes a silencing suppressor protein. In the next step, DNA fragments corresponding to the 129 K protein of strains SH and SH33b were PCR-amplified and inserted into the plant expression vector plasmid, respectively. Each plasmid was added to the transient RNA silencing induction system by particle bombardment with a mixture of two types of GFP-carrying plasmids: one with a single copy of GFP and the other with an inverted repeat of GFP to produce dsRNA, using an onion bulb. It was shown that GFP silencing was suppressed in the presence of the 129 K protein from SH but not from SH33b. Furthermore, only the methyltransferase domain of the 129 K protein was also expressed using the expression vector plasmid. The methyltransferase domain of the SH 129 K protein suppressed RNA silencing, while that of SH33b did not. Since there is only one amino acid substitution, glutamic acid (SH) to glycine (SH33b), in this region, it may be responsible for decreasing the suppression.

37.105 COMPARISON OF ECOPHYSIOLOGICAL AND PHYTOPATHOLOGICAL RESULTS OF *ALNUS GLUTINOSA* TREES INFECTED NATURALLY OR ARTIFICIALLY WITH *PHYTOPHTHORA ALNI*. W. Oßwald, C. Clemenz, F. Fleischmann and R. Matyssek. *Section Phytopathology of Woody Plants, Technische Universität München, Am Hochanger 13, 85354 Freising, Germany. Email: osswald@wzw.tum.de*

Ecophysiological and phytopathological data on 25-year-old *Alnus glutinosa* trees infected with *Phytophthora alni* subsp. *alni* and of three-year-old alder saplings inoculated with the same pathogen were compared. Young and old infected plants showed significantly reduced rates of photosynthesis at comparable rates of transpiration. Young plants exhibited large variability regarding pathogen development, ranging between high susceptibility and resistance. Infection resulted in strong growth retardation, and finally 75 per cent of all infected saplings had died after two years of infection. A linear regression was found, when all girdling values of inoculated saplings were plotted versus the corresponding mean values of photosynthesis during the first year of infection, proving the inhibitory systemic effect of stem girdling on photosynthesis. Starch levels of leaves of young and old infected plants were significantly increased compared to controls, possibly indicating destruction of the bark tissue by the pathogen to cause blockage of phloem transport from leaves to roots. Given such a scenario, stomatal closure appears to result from product-inhibited photosynthesis upon phloem disruption, preventing WUE to decline. Water consumption of mature infected trees was significantly lower compared to controls. All these trees showed severe secondary infections with wood-decaying fungi, besides destruction of phloem and cambium tissue caused by primary infections with *P. alni*. We conclude that *P. alni*-infected trees in the field die due to the primary infection, and because of secondary pathogens which are specialists causing final decay of woody tissue.

37.106 ANALYSIS OF THE AMINO-TERMINAL REGION OF *PLANTAGO ASIATICA MOSAIC VIRUS* COAT PROTEIN, RESPONSIBLE FOR VIRAL CELL-TO-CELL MOVEMENT. J. Ozeki, H. Senshu, M. Himeno, K. Maejima, K. Komatsu, Y. Yamaji and S. Namba. *Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. Email: aa67004@mail.ecc.u-tokyo.ac.jp*

Successful establishment of systemic infection of plant viruses requires at least three steps: virus replication, cell-to-cell movement, and long-distance movement. Cell-to-cell movement requires virus-encoded proteins, movement proteins (MPs), and host proteins. Potexviruses encode three MPs, referred to as triple gene block protein (TGBp) 1, 2 and 3. Besides the TGBps, coat protein (CP) is also required for cell-to-cell movement; thus potexvirus CPs are bifunctional, required for cell-to-cell movement and virion formation. However, the details of these functions remain unclear. The central region of the potexvirus CP is highly conserved, but the amino- and carboxyl-terminal regions are quite variable and show low levels of similarity among potexviruses. *Plantago asiatica mosaic virus* (PlAMV, *Potexvirus*) has two in-frame AUG codons located at or near the 5' terminus of the CP ORF (AUG1 and AUG2 respectively). Using PlAMV mutants, we found that CP translated from AUG1 is important for the systemic infection of PlAMV. We further found that the amino-terminal is important for cell-to-cell movement of PlAMV. We compare these findings to previous studies on the potexvirus CP, and discuss its role in cell-to-cell movement and particle formation.

37.107 MAPPING OF DETERMINANTS FOR RESISTANCE AND TOLERANCE TO *CUCUMBER MOSAIC VIRUS* IN *ARABIDOPSIS THALIANA*. I. Pagán, C. Alonso-Blanco and F. García-Arenal. *Departamento de Biotecnología, E.T.S.I. Agrónomos, and Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid, 28040 Madrid, Spain. Email: jesusisrael.pagan@upm.es*

Screening of genetic variation among *Arabidopsis thaliana* has been used to analyse resistance and tolerance components in the interaction *Cucumber mosaic virus* (CMV) – *A. thaliana*. We challenged 21 *Arabidopsis* accessions with three different CMV strains. Virus accumulation, symptom development, plant growth, and seed production and viability were quantified. The three CMV isolates infected all the accessions, but there were differential interactions in all traits measured. Thus, *Arabidopsis* responses to CMV infection depend on host-pathogen genotype × genotype interactions. One accession (Co-1) developed systemic veinal necrosis upon infection with CMV isolates in subgroup I. Analysis of the F2 population from a cross with an accession that did not develop necrosis showed that this syndrome was determined by a single dominant gene. Fine mapping indicated that this gene was *RCY-1*, involved in hypersensitive response in *Arabidopsis* accession C-24 against CMV-Y. Sequencing of *RCY-1* in Co-1 showed various deletions relative to the sequence of *RCY-1* from C-24, which may explain the different phenotype. The rest other accessions showed different degrees of resistance and tolerance to the different virus isolates. We mapped quantitative trait loci for resistance and tolerance using RILs derived from parentals with extreme phenotypes. While no significant QTLs for resistance were identified, five QTLs explained 50% of the variance for tolerance to viral infection. The involvement of these QTLs in plant architecture and resource allocation was explored, illustrating on the mechanisms of tolerance of *Arabidopsis* to CMV.

37.108 INFLUENCE OF THE *BEET NECROTIC YELLOW VEIN VIRUS* P25 PROTEIN UPON REGULATION OF CELLULAR RNA. C. Peltier, L. Schmidlin, E. Klein and D. Gilmer. *IBMP, 12 Rue du Général Zimmer, 67084 Strasbourg Cedex, France. Email: claire.peltier@ibmp-ulp.u-strasbg.fr*

Rhizomania is caused by *Beet necrotic yellow vein virus* (BNYVV) which induces general stunting of the beet and proliferation of lateral rootlets. These symptoms are mainly caused by the virulence factor p25 encoded by the viral RNA 3. Although this virus has been well studied, the mechanisms of its virulence are still unknown. In order to better understand the molecular aspects of p25 function, we created plants that constitutively express p25. Several p25-expressing *Arabidopsis thaliana* transgenic lines were obtained. Root proliferations and increased sensitivities to auxin were found in all transgenic lines, which were resistant to high concentrations of hormones such as methyl-jasmonate and abscissic acid. Furthermore, expression of p25 allowed viral accumulation in plants. Microarray analysis of three independant single-copy transgenic and wild-type plants revealed modulation of more than a thousand cellular genes. To overcome growth kinetic variations that may differ between lines, we created new inducible trangenic lines using an oestradiol-sensitive promotor. RNA profiling of induced and non-induced plants will allow better characterization of up- and down-regulated genes. Expression profiles of candidate genes (orthologs) will then be assessed in infected and uninfected sugar beets and their effects on viral accumulation will be studied by knock-out (RNAi).

37.109 IDENTIFICATION OF GENES INVOLVED IN *SCLEROTINIA SCLEROTIORUM* RESISTANCE IN *ARABIDOPSIS*. L. Perchepied, P. George , M. Barthes, C. Claudel, B. Grezes-Besset, D. Roby and C. Balagué. *Laboratory of Plants-Microorganisms Interactions, B.P. 52627, CNRS/INRA 2594, 31326 Castanet-Tolosan, France. Email: laure.perchepied@toulouse.inra.fr*

Sclerotinia sclerotiorum is a major fungal parasite of both oilseed rape and sunflower. Inoculum remains viable in soil for up to ten years and consequently the pathogen may become a limiting factor for such crops in infected areas. In addition, chemical control is of limited efficiency. The worldwide importance of this pathogen has led to genomic studies of resistance in oilseed rape and in sunflower. In these crops, resistance QTLs have been characterized, and gene expression profiling has led to the identification of a large number of candidate genes. In order to complement these approaches, *Arabidopsis thaliana* has been used as a model host in the interaction with *Sclerotinia*. Several approaches are used. First, *Arabidopsis* signalling mutants impaired in resistance to different pathogens are tested, in order to determine if known signalling components of resistance are involved in resistance to *Sclerotinia*. Preliminary data indicate a major role of the hormone ethylene in this resistance. Second, functions of candidate genes previously identified in oilseed rape and in sunflower are being validated in *Arabidopsis*. Results from these different approaches will be presented.

37.110 GRAPEVINE GENE EXPRESSION IN RESPONSE TO *PLASMOPARA VITICOLA* ANALYSED USING COMBIMATRIX MICROARRAYS. M. Polesani, D. Glissant, A. Ferrarini, F. Desario, M. Pezzotti, M. Delledonne and A. Polverari. *Dip. Scienze Tecnologie e Mercati della Vite e del Vino, Università di Verona, 37029 San Floriano di Valpolicella (VR), Italy. Email: annalisa.polverari@univr.it*

Transcriptional changes associated with *Plasmopara viticola* infection in grapevine genotypes either susceptible (*Vitis vinifera* cv. Pinot Noir) or resistant (*Vitis riparia* cv. Gloire de Montpellier) were analysed using microarrays, at different times after infection. We used the newly developed Combimatrix platform at Verona University, on a Grape chip carrying 24562 specific probes in triplicate from assembly of the Tentative Consensus of the last TIGR *Vitis vinifera* Gene Index release 5.0, and from non-reduntant genome sequences produced by genome annotation in the International Grape Genome Project. Combimatrix technology employs exclusive in situ oligo (up to 40 mers) synthesis driven by electrochemistry, and the microarray is reusable, factors that confer high flexibility on the system and reduce drastically the costs of analysis. Leaves of resistant and susceptible grape plants grown *in vitro* were infected with *P. viticola* or treated with distilled water as a control, and collected at 12 and 24 h post-inoculation. Hybridisations were done with samples from three independent biological replicates. Differentially expressed genes were selected using the multi-experiment Significance Analysis of Microarray test, and gene clustering was analysed using Genesis software.

37.111 SORGHUM IS A SUITABLE BREAK CROP TO MINIMIZE *FUSARIUM PSEUDOGRAMINEARUM* AND *GIBBERELLA ZEAE* IN ANY LOCATION. S.A.J. Quazi, L.W. Burgess and J. Smith-White. *Plant Pathology Division, Bangladesh Rice Research Institute, 1701 Gazipur, Bangladesh. Email: q.shireen@bdonline.com*

The susceptibility of grain sorghum (*Sorghum bicolor)* to infection and colonisation by *Fusarium pseudograminearum* (Fpg) and *Gibberella zeae* (Gz) was assessed by isolation studies involving plants grown in field plots and commercial fields. Fpg was isolated at low frequency from plants sampled from an experimental trial site at Livingston Farm, Moree which harboured varying levels of stubble-borne inoculum. In this trial site 1000 plants were analysed at pre- and post-senescence from four replicate plots in each of five residue management treatments. Fpg was detected at insignificant levels in sorghum from the different treatments and inoculum levels. No Gz was isolated from this trial site. The effect of agro-climate factors on the occurrence of Fpg and Gz in mature sorghum stems was assessed at 31 commercial sites representing two climatic regions, Goondiwindi/ Moree and the Liverpool Plains. Fpg was rarely isolated from the commercial crops, which provides further evidence that modern hybrids are resistant to infection and colonization. In contrast, Gz was isolated at low to medium levels from the Liverpool Plains region where the pathogen is relatively common, causing *Fusarium* head blight of wheat, in some seasons. However, Gz was not isolated from sorghum stalks from Goondiwindi/Moree region. In addition, Gz was more frequently isolated from sorghum crops where the wheat residues were known to be infested with Gz.

37.112 *PSEUDOMONAS SYRINGAE AVRB* SUPPRESSES DEFENSE RESPONSES IN SUSCEPTIBLE SOYBEAN. O. Radwan, J. Zou and S.J. Clough. *1101 W. Peabody Dr., Urbana, IL 61801 USA. Email: oradwan@uiuc.edu*

Bacterial effector proteins secreted through type III secretion systems play a crucial role in establishing plant and human diseases. Type III effectors have been shown to trigger defense responses when recognized by resistant plants, and to suppress defense responses in susceptible host plants. Here we examined the hypothesis that *Pseudomonas syringae* pv. *glycinae* (*Psg*) carrying the avirulence gene *avrB* suppresses soybean defense responses from a soybean host that lacks the corresponding *R* gene (*RPG1*). We inoculated *rpg1* recessive soybean plants with *Psg* with or without *avrB*, and compared gene expression profiles to expression response from soybean dominant for *RPG1*. Gene expression profiling using soybean oligo microarrays consisting of approximately 38,000 different genes, indicated that while defense genes, transcription factors, genes involved in the phenoproponoid pathway and signal transduction components were upregulated in the *Psg* incompatible reaction, they are down in abundance when comparing *rpg1* susceptible plants inoculated with *Psg* (*avrB*) compared to an *Psg (avrB$^-$)* control. The expression profiling results support the hypothesis that avirulence genes have a 'dual agent' character and can trigger rapid defense in incompatible interactions, and can promote bacterial multiplication by suppressing plant defenses and enhancing access to nutrients trapped in plant cells in susceptible interactions.

37.113 RNA-DEPENDENT RNA POLYMERASE-1 CAN PLAY A DEFENSIVE ROLE IN SYSTEMIC VIRAL INFECTION. F. Rakhshandehroo, J. Squire and P. Palukaitis. *Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran 14515-775, Iran, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK. Email: rakhshandehroo_fa@srbiau.ac.ir*

RNA-dependent RNA polymerases are amongst the most conserved enzymes within eukaryotic cells. The aim of this study was to evaluate the role of the tobacco (*Nicotiana tabacum* cv Samsun NN) RdRp-1 in response to infection by *Potato virus Y* strain O. We used two lines of transgenic tobacco in which the RdRp-1 gene was silenced moderately (R-14-1) or highly (R-5-1). Examination of the expression level of RdRp-1 by semi-quantitative RT-PCR indicated a higher level of gene expression after infection with PVY in all inoculated plants compared with the uninoculated control plants. After inoculation with PVY, the RdRp-1 gene was expressed to a higher level in the non-transgenic plants than in the RdRp-1-silenced lines; however, PVY infection could also induce the expression of the RdRp-1 gene in both RdRp-1-silenced transgenic lines, although to a lesser extent than in the non-transgenic plants. Analysis of the plants using Western blotting for PVY coat protein and semi-quantitative RT-PCR for PVY RNA, showed a lower level of virus accumulation in non-transgenic plants after inoculation with PVY in comparison to both RdRp-1-silenced transgenic lines. We also found a higher level of expression of the gene encoding the so-called inhibitor of virus replication in non-transgenic plants than in either of the two transgenic lines after PVY infection. Therefore, our data indicate that the RdRP-1 enzyme is involved in the expression of host resistance responses to viral infection even during a compatible virus-host interaction leading to systemic infection by PVY in tobacco plants.

37.114 THE INFLUENCE OF SILICON ON COMPONENTS OF RESISTANCE TO ANTHRACNOSE IN SUSCEPTIBLE AND RESISTANT SORGHUM LINES. R.S. Resende, F.Á. Rodrigues, J.M. Soares and C.R. Casela. *Viçosa Federal University, Department of Plant Pathology, Laboratory of Host-Parasite Interaction, Viçosa, MG, 36570-000, Brazil. Email: fabricio@ufv.br*

This study aimed to evaluate the effects of silicon (Si) on some components of resistance to anthracnose (*Colletotrichum sublineolum*) on sorghum. A 5×2 factorial experiment, consisting of five Si rates (0, 0.06, 0.12, 0.24 and 0.30 g/kg of soil) and two sorghum lines ('BR009'; susceptible; 'BR005'; resistant) was arranged in a randomized design with three replications. Plants from both lines were inoculated 30 days after emergence. The incubation period (IP), latent period (LP_{60}), relative infection efficiency (RIE), area under anthracnose progress curve (AUAPC), real disease severity (RDS) estimated with the software QUANT and final disease severity (FDS) were evaluated. A positive quadratic regression model best described the effect of Si rates on both PI and LP_{60} for the susceptible line. For the susceptible line, the variables RIE, AUAPC, RDS and FDS decreased as the Si rates increased with the lowest values occurring at 0.25 g of Si/kg of soil. The LP_{60} for the resistant line was unaffected (absence of acervulae) by the Si rates. The IP for the resistant line was not affected by the Si rates. Si rates had a significant effect on AUAPC, RDS and FDS for the resistant line. Si content in sorghum tissue increased relative to the control by 55 and 58%, respectively, for the susceptible and resistant lines. There was no significant change in calcium in sorghum tissue for either line tested. In conclusion, the results underscore the importance of Si in resistance to sorghum anthracnose particularly for the susceptible line. Financial Support: FAPEMIG.

37.115 HOST RESPONSES TO INFECTION BY SOUTH AFRICAN *CASSAVA MOSAIC VIRUS*. M.E.C. Rey and E.J. Pierce. *School of Molecular and Cell Biology, Univerisy of the Witwatersrand, Johannesburg, South Africa. Email: chrissie.rey@wits.ac.za*

Establishment of disease by viruses may often be regarded as a "time-dependent competition" between the induction of stress responses and viral suppression of plant host genes to enhance its replication and movement. Microarray studies were conducted in order to elucidate host gene responses to *South African cassava mosaic virus* (SACMV) in a model fully-susceptible host, *Arabidopsis thaliana*, at 3 time points post virus inoculation (dpi). Infectivity studies revealed that *Arabidopsis* was fully symptomatic at 35 dpi with the appearance of characteristic symptoms such as leaf distortion, chlorosis and stunting. In a preliminary study, the 35-dpi plants were used for hybridization to a custom-made *Arabidopsis* slide containing 7200 cDNAs (cell-signaling and some defence-related genes). Intensity values for Cy3 and Cy5 channels on the hybridized slides were obtained using GenePix Pro 5.1. This was followed by analysis using the TIGR TM4 Microarray Software Suite. Normalization was performed by applying a LOWESS algorithm to the data set followed by standard deviation (SD) regularization and a flip-dye consistency checking parameter. Differentially expressed genes were identified at 95% CI. Results were confirmed by real time RT-PCR. Forty eight up-regulated and 38 down-regulated genes, associated with metabolism, transcription and transport, were differentially expressed. Elongation Factor 1-Alpha (EF-1-Alpha) was the most up-regulated gene, which interestingly has also been reported in cassava. Significance and results of other differentially expressed genes at the two other time points, using Limma analysis, as well as full *Arabidopsis* genome slide hybridizations, will be discussed.

37.116 VIRUS-INDUCED GENE SILENCING OF *NICOTIANA BENTHAMIANA* SERINE PALMITOYLTRANSFERASE AFFECTS GROWTH, DEVELOPMENT AND DEFENCE RESPONSES. **M. Rivas-SanVicente, M. Gavilanes-Ruíz and J. Plasencia.** *Depto. de Bioquímica, Fac. de Química, Universidad Nacional Autónoma de México, C.P. 04510, Mexico. Email: lore_mariana@yahoo.com*

Serine palmitoyltransferase (SPT) is a heterodimer, composed of subunits LCB1 and LCB2, that catalyzes the first reaction of sphingolipid biosynthesis to yield sphingoid bases. Besides their structural function in cell membranes, sphingolipids are signal transducers of many cellular processes. Recent reports in plants suggest their possible role as signalling mediators in plant defence. In this work, we used virus-induced gene silencing (VIGS) of SPT subunit LCB2 to assess the sphingolipid role in plant defence response against pathogens. We cloned a 602-bp NbLCB2 cDNA fragment into the *Tobacco rattle virus*-based VIGS vector pTV00 and infiltrated *Nicotiana benthamiana* plants with a culture of *Agrobacterium tumefaciens* containing the plasmid. VIGS of SPT LCB2 with this construct (pTV:SPT602) resulted in a phenotype with reduced growth, abnormal morphology of leaves and flowers as well as premature senescence of these organs, and notably reduced seed production. The control plants agroinfiltrated with the empty plasmid showed a normal phenotype and discrete symptoms associated with the viral infection. When plants were challenged with the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (Pst), no differences were observed in the temporal and spatial induction of the hypersensitive reaction (HR) between pTV00 and pTV:SPT602 plants. However, lower levels of phenylalanine ammonia lyase (PAL) and PR-1 transcripts were detected in pTV:SPT602 plants as compared to pTV00 control plants. Our results suggest that sphingoid bases might participate in the signal transduction pathway leading to defence gene expression.

37.117* EXPRESSION OF RGS-CAM SUPPRESSOR OF RNA SILENCING IN CITRUS INOCULATED WITH *CITRUS VIROID III.* **S. Rizza, D. Raspagliesi, A. Catara and E. Conte.** *Department of Phytosanitary Sciences and Technologies, University of Catania, Via S. Sofia 102, 95123 Catania, Italy. Email: srizza@unict.it*

Post-transcriptional gene silencing (PTGS) is a mechanism which higher plants have evolved to defend against viral infection in addition to regulation of gene expression for growth and development. It has been shown that viroids can act both as inducers and as targets of the PTGS mechanism. As a counterdefence, many plant viruses have also evolved RNA silencing suppressors (RSS) that interfere with various steps of the silencing pathway. One of them is a calmodulin-related protein termed rgs-CaM (regulator of gene silencing–calmodulin-like protein). Therefore, particularly intriguing appear our results of differential display RT-PCR analysis on Etrog citron infected by *Citrus viroid-III* (CVd-III), a dwarfing viroid affecting some specific citrus rootstock/scion combinations, without any significant detrimental effect on fruit size and yield. In fact the results show that among the 18 genes identified, the expression level of rgs-CaM gene is enhanced compared to the healthy control. In order to investigate a possible role of rgs-CaM in viroid-induced RNA silencing pathway, in this study we developed a reverse-transcriptase quantitative real-time PCR assay (RT-qPCR) by which we evaluated rgs-CaM gene expression in different citrus species inoculated with CVd-III. Viroid accumulation was also quantitavely evaluated by RT-qPCR. We showed that rgs-CaM is over-expressed in trifoliate orange, Troyer citrange and Etrog citron plants infected by CVd-III, whereas it is repressed in lemon and sour orange. However, this plant-specific pattern of rgs-CaM gene regulation seems not to be correlated with viroid load.

37.118 THE INFLUENCE OF SILICON ON COMPONENTS OF RESISTANCE TO ANTHRACNOSE IN SUSCEPTIBLE AND RESISTANT SORGHUM LINES. **F.Á. Rodrigues, R.S. Resende, J.M. Soares and C.R. Casela.** *Viçosa Federal University, Department of Plant Pathology, Laboratory of Host-Parasite Interaction, Viçosa, MG, CEP 36570-000, Brazil. Email: fabricio@ufv.br*

This study aimed to evaluate the effects of silicon (Si) on some components of resistance to anthracnose (*Colletotrichum sublineolum*) on sorghum. A 5×2 factorial experiment, consisting of five Si rates (0, 0.06, 0.12, 0.24 and 0.30 g/kg of soil) and two sorghum lines ('BR009'; susceptible; 'BR005'; resistant) was arranged in a randomized design with three replications. Plants from both lines were inoculated 30 days after emergence. The incubation period (IP), latent period (LP_{60}), relative infection efficiency (RIE), area under anthracnose progress curve (AUAPC), real disease severity (RDS) estimated with the software QUANT and final disease severity (FDS) were evaluated. A positive quadratic regression model best described the effect of Si rates on both PI and LP_{60} for the susceptible line. For the susceptible line, the variables RIE, AUAPC, RDS and FDS decreased as the Si rates increased with the lowest values occurring at 0.25 g of Si/kg of soil. The LP_{60} for the resistant line was unaffected (absence of acervuli) by the Si rates. The IP for the resistant line was not affected by the Si rates. Si rates had a significant effect on AUAPC, RDS and FDS for the resistant line. Si content in sorghum tissue increased relative to the control by 55 and 58%, respectively, for the susceptible and resistant lines. There was no significant change in calcium in sorghum tissue for either line tested. In conclusion, the results underscore the importance of Si in resistance to sorghum anthracnose particularly for the susceptible line. Financial Support: FAPEMIG.

37.119 INFLUENCE OF MICROELEMENTS ON INTERACTIONS WITHIN THE WHEAT SEEDLING-*BIPOLARIS SOROKINIANA* SYSTEM. **V. Roginskaya and L. Roguinski.** *Department of Ecology and Plant Biotechnology, Krasnoyarsk State Agrarian University, Prospect Mira 90, Krasnoyarsk, Russia. Email: roginskaya48@yahoo.com*

The influence of microelements on common root rot caused by *Bipolaris sorokiniana* Schoem. and the abilities of young wheat seedlings to resist the disease were observed in multifactor experiments. Addition of Zn^{2+} (17mg/l) or Mn^{2+} (16 mg/l) to Czapek media hindered the pathogen growth over the first 5 days, but addition of Cu^{2+} (14 mg/l) had no effect. Nevertheless, presence of chlamidospores in all treatments indicated that the fungus had experienced unfavorable conditions. It is known that field germination greatly depend on eco-conditions in the sowing–seedling period. Pre-sowing treatment of seeds with microelements caused 43.5–61.0% suppression of germination. In presence of infection, seed treatment leveled the negative influence of the pathogen. An inhibiting effect of microelements on the pathogen is suspected. On the contrary, treatment with copper ions increased the negative influence of infection. The mass of roots per unit of the shoot mass is a crucial criterion for evaluation of resistance to drought and common root rot. In the infection-free variant with Cu^{2+} that ratio exceeded the control by 47.3% and reached 1.142 (in control 0.756). Therefore increased seedlings drought resistance could result from pre-sowing treatment with copper. Nevertheless, in the presence of infection, that treatment reduced root mass by 23.1%. Therefore, at a high level of substrate inoculum potential, the positive influence of Cu^{2+} does not manifest itself; hence the presence of the soil pathogen must be taken into account prior to seed treatment with copper salts.

37.120 STUDY ON PATHOGENICITY OF *MAGNAPORTHE GRISEA* SPECIES COMPLEX. **J.H. Roh, S.S. Han, Y.C. Cho, I.S. Oh, J.H. Choi and W.B. Choi.** *Crop Environment & Biotechnology Division, NICS, RDA 209 Seodundong, Suwon, 441-857, Republic of Korea. Email: rohjh62@rda.go.kr*

The blast fungus *Magnaporthe grisea* is known as a species complex causing diseases on a wide range of gramineous hosts, not only cultivated rice, but also other cereals and weeds. One hundred fifty two isolates collected from ten weed species in Korea were screened to identify their pathogenicity spectrum to rice using the cultivars LTH and Nagdongbyeo. Pathogenicity tests revealed that 68% of 152 isolates from crabgrass caused blast lesions on rice cultivar, Nagdongbyeo, while 76.8% of 55 isolates from crabgrass caused blast lesions on rice 'LTH'. In cross-infection tests between rice and crabgrass using two isolates tagged with green fluorescent protein (GFP), it was proved that two isolates collected from rice and crabgrass had reciprocal infectivity, and each isolates collected from crabgrass had different pathogenicity. Through inoculation tests of a number of isolates of *M. grisea* to different rice cultivars, each isolate showed different pathogenicity. These results indicated that isolates collected from rice and crabgrass had cross-infectivity on their opposite hosts.

37.121 DEFENCE RESPONSES AND SIGNALLING PATHWAYS IN *ARABIDOPSIS THALIANA-PHYTOPHTHORA CINNAMOMI* INTERACTIONS. **J.E. Rookes, M.L. Wright and D.M. Cahill.** *School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Victoria 3217, Australia. Email: james.rookes@deakin.edu.au*

Phytophthora cinnamomi causes significant destruction to many natural ecosystems and agricultural crops world wide. The threat it poses to the native Australian environment is so severe that it has been identified as a 'key threatening process' by the Australian Government under the Environment Protection and Biodiversity Conservation Act 1999. Much research has been conducted on *P. cinnamomi* and the disease it causes, however relatively little is known regarding the responses of plants challenged by the pathogen. In this study the interaction between the model research plant *Arabidopsis thaliana* (Arabidopsis) and *P. cinnamomi* has been investigated. The interaction of Arabidopsis and *P. cinnamomi* was examined using plants grown in soil and in specially developed vertical growth trays to facilitate root inoculations. Arabidopsis defence responses against *P. cinnamomi* were assessed using microscopy, and biochemical and molecular approaches. In addition, a suite of Arabidopsis defence-related mutants were obtained to investigate the involvement of known defence responses/signalling pathways against *P. cinnamomi*. Defence responses found to be involved included the activation of secondary metabolite pathways, the accumulation of callose and reactive oxygen species, and restricted cell death. Of the Arabidopsis defence mutants tested, no single mutation caused a major change in infection levels or processes when compared with wild-type plants, but some mutant lines showed increased hyphal penetration. Gene expression analysis showed induction of several defence-related genes including the plant defensin gene PDF1.2. Identified defence mechanisms can be employed to develop strategies to protect susceptible species against *P. cinnamomi*.

37.122 MORPHOLOGICAL VARIABILITY OF THE WHEAT POWDERY MILDEW PATHOGEN UNDER THE ACTION OF EXOGENOUS CYTOKININS. **A.S. Ryabchenko, A.V. Babosha, T.V. Avetisyan.** *N.V. Tsitsin Main botanical garden RAS, 127276 Moscow, Botanicheskaya, 4, Russia. Email: marchellos@yandex.ru*

The impact of exogenous zeatin on susceptibility of wheat and an *Aegilops* line (*Triticum aestivum* × *Aegilops speltoides*) to *Erysiphe graminis* DC. f. sp. *tritici* Marchal. (syn. *Blumeria graminis*), a causal organism of wheat powdery mildew, was investigated. The aim of the work was to determine the effects of different cytokinin concentrations on morphological variability of the pathogen at early stages of infection. Different concentrations of exogenous cytokinin had complex effects on different parameters of fungal development. Treatment with 1 and 4.5 mkM zeatin essentially enlarged the halo at pathogen penetration sites 24 h after pathogen inoculation, but 1.5 mkM zeatin had no effect. The dose response curves of numbers of microcolonies, normal appressoria and ungerminated conidia also showed two regions of up-regulation. Thus the maximums and minimums for different parameters were observed on the curves at different zeatin concentrations. In the case of abnormal appressoria with long growth tubes the curve had a single maximum at low zeatin concentration. The high zeatin concentration peak on the curve for number of microcolonies corresponded to the maximum for normal appressoria and minimum for ungerminated conidia. The low zeatin concentration peak correlated with maximum for abnormal appressoria, but not with the extremes on concentration curves for normal appressoria and ungerminated conidia. Thus bidirectional immunomodulation properties of cytokinins appeared at early stages of pathogen development. The data do not exclude the hypothesis that cytokinins may have effects at different stages of pathogen development.

37.123 KEY METABOLIC AND OXIDATIVE ENZYME ACTIVITIES IN TOMATO AFFECTED BY BACTERIAL SPOT. **T. Sadunishvili, M. Betsiashvili, N. Kuprava, N. Amashukeli and N. Dzamukashvili.** *Durmishidze Institute of Biochemistry and Biotechnology, David Agmasheneblis Kheivani 10 km, Tbilisi 0159, Georgia. Email: tinatin_sadunishvili@yahoo.com*

Bacterial spot caused by *Xanthomonas vesicatoria* is a widespread bacteriosis of tomato. Damage may range from light leaf spotting to almost complete defoliation, with corresponding impacts on the plant's morphological, structural and physiological/biochemical functions and production potential. To develop an effective disease management strategy, it is important to understand the mechanism of pathogenic bacterial action and changes occurring in plant cells at the level of main metabolic processes. Tomato plants and fruits affected by black bacterial spot at different stages of disease were collected from western and eastern regions of Georgia. There was significant stimulation (200–300%) of glutamate dehydrogenase activity both in amination and deamination reactions in leaves of diseased plants at the flowering stage, sampled from different regions. Malate dehydrogenase activity increased less (110%). With the oxidative enzymes, there was significant stimulation of peroxidase activity and inhibition of phenoloxidase activity as a response to bacterial infection. The increase in peroxidase activity was expressed in the appearance of four new isoforms on the zymogram of affected leaves. At a later stage of plant development (green fruit) stronger stimulation of all enzymes studied was observed in damaged parts of affected leaves. Differential enzyme responses were also found in diseased green and ripe fruits. Participation of glutamate dehydrogenase along with peroxidase and phenoloxidase in plant defence response against bacterial infection is discussed.

37.124 SCREENING LENTIL ACCESSIONS FOR RESISTANCE TO AUSTRALIAN ISOLATES OF *ASCOCHYTA LENTIS*. **P. Sambasivam, P.W.J. Taylor, E.C.K. Pang and R. Ford.** *Biomarka, School of Agriculture & Food Systems, Faculty of Land and Food Resources, The University of Melbourne, Australia. Email: p.thanjavursambasivam@pgrad.unimelb.edu.au*

Lentil (*Lens culinaris* ssp. *culinaris*) is one of the most important pulse crops in the world and Australia is the third largest exporter and fifth largest producer (FAOSTAT). Various biotic and abiotic factors limit lentil production, and Ascochyta blight caused by *Ascochyta lentis* Vassilievsky is one of the most widespread and economically important fungal diseases. The use of fungicides may reduce disease incidence but the most economically viable and promising way to control Ascochyta blight is via plant host resistance in combination with other cultural practices. In order to maintain an efficient breeding programme, there is a need to understand the variation in lentil germplasm for resistance to *A. lentis*. To investigate this, six differential lentil accessions were evaluated in glasshouse tests for their reaction to nine Australian isolates of *A. lentis*. The objective of the study was to characterize the disease response of differential lentil genotypes to *A. lentis* isolates at the seedling stage. The bioassay consisted of 54 treatments (6 genotypes × 9 isolates). For each treatment 15 plants (14-day-old) were inoculated with a fine mist of inoculum until run off, and disease was assessed at 7, 14 and 21 days after inoculation (1-9 scale). Analysis of variance showed highly significant differences between lentil genotypes and *Ascochyta* isolates. The genotype × isolate interaction was also considered to be significant at $P \leq 0.05$.

37.125 PHYSIOLOGICAL SPECIALIZATION OF *STAGONOSPORA NODORUM*. **A.A. Sanina, E.V. Paholkova and X. Chen.** *All-Russia Research Institute of Phytopathology, Moscow, Russia. Email: saa@vniif.rosmail.com*

Septoriosis is a harmful disease of wheat, widespread all over the world, including Russia. *Stagonospora nodorum* (Berk.) Castellani and E.G. Germano is one of the main agents of *Septoria* wheat diseases. Today there is no information on physiological specialization of this pathogen. Not many authors studying cultivar × isolate interactions differentiate *S. nodorum* isolates into races, in consequence of the absence of different reactions of the hosts to the pathogen. Authors have emphasized only the degree of infection of plants as *S. nodorum* does not sporulate on plants under artificial growing conditions. We have worked out ways to induce the fungus to sporulate. Intensity of sporulation in vivo and degree of infection of the plants were used as parameters for determination of *S. nodorum* races. The morphological characteristics of isolate colonies on nutrient medium were taken into account. Isolates were divided into three main types and seven morphotypes. Twenty wheat cultivars were inoculated with 21 *S. nodorum* isolates from various regions and morphotype groups. Using the method of complete connection of cluster analysis, six cultivars with specific reactions to individual pathogen isolates were selected. From the 21 isolates, 17 races were identified.

37.126* OVEREXPRESSION OF PUTATIVE TRANSCRIPTIONAL COACTIVATOR KELP INTERFERES WITH VIRUS MOVEMENT. **N. Sasaki, T. Ogata, M. Deguchi, S. Nagai, A. Tamai, T. Meshi, S. Kawakami, Y. Watanabe , Y. Matsushita and H. Nyunoya.** *Gene Research Center, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan. Email: chaki@cc.tuat.ac.jp*

The genomes of plant viruses encode one or more movement proteins (MPs) that are required for virus movement through plasmodesmata. It is proposed that the movement is facilitated or blocked through interactions of the MP with various host factors. We previously demonstrated that a putative transcriptional coactivator of *Arabidopsis thaliana*, KELP, could bind to the MP of *Tomato mosaic virus* (ToMV) *in vitro*. In this study, to investigate roles of KELP on virus infection, we carried out *in vivo* analyses using *Nicotiana benthamiana*, an experimental host for ToMV. In bombardment experiments with expression plasmids for GFP-expressing ToMV and untagged or DsRed-tagged KELP, we found that movement of the GFP-expressing ToMV was inhibited when KELP was coexpressed. On the other hand, ToMV multiplied normally in protoplasts prepared from tissues transiently expressing KELP. These results indicated that overexpression of KELP affected virus movement but not virus multiplication in individual cells. Subcellular localization analysis showed that GFP-tagged KELP was localized at the nucleus and the cytoplasm. In addition, YFP-tagged MP was largely distributed in the cytoplasm or was associated with cytoplasmic aggregates or filamentous structures when expressed with DsRed-tagged KELP. Meanwhile, the tagged MP was exclusively localized in discrete spots on the cell periphery with expression of DsRed. These results suggested that interaction with KELP interfered with the localization of ToMV MP to plasmodesmata. In conclusion, we demonstrate that KELP can function as an inhibitory factor for virus movement when overexpressed.

37.127 UPTAKE AND METABOLISM OF BACTERIAL HOMOSERINE LACTONE SIGNAL MOLECULES IN PLANTS. **P. Schröder, C. Götz, M. Matucha and A. Hartmann.** *Department of Microbe–Plant Interactions, GSF–Research Centre for Environment and Health, Ingolstädter Landstraße 1, D-85764 Neuherberg, Germany. Email: peter.schroeder@gsf.de*

Bacterial intra- and inter-species communication in the rhizosphere is mediated by diffusible signal molecules. Many Gram-negative bacteria use N-acyl-homoserine lactones (AHLs) as autoinducers in the "quorum sensing" response. While bacterial signalling is well described, the fate of AHLs in contact with plants is much less known. Thus, adsorption, uptake and translocation of homoserine lactones were studied in axenic systems with barley (*Hordeum vulgare* L.) and the tropical legume *Pachyrhizus erosus* L. Losses of AHL due to abiotic adsorption or degradation were tolerable under the experimental conditions. The presence of plants enhanced AHL decline in media depending on the compounds' lipophilicity, and AHLs were traceable in root extracts of plants. Tritium-labelled AHLs were used to determine short-term uptake kinetics. These results indicate substantial differences in uptake and degradation of different AHLs in the selected plant species and shed new light on plant-microbe communication.

37.128 TRACKING THE GROWTH OF PHYTOPATHOGENIC FUNGI AND USE OF DIGITAL IMAGE PROCESSING IN DIAGNOSTICS. **J. Sedlář, M. Sedlářová and J. Flusser.** *Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71 Olomouc-Holice, Czech Republic. Email: michaela.sedlarova@upol.cz*

Aside from molecular markers, microscopic investigation is still an indispensable method for plant disease diagnostics. Mathematical modeling of solid media-based filamentous specimens elongating over time, such as microfungi, was tested. In phytopathology, growth parameters are frequently examined features which are measured in sequence at defined times, leaving the growth pattern as a whole undocumented. Reconstruction of intermediate images from those acquired is possible by means of image warping. In this method, the parameters of the geometric transformation are estimated by means of object tracking based on the morphological skeleton. A second aspect of our work was to test utilisation of automatical digital microimage processing for diagnostics. Microscopical platforms have been previously developed for large-scale data acqusition and analysis to automate sample processing, especially in medical research. A limitation for automatic structure recognition and category selection lies in software specifications, e.g. defined taxonomical features of disease causal agents. A case study was conducted on downy mildews (Peronosporaceae). The work was supported by projects GAUK 148207 (Grant Agency of Charles University in Prague) and MSM 6198959215 (Ministry of Education, Czech Republic).

37.129 THE ROLE OF NITRIC OXIDE IN DEVELOPMENT OF BIOTROPHIC PATHOGENS AND THEIR INTERACTION WITH HOST CELLS. **M. Sedlářová, M. Petřivalský, L. Luhová, M. Hašová, J. Hofman, J. Kočířová and A. Lebeda.** *Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71 Olomouc-Holice, Czech Republic. Email: michaela.sedlarova@upol.cz*

Nitric oxide (NO) is a ubiquitous reactive molecule involved in plant signalling and defence mechanisms. The importance of NO for the initial stages of host-pathogen interactions was compared for three pathosystems (*Lactuca sativa–Bremia lactucae*, *Cucumis* spp. – *Golovinomyces cichoracearum* and *Lycopersicon* spp. – *Oidium neolycopersici*). Distribution of the molecule in host cells and pathogen infection structures was examined by confocal scanning microscopy (Olympus FV1000) using the NO probe DAF-2DA. The strongest accumulation of NO occurred in cells of susceptible *L. sativa* with developed *B. lactucae* haustoria or in *Cucumis* spp. and *Lycopersicon* spp. cells undergoing hypersensitive reaction due to powdery mildew infection. Differing in intensity, the NO signal was also detected in fungal oomycete and pathogen hyphae. For each pathosystem, the effects of several compounds modulating NO metabolism (SNP, PTIO, L-NAME, sodium tungstate, rutin) on pathogen development and host-cell response were studied in detail. During the course of 48 h after inoculation, exogenous application of NO donors and scavengers on host tissues influenced timing and rate of pathogen infection structure formation as well as expression of hypersensitive reaction within host tissues. To summarize, our results showed that NO plays an indispensable role both in host responses to pathogen challenge and in pathogen metabolism during germination and penetration. The work was supported by MSM 6198959215 (Ministry of Education, Czech Republic).

37.130 HOST-PATHOGEN INTERACTION BETWEEN VEGETATIVE AND MYCELIAL PHASE OF *AGARICUS BISPORUS* AND *VERTICILLIUM FUNGICOLA*. **A. Shamshad, A. Clift and S. Mansfield.** *Faculty of Agriculture, Food and Natural Resources, The University of Sydney, NSW 2006, Australia. Email: a.shamshad@usyd.edu.au*

Verticillium fungicola is a casual agent of dry bubble disease, the most common and serious fungal disease of cultivated mushrooms. This disease has been a continuing problem for *A. bisporus* mushroom farmers for many years and has developed world wide resistance against benomyl which belongs to the benzimidazole group of fungicides. To investigate the host-pathogen interaction at the mycelial and sporophore stage, transmission and scanning electron microscopy of the host (*A. bisporus*) and pathogen (*V. fungicola*) was conducted. Transmission electron micrographs showed that the pathogen mycelium grows very close to the host mycelium. Scanning electron micrographs of the necrotic tissue of the diseased mushroom showed clusters of phialospores and hyphae of the pathogen in large numbers.

37.131 GLYCOPROTEINS SECRETED BY GERMINATING SPORES OF *MAGNAPORTHE ORYZAE* DETERMINE SPECIFICITY LIKE A SUPPRESSOR IN RICE PLANT-BLAST INTERACTION. **A. Shinjo, Y. Okamoto, A. Kadoiri, T. Koizumi, T. Arie and T. Teraoka.** *Graduate School of Agriculture, Tokyo University of Agriculture & Technology 3-5-8 Saiwai-cho, Fuchushi, Tokyo, Japan. Email: 50007951016@st.tuat.ac.jp*

In the rice plant-blast interaction, the incompatible reaction is not dependent only on the cultivar, and also the compatible reaction is not dependent only on the pathogenic race. The reaction depends on the combination of the cultivar and the attacking race. Induced resistance and induced susceptibility have been noted in the interaction. Similar effects in a cultivar were triggered by the fluid of germinating spores in some isolates. Novel glycoproteins, which have potential suppressor activity to induce susceptibility and also to enhance the growth of invasive hyphae,

were found in the fluid. These glycoproteins were fractionated by Concanavalin A affinity chromatography and analyzed by 2-dimensional electrophoresis. Both the sugar moiety and the peptide chain were involved in the activity. The other fraction in the fluid not bound to the column had activity to elicit some events associated with the resistant reaction. These glycoproteins had ability to interact with the mannose-binding rice lectin.

37.132 PROMOTER ENGINEERING: CONSTRUCTION OF PLANT-PATHOGEN INDUCIBLE PROMOTERS. **F. Shokouhifar, M. Motallebi, M.R. Zamani, M. Malboobi and M. Mousavi.** *National Institute of Genetic Engineering and Biotechnology, Iran. Email: shokouhifar@nigeb.ac.ir*

Regulation of transgene expression is very important to produce resistant transgenic plants. A variety of promoters is used to express resistant transgenes in plants, but they have faults in comparison with an ideal promoter. Recently there have been efforts to design and synthesise new promoters with high flexibility and inducibility to express transgenes temporally and locally only at the site of pathogen infection. In order to construct suitable promoters to regulate a resistance transgene in canola, we selected and synthesized some pathogen-inducible elements as regulatory blocks that interact with some transcription factors involved in plant defence pathways. The blocks were cloned in the Amp resistance gene of vector pACYC177 and using the insertional inactivation technique, positive colonies were selected. Then the segments were cloned in vector pSH4. The regulatory cassettes fused with GUS were sub-cloned in vector pGPTV. The constructs were checked in cloning steps using PCR, digestion and sequencing procedures. Finally nine synthetic promoters and positive and negative controls have been constructed that will be checked for their stringency and inducibility in canola using transient expression experiments.

37.133 THE TRANSCRIPTIONAL RESPONSE OF ARABIDOPSIS TO INFECTION WITH *CUCUMBER MOSAIC VIRUS*. **L. Shuey, S.T. Hall, R. Sintrajaya, K. McGrath, K. Kazan and P. Schenk.** *School of Integrative Biology, The University of Queensland, St Lucia Campus, Brisbane, QLD 4072, Australia. Email: l.shuey@uq.edu.au*

Monitoring the progression of pathogenic and parasitic organisms is an import part of the management and control of infectious and parasitic disease. Traditional methods of monitoring plant disease include the laborious and potentially variable methods of spore and colony counting, and symptom measurement and scoring. We have developed a suite of quantitative real time PCR assays for the detection and quantification of diverse plant pathogens and parasites that are highly specific. These tests not only enable the monitoring of disease progression but allow direct quantification of the pathogen in pre-symptomatic phases, providing a greater understanding of the correlation with the diseased state of the plant. This method has been used to monitor the transcriptional response of *Arabidopsis* to *Cucumber mosaic virus* (CMV) in the early stages of infection. The preliminary results show that a number of genes are differentially regulated after infection with the virus, in comparison to mock inoculated plants. Additionally, it indicates that the technique can successfully detect changes in gene expression following inoculation. Our studies show this quantitative real time PCR technique can be adapted to potentially any disease agent ranging from bacterial and viral to fungal and animal.

37.134 *ARABIDOPSIS THALIANA* NON-HOST RESISTANCE RESPONSES TO THE COFFEE ORANGE RUST FUNGUS *HEMILEIA VASTATRIX*. **M.C. Silva, H.G. Azinheira, L. Bernier, C. Medeira, A-S Petitot, I. Maia, M. Nicole and D. Fernandez.** *Centro de Investigação das Ferrugens do Cafeeiro/Instituto de Investigação Científica Tropical, Quinta do Marquês, 2784-505 Oeiras, Portugal. Email: mariaceudasilva@gmail.com*

The plant rusts, caused by Basidiomycetes, are among the most destructive plant diseases, especially of economically important crops such as Arabica coffee (*Coffea arabica* L.). The model plant *Arabidopsis thaliana* is naturally immune to the rust pathogens, but the mechanisms underpinning non-host disease resistance remain relatively unexplored. Leaf rust of coffee, caused by *Hemileia vastatrix*, is of central economic significance, and thus insights into the expression of non-host resistance against this pathogen may be of particular importance. The non-host pathosystem involving *A. thaliana* (Col-0) and *H. vastatrix* (race II) was characterized at the cytological and molecular levels. By 24 h after inoculation, 67% of *H. vastatrix* urediospores had germinated on the upper epidermis of the leaves and 46% had differentiated appressoria on stomata. The majority (80%) of appressoria penetrated but failed to successfully form haustoria. The hypersensitive response was detected, particularly in the stomatal cells. Cell wall modifications, such as deposition of phenolic-like compounds and callose seemed to act as a barrier preventing haustoria formation. The *A. thaliana* molecular resistance responses to the rust were investigated by monitoring the relative expression of a variety of defence genes including *WRKY*, pathogenesis-related (PR), defensins, lipoxygenases and peroxidase genes. Inoculation of *H. vastatrix* on to *Arabidopsis* triggered the rapid induction of defence genes. Results were compared to the *A. thaliana* race-specific resistance responses when challenged with *Pseudomonas syringae* strain DC3000 carrying the avrRpt2 gene. This provided significant new insight into the expression of non-host resistance.

37.135 GENETIC DIVERSITY OF *GUIGNARDIA CITRICARPA* ISOLATED FROM DIFFERENT CITRUS GENOTYPES. **A.C.O. Silva-Pinhati, A. Goes, R.L. Boscariol-Camargo and M.A. Machado.** *Centro APTA Citros Sylvio Moreira-IAC, C.P. 04 CEP. 13490-970 Cordeirópolis, SP, Brazil. Email: raquel@centrodecitricultura.br*

Citrus black spot, caused by the fungus *Guignardia citricarpa* Kiely, causes an economically important disease in Brazil and other regions of the world, affecting various citrus species. The disease is characterized by the occurrence on the fruits of necrotic lesions that can vary in colour, shape and size. The disease also causes early maturation and premature drop, with significant production losses. The main objective of this work was to evaluate the genetic diversity of this fungus, particularly isolates associated with different types of symptoms and different citrus hosts. *G. citricarpa* mycelia were isolated from different citrus genotypes presenting various black spot symptoms. Genetic diversity was assessed using molecular markers based on the fungal ITS region. A total of 37 isolates from 29 genotypes from the Citrus germplasm active bank of the Centro APTA Citrus Sylvio Moreira-IAC, Cordeirópolis, SP, Brazil, were analyzed. Preliminary phylogenetic analyses, based on ITS 1 and ITS 2 of the fungus, suggest high similarity among the isolates and very small intraspecific genetic diversity. However, we detected three distinct groups based on different host species, but not on different symptoms. Our data suggest that there may be some specificity between *G. citricarpa* isolates and citrus genotypes. This is an ongoing project and new molecular markers will be used in order to confirm our results.

37.136 SIGNIFICANCE AND OCCURRENCE OF THE TEMPERATE RIBOTYPES OF *POLYMYXA* SPECIES. **M.J. Smith, E. Ward, J.A. Walsh and M. Adams.** *Centre for Sustainable Pest and Disease Management, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK. Email: madeleine.smith@bbsrc.ac.uk*

Polymyxa graminis and *P. betae* are obligate, intracellular, root-infecting organisms of cereals (*P. graminis*) and of members of the Chenopodiacae (*P. betae*). Between the two species, they are known to transmit approximately 15 economically important plant viruses. These include *Soil-borne cereal mosaic virus*, *Barley yellow mosaic virus* and *Beet necrotic yellow vein virus* and together, cover a world-wide distribution. Recent ribosomal DNA (rDNA) sequence data has shown that temperate isolates of *P. graminis* belong to two groups or 'ribotypes' based on sequence differences in the internal transcribed spacer (ITS) region. These ribotypes appear to differ in host range and ability to transmit viruses. It is hypothesised that particular ribotypes have different host specificities or preferences and are involved in the transmission of specific viruses. We have undertaken a number of studies to better understand the distribution and viral associations of the temperate ribotypes and to further clarify their taxonomic position within the order Plasmodiophorales and also their wider phylogenetic affinities.

37.137 NUTRIENT ACQUISITION STRATEGY IN COMPATIBLE *PERONOSPORA VICIAE* INFECTIONS. **P.T.N. Spencer-Phillips.** *School of Biosciences, University of the West of England, Coldharbour Lane, Bristol, BS16 1QY, UK. Email: peter.spencer-phillips@uwe.ac.uk*

Peronospora viciae is an endophytic oomycete that causes downy mildew of pea (*Pisum sativum*) and other legume hosts. It employs biotrophic infection strategies, where plant tissues remain alive for prolonged periods after initial colonisation. Intercellular hyphae of *P. viciae* isolated from leaves accumulate carbon from glucose (but not fructose and sucrose) *in vitro*, and this ability explains nutrient acquisition in non-haustorial infections of some hosts. The hyphae display a topology that is intermediate between herringbone and random up to 60 h post-infection, but which becomes more dichotomous at later stages of colony development. This is interpreted to reflect an initial strategy for overcoming host resistance and out-competing other pathogens through rapid colonisation and resource capture (the exploration phase), followed by formation of an increased surface area in contact with plant cells at the time of peak demand for nutrients immediately prior to sporulation (the exploitation phase). Proteome analysis of infected tissues has revealed an altered abundance of proteins that may mediate changes in nutrient acquisition strategy and the outcome of infection. Comparative analysis of the proteome of spores, germlings and appressoria provides evidence for pre-invasion growth processes and infection strategies.

37.138 ROOT INFECTION OF *BRASSICA NAPUS* BY *LEPTOSPHAERIA MACULANS* IN RELATION TO GLUCOSINOLATE DISTRIBUTION. **S.J. Sprague, J.A. Kirkegaard, M. Watt and B.J. Howlett.** *CSIRO Plant Industry, P.O. Box 1600, Canberra, ACT 2601, Australia. Email: susan.sprague@csiro.au*

Leptosphaeria maculans causes blackleg or *Phoma* stem canker of *Brassica napus*. This fungus invades cotyledons and leaves where it forms lesions. It then grows systemically to form cankers at the stem base and also grows into the root. The pathway of root infection has been determined by inoculating petioles with an *L. maculans* isolate expressing GFP. Fluorescence microscopy showed hyphae in all tissues of the stem and hypocotyl. In comparison, hyphae were mainly restricted to xylem tissue in roots. *B. napus* roots contain high levels of glucosinolates (sulphur-containing compounds), particularly 2-phenylethyl glucosinolate (2-PE GSL). Upon tissue disruption, this glucosinolate is hydrolysed to 2-PE isothiocyanate, which is toxic *in vitro* to *L. maculans* and other fungi. 2-PE GSL has been implicated in the interaction of soilborne organisms with brassicaceous species which contain this glucosinolate. The relationship between 2-PE GSL and root rot caused by *L. maculans* was determined by assessing disease severity in *B. napus* cultivars with high and low concentrations of 2-PE GSL. There was no consistent relationship between the concentration of 2-PE GSL in whole roots and root rot severity. However, 2-PE GSL was localised to the outer tissues of canola roots, as shown by destructive analysis. Although there is a correlation between high concentrations of 2-PE GSL in the outer root tissues and reduced hyphal density of *L. maculans*, the role of this glucosinolate in containment of the pathogen is unknown.

37.139 POPULATION STRUCTURE OF THE BLAST PATHOGEN *MAGNAPORTHE GRISEA* ON CEREAL HOSTS IN AFRICA. **S. Sreenivasaprasad, J.P. Takan, S. Muthumeenakshi, J. Chipili, N.J. Talbot, E.O. Manyasa, Y. Sere and A.E. Brown.** *University of Warwick, Warwickshire, CV35 9EF, UK. Email: s.prasad@warwick.ac.uk*

Blast caused by *Magnaporthe grisea* is a major disease of finger millet and rice in sub-Saharan Africa. We have used molecular markers such as MGR586 fingerprints and AFLPs, mating assays and pathogenicity tests to characterise the pathogen populations on these hosts. A high diversity of the blast pathogen haplotypes was recorded at both macro- and micro-geographic scales on finger millet in East Africa, the centre of origin and crop domestication. The pathogen populations revealed continuous variation (lack of clonality) reflecting sexual recombination. In the overall population, near equal distribution of the two mating types *MAT1-1* and *MAT1-2* was observed. Pathogen isolates from various locations revealed high fertility (70-100%) and more than 60% of them were hermaphrodites. All isolates tested showed basic compatibility to a range of finger millet varieties and only showed differences in aggressiveness suggesting polygenic quantitative interactions. On the other hand, in West Africa, where intensive rice cultivation is relatively recent (less than 500 years), blast pathogen populations showed a typical lineage-based structure with 2-3 dominant lineages in each country. Skewed distribution of the mating types, high female sterility and low fertility were observed. Pathogen isolates revealed clear differences in compatibility on rice differentials exhibiting R gene-type interactions, and a diverse range of pathotypes were recorded. Thus, *M. grisea* populations on finger millet and rice in sub-Saharan Africa appear to have adopted different evolutionary patterns related to crop domestication and agricultural intensification.

37.140 MOLECULAR ANALYSIS OF POPULATION DIVERSITY AND PATHOGENICITY LIFESTYLES IN *COLLETOTRICHUM* SPECIES. **S. Sreenivasaprasad, P. Talhinhas, S. Muthumeenakshi, João Neves-Martins and H. Oliveira.** *University of Warwick, Warwickshire, CV35 9EF, UK. Email: s.prasad@warwick.ac.uk*

Colletotrichum acutatum is a cosmopolitan pathogen causing

anthracnose diseases on a wide range of hosts including strawberry, citrus, olive, peach and almond. We are using a range of molecular approaches to understand the population diversity and pathogenicity lifestyles in this pathogen. Based on ITS, *tub2* and *his4* sequences and PCR-based markers, *C. acutatum* isolates from olive in Portugal comprised five molecular groups, A2-A6. Among these, A2 was dominant, mainly associated with intensive olive cultivation and heavy incidence of anthracnose. Isolates in group A2 were also more aggressive than others, in pathogenicity assays. A spatio-temporal survey over a 3 year period also enabled an understanding of the dynamics of these populations. On a global scale, phylogenetic analysis of the ITS sequences of nearly 300 *C. acutatum* isolates from various hosts, revealed nine molecular groups, A1-A9, with varying bio-geographic association patterns. PCR tests based on ITS and *tub2* sequences enabled rapid and reliable detection and differentiation of *C. acutatum* populations. These tests were also useful in epidemiological investigations, particularly with reference to olive anthracnose. *C. acutatum* exhibits different pathogenic strategies on various hosts, but the components regulating these processes are only beginning to be understood. We are using forward and reverse genetic approaches as well as a novel *Colletotrichum-Arabidopsis* model system to investigate pathogenicity lifestyles in *Colletotrichum*. A collection of transformants generated by *Agrobacterium* T-DNA insertional mutagenesis is being tested for alternations in pathogenicity. Further, identification and characterisation of genes involved in these interactions is underway.

37.141 THE BIOCHEMICAL EFFECT OF PHOSPHITE ON THE VIRULENCE OF *PHYTOPHTHORA CINNAMOMI*. **P.M. Stasikowski, J.A. McComb, G.E.StJ. Hardy and P.A. O'Brien.** *Centre for Phytophthora Science & Management, School of Biological Sciences & Biotechnology, Murdoch University, Murdoch WA 6150, Australia. Email: p.stasikowski@murdoch.edu.au*

Phytophthora cinnamomi is a broad-host-range necrotrophic oomycete pathogen responsible for the destruction of native flora in many parts of Australia. Disease symptoms and pathogen spread can be contained by treatment with phosphite as a foliar spray, trunk injection or soil drench. It has been proposed that phosphite, an analogue of phosphate, exerts its fungistatic effect either by direct inhibition of pathogen growth at the point of ingress, or indirectly by stimulating host-plant defence responses. However the biochemical processes that underlie and mediate these effects are not known. We have developed a *Lupinus angustifolius – P. cinnamomi* hydroponic bioassay to assess the action of various specific biochemical inhibitors on the pathways expected to be involved in pathogenesis. The direct application of 3mM phosphite to cultures of *P. cinnamomi* alone is sufficient to completely inhibit the development of disease symptoms on 5 day-old lupins without affecting pathogen viability. Pathogen virulence is also significantly altered by the guanine nucleotide exchange inhibitor, brefeldrin A; the serine/threonine phosphatase inhibitor, okadaic acid; and the phospholipase D inhibitor, butan-1-ol. Results are presented within the context of the effect of phosphite on the signal transduction pathways involved in pathogenesis.

37.142 ADHESION OF INFECTION STRUCTURES FORMED BY *VENTURIA INAEQUALIS* CONIDIA. **U. Steiner and E.-C. Oerke.** *Institute of Crop Science and Resource Conservation, University of Bonn, Germany. Email: u-steiner@uni-bonn.de*

Adhesion of early infection structures to the host plant surface is the first step for successful pathogen development and has been described as being a passive or active processes. Conidia of *V. inaequalis* adhered to wet hydrophobic surfaces immediately after contact to the surface hours before the initiation of germination. The attachment of non-germinated conidia was much better on hydrophobic surfaces. Conidia released adhesive material localized in a droplet at the spore apex which interacted with a surface only when water was present, leading to fixation at a point. Histochemical investigations suggested the presence of proteins and carbohydrates in this glue. Protein biosynthesis inhibitors did not affect adhesion of conidia indicating that the adhesive material was preformed. With the beginning of germ tube elongation up to formation of the appressorium, mucilage-like material was released. As a result the infection structures were embedded in a protein- and carbohydrate-containing matrix spreading over the contact surface. Additionally a dark brown ring structure was formed at the base of the appressorium. This melanized appressorial ring was attached to the leaf surface like a sealing ring and formed the fungus-plant interface; it is required for pathogen penetration of the cuticle. Transmission electron microscopy confirmed a localized melanization of the cell wall around the penetration pore and melanin was incorporated into all layers of the fungal wall. This ring may be a means to focus the activity of hydrolytic enzymes during cuticle penetration but is not involved adhesion.

37.143 GLOBAL EXPRESSION ANALYSIS OF *FUSARIUM GRAMINEARUM* GENES DURING CROWN ROT DISEASE OF WHEAT. **A.E. Stephens, D.M. Gardiner, K. Kazan, A.L. Munn and J.M. Manners.** *Commonwealth Scientific and Industrial Research Organisation (CSIRO), Plant Industry, 306 Carmody Rd, St Lucia, 4067, Queensland, Australia. Email: donald.gardiner @csiro.au*

Fusarium head blight (FHB) is one of the most important diseases of wheat worldwide. In Australia, *F. graminearum* and the closely related fungus *F. pseudograminearum*, cause sporadic head blight epidemics and are also responsible for crown rot (CR) disease in wheat. These diseases can lead to substantial yield losses and accumulation of mycotoxins in grain, particularly trichothecenes, which adversely affect human and animal health when consumed. The two diseases, although potentially caused by *F. graminearum* show very different disease progression kinetics. These differences are probably associated with the type of tissue (head vs crown) infected and this is particularly reflected in estimations of fungal biomass during CR and FHB infections. During FHB, the fungus rapidly proliferates within the head tissue following initial infection. In contrast, during CR disease, there appears to be at least three distinct phases of infection; initial infection of stem base, an extended period (up to 4 weeks) of limited fungal proliferation, followed by a rapid increase in fungal biomass. The third stage of the infection process also coincides with the development of necrosis in the infected tissue. We have used confocal microscopy and *Fusarium* Affymetrix gene chip analyses to characterise the process of CR disease of wheat. A number of candidate genes found to be altered in expression during different stages of infection are being mutated in the fungus to determine their roles.

37.144 NOVEL GENETIC MODELS FOR ANALYZING INTERACTIONS WITH *SCLEROTINIA SCLEROTIORUM*. **H.U. Stotz, A.T. Bakalinsky and X. Guo.** *Departments of Horticulture and Food Science and Technology, Oregon State University, Corvallis, OR 97331, USA. Email: stotzhe@hort.oregonstate.edu*

Sclerotinia sclerotiorum causes white mould and other diseases on hundreds of host plants, including economically important food and fuel crops. The National *Sclerotinia* Initiative, under leadership of the USDA-ARS, aims to neutralize *S. sclerotiorum* on sunflower, soybean, canola, edible dry beans, chickpeas, lentils, and dry peas. Because host genes that control or facilitate interactions with this ascomycete have not been identified, we initiated genetic screening in *Arabidopsis thaliana*. In addition, a genome-wide screen for oxalate sensitivity was carried out in the yeast *Saccharomyces cerevisiae* to discover genes whose orthologs may protect the host from the adverse effects of this virulence factor. Exposure to oxalic acid was lethal to the *S. cerevisiae* mutants rib4Δ, drs2Δ, vps16Δ, vps51Δ, and ric1Δ. With the exception of rib4Δ, all of these mutants are impaired in vesicle-mediated transport. The *Arabidopsis* ortholog of the riboflavin biosynthetic gene RIB4 is COS1, which contributes to jasmonate signaling. Based on the connection between riboflavin and jasmonate, we have focused attention on induced defence response pathways and demonstrated that defence against *S. sclerotiorum* in *Arabidopsis* depends on jasmonate, salicylic acid, and ethylene signaling. Based on analysis of coi1, npr1, and ein2 mutants, defence against *S. sclerotiorum* is independent of oxalic acid. On the other hand, susceptibility is oxalate-dependent and we have developed guard cells as a model system to analyze genes that facilitate interactions with *S. sclerotiorum*.

37.145 EVALUATION OF WOODY INDICATORS FOR PEAR CERTIFICATION IN THE CLIMATIC CONDITIONS OF THE CZECH REPUBLIC. J. Suchá and L. Talácko. *Research and Breeding Institute of Pomology Holovousy Ltd., Holovousy 1, Horice, Czech Republic. Email: sucha@vsuo.cz*

Woody indicators for pear recommended by EPPO, were tested for certification schemes of fruit species in their second cycle for diagnostic improvement. Seven indicator cultivars were tested (*Pyrus communis* 'LA 62', 'A 20', 'Noveau Poiteau', 'Hardy', 'Williams', *Pyronia veitchii* and *Malus pumila* 'Virginia Crab'). Double chip budding was used to inoculate the indicators. The presence of viruses in indicators was verified by laboratory diagnostic tests. Woody indicators were tested in the field for 3 years. The following indicators were selected for conditions of the Czech Republic on the basis of symptom intensity: *Pyrus communis* cultivars Hardy and Noveau Poiteau for *Apple chlorotic leaf spot virus* and *Pyronia veitchii* for *Apple stem-grooving virus* and for *Apple stem pitting virus*. *P. veitchii* also showed the best results for '*Candidatus* Phytoplasma pyri'. The results agreed with EPPO standards. The research was funded by the NAZV Grant Agency No. QG60123.

37.146 DISSECTING RESISTANCE TO SPOT BLOTCH (*BIPOLARIS SOROKINIANA*) IN BARLEY. M.W. Sutherland, N.L. Knight, J.H. Bovill, A. Lehmensiek, E.S. Mace and G.J. Platz. *Centre for Systems Biology, University of Southern Queensland, Toowoomba, QLD 4350, Australia. Email: marksuth@usq.edu.au*

Spot blotch (SB) of barley, due to infection by the fungus *Bipolaris sorokiniana* (teleomorph *Cochliobolus sativus*), is an economically important foliar pathogen in many barley growing regions of the world. In Australia it causes significant losses in the warmer and often humid spring conditions of north-eastern NSW and Queensland. We have been identifying and characterising sources of resistance to this disease for deployment in breeding programs, as current Australian cultivars are susceptible. Breeding for host resistance to SB is currently being hampered by our lack of understanding of both the race structure of the pathogen and of the effectiveness of the current resistant sources against the range of pathogen genotypes present in Australia. To date several North American sources of resistance are the most promising and we have identified molecular markers for these resistances. AFLP analysis of an extensive collection of fungal isolates from spot blotch - infected leaves and from root materials with common root rot symptoms, also caused by *B. sorokiniana*, indicate genetic clustering of isolates according to host tissue source. Fungal isolates from common root rot infections did not produce extensive spot blotch symptoms when applied to susceptible barleys. We are currently examining the virulence of a range of isolates from spot blotch infections across a differential set of cultivars to determine pathotype variability.

37.147 A NEW *IN VITRO* METHOD TO IDENTIFY RED ROT-SUSCEPTIBLE CLONES OF SUGARCANE. N. Swarnakumari and M.C. Gopinathan. *R & D Centre, E.I.D. Parry (India) Ltd., Nellikuppam, Cuddalore district, Tamilnadu, India. Email: swarnakumarin@parry.murugappa.com*

Red rot is a serious disease of sugarcane in India, caused by *Colletotrichum falcatum* Went. It is distributed world wide. Several promising varieties were deteriorated due to frequent appearance of new virulent biotypes of *C. falcatum*. Thus, testing of new clones for their reaction to red rot becomes inevitable. Several methods were described elsewhere for screening at field level. Most of them require 8-9 months for evaluation. Hence there is an immediate need to develop a simple method. Consequently, a new technique was developed with the aim of identifying susceptible clones under *in vitro* conditions. Healthy cane stalks of different varieties with a range of reactions to red rot were selected. The stalks were surface sterilized and inoculated with spore suspensions and then incubated for two months under *in vitro* conditions. Lesion development was recorded on the 30th and 60th day after inoculation. The lesion volumes (range 22.3-242.3 cm^3) of tested clones were compared to standards and found to be on a par with field data. Highly susceptible clones could be rated within a month since the lesion crossed four nodes on the 30th day after inoculation. A positive correlation existed between lesion length and volume (r = 0.98). This method should help to evaluate more clones in a short period, and is also repeatable. The detailed methodology and results will be furnished in this paper.

37.148 APHID TRANSMISSIBILITY AND INTERACTIONS WITH HOST PLANTS OF NOVEL ISOLATES OF *POTATO VIRUS Y*. J. Syller, K. Golnik and A. Kaliciak. *Plant Breeding & Acclimatization Institute, 05-831 Mlochow, Poland. Email: j.syller@ihar.edu.pl*

Potato virus Y (PVY) is one of the most destructive pathogens attacking solanaceous crops. The virus is non-persistently transmitted by aphids, *Myzus persicae* being its chief vector. In the last 20 years, the increasing spread of new, more infective, necrotic PVY isolates has frequently been reported. Novel virus variants have been classified into substrains of the PVY^N strain. The results of timed probe and arena experiments using *Nicotiana tabacum*, *Physalis floridana* and *Solanum nigrum* assay plants showed that transmissibility by *M. persicae* of several geographically different PVY isolates belonging to PVY^{NTN} and PVY^N-W substrains was significantly higher than that of the isolates representing the long-established PVY^N and PVY^0 strains. Unexpect-

edly high transmission rates of PVY acquired by aphids during sustained feeding on infected leaves, as opposed to virus acquisition during short feeding probes, were recorded. The effects of virus acquisition mode were found to depend on the species of assay plant. To *N. tabacum*, PVY was transmitted efficiently following either acquisition pattern, whereas to *P. floridana* the virus acquired during short probes was transmitted relatively better. Thus, PVY transmissibility by *M. persicae* was shown to depend on the effects of viral genetic variability, host genotype and mode of acquisition. Three new natural hosts of PVY were found among arable weeds in the Asteraceae and Geraniaceae, common in Europe and some parts of Asia.

37.149 MULTIPLICATION OF *RICE STRIPE VIRUS* IN THE SHOOT APEX IS THE FIRST STEP TOWARD SYSTEMIC SPREAD. **M. Takahashi, K. Ishikawa, Y. Hayano-Saito and K. Tsuchiya.** *National Agricultural Research Center, Inada 1-2-1, Jouetsu, Niigata 943-0193, Japan. Email: mamitaka@affrc.go.jp*

Rice stripe virus (RSV), which is transmitted by the small brown planthopper (SBPH), systemically infects gramineous plants. To test the hypothesis that RSV multiplies in the shoot apex, we conducted experimental and observational studies. In the experimental component, we exposed rice plants at the 1.5th leaf stage to viruliferous SBPHs for durations of 1 hour to 3 days, then removed all of the leaf sheaths and leaves from half of the inoculated plants, leaving the shoot apex and root system intact. The plants were incubated for 2 weeks, then the leaf sheaths were examined for viral infection. Even after inoculation for only 1 hour, RSV was detected in the leaf sheaths that developed thereafter, and the proportions of RSV-positive plants were similar for the groups of plants from which the leaf sheaths and leaves had and had not been removed for each inoculation duration. These results suggest that RSV particles are immediately transferred to the shoot apex, and that only RSV that is thus transferred successfully causes infection. Next, we observed the shoot apex of infected wheat by immunogold electron microscopy using RSV-specific antibody. Gold labeling revealed that many cells in the apical domes and leaf primordia were infected with RSV. Gold label was also found on both sides of the cell walls between two sister cells. Taken together, our experimental and observational data indicate that RSV multiplies in the shoot apical meristems and is spread systemically by active cell division.

37.150 SPATIAL ANALYSIS FOR MIXED INFECTIONS OF DIFFERENTLY LABELLED *CUCUMBER MOSAIC VIRUS* AND *TURNIP MOSAIC VIRUS* VECTORS. **M. Takeshita, E. Hirano, M. Noguchi, K. Sueda, N. Suehiro, T. Natsuaki, C. Masuta, K. Ohshima, N. Furuya, K. Tsuchiya and Y. Takanami.** *Laboratory of Plant Pathology, Division of Applied Genetic and Pest Management, Graduate School of Kyushu University, Fukuoka, Japan. Email: minorutk@agr.kyushu-u.ac.jp*

Mixed infections of some plant viruses in *Nicotiana benthamiana* were examined using differently labelled CMV vectors designated C2-A1-DsRed2 (virus: CMV-DsRed2) and C2-A1-EGFP (virus: CMV-EGFP), which have been constructed on the basis of CMV2-A1 CMV vector (Otagaki *et al.*, 2006). Mixed infection of CMV-DsRed2 and CMV-EGFP resulted in spatial separation between EGFP and DsRed2 fluorescent foci in the plants. Pre-infection of CMV-DsRed2 (or CMV-EGFP) efficiently inhibited challenge infection of CMV-EGFP (or CMV-DsRed2). The CMV vectors also facilitated characterization of spatial dynamics in co-infection with wild-type or EGFP-expressing TuMV vectors (virus: TuMV or TuMV-EGFP, Suehiro *et al.*, 2004). Co-infection of the plants with CMV-DsRed2 and TuMV-EGFP caused a very destructive disease (leaf necrosis and plant death). Synchronous infection and enhancement of virus spread, however, were not observed in the plants co-infected with CMV-DsRed2 and TuMV-EGFP. Interestingly, CMV-DsRed2 and CMV-EGFP populations showed fluorescent signals in discrete area in mixed infection with TuMV, indicating that the competitive interactions between CMV-DsRed2 and CMV-EGFP were not compromised by the synergistic interactions between CMV and TuMV. Furthermore, C2-H1 CMV vector from which the *2b* gene was removed (Matsuo *et al.*, 2007) demonstrated that the *2b* gene was dispensable for the CMV/TuMV synergism in the plants.

37.151 *ARABIDOPSIS* ETHYLENE-RESPONSIVE ELEMENT-BINDING PROTEIN, ATEBP, NEGATIVELY REGULATES THE HYPERSENSITIVE CELL DEATH RESPONSE THROUGH THE FUNCTION OF BAX INHIBITOR-1. **K. Tamura, T. Ogawa, M. Kawai-Yamada and H. Uchimiya.** *Institute of Molecular and Cellular Biosciences, University of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan. Email: ktamura@iam.u-tokyo.ac.jp*

Arabidopsis ethylene-responsive element-binding protein (AtEBP) is a transcriptional activator belonging to the plant specific AP2/EREBP family. This factor mediates ethylene signalling possibly downstream of EIN2 but not EIN3, and confers resistance to murine Bax- and abiotic stress-induced plant cell death. To further understand the function of AtEBP in stress response, we examined the role of this protein in the regulation of hypersensitive response (HR) elicited by incompatible plant-microbe interactions. Expression of AtEBP in *Arabidopsis* leaves was upregulated during elicitation of HR by *Pseudomonas syringae* pv. *tomato* (Pst) DC3000 carrying either *avrRpm1* or *avrRpt2* as well as by treatment with 1-aminocyclopropane-1-carboxylic acid as the precursor of ethylene. When *Arabidopsis* leaves were infiltrated with serially diluted inocula of Pst (*avrRpm1*), the AtEBP-knockout (KO) mutant exhibited increased sensitivity to HR induction, whereas transgenic AtEBP-overexpressing lines were less sensitive than wild type. Measurement of electrolyte leakage and ROS generation verified the enhanced inductivity of HR in the AtEBP-KO line. To further examine the HR suppressive action of AtEBP, we analyzed its functional linkage with *Arabidopsis* Bax Inhibitor-1 (AtBI-1), an antiapoptotic factor. RNA blot analysis of the AtEBP-KO line showed attenuation of AtBI-1 expression, which in wild type was upregulated by elicitation of HR. Like AtEBP-KO, AtBI-1-RNAi lines were more sensitive to HR induction compared to wild type, suggesting that AtBI-1 also functions negatively in the modulation of HR. Together, these results demonstrate that AtEBP plays a role in the negative regulation of HR mediated through the cell death suppression activity of AtBI-1.

37.152 THE ALDEHYDE DEHYDROGENASE GENE LOCATED IN THE PATHOGENICITY ISLAND IS INVOLVED IN VIRULENCE OF *PSEUDOMONAS CICHORII*. **M. Tanaka, M. Koyanagi, H. Hojo, S. Kajihara, K. Ohnishi, A. Kiba and Y. Hikichi.** *Laboratory of Plant Pathology & Biotechnology, Kochi University, Kochi, Japan. Email: b4mb020@s.kochi-u.ac.jp*

Pseudomonas cichorii SPC9018 causes necrotic lesions and rot on leaves of eggplant and lettuce plants, respectively. The nucleotide sequences of the pathogenicity island (PAI) including the *hrp* genes of SPC9018 and its genetic structure are homologous

to those of *P. viridiflava* AS group strains. The consensus *hrp*-box (GGAACC-N_{15-16}-CCANNCA) was identified at putative promoters of *hrpA, hrpF, hrpW, avrE, avrF* and *hrpJ*. RT-PCR analysis showed that expression of *hrp* genes was dependent on HrpL. Interestingly, pathogenicity of SPC9018 on eggplant but not lettuce plants is *hrp*-dependent. In the PAI of SPC9018, the aldehyde dehydrogenase (Aldh) gene is located near *hrpL* and shows high homology to not only to that of *P. syringae* pv. *tomato* strain DC3000 but also that located in PAIs of *P. viridiflava* AS group strains and that located in the PAI of virulent *P. aeruginosa* strain X24509. Recombinant Aldh protein showed $NADP^+$-dependent *in vitro* activity of aldehyde dehydrogenase. The *aldh*-deficient mutant of SPC9018 lost its virulence on eggplants, but retained its virulence on lettuce plants. Complementing the *aldh*-deficient mutant with *aldh* from SPC9018 allowed the mutant to cause disease on eggplant. Interestingly, *aldh* from *P. syringae* pv. *tomato* strain DC3000 also allowed the mutant to complement its virulence on eggplant. RT-PCR analysis showed that expression of *aldh* is independent of HrpL. These results suggest requirement of *aldh* for virulence of SPC9018 on eggplant and functional conservation of *aldh* among *Pseudomonas* species.

37.153 IDENTIFICATION OF RESISTANCE AGAINST DIVERSE PATHOTYPES OF *SCLEROSPORA GRAMINICOLA* IN PEARL MILLET GERMPLASM ACCESSIONS. **R.P. Thakur, V.P. Rao and R. Sharma.** *International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India. Email: r.thakur@cgiar.org*

Downy mildew caused by *Sclerospora graminicola* (Sacc.) Shroet. is a major biotic constraint to pearl millet production in the semi-arid tropics. The pathogen is heterothallic, and frequent recombinations lead to genotypic diversity and evolution of virulent populations. Identification of resistance against new virulent populations is a prerequisite for managing the disease through resistance breeding. One hundred twenty-nine pearl millet germplasm accessions from 16 countries that were identified as highly resistant (≤10% incidence) in the downy mildew nursery at ICRISAT, India, during 1990-93 were re-evaluated against the new population of *S. graminicola* in the downy mildew nursery as well as in the greenhouse during 2006. Of the 129 accessions, 60 were found resistant in the field and 25 in the greenhouse screens. Twenty-one lines that showed resistance both under field and greenhouse conditions were further evaluated in the greenhouse against 11 virulent pathotypes of *S. graminicola* collected from different pearl millet growing areas in India and maintained at ICRISAT. Differential reactions for downy mildew resistance were observed among the accessions against the pathotypes. None of the 21 accessions showed resistance to all the 11 pathotypes. However, 3 accessions (IP 18295, P 1449-2 and YL-18) were resistant to 10 pathotypes, 2 to 9 pathotypes, 3 to 8 pathotypes, 1 each to 6 and 7 pathotypes, and 2 each to 5 and 4 pathotypes. We conclude that virulence changes in *S. graminicola* and resistance in pearl millet should be closely monitored for managing this disease through resistance breeding.

37.154 ENZYME ACTIVITIES IN WHEAT LEAVES INFECTED BY *SEPTORIA TRITICI* AND THE RELATIONSHIP TO DISEASE SEVERITY FROM BA-ELISA IN WHEAT VARIETIES. **S.M. Tian, J. Weinert, Q.H. Zhao and G.A. Wolf.** *Institute of Plant Pathology and Plant Protection, Georg-August-University Goettingen, D-37077 Goettingen, Germany. Email: jweinert@gwdg.de*

Investigation of enzyme activities in extracts from wheat leaves inoculated with *Septoria tritici* revealed activities of the following enzymes: xylanase, cellulase, protease and α-amylase. Levels of xylanase, protease and cellulase activity were closely related to fungal antigen levels as measured by BA-(biotin-avidin) ELISA and to visual disease assessments on infected leaves from greenhouse experiments and from field trials at growth stage 75. For the wheat cultivars tested, levels of the three enzymes also showed good correlation ($p<0.01$) with susceptibility ratings from the *Bundessortenamt* (Federal Office of Plant Variety) recommended cultivar list, which was based on visual assessment. Protease, like xylanase and cellulase, is suggested to play important roles in plant pathogenesis by *S. tritici*. Enzyme activity may serve as a measure of disease severity for assessing the resistance of wheat cultivars to *S. tritici*.

37.155 TRANSLOCATION AND CHAPERONE INTERACTION OF THE *ERWINIA AMYLOVORA* SECRETED EFFECTOR DSPE. **L.R. Triplett, M. Melotto, S.Y. He and G.W. Sundin.** *103 CIPS, Michigan State University, East Lansing, MI, 48824, USA. Email: endersli@msu.edu*

Many plant pathogenic bacteria require a type III secretion system (T3SS) to introduce bacterial effector proteins into host cells and cause disease. Effector-specific chaperones are often required for T3SS secretion, although the exact role of these chaperones is unclear. We have characterized the interaction between the pathogenicity factor DspE and its T3SS chaperone DspF in *Erwinia amylovora*, the causal agent of fire blight of apple and pear. An adenylate cyclase reporter system was used to map the portion of DspE required for translocation into tobacco cells. Results showed that the first 51 amino acids of DspE are sufficient for minimal protein translocation into the host cell, and that translocation levels increase with increasing DspE fragment length. Yeast-two-hybrid experiments identified a putative chaperone binding domain within residues 51–100 of DspE. To further characterize the DspE-DspF interaction, computational methods were used to create a homology-based structural model of DspF. Based upon this model, 13 amino acids of DspF were identified as putative exposed substrate-binding residues. Site-directed mutagenesis was used to replace codons for candidate residues with alanine on a plasmid clone of *dspF*, yielding 13 single-site *dspF* mutant clones. In virulence assays on immature pears, 5 *dspF* mutants failed to complement the virulence phenotype of the *dspF* knockout strain Ea1189Δ*dspF*:kn and abolished DspE-DspF binding in a yeast-two-hybrid assay. Studies are underway to identify specific residues of DspE required for binding to DspF, and the results will be used to model the DspE-DspF interaction.

37.156 STUDYING THE ROLE OF THE G PROTEIN βSUBUNIT IN PATHOGENESIS OF *VERTICILLIUM DAHLIAE*, THROUGH RNAI AND GENE REPLACEMENT. **A. Tzima, E.J. Paplomatas and S. Kang.** *Laboratory of Plant Pathology, Agricultural University of Athens, 118 55 Athens, Greece. Email: aliki@aua.gr*

To gain insight on the role of G protein signaling in virulence of the soilborne fungus *Verticillium dahliae*, RNAi and gene replacement technology were employed. For silencing gene expression via RNAi, part of the β subunit of G protein was cloned, a hairpin was constructed and successfully integrated into the *V. dahliae* genome. However, in transformants screened so far, low levels of silencing were detected. This obviated the need to demonstrate the presence

of an operational RNAi mechanism in *V. dahliae*. In isolates already expressing GFP, a hairpin of this reporter gene was integrated into the genome. As evidenced for Gb silencing, no significant down-regulation of GFP was observed in about 60 transformants screened by UV microscopy. The functionality of the constructed GFP hairpin will be examined in other GFP expressing fungi, known to possess RNAi mechanisms. Moreover, a new hairpin with spacer sequences of unrelated origin is under construction. To compare gene knock-down with the more robust technique of gene replacement, a Gb mutant allele (generated by disrupting part of the Gb gene with the geneticin resistance cassette) was inserted via ATMT into the *V. dahliae* genome. Despite the use of the HSVtk-negative selection marker for ectopic integration, replacement of the Gb gene did not occur, probably due to the small gene fragments flanking the geneticin resistance cassete To achieve gene replacement in *V. dahliae*, longer flanking sequences of the Gb gene are being isolated using inverse PCR. Likewise, other pathogenicity genes are being studied in *V. dahliae*.

37.157 DETERMINATION OF REACTION MODE IN SOME OIL SEED RAPE CULTIVARS TO CANOLA WHITE STEM ROT, *SCLEROTINIA SCLEROTIORUM* IN IRAN. Z. Vakili-zarj and K. Rahnama. *Department of Plant Protection, College of Crop Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Email: Kamran_ra @Yahoo.com*

Canola white stem rot, causal agent *Sclerotinia sclerotiorum*, is the most serious canola disease in northern Iran. Common cultivars of canola (Amica, Sarigol, RGS 003, Option 500, Hyola 401, Hyola 60, Hyola 420, Hyola 330, Hyola 308, Kimberly, RGS 3006, PR-401/16, PP-401/15E, PP-308/3, PP-308/8, S-3.) and four isolates of the fungus from canola fields (Ss_2, Ss_5, Ss_8, Ss_{18}), were field-tested during 2004-2005. The experimental design was a randomized complete block design with three replicates (in each replicate 10 plants) for each cultivar inoculated by each fungal isolate. The stems were inoculated by a small block of 3 day-old fungal mycelium on PDA (10-mm diameter) placed around the stem and wrapped with parafilm. Results showed that isolate Ss_{18} produced smaller lesions (8-cm long) in most cultivars, and was significantly less pathogenic than other isolates when tested on cultivars Hyola 401and RGS 003, with mean lesion lengths of 7.45 and 8.28 cm. The oxalic acid production test needed longer to produce colour change in semi-selective medium (indicating lower oxalic acid production) with Ss_{18}, compared with Ss_2, and Ss_5. The greatest lesion lengths were observed on 'Amica' infected by isolate Ss_5 (34.73 cm) and 'Hyola 420' infected by isolate Ss_2 (34.63 cm). Thus, because of the risk of stem breaking in the field, we recommend sowing the tolerant cultivars RGS 3006 and Sarigol rather than Option 500, Hyola 330, and Hyola 420, which are common sensitive cultivars.

37.158 A CULTURE FILTRATE OF *PHYTOPHTHORA INFESTANS* PRIMES DEFENSE REACTION IN POTATO CELL SUSPENSIONS. F. Val, S. Desender, K. Bernard, P. Potin, G. Hamelin and D. Andrivon. *INRA, Agrocampus Rennes, UMR1099 BiO3P, Biology of Organisms and Populations applied to Plant Protection, F-35042 Rennes, France. Email: didier.andrivon@agrocampus-rennes.fr*

Priming of defence reactions by an elicitor results in enhanced ability of the plant to respond to subsequent pathogen challenge. We previously showed that application of lipopolysaccharides (LPS) to potato cell suspensions causes apoplastic acidification, but does not stimulate lipoxygenase (LOX) activity. Here, we tested the ability of various elicitors to prime and elicit defence reactions in potato cell suspensions. Adding 20 µg ml^{-1} LPS, laminarin, harpin N or a concentrated culture filtrate of *Phytophthora infestans* (CCF) to cell cultures 18 h before a second elicitation with LPS did not alter the intensity of apoplastic acidification, compared with a single LPS application. Conversely, high concentrations (200 or 400 µg ml^{-1}) of LPS, laminarin and harpin N could activate LOX in cells pre-treated with 1 µg ml^{-1} CCF, but not in cells pre-treated with LPS, laminarin, or harpin N. LOX response was maximal in pre-treated cells of potato cv. Bintje when the second elicitation occurred 18-24 h after CCF application. These results showed that LOX activation is primed in potato cells by CCF, but not by LPS, harpin N or laminarin. Finally, bioassays showed a slightly greater reduction of rot weight in half tubers treated with CCF followed by LPS before inoculation with *Pectobacterium atrosepticum* than in half tubers treated with either preparation alone.

37.159 GENES UP-REGULATED IN TOLERANT CAVENDISH BANANA ROOTS IN RESPONSE TO *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* INFECTION. N. van den Berg, I. Hein, P. Birch, D. Berger, M. Wingfield and A. Viljoen. *Dept. Microbiology & Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa. Email: noelani.vdberg@fabi.up.ac.za*

Fusarium oxysporum f. sp. *cubense* (Foc) , is regarded as one of the most destructive diseases of banana. Cavendish bananas are highly susceptible to Foc race 4 and no control strategy exists other than replacing susceptible cultivars with resistant bananas. The aim of this study was to study the regulation of defence-related genes in the tolerant banana GCTCV-218. A cDNA library was constructed using suppression subtractive hybridisation (SSH). The library was screened using DNA microarray technology, and 334 potentially induced clones were selected and sequenced. Four defence-associated genes, *catalase 2*, pectin acetyl esterase (*PAE*), *PR-1* and endochitinase (*PR-3*) were selected for expression-profile analysis using real-time reverse transcriptase PCR. GCTCV-218 showed significantly lower disease severity and incidence compared to the susceptible Cavendish cultivar Williams in replicated greenhouse and field trials. Several defence-related transcripts were identified by BLASTX searches, including genes coding for PR1, pectin acetylesterase, xylanase inhibitor, peroxidase, catalase 2, metallothionein, isoflavone reductase and tripsin inhibitor. Quantitative RT-PCR results confirmed that all four genes were differentially expressed in GCTCV-218 at 3 and 6 h after infection, confirming SSH results. *PR-1* and *PAE* were induced very early (3 h after infection) in the GCTCV-218, while *PR3* and *catalase 2* followed with a significant induction at 6 h after infection. We concluded that GCTCV-218 is able to respond rapidly in response to Foc infection by activating both a biochemical and a structural defence. The tolerance of GCTCV-218 was further linked to a significant increase in the induction of cell wall-associated phenolic compounds.

37.160 DID PATHOGENS EMERGE WITH CROP DOMESTICATION? M.M. van der Merwe and C.C. Linde. *School of Botany and Zoology, Building 116, The Australian National University, Daley Rd, Canberra, 0200, Australia. Email: celeste.linde@anu.edu.au*

Some evidence suggests that agriculture contributed to the emergence and divergence of fungal crop diseases. Published

studies suggest that while barley scald and *Mycosphaerella graminocola* on wheat emerged at or after the time of domestication of the host, smuts of maize and sorghum diverged with the origin of the host lineages. However, the dating methods and data used differed widely, making comparisons difficult. We used published DNA sequence data of 12 crop pathogens and Bayesian methods to test the hypothesis that domestication played a significant role in the emergence and divergence of crop diseases. First we estimated the time since the host-specific isolates shared a common ancestor by using a relatively fast fixed mutation rate. With this approach our hypothesis could not be rejected if the estimated date overlapped with the time of domestication of the host crop. Secondly we constrained the pathogen lineage to be no older than the time since the host crop was domesticated. This approach provided an estimated mutation rate that was compared to published mutation rates for the particular loci in order to assess the likelihood of the scenario. Results are discussed in reference to the type of data used, the estimates of divergence date and mutation rate, and the overall trend in pathogen divergence in relation to the domestication of these monocot hosts.

37.161 GENETIC ANALYSIS OF A NATURALLY-INDUCED MILD *PLUM POX VIRUS* ISOLATE. N. Vassilakos and C. Varveri. *Benaki Phytopathological Institute, 8 Stefanou Delta Str., Kifissia, Attica, 14561, Greece. Email: n.vassilakos@bpi.gr*

A mild isolate of *Plum pox virus* (PPV) was obtained after low-temperature treatment of the isolate PPV-D-GR in *Nicotiana benthamiana* and was designated PPV-B2. PPV-B2 conferred total cross-protection against the highly pathogenic PPV-M in *N. benthamiana* and *N. clevelandii*. It induced mild symptoms in *N. benthamiana* where it multiplied at a lower rate than the wild type isolate; it induced practically no symptoms in *N. clevelandii* where it multiplied very poorly and was not aphid transmissible. Thus far, sequence analysis of the full genome of PPV-B2 has revealed two substitutions in the HC-Pro (Thr85Ile and Thr400Ala) and two in the P3 (Thr123Ser and Asp176Glu) protein. The HC-Pro FRNK domain, linked with aggressiveness in other potyviruses, was intact in PPV-B2 indicating that this domain is not solely associated with potyvirus symptomatology. Although PPV-B2 was not aphid transmissible, it retained the "DAG" motif in the CP and "PTK" in the HC-Pro. The genetic basis for the diverse symptomatology, aphid transmissibility and host adaptability of PPV-B2 is discussed.

37.162 DIFFERENCES IN MORPHOLOGY AND VIRULENCE OF *MYCOSPHAERELLA GRAMINICOLA*, CAUSAL AGENT OF SEPTORIA TRITICI BLOTCH ON WHEAT. L. Vechet and E. Vydrova. *Crop Division of Plant Genetics, Breeding and Product Quality, Drnovska 507, Crop Research Institue, Prague-Ruzyne, Czech Republic. Email: vechet@vurv.cz*

Eighteen isolates of *Mycosphaerella graminicola* from winter wheat cultivars in Plzen, Prague, Usti n. Orlici and from individual blotches were tested for virulence on thirteen selected wheat cultivars, including 'Cleo' (Stb 4), 'Arina' – medium resistance, and 'Hereward' (Stb 6). The isolate from 'Chul' was strongly virulent to 'Hereward'. There were differences in virulence of individual isolates collected from one cultivar obtained from the same area and from different areas. There were also differences in virulence in isolates derived from a single blotch, which was found to carry some genotypes of *M. graminicola* distinguished from each other in coloration and morphology i.e. fringe, internal structure and surface. Of the cultivars tested, the most susceptible were 'Sarka' and 'Barroko'.

37.163 CHARACTERIZATION OF LEAF RUST POPULATION GENETICS IN THE NORTH-CAUCASIAN REGION OF RUSSIA IN 2006. G.V. Volkova, L.K. Anpilogova, O.A. Kudinova and O.J. Kremneva. *All-Russia Research Institute of Biological Plant Protection, Krasnodar-39, Russia. Email: volkova1@mail.kubtelecom.ru*

Virulence analysis of a collection of *Puccinia triticina* isolates was made using 16 isogenic lines – carrying the following *Lr* genes: *1*, *2a*, *2c*, *3*, *9*, *16*, *24*, *26*, *3ka*, *11*, *17*, *30*, *B*, *10*, *14a* and *18*. Single-pustule isolates derived from the 2006 urediniospore population showed high heterogeneity in the North Caucasus. Among 330 analyzed isolates 283 virulence phenotypes were identified, 255 of which were represented only by one isolate and only 28 phenotypes by 2-9 isolates. In the population the phenotype KHTT occurred more frequently (2.7%) and was identified in the West Azov zone, Central zone, East Steppe zone. The phenotypes KHTR and THTT were also identified in these zones with frequencies of 1.51% and 1.2%, respectively. Statistical analysis showed that phenotypic diversity was very high in the leaf rust populations of all the zones tested (Sh from 3.14 to 4.35). None of the phenotypes identified was clearly predominant except for FCRR in the Southern Submontane zone (6.25%), PHTT in the West Azov zone (5.00%) and KHTT in the East Steppe zone (12.00%). The leaf rust populations from different agroclimatic zones differed significantly in their phenotypic composition. The difference level in all cases exceeded 0.91, and the populations from the Central zone and North zone; East Steppe zone and North zone had no common phenotypes at all. As a rule, the most nearly isogenic wheat lines had high percentages of virulent isolates (from 41.8% to 88.5%). As before, no virulence to the *Lr9* gene carrier was identified; and as for the gene *Lr24*, 1.3% of virulent isolates were identified in the Central zone and 1.7% in the North zone.

37.164 IMPACT OF COTTON ON TEMPORAL VARIATION IN A *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* POPULATION. B. Wang, C.L. Brubaker, P.H. Thrall and J.J. Burdon. *CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia. Email: bo.wang@csiro.au*

Temporal variation in *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) was observed in a field at Boggabilla, NSW. 157 Fov isolates were recovered from diseased plants in 3 successive growing seasons; among these 4 AFLP genotypes were detected. In 2002, the population was dominated by genotype 11-A, that accounted for 89% of the isolates, while genotype 11-B occurred at a frequency of only 2%. In 2004, the frequency of 11-A declined to 36%, while that of 11-B increased to 48%. In 2006, 11-B dominated the population, accounting for 77% of isolates, while 11-A was undetectable. How genotypes 11-A and 11-B interact on different cotton cultivars (susceptible Siokra 1-4 and tolerant Sicot 189) was assessed experimentally. Seedlings were inoculated by dipping roots into a 50:50 mixed spore suspension. Ten single spores were subcultured from each of 20 randomly chosen symptomatic plants, and cultures were genotyped. Results show that disease was caused by infection of a single genotype on 17 of the 20 Siokra 1-4 plants and all Sicot 189 plants, suggesting that infection of a host plant by one genotype can exclude infections by others. In addition, the two Fov genotypes had different frequencies of occurrence on the two cotton cultivars. Genotype 11-A had a lower incidence on Sicot

189 while genotype 11-B showed the reverse. This is consistent with the correlation observed between the increasing incidence of 11-B and use of tolerant cotton cultivars in the field, suggesting that cotton may play an important role in causing temporal variation of Fov populations.

37.165 ISOLATION OF SPORE GERMINATION INHIBITORS FROM *PESTALOTIOPSIS NEGLECTA*. **K. Watanabe, M. Natsume, H. Kitahara, J. Ota, Y. Okazaki, T. Otuki, M. Kinoshita, T. Teraoka and T. Hibi.** *Faculty of Agriculture, Tamagawa University, Machida, Tokyo 194-8610, Japan. Email: wkyoko@agr.tamagawa.ac.jp*

Fungal growth is often controlled by its own metabolic products. In *Pestalotiopsis neglecta*, a necrotrophic fungus, the mucilaginous spore matrix is formed by accumulating metabolic products with functions such as self-inhibition of germination, antagonism toward Coelomycetes, and adhesion to substrates (Watanabe *et al.*, 2000a, b). Isolation and characterization of the self-inhibitors could be useful in developing novel control strategies based on the host-fungus interaction. In order to isolate the self-inhibitors, *P. neglecta* (TACP99M111) was cultured on a barley medium (150 ml flask containing 10 g of barley seeds and 10 ml of YT solution (5 g yeast, 12 g tryptone, 2.5 g NaCl/l)) at 25 ^{o}C under fluorescent lights. The mucilaginous spore matrix from one-week cultures was mixed with 10% MeOH, and centrifuged at 3000 rpm for 10 min. The water phase was then mixed with an equal volume of ethyl acetate, and the ethyl acetate fraction was evaporated to dryness (yield; 29.6 mg/flask). The residue was then purified using a series of column chromatography steps as follows: 2 steps of SiO_2-column chromatography (eluent: An-Bz solvent and $CHCl_3$ solvent), 2 steps of C18-column chromatography (eluent: AcN-H_2O solvent and MeOH-H_2O solvent), and C18-column-HPLC (eluent: AcN-solvent). The analyses of chemical structure and activity of the final purified specimen are in progress.

37.166 ALR1, RELATED TO *ARABIDOPSIS* RESISTANCE TO INFECTION BY *ALTERNARIA PORRI*. **Q.Y. Weng, J.H. Xing, Y.C. Liu and J.G. Dong.** *Mycotoxin Laboratory, Agricultural University of Hebei, Baoding 071001, P.R. China. Email: wengqiaoyun@126.com*

The molecular and cellular mechanisms involved in plant resistance to *Alternaria porri* and their genetic control are poorly understood. When inoculated with *A. porri*, transgenic NahG and WS-2 ecotype showed resistance, while Shakh-dara ecotype was susceptible. Using resistant and susceptible *Arabidopsis* inoculated with *A. porri* at different times, differentially expressed genes were studied using differential display reverse transcription-PCR (DDRT-PCR). Comparison of uninoculated with inoculated plants revealed many differentially expressed gene fragments, related to the resistance of *Arabidopsis* to *A. porri* infection. Cloning and sequencing of positive fragments indicated that genes related to respiration, cytoskeleton assembly, control protein and proteolysis were induced. The 5' sequence of one fragment, obtained by the method of rapid amplification of cDNA ends (RACE), identified it (99%) with *Arabidopsis* gene ATCG01180.1, a chloroplast-encoded 23S ribosomal RNA involved in translation. This gene was named *ALR1* (*Alternaria* resistance 1). Our result suggests that *ALR1* may play a role in *Arabidopsis* resistance against *Alternaria porri* infection.

37.167 SECONDARY METABOLITES WITH ANTIFUNGAL ACTIVITY AGAINST *GANODERMA PHILIPPII* FROM *ACACIA MANGIUM* AND *ACACIA AURICULIFORMIS*. **S.M. Widyastuti, S. Wahyuono, Yuniarti and T. Satriadi.** *Faculty of Foresty, Gadjah Mada University, Yogyakarta, 55281, Indonesia. Email: smwidyastuti@yahoo.com*

Ganoderma philippii causes red root rot, one of major diseases of *Acacia* plantations in Asian countries. The objective of this study was to isolate and identify secondary metabolites of *A. mangium* and *A. auriculiformis* having antifungal activity against this fungus *in vitro*. This study is a part of initial attempts to identify resistance factors of these *Acacia* species to *G. philippii*. Healthy and infected tissues of sapwood and heartwood from *A. mangium* or *A. auriculiformis* were separately macerated with n-hexane followed by methanol to give n-hexane and methanol extracts respectively upon solvent evaporation. Antifungal activity of these extracts was tested on germinated spores of *Fusarium* sp. Crude (methanol/n-hexane) extracts of infected heart wood of *A. mangium* and *A. auriculiformis* showed 71% and 64% inhibition at a concentration of 500 mg ml^{-1}. Upon bioactive guided isolation, an active substance was obtained and identified as a phenolic compound. Antifungal activity was tested on *G. philippii* using a modified cup plate method. A fraction from *A. mangium* and from *A. auriculiformis* inhibited *G. philippii* hyphal growth at a minimum concentration of 500 mg ml^{-1}. The selected fraction of *A. mangium* was identified partially as a glycoside of an aromatic compound, whereas the active fraction from *A. auriculiformis* appeared as to be a mixture of 4-hydroxy-benzaldehide (3%), p-propylphenol (83%) and 1,2-benzenedicarboxylic acid (14%).

37.168 CLONING AND ANALYSIS OF TOMATO PROMOTERS INDUCED BY *GLOBODERA ROSTOCHIENSIS*. **A. Wiśniewska, J. Dąbrowska, E. Łuniewska, A. Wilkowska, K. Woroniecka, J. Matusiak and M. Filipecki.** *Department of Plant Physiology, Warsaw University of Life Sciences, Nowoursynowska 166, 02-787 Warsaw, Poland. Email: anita_wisniewska@sggw.pl*

Plant-parasitic cyst nematodes are very important pests of many crops, causing substantial agricultural damage. Reproductive success of nematodes depends on induction and development of a feeding site (syncytium) composed of modified plant cells. In previous research, more than 300 genes from tomato were found to be induced during *G. rostochiensis* migration and syncytium development, and the expression levels and functions of some of them were analysed. To characterize the regulatory mechanisms controlling transcription of the chosen genes we isolated promoter regions. Seven promoter sequences were cloned by inverted PCR: pPUTKIN, pFLV, pGTPBP, pP450, pDFR, pPMEI and pEXPA5. Analysis using the PLACE program showed that these promoters contain some putative cis-acting elements related to pathogen response: WRKY71Os, WBBoxPcWRKY1, WBoxAtNPR1, *W*BoxHvISO1, WBoxNtERF3 and others. Constructs for plant transformation containing the GUS (uidA) reporter gene under control of isolated promoters were made and introduced to tomato and potato by *Agrobacterium rhizogenes*, inducing transgenic hairy roots. Hairy root culture is an easy and reproducible system for studying the interaction of plants with root pathogens. GUS activity in hairy roots was examined for each promoter. We observed different expression profiles for each promoter before and after nematode infection and also after wounding and hormone treatments. This work was supported by the Polish Ministry of Science and Higher Education (grant no. 2P06A03230).

37.169 YEAST-TWO-HYBRID SCREENING OF INTERACTIONS BETWEEN *HIBISCUS CHLOROTIC RINGSPOT VIRUS* CP AND PROTEINS IN KENAF HOST PLANTS. Z. Xin and W.S. Man. *Science Drive 4, S1A, 06-05 , Department of Biological Sciences, National University of Singapore, Singapore. Email: g0600436@nus.edu.sg*

Hibiscus chlorotic ringspot virus (HCRSV) infection affects hibiscus cultivars and species used in the wood pulp industry globally. Numerous studies focus on the properties of the virus but not on the response of the host plants to infection. This project examines the proteins which are produced by a host plant, kenaf, in response to HCRSV infection. We aimed to screen for proteins in kenaf which can interact with the HCRSV coat protein (CP) through the yeast-two-hybrid assay. Such proteins are most likely to be involved in the mechanisms of plant virus infection. The positive protein-protein interactions were examined further. One of the cDNAs coding for an interacting protein in kenaf was sequenced and the protein was inferred to be sulfite oxidase. The initial steps in a larger screening process to identify specific proteins in kenaf which interact with the HCRSV CP are completed. We are now trying to confirm the protein-protein interactions by other methods, such as co-localization and pull down assay. This should contribute towards better understanding the mechanisms of plant viral infection. From the results of bioinformatics programs, it was inferred that the cDNA may code for sulfite oxidase in kenaf, and this protein was able to interact with the HCRSV CP. Sulfite oxidase in plants may be important in detoxification of excess sulfite and consequently in the protection of plant cells against sulfite damage or sulfitolysis (Hansch and Mendel, 2005).

37.170 INTERACTION BETWEEN *TOBACCO MOSAIC VIRUS* RNA-DEPENDENT RNA POLYMERASE AND HOST TRANSLATION ELONGATION FACTOR 1A. Y. Yamaji, S. Namba and T. Hibi. *Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan. Email: ayyamaji@mail.ecc.u-tokyo.ac.jp*

RNA viruses have a quite limited number of genes, which encode proteins responsible for replication, and other infection processes. Therefore, RNA viruses also require host proteins for their efficient infection throughout the host. Evidence has been obtained that host eukaryotic translation elongation factor 1A (eEF1A) or its counterpart in bacteria (EF-Tu) participates in the replication of RNA viruses infecting animals, plants and bacteria (Lai, 1998). Both the interactions between eEF1A (or EF-Tu) and the viral RNA-dependent RNA polymerase (RdRp) and the interactions between eEF1A (or EF-Tu) and the viral RNA have been reported. This interaction can be a model to study the molecular mechanism of host-virus interactions. eEF1A from wheat germ and *Nicotiana benthamiana* interacted with the 3' untranslated region (3'-UTR) of the *Tobacco mosaic virus* (TMV) RNA genome, indicating that eEF1A is also involved in the replication of TMV (Zeenko et al., 2002). We further investigated the interaction between eEF1A and replication components of TMV. We demonstrated the interaction between eEF1A and TMV RdRp in vivo by immunoprecipitation. The tobacco eEF1A interacted not only with the 3'-UTR of TMV RNA but also directly with the RdRp without mediation by the 3'-UTR. The methyltransferase domain of TMV RdRp was responsible for the interaction with eEF1A. Our data together with previous reports indicate that RNA viruses utilize eEF1A in various ways to support their infections.

37.171 THE *L1A* GENE CONFERS TEMPERATURE-INSENSITIVE TOBAMOVIRUS RESISTANCE IN *CAPSICUM* PLANTS. Y. Yamane, K. Matsumoto, H. Sawada, S. Takeuchi, H. Hamada, K. Kobayashi, T. Okuno, K. Suzuki, A. Kiba and Y. Hikichi. *Laboratory of Plant Pathology & Biotechnology, Kochi University, Nankoku, Kochi 783-8502, Japan. Email: yamanenne626@yahoo.co.jp*

Tobamovirus resistance in *Capsicum* plants is conferred by four allelic genes at loci *L+*, *L1*, *L2*, *L3* and *L4*, which provide increased protection against different kinds of tobamovirus: pathotypes P0, P1, P1,2 and P1,2,3. This *L*-mediated tobamovirus resistance is temperature sensitive. However, the *L1a* gene, a newly identified tobamovirus resistance gene that maps to the *L* locus, confers temperature-insensitive resistance against the P0 pathotype of tobamovirus. To identify the viral elicitor that activates the *L1a* gene-mediated resistance, several chimeric viral genomes were constructed between *Tobacco mosaic virus*–L (P0 pathotype), *Tobacco mild green mottle virus*-J (TMGMV-J, P0 pathotype), *Paprika mild mottle virus*-J (PaMMV-J, P1 pathotype) and *Pepper mild mottle virus*-J (P1,2 pathotype). Infection patterns of these chimeric viruses in L1a-harboring plants revealed that the *L1a* gene-mediated resistance was not only temperature-insensitively activated by the CP of P0 but also temperature-sensitively activated by the CP of P1. Infection patterns of these chimeric viruses involving intramolecular recombination of the CP gene between TMGMV-J and PaMMV-J in *L1a*-harboring plants showed that Ala122Ser and Ser126Cys amino acid substitutions of TMGMV-J CP resulted in temperature-sensitive resistance. Three-dimensional structural analysis using Swiss-PdbViewer showed that these amino acids are located on the surface of the CP. These results suggest that difference between the surface features of the P0 and P1 tobamovirus CPs may be involved in the temperature sensitivity of *L1a* gene-mediated resistance.

37.172 THE ROLE OF PRIMARY GERM TUBES IN THE LIFE CYCLE OF *BLUMERIA GRAMINIS*: THE PRIMARY GERM TUBE IS RESPONSIBLE FOR THE SUPPRESSION OF RESISTANCE INDUCTION OF A HOST PLANT CELL. N. Yamaoka, T. Ohta, N. Danno, S. Taniguchi, I. Matsumoto and M. Nishiguchi. *Laboratory of Plant Pathology, Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan. Email: yamaokan@agr.ehime-u.ac.jp*

When the conidia of *Blumeria graminis* f.sp. *hordei* (Bgh) are inoculated on barley coleoptile cells they produce short germ tubes called primary germ tubes (PGTs). We evaluated the role of a PGT for induced accessibility of the host cell under the germ tube. When an appressorium (APP) penetrates the same cell on which a PGT is present, penetration efficiency (PE) is significantly higher than when an APP penetrates the cell adjacent to that on which a PGT is present. When an APP had penetrated the laterally adjacent cell on which a PGT was present, we killed the cell under the PGT with a microneedle and then investigated the PE at the cell adjacent to the killed cell. As a control we killed the longitudinally adjacent cell to the one on which a PGT was present and investigated the PE of the laterally adjacent cell. The results showed that the PE of the former was significantly lower than that of the latter. This suggests that some factor of accessibility might transfer from a cell on which PGT is present to a laterally adjacent cell. Moreover, when a Bgh germling was removed 6 h after inoculation and another germling was transferred to the same cell, the PE was significantly higher than that of control. As a control, a Bgh germling was transferred to a cell on which no germling was present. These results suggest that the presence of a PGT induces accessibility (suppresses resistance) of a host cell when Bgh penetrates.

37.173 N-GLYCANS ARE ESSENTIAL FOR THE ENZYMATIC ACTIVITY AND THERMOSTABILITY OF PPPG1, AN ENDOPOLYGALACTURONASE FROM *PHYTOPHTHORA PARASITICA*. **H.-Z. Yan and R.-F. Liou.** *Department of Plant Pathology and Microbiology, National Taiwan University, Taipei 106, ROC. Email: rfliou@ntu.edu.tw*

Our previous study suggests the importance of *pppg1* in the process of plant infection by the oomycete pathogen *Phytophthora parasitica*. It encodes an endopolygalacturonase with 11 putative N-glycosylation sites (N1-N11). Expression in the yeast *Pichia pastoris* followed by in-gel activity assay indicated that the recombinant protein of *pppg1* is differentially glycosylated, with a molecular mass ranging from 75 to 200 kDa. To determine the functional significance of N-glycosylation on the enzymatic activity of PPPG1, a site-directed mutagenesis strategy was employed to generate a series of single and multiple mutations at the N-glycosylation sites. Mutated proteins were expressed in *P. pastoris*, and their biochemical characteristics were analyzed. The results indicated that mutations in each of the N-glycosylation sites, especially N2 and N8, resulted in a significant loss of endoPG activity when compared to that of the wild-type protein, suggesting the importance of N-glycosylation on the structure and thereby the enzymatic function of PPPG1. Besides, thermal treatments reduced the enzymatic activity of each mutant to various extents. Dramatic changes were detected in proteins harboring a single mutation in N4, N6, N7, N8, or N9. It is thus suggested that N-glycosylations also play a key role in preventing the protein from the deleterious effects of heat. Moreover, mutations were generated in 6 amino acid residues which are highly conserved among endoPGs. Analyses of the biochemical properties of these mutants indicated that residues Asp_{209}, Asp_{230}, Asp_{231}, His_{252}, and Lys_{290} play important roles in the function of PPPG1.

37.174 THE GENETIC STRUCTURE OF *MAGNAPORTHE GRISEA* IN RELATION TO DISTRIBUTION OF RICE VARIETIES. **X. Yang, J. Yuan, H. He and Y. Wang.** *Institute of Plant Protection, Guizhou Academy of Agricultural Sciences, Guiyang 50006, P. R. China. Email: yuanjgz@yahoo.com.cn*

Two hundred isolates of the rice blast fungus collected from the Guizhou Province of China were analyzed for genetic structure by using the Rep-PCR fingerprinting technique. The result showed that there were 87 different haplotypes, and 17 genetic lineages at 0.83 similar linkage distance. It was remarkable that the rice blast population genetic lineages was related to distribution of rice varieties in different areas of Guizhou. In some areas like Zunyi, Qiandongnan, Qianxinan, where the most popular varieties planted by farmers are hybrid, genetic lineages of *Magnaporthe grisea* were relatively simple and most of them were predominant lineages. In those where traditional varieties and *japonica* rices are popularly planted by farmers, as in Liupanshui and Bijie, genetic lineages of *M. grisea* were spread around and the proportion of different lineages had little difference. No predominant or hypo-predominant lineages appeared. All this showed that the *M. grisea* population in Guizhou province displayed abundant genetic diversity. Therefore, hybrid rices planted on a large scale would make the genetic background of *M. grisea* population become consistent and would risk a rice blast disaster.

37.175 ANALYSIS OF *PAPAYA RINGSPOT VIRUS* PATHOGENESIS. **Y.-K. Yap and S. Panyim.** *Institute of Molecular Biology and Genetics, Mahidol University, Salaya, Nakhon Pathom 73170, Thailand. Email: fryky@mahidol.ac.th*

Papaya Ringspot Virus (PRSV), a member of the *Potyviridae* family, consists of a (+) RNA genome of approximately 10 kb. The genome is first translated into a single polypeptide which is then cleaved by 3 virus-encoded proteases to give 10 gene products. We have constructed a PRSV cDNA infectious clone which is able to infect zucchini and pumpkin plants through particle gun bombardment. The symptoms on both hosts are indistinguishable from those produced by PRSV virions. In this study, we investigated the role of the Hc-Pro gene in PRSV pathogenesis (infectivity and symptom severity). Hc-Pro, a multifunctional protein, has been proposed to play a role in aphid transmission, viral movement, genome amplification and suppression of host anti-viral post-transcriptional gene silencing. Various Hc-Pro regions of the full-length cDNA clone will be deleted in individual construct and these Hc-Pro mutants will be introduced into zucchini plants. Plants will be evaluated for symptom development and RT-PCR will be used to determine the presence of the PRSV genome and at the same time to semi-quantify the amount of PRSV in the plant tissues. We have tested the complete Hc-Pro deleted cDNA clone, and found it not infectious in zucchini. Therefore, Hc-Pro is indispensable for PRSV infection in zucchini. Further investigation to determine functional domains essential for zucchini infection is underway.

37.176 DETERMINATION OF GENES FOR RESISTANCE TO LEAF RUST IN VARIOUS WHEAT CULTIVARS. **A. Zhemchuzhina, N. Kurkova and H. Bockelman.** *All-Russia Research Institute of Phytopathology, Moscow, Russia. Email: nsgchb@ars-grin.gov*

We identified genes for resistance to leaf rust in 80 wheat cultivars received from Germplasm Resources Information Network (GRIN, USA). Cultivars were characterized by resistance in field tests in an infectious nursery. Lr-genes were identificated by comparison of monogenic lines and cultivar responses on separate pathogen test isolates. Twenty three cultivars were resistant, and 13 were susceptible to 10 pathogen test isolates. Resistance genes (*Lr 2a*, *Lr 3ka*, *Lr 10*, *Lr 14b*, *Lr 15*, *Lr 16*, *Lr 21*, *Lr 26*, *Lr 27+31*, *Lr 28*, *Lr 32*, *Lr 39*, *Lr 39*, *Lr 40*, *Lr 46*) or their combinations with known and unknown genes were postulated in other cultivars. One group of cultivars had one known and one unknown *Lr*-genes. The other group of cultivars had one known and two or three unknown resistance genes. In some cultivars a combination of two known *Lr*-genes was found, and in others a combination of two known and one unknown Lr-genes was found. Yugoslavian samples with genes *Lr* 28, *Lr* 39 and the American sample with gene *Lr* 46 represent the greatest value, as complementary virulence genes are seldom encountered in populations of leaf rust in Russia.

INDUCED RESISTANCE

32.1 SYSTEMIC INDUCED RESISTANCE IN AN INSECT-FUNGUS-PINE TRIPARTITE INTERACTION OVER VARYING SOIL FERTILITY. **A. Eyles, R. Chorbadjian, C. Wallis, R. Hansen, D. Cipollini, B. Mcspadden Gardener, K. Riedl, D. Herms and P. Bonello.** *Cooperative Research Centre for Forestry, TIAR/University of Tasmania, Private Bag 12, Hobart TAS 7001, Australia. Email: aeyles-ts@csiro.au*

Cross-effects of systemic induced resistance were examined in

a *Pinus nigra* (Austrian pine) – *Diplodia pinea* (a fungal pathogen) – *Neodiprion sertifer* (European pine sawfly) tripartite model system over varying soil fertility. Independent of soil fertility, larval feeding induced systemic resistance to subsequent fungal infection in 2006 but not in 2005. A negative systemic effect of fungal infection on larval growth was detected in 2005 only. Larval survival was affected by a significant interaction between induction treatment and fertility levels in 2006. Consistent induction of systemic resistance by fungal infection against the same fungus was found in both years, even under varying soil fertility. Concomitant studies of host secondary metabolism revealed that the induced resistance phenotypes were characterized by specific changes in the shikimic and mevalonic acid metabolic pathways.

32.2 INDUCED RHIZOCTONIA WILT RESISTANCE IN COTTON SEEDLINGS BY *TRICHODERMA* SPP. M.A. Al-Hamdany, F.R. Hameed and F.A. Fattah. *Agricultural Researches Office, Ministry of Science and Technology, P.O. Box 765, Baghdad, Iraq. Email: ma_alhamdany@yahoo.com*

Studies were carried out on 15 isolates of *Trichoderma* spp. TH, TV, T1, T2, T3, T8, T11, T160, T162, T191, T194, T195, T197, T211, and T212 aimed to detect the mechanism of resistance induction through raising peroxidase activity in cotton seedlings (cultivar Coker 310), along with the role of this mechanism in biocontrol of *Rhizoctonia solani*, the causal agent of disease in cotton seedlings. Studies also included the identification of any substance or substances produced by *Trichoderma* isolates which induced resistance in cotton seedlings. The results indicated that T211, TV, T160, T191, T11, T194, and T195 tended to induce disease resistance in cotton seedlings, reflected in a significant increase of peroxidase activity in the seedlings following treatment of the seed seed with these isolates. Disease incidence of cotton seedlings in the greenhouse was significantly reduced by certain isolates of *Trichoderma* spp. Results of gas chromatography of culture atmospheres of many isolates of *Trichoderma* spp. indicated that isolates TV, T160, and T194 produced ethylene while no ethylene was produced by isolates T2 and T3. However, in contrast to T2 and T3, isolates TV, T160, and T194 caused significant increase in peroxidase activity.

32.3 INFLUENCE OF SILICON ON *PYTHIUM*-INDUCED DAMPING-OFF DISEASE OF CUCUMBER (*CUCUMIS SATIVUS*). A.M. Al-Sa'di, A. Drenth, M.L. Deadman, F.A. Al-Said, I. Khan and E.A.B. Aitken. *P.O. Box 34, AlKhoud 123, Oman. Email: alsadi@squ.edu.om*

Laboratory and greenhouse experiments were conducted to study the influence of supplementing cucumber seedlings with silicon on improvement of defence responses to *Pythium*-induced damping-off disease. Supplementing cucumber seedlings with different concentrations of silicon (0-2500ppm) for 7 or 14 days before inoculation with *P. aphanidermatum* isolates differing in the level of aggressiveness did not result in a significant reduction in seedling mortality at 20 and 25°C. In addition, greenhouse experiments over two seasons showed no improvement of damping-off control using silicon. This could be related to the rapid infection by *Pythium* of cucumber seedlings in relation to a slower rate of induction of defence responses using silicon.

32.4* NATURAL SUBSTANCES OF PLANT ORIGIN AS INDUCERS OF SYSTEMIC RESISTANCE FOR THE MANAGEMENT OF VIRAL DISEASES OF CROPS. L.P. Awasthi and H.N. Verma. *Department of Plant Pathology,Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad-224 229 (U.P.), India. Email: lpawasthi@sify.com*

A large number of phytopathogenic viruses infect a wide range of crops and cause great economic losses every year throughout the world. Insecticides may kill the insect vectors and thus may prevent the spread of vector-borne viruses but they are very costly and after indiscriminate use for long times they leave residues in crop produce, soil, water and the environment. Botanicals (*Boerhaavia diffusa* root extract and/or *Clerodendrum aculeatum* leaf extract) as antiviral agents could prevent the infection and spread of many viruses. The glycoprotein isolated from *B. diffusa* or a protein from *C. aculeatum* induced systemic resistance in many hosts against a number of plant pathogens viruses. The resistance induced by *B. diffusa* and/or *C. aculeatum* is of systemic nature. The antiviral substances (proteins) inducing systemic resistance in hosts following the treatment may move from the site of synthesis and be translocated through whole of the plant and thus protect the plant against virus infection. The systemic resistance induced by *B. diffusa*/*C. aculeatum* was reversed by the application of actinomycin-D (an inhibitor of DNA-dependent RNA synthesis). Further, we have demonstrated that infection by tomato leaf curl, chilli mosaic, cucumber mosaic, begomoviruses and papaya ringspot viruses may be prevented by seed treatment followed by nursery treatments and sprays in the field.

32.5 BION® OR REZIST® BUT NOT MILSANA® CONTROLS POWDERY MILDEW OF CUCURBITS AND INDUCES CHITINASE AND PEROXIDASE AND PHENOLIC ACCUMULATION. A.I. Bokshi, R.M. McConchie and J. Jobling. *Faculty of Agriculture, Food and Natural Resources, University of Sydney, NSW 2006, Australia. Email: a.bokshi@usyd.edu.au*

Several glasshouse and field experiments were conducted with the claimed elicitors Bion®, Rezist® and Milsana® for the control of powdery mildew of cucurbits through induction of systemic resistance. Application of each agent diminished the disease. Treatment with Bion® provided a more consistent and longer lasting protection than Milsana® or Rezist®. Protection by Milsana® or Rezist® was variable in different cucurbits and during different seasons. Increased activities of chitinase and peroxidase were induced by Bion® or Rezist® in glasshouse and field crops of cucumber and zucchini but not by Milsana®. The onset and the level of systemic protection against powdery mildew were correlated with elevated enzyme activities due to treatment with Bion® or Rezist®. Phenolic compounds accumulated systemically at infection sites from Bion® or Rezist® application but not Milsana®. Repeated sprays of Bion® or Rezist® caused a longer lasting systemic induction of increased enzyme activities and a more durable protection against powdery mildew than a single spray. Although no systemic induction of resistance was caused by Milsana®, spore germination was inhibited on the treated leaves. Some protection against powdery mildew by Milsana® may be caused by direct effects on spores on the leaf surfaces thus minimising establishment of the pathogen. Further studies are in progress on the mode of action of Milsana® in control of powdery mildew of cucurbits.

32.6* SEEING THE LIGHT: UV-GENERATED HOST DNA DAMAGE INDUCES BIOTROPHIC RESISTANCE IN *ARABIDOPSIS*. **D.M. Cahill, P. Dando, D. Grice, S. Grimley, P. Schenk, S. Thomas-Hall and B. Kunz.** *School of Life and Environmental Sciences, Deakin University, Waurn Ponds campus at Geelong, 3217 VIC, Australia. Email: david.cahill@deakin.edu.au*

Ultraviolet (UV) light is a ubiquitous component of the environment to which all plants are exposed. Light in the UV range damages cellular components, proteins and DNA but plants have shielding and repair mechanisms that enable them to tolerate these effects. UV-C treatment of *Arabidopsis thaliana* induced resistance to virulent isolates of the biotrophic pathogen *Hyaloperonospora parasitica*, and experiments using repair-deficient mutants indicated that UV-induced DNA photoproducts are involved. To further examine the role of DNA damage we analysed the effect of mutations in nucleotide excision repair, photoreactivation of cyclobutane pyrimidine dimers, flavonoid production and components of the RAR1/SGT1 R protein-linked signalling complex on the resistance of *Arabidopsis* to the pathogen, with or without pre-inoculation treatment with UV-C. In wild-type plants UV-C-induced resistance was dose and time dependent and results suggested that in addition to UV photoproducts, an accumulation of endogenous oxidative DNA damage may also trigger resistance to the pathogen. The responses observed for the biotroph differed from that found for a necrotroph *Alternaria brassicicola*, where there was no increase in resistance following UV treatment. This suggests that specific resistance signalling pathways are activated by UV exposure. We examined UV-induced resistance using microscopy, measurement of the activity of a suite of defence-related enzymes, and whole-genome gene expression analysis. UV-induced resistance appears to be systemic and we are currently testing other DNA repair-deficient and signalling mutants and quantifying UV-C-induced DNA damage to assess further the relationship between damage levels and the level of resistance.

32.7 INDUCTION OF RESISTANCE WITH SYNTHETIC SIGNAL MOLECULES AGAINST BROWN LEAF SPOT OF RICE. **S.S. Chahal, A. Satija and P.P.S. Pannu.** *43-H, BRS Nagar, Ludhiana-141012, Department of Plant Pathology, Punjab Agricultural University, Ludhiana 141 004, India. Email: chahalsspau@yahoo.com*

The effect of chemical compounds known as synthetic signal molecules (SSMs) has been studied for inducing resistance in rice plants against brown leaf spot caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker. All of the five SSMs tested, viz. Bion 50 WG, β-amino-n-butyric acid, calcium chloride, methyl salicylate and salicylic acid, with their different concentrations, significantly reduced the disease as compared to controls. Minimum disease severity (4.2%) was observed on plants treated with 5 ppm calcium chloride, whereas maximum severity (24.3%) occurred with 1 ppm methyl salicylate treatment Bion 50 WG, a commercial product at 100 and 200 ppm, and salicylic acid at 3 ppm concentration reduced the disease to approximately 1/3 level, compared to the control. There were significant differences in efficacy of different methods applied for different treatments. Foliar spray combined with seed treatment provided maximum disease control. Individually, however, the effect of calcium chloride at 25 and 5 ppm and β-amino-n-butyric acid 75 ppm was almost the same with foliar spray and seed treatment plus foliar spray. Calcium chloride treatment at 50 ppm provided maximum induction of resistance, as high as 93% of the disease control. Methyl salicylate at 1 ppm applied as a root dip was least effective of all the treatments. The results provide an evidence that the SSMs can induce resistance to brown leaf spot in rice, though their effect depends upon the concentration, method of application and plant age.

32.8 PLANT EXPRESSION OF SINGLE-CHAIN ANTIBODIES AGAINST AN OLIVE TREE VIRUS. **A. Custodio, C. Novo, L. Torrance and A. Ziegler.** *Unit of Protein and Monoclonal Antibodies Technologies, National Institute of Engineering Technology and Innovation, Estrada do Paco do Lumiar, 1649-038 Lisboa, Portugal. Email: ana.custodio@ineti.pt*

Viral diseases are a major concern in agriculture. Olive (*Olea europaea* L.) is a common and widespread Mediterranean crop, with a high economic importance in Portugal. Its demonstrated susceptibility to viral infection justifies research leading to accurate virus detection and improved strategies for control. Immunomodulation with recombinant antibodies is being used as way to interfere with pathogen infectivity and has great potential to prevent viral infections. Different rABs were successfully expressed in transgenic plants, and methods were developed for their accumulation in different plant organs and cell compartments. We are trying to achieve stable cytoplasmic expression of the previously selected phage display-derived scFv able to recognize OMMV, for inducing resistance against this pathogen.

32.9 BACTERIAL TRAITS AND HOST DEFENCE RESPONSES UNDERPINNING RHIZOBACTERIA-INDUCED SYSTEMIC RESISTANCE IN RICE. **D. De Vleesschauwer and M. Höfte.** *Laboratory of Phytopathology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium. Email: david.devleesschauwer@ugent.be*

Selected strains of plant growth-promoting rhizobacteria can reduce disease in above-ground plant parts through the induction of defence commonly referred to as induced systemic resistance (ISR). Compared to the vast body of information on induced resistance in dicots, our understanding of biologically induced defence responses in monocot crops is still rudimentary. Aiming at developing a model system for studying induced resistance in monocots, we screened several rhizobacterial strains for their ability to elicit ISR against various rice pathogens. Here, we report on ISR triggered by *Pseudomonas fluorescens* strain WCS374r against *Magnaporthe oryzae*. Bioassays with various transgenic and mutant rice lines coupled to PR transcript profiling revealed that WCS374r-mediated ISR is independent of salicylic acid accumulation and PR gene expression but is responsive to ethylene. Extensive bacterial mutant analysis identified the siderophore pseudobactin as a crucial determinant of WCS374r-elicited ISR. Root application of pure pseudobactin was shown to prime rice for potentiated activation of a pathogen-inducible multifaceted cellular defence response comprising accumulation of phenolics at appressorial interaction sites, the development of infection peg-embedding tubules, and a timely oxidative burst driving cell wall reinforcements and protein cross-linking. By contrast, purified pseudobactins from other ISR-triggering strains such as *P. aeruginosa* 7NSK2 did not increase blast resistance. Several lines of evidence suggest that the ISR-triggering potential of specific pseudobactins is related to their ability to deprive rice seedlings of iron, leading to intracellular iron depletion. The putative involvement of the methionine pathway in pseudobactin-induced resistance will be discussed.

32.10 INDUCED RESISTANCE IN TOMATO PLANTS AGAINST THE NECROTROPHIC PATHOGEN *ALTERNARIA ALTERNATA*. **M. Egusa, H. Otani, T. Tsuge and M. Kodama.** *Laboratory of Plant Pathology, Tottori University, Tottori, Japan. Email: mk@muses.tottori-u.ac.jp*

The effectiveness of induced resistance in tomato plants against *A. alternata* tomato pathotype (host-specific AAL-toxin producer) and the involvement of salicylic acid (SA) and jasmonic acid (JA) signaling pathways in the induction processes were assessed in this study. The susceptible tomato cultivar Aichi-first (AF) was inoculated with non-pathogenic *A. alternata* (O-94) or treated with the elicitor from O-94. After inoculation or treatment, AF leaves were inoculated with spores of HST producing *A. alternata* tomato pathotype (As-27). Lesion development and infection hypha formation by As-27 were clearly decreased on leaves previously inoculated with O-94 spores or treated with the elicitor. When AF plants were treated with SA or methyl JA (MeJA) prior to inoculation with As-27 spores, typical lesions were formed on the inoculated leaves. Total RNAs were extracted from tomato leaves after the several treatments. Expression of PR-protein genes was analyzed by real-time PCR. Genes for PR-1 and proteinase inhibitor, which are marker genes for SA and JA signalling pathways, respectively, were expressed in the leaves treated with SA or MeJA. Furthermore, O-94 could not infect leaves of SA- and JA-related mutants, NahG and def1. These results indicate that SA/JA/PR protein systems are not involved in the non-pathogen/elicitor-induced resistance in tomato against the necrotrophic and toxigenic pathogen, *A. alternata*. Specific genes induced in tomato by infection with non-pathogenic *A. alternata* were identified using a subtractive hybridization procedure. The expression of candidate genes for induced resistance in tomato is being invstigated in detail by real-time PCR.

32.11 DIFFERENTIAL RESPONSES IN CANOLA CULTIVARS TO VARIOUS PATHOGENICITY GROUPS OF *LEPTOSPHAERIA MACULANS* INVOLVE THE INDUCTION OF SPECIFIC HYDROXYCINNAMIC ACIDS. **A. El Hadrami, D.W.G. Fernando and F. Daayf.** *University of Manitoba, Department of Plant Science, 222 Agriculture Building, Winnipeg, Manitoba, R3T 2N2, Canada. Email: elhadram@cc.umanitoba.ca*

Phoma stem canker (blackleg) caused by the ascomycete *Leptosphaeria maculans* (anamorph: *Phoma lingam*) represents the most threatening disease to Brassicaceae including canola. Controlling this disease often relies on the use of resistant cultivars along with fungicide application and crop rotation. *L. maculans* displays differential interactions with canola cultivars ranging from HR-like reaction to partial resistance and susceptibility. Five pathogenicity groups (PG1, 2, 3, 4 and T) have been characterized. PG1 isolates are hypo-aggressive and emerge only late in the season while plants are senescent. Other PGs can be found throughout the season and mainly include highly aggressive isolates. PG2 represents the most prevalent group in Western Canada in the last two decades. Our investigation examines the possibility of using induced resistance (PG1 or its components) to control blackleg in canola. Constitutive and induced phenolics in canola leaves interacting with either hypo- (PG1) or hyper-aggressive isolates (other PGs) were analyzed. Both qualitative and quantitative differences in hydroxycinnamic acids were observed among treatments in response to either PG1 or other PGs. Our results suggest that these compounds get incorporated into the lignin matrix around the infection site as well as into phenolamide phytoalexins. The speed by which the accumulation occurs and the accumulated amounts may explain the differential responses of the cultivars.

32.12 EFFECTIVE BIOCONTROL OF *VERTICILLIUM DAHLIAE* IN POTATO PLANTS CORRELATES WITH HIGH LEVELS OF INDUCED RUTIN. **A. El Hadrami, A.K. Uppal, L.R. Adam and F. Daayf.** *University of Manitoba, Department of Plant Science, 222 Agriculture Building, Winnipeg, Manitoba, R3T 2N2, Canada. Email: elhadram@cc.umanitoba.ca*

Verticillium wilt, caused by *Verticillium dahliae*, is one of the main constraints to potato cultivation worldwide. Over the years, progress in disease control strategies has shown that biocontrol may be an eco-friendly alternative in controlling this disease. Many biocontrol agents reduce diseases through induction of host defense mechanisms against invading pathogens. In an earlier study, we selected a set of bacteria and plant extracts that are able to reduce potato *Verticillium* wilt. In the current study, we investigated the mechanisms by which these biocontrols were successful. Our analyses revealed a high induction of rutin synthesis/accumulation in response to the effective treatments, but treatments with low biocontrol ability induced low synthesis/accumulation. Overall, increase in rutin content correlated with a decrease in disease under both controlled and field conditions. Complementary experiments showed that the induced rutin derivative was able to inhibit the growth of *V. dahliae* equally to a pure standard of rutin calibrated at ≥100 µg/ml. Lower than this threshold, the induced compound was ineffective against the pathogen, which was able to use it as a carbon source.

32.13 THE ROLE OF PHOSPHITE IN INDUCING RESISTANCE TO *PHYTOPHTHORA* SPECIES IN *ARABIDOPSIS THALIANA*. **L. Eshraghi, J. McComb, G.E.St. J. Hardy and P. O'Brien.** *Centre for Phytophthora Science and Management, School of Biological Science and Biotechnology, Murdoch University, Murdoch WA 6150, Australia. Email: l.eshraghi@murdoch.edu.au*

Application of phosphite (phosphoric acid) to plants induces resistance to infection by oomycete pathogens (*Phytophthora, Pythium, Bremia, Peronospora*). In this study, the mechanism(s) underlying the ability of phosphite to protect plants against infection by *Phytophthora brassicae* and *Phytophthora cinnamomi* was investigated using *Arabidopsis thaliana* as a model. Resistance was assessed by comparing indicators such as callose formation, hyphal length, and production of hydrogen peroxide. Induction of host defence gene expression was measured with quantitative PCR using PPR and EF1α as housekeeping genes. Application of phosphite to the plants resulted in a greater than 2.5 fold increase in expression of the PR1 gene. The results of analysing other defence genes will be presented.

32.14 THE EFFECTS OF VARIOUS CONCENTRATIONS OF SALICYLIC ACID ON RESISTANCE IN WHEAT SEEDLING ROOTS AGAINST THE TAKE-ALL FUNGUS, *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI*. **H.R. Etebarian, E. Sari, A. Roustaei and H. Aminian.** *Department of Plant Protection, Abourayhan Campus, University of Tehran, P.O. Box 11365/4117, Tehran, Iran. Email: etebar@chamran.ut.ac.ir*

This study evaluated the protective effects of salicylic acid (SA) application in induction of wheat defense mechanisms against *Gaeumannomyces graminis* var. *tritici* (Ggt) infection. The effects of 0.1, 0.2, 0.5 and 1 mM SA on the hyphal growth of Ggt were studied in vitro. Also, the effects of these concentrations applied as root drench on the susceptibility of wheat seedlings to Ggt were studied in the glasshouse. The activities of soluble

(SPOX) and ionically cell wall-bound (CWPOX) peroxidases and phenolic contents of the roots were also determined. The results indicated that SA concentrations more than 0.5 mM possessed direct activity against take-all fungus. The severity of take-all in 0.2 and 0.5 mM SA-treated roots was significantly less than in pathogen control roots without SA. The levels of SPOX and CWPOX were increased in the roots treated with 0.2 and 0.5 mM SA in the presence of pathogen challenge. In these treatments, we recorded maximum SPOX activity on day 4 and CWPOX activity on day 6 after pathogen challenge. Also, the wheat roots treated with these concentrations showed increased levels of phenolic compounds on day 2 after pathogen challenge. The maximum level of phenolic compounds was recorded on the 0.5 mM SA-treated roots. Isoform analysis of the SPOX revealed greater accumulation of the P1 (Rf =0.04), P2 (Rf=0.13) and P3 (Rf=0.27) isozymes in the 0.2 mM SA-treated wheat roots challenged with pathogen than in the other treatments. The results suggest that the inhibitory effect of SA on take-all may be related to its ability to enhance defense responses in the wheat roots, and that the effect is dose-dependent.

32.15 DEFENCE RESPONSES INDUCED IN TOBACCO CELLS BY *BACILLUS* LIPOPEPTIDES ACTING AS ELICITORS OF SYSTEMIC RESISTANCE IN PLANTS. G. Henry, E. Jourdan, P. Thonart and M. Ongena. *Unité de Bioindustries, Faculté des Sciences Agronomiques, B-5030 Gembloux, Belgium. Email: ongena.m@fsagx.ac.be*

The beneficial rhizobacterium *Bacillus subtilis* can stimulate plant growth and produce a broad range of antimicrobial compounds that may be involved in the biocontrol of diseases caused by microbial pathogens. Among them, cyclic lipopeptides from the surfactin and fengycin families have been recently shown to stimulate the plant immune system and initiate induced systemic resistance (ISR). These compounds thus constitute a novel class of elicitors of plant host resistance. However, nothing is known about the molecular interactions with plant cells that govern the induction of ISR. In this study on the interaction between surfactin and cultured tobacco cells, we highlighted early perturbations in the plant cell metabolism upon treatment with the lipopeptide. Surfactins induce a rapid and temporary alcalinization of the extracellular medium and a production of hydrogen peroxide (oxidative burst). Our results suggest that both phenomena are Ca^{2+} dependant. These responses, occurring within minutes of surfactin addition, are followed by the stimulation of typical defence enzymes (phenylalanine ammonialyase and lipoxygenase) and by the accumulation of phenolics deriving from secondary metabolism. The lipopeptides did not induce leakage or death of tobacco cells despite their detergent properties. These results suggest that these molecules may interact with plant cells without forming irreversible pores but in a way sufficient to induce some disturbance or transient channelling in the plasma membrane. This could in turn activate a cascade of molecular events leading to defensive responses.

32.16 A NEW EVALUATION METHOD FOR PLANT DEFENCE ACTIVATORS BASED ON POTENTIATION OF ELICITOR-RESPONSIVE PHOTON EMISSIONS (ERPE) IN PLANT CELLS. H. Iyozumi, H. Nukui and K. Kato. *Shizuoka Prefectural Research Institute for Agriculture and Forestry, 678-1, Iwata, Shizuoka 438-0803, Japan. Email: kimihiko1_kato@pref.shizuoka.lg.jp*

Organisms generate ultraweak photon emissions, so-called biophotons (a few photons/sec/cm^2) as a by-product of their metabolism. Plants, including cultured cells, transiently generate high levels of biophotons during the response to pathogen attack or elicitor treatment. In the case of rice and chitin elicitor, the elicitor-responsive photon emission (ERPE) was generated through the phosphatidylinositole (PI) pathway. Phosphatidic acid functions as a second messenger in this system, as in the pathogen-responsive ROS generation system. We found that when rice cells were "primed" for defence by pretreatment with a plant defence activator, such as probenazole, ERPE in rice cells was accelerated and potentiated. This priming effect on ERPE was found among a variety of plant defence activators and elicitors. Potato cells also showed potentiated generation of ERPE when primed for defence. Based on these facts, we developed a new evaluation method for plant defence activators as follows: I) dispense a suspension culture into dishes, II) add each candidate or solvent control to the cells, III) incubate treated cells for 2-4 h and check if each chemical has any disturbance on cell metabolism by photon counting, IV) add elicitor solution eg. chitin hexamer for rice, or arachidonic acid for potato, V) measure ERPE from cells, VI) estimate the potentiation rate of photon emission from chemical-treated cells to that from solvent-treated cells. VII) Select chemicals, which showed high potentiation rate of ERPE. Now we are developing this evaluation method for other crops.

32.17 ROLE OF PHENOLIC COMPOUNDS IN RESISTANCE OF CHILLI TO WILT CAUSED BY *FUSARIUM PALLIDOROSEUM*. N. Jabeen, N. Ahmed, M.Y. Ghani and S.H. Khan. *Division of Olericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar, Sriangar-191121, Jammu & Kashmir, India. Email: nayeema_jabeen@yahoo.co.in*

Two resistant and six susceptible chilli genotypes and their twelve hybrids showing variable degree of resistance to wilt caused by *Fusarium pallidoroseum* (Cooke) Sacc. were analyzed for phenols and phenolic enzymes, both under uninoculated and inoculated conditions at different phenological stages. In general total phenols, O.D. phenols and the activity of enzymes were invariably high in resistant parents and hybrids irrespective of stages, while, in susceptible parents the content of phenols and enzyme activities were comparatively less. There was a positive correlation between resistant hosts and higher amounts of phenols and increased enzyme activities, while it was almost the reverse in susceptible hosts. The positive association of higher phenols and enzymes with resistance could be of immense value for early and quick identification of resistant genotypes during screening of large populations generated by crossing resistant and susceptible parents.

32.18 SCREENING OF HOT PEPPER GERMPLASM FOR RESISTANCE TO WILT CAUSED BY *FUSARIUM PALLIDOROSEUM*. N. Jabeen, N. Ahmed, M.Y. Ghani and S.H. Khan. *Division of Olericulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Sriangar-191121, Jammu & Kashmir, India. Email: nayeema_jabeen@yahoo.co.in*

A set of 105 chilli (*Caspsicum annuum* L.) genotypes were screened for their resistance to *Fusarium pallidoroseum* (Cooke) Sacc. under both controlled and field conditions. Only two genotypes, 'Arka Lohit' and 'Roshini' showed inbuilt immunity under both screening procedures. Most of the lines/cultivars which were found resistant under artificial soil inoculation exhibited

more or the less same degree of resistance under field conditions. In general, there were 38 genotypes which showed an acceptable degree of resistance under both conditions, and these included 'Arka Lohit' and 'Roshini' (immune), SH-C-404, SH-C-1154, SH-C-1155 (highly resistant.), SH-C-83, SH-C-89, SH-C-206, SH-C-266, SH-C-277, SH-C-808, SH-C-815, SH-C-825, SH-C-910, SH-C-916, SH-C-1111, SH-C-PE-14 (resistant) SH-C-23, SH-C-59, SH-C-100, SH-C-103, SH-C-304, SH-C-304, SH-C-305, SH-C-345, SH-C-403, SH-C-402, SH-C-406, SH-C-503, SH-C-504, SH-C-579, SH-C-607, SH-C-864, SH-C-915, SH-C-962, SH-C-963, SH-C-1009, SH-C-1151 and SH-C-PC-2 (moderately resistant).

32.19 INDUCTION OF RESISTANCE IN CAULIFLOWER AGAINST *ALTERNARIA* BLIGHT USING POTASSIUM AND PHOSPHORUS SALTS. P.L. Kashyap and J.S. Dhiman. *Department of Plant Pathology, Punjab Agricultural University, Ludhiana 141004, India. Email: premlal_kashyap@yahoo.co.in*

We studied the effect of exogenous application of seven potassic and phosphonic resistance-inducer salts viz. KH_2PO_4, H_3PO_4, K_2HPO_4, K_2SO4, salicylic acid, $NH_4H_2PO_4$ and KOH at 0.5, 1.0, 2.0 and 4.0 ml/l on cauliflower (cv. PG-26) leaves on induction of resistance against *Alternaria* blight caused by *Alternaria brassisicola* of cauliflower. The resistance inducers tested were found to suppress the disease to varying levels. Protection was in the order of KH_2PO_4 > H_3PO_3> K_2HPO_4 > K_2SO_4 > salicylic acid > $NH_4H_2PO_4$ > KOH; irrespective of the salt, protection was maximum up to three days of treatment, and became less that with the passage of time, till 15 days. Smaller lesions of *Alternaria* were produced in the case of KH_2PO_4 (2.45 mM) followed by H_3PO_3 (3.17 mM) However, fewer lesions were found in the case of KH_2PO_4 (1.46 p.s.i.) followed by K_2HPO_4 (2.03 p.s.i.). The levels of total phenols, ortho-dihydroxy phenols, and flavonols was higher in the case of KH_2PO_4 and H_3PO_3 than the other salts. The treated leaves developed a dark green and glabrous texture due to the rich content of chlorophyll a and b. None of the salt solutions tested was toxic to the foliage. The study provides preliminary information that may facilitate the standardization and use of immunization technology using these potassium and phosphoric salts for the protection of plants in the field or greenhouses.

32.20 BACTERIAL RNA ELICITED-PLANT INNATE IMMUNITY. B. Lee, S. Lee, J.W. Yang and C.M. Ryu. *Systems Microbiology Research Center, KRIBB, Republic of Korea. Email: cmryu@kribb.re.kr*

Plants need to protect themselves against multiple pathogens at the infected site as well as consequently to elicit defense responses in distal parts as innate immunity. It is recently reported that plants perceive general microbial cues referred to as pathogen-associated molecular patterns (PAMPs). Plant innate immunity elicited by recognition of PAMPs such as fllagellin, elongation factor, and surface structural materials from microbes has been intensively studied. Here we provide evidence that bacterial genetic material can constitute a PAMP to induce plant defense responses against bacterial pathogens. Infiltration of total RNA from *Pseudomonas syringae* pv. *tomato* DC3000 resulted in enhancing resistance to subsequent infection by this pathogen compared to the water control on *Arabidopsis thaliana*. The 50K *Arabidopsis* Affymetrix microarray was employed to analyze unique RNA transcripts from *Arabidopsis* plants following challenge of bacterial RNA from *P. syringae* pv. *tomato* DC3000. The transcriptional expression of plant defense-related ribonuclease, and its transcription factor genes was up-regulated. The role and characterization of up-regulated genes will be presented. Our results indicate that bacterial genetic material can be a potential PAMP.

32.21 THE EFFECT OF *STREPTOMYCES LYDICUS* A02 ON INDUCED RESISTANCE OF TOMATO AGAINST *BOTRYTIS CINEREA*. J.H. Liu, Y. Liu, W.C. Liu, J.Y. Qiu, T. Liu and G.J. Tang. *Institute of Plant & Environment Protection, Beijing Academy of Agriculture & Forestry Sciences, Beijing 100097, P. R. China. Email: ljh0779@sina.com*

We studied the induced resistance effect of an antifungal strain, *Streptomyces lydicus* A02, in tomato seedlings inoculated with grey mould. The seedlings were pot-cultured in a greenhouse. At the squaring stage, the seedlings were sprayed according to one of the following three designs: (1) only with broth filtrate of strain A02, (2) with the filtrate, at 24 h after inoculating with spore suspension of *Botrytis cinerea*, (3) with the filtrate, at 24 h before inoculating with the spore suspension. Changes in the quantities of 4 defence enzymes (phenylalanine ammonialyase, peroxidase, catalase and polyphenoloxidase), 2 pathogenesis-related proteins (chitinase and β-1,3-dextranase), and the total phenols contents of tomato leaves before and after the treatments were assayed by ultraviolet-visible absorption spectroscopy. The results showed that each of the three treatments could induce an increase of the enzymatic activities and total phenolics. The peak values of the inducing activities appeared 24-72 h after treatment, which was earlier than in the blank control. Treatment (3) of inoculating after inducing gave the best inducing effect. Correspondingly, the good control (up to 94.92%) of grey mould was obtained in greenhouse with this treatment. We suggest that *S. lydicus* A02 culture filtrate could induce more effectively resistance of tomato seedlings against *B. cinerea* if used before mass outbreak or at the initial phase of the disease.

32.22 POTENTIAL OF INDUCED RESISTANCE IN POSTHARVEST DISEASE CONTROL OF HORTICULTURAL CROPS. C. Mahendranathan. *Department of Botany, Eastern University, Chenkalady, Sri Lanka. Email: cmahendranathan@yahoo.com*

Diminished use of conventional fungicides due to pathogen resistance and general unacceptability in terms of public and environmental risk have favoured the introduction of integrated pest management (IPM) programmes. Induction of natural disease resistance (NDR) in harvested horticultural crops using physical, biological and/or chemical elicitors has recently received increasing attention, it being preferred as a disease management strategy. This study reviews the enhancement of inducible antifungal compounds and suppression of anthracnose diseases in aubergine through bio-elicitors. Experiments were conducted to examine the possibility of elicitation of host natural resistance in aubergine against anthracnose, a problem disease in the study area, using a relatively weaker pathogen in aubergine, *Fusarium solani*. The results showed that pre-inoculation with *F. solani* gave greater phytoalexin accumulation, sufficient to prevent lesion development by *Colletotrichum capsici*, agent of aubergine anthracnose. Thus, *F. solani* appears to be an effective elicitor of natural host resistance in aubergine. The mechanism of induction of this resistance after inoculation with *F. solani* appears to be associated with phytoalexin accumulation. Purification of the phytoalexin using flash chromatography showed that the compound is lubimin.

32.23 SYSTEMIC RESISTANCE INDUCTION IN TOMATO BY *PSEUDOMONAS PUTIDA* BTP1: INVESTIGATION OF DEFENSE PATHWAYS. **M. Mariutto, F. Duby, M. Ongena, E. Jourdan, P. Thonart and J. Dommes.** *Laboratory of Plant Molecular Biology and Biotechnology, University of Liège, Bvd du Rectorat, 27, B-4000 Liège, Belgium. Email: martin.mariutto@student.ulg.ac.be*

Some non-pathogenic rhizobacteria can induce systemic resistance in plants that differs from pathogen induced-systemic acquired resistance (SAR). Despite extensive work, molecular events leading to that rhizobacteria-mediated resistance, termed ISR (induced systemic resistance) are less well understood that in the case of SAR. We have studied the protective effects of *Pseudomonas putida* strain BTP1 against grey mould caused by *Botrytis cinerea* in tomato. The activities of phenylalanine ammonia-lyase (PAL) and lipoxygenase (LOX), two key enzymes of the phenylpropanoid and lipoxygenase defense pathways respectively, were compared in leaves of control plants and plants treated with growth-promoting rhizobacteria. No significant differences were detected for PAL activities. On the contrary, LOX activity was significantly stimulated in *P. putida* BTP1-inoculated plants before and two days after challenging with the pathogen. To determine the contribution of each LOX isoform to the enzymatic activity increase, the expression level of five Lox genes was analysed. Whereas *LoxA*, *LoxB* and *LoxC* were not stimulated in response to the treatment, *LoxD*, which leads to the synthesis of octadecanoid defense compounds, and *LoxF*, a recently identified gene, showed consistent stimulation of their transcription level. These results strongly suggest that the resistance conferred by *P. putida* BTP1 is associated in tomato with stimulation of the oxylipin pathway.

32.24 THE EFFECT OF A PLANT ACTIVATOR AND NEW CUPRIC FUNGICIDES FOR CITRUS CANKER CONTROL. **M. Mitidieri, E. Piris, V. Saliva, V. Brambilla, R. Celié, M. Piris and J. Diz.** *EEA INTA San Pedro, Ruta 9 km 170 CC 43 CP 2930, San Pedro, Buenos Aires, Argentina. Email: mmariel@correo.inta.gov.ar*

Citrus canker, caused by *Xanthomonas axonopodis* pv *citri* is responsible for direct losses and difficulties for fruit exportation to European Union countries. The objective of this work was to evaluate the effect of different concentrations of a plant activator alone or in combination with a cupric fungicide. The trial was performed in a commercial nursery grafted in January 2005. The genetic material was lemon 'Genova'. The treatments evaluated were: copper oxychloride (OXI) (WP 84% metallic copper: 300 g/hl), acybenzolar-s-methyl CGA 245704 (BION) (WP 50% : 160, 80, 40, 20 g/hl), copper hydroxide + copper oxychloride (AIRONE) (SC 27.2%: 200 ml/hl) and copper oxychloride (PASTA ISAGRO) (SC 37.7%: 200 ml/100 hl). The products were sprayed 8 times from January to May. Significant differences were obtained between treatments for number of leaves with cankers (incidence) and number of cankers per leaf (severity) for the evaluations made in February and April. While the untreated control showed citrus canker incidence of 64.9% in April, BION combination (160 and 80 g /hl) + OXI, AIRONE and PASTA ISAGRO showed lower incidence values (36.8%, 36.9%, 15.8%, 27.3% respectively). Leaves treated with BION 160 g/hl showed lower percentages of affected tissue after *in vitro* inoculations (10% vs. 40.6% for the untreated control and 9% for copper oxychloride). The use of a plant activator in combination with cupric fungicides could contribute to integrated citrus canker management.

32.25 INVESTIGATING EFFICACY OF MILSANA AND ITS MODE OF ACTION IN CONTROLLING POWDERY MILDEW IN KABOCHA SQUASH IN AUSTRALIA AND TONGA. **J. Morris, J. Jobling and R. McConchie.** *The Faculty of Agriculture, Food and Natural Resources, University Of Sydney, NSW, Australia. Email: jmor7815@usyd.edu.au*

Powdery mildew in Kabocha squash is difficult to control. The use of hard chemicals with a single-site mode of action has led to the development of resistance within powdery mildew populations. Alternative methods for disease control are needed. One option is treatment with Milsana, a plant extract derived from *Reynoutria sachalinensis*. This plant bio-protectant has been shown to reduce disease severity. A field trial in Sydney has shown that it reduces powdery mildew. The best control strategy was to alternate sprays of Milsana and a systemic fungicide. This combination reduced disease more than the treatments used on their own. In Tonga similar treatments were ineffective due to high disease severity and conditions conducive to plant disease. Understanding the mode of action of Milsana in this plant-pathogen interaction will help to improve the effective application of this control method in Tongan crops. Currently, its mode of action in terms of inducing systemic resistance is in question. Our experimental results based on enzyme assays and tests for total phenolics indicate that Milsana does not induce a response to disease in plant tissue of Kabocha Squash or cucumber. Further research using fluorescence microscopy will be done to try and elucidate it mode of action. It is hoped this understanding will improve the efficacy of its use in controlling powdery mildew in Kabocha squash and cucumber.

32.26 PGPR INDUCED HISTOCHEMICAL CHANGES DURING INDUCTION OF RESISTANCE IN PEARL MILLET. **S. Niranjan Raj and H. Shekar Shetty.** *DOS in Applied Botany and Biotechnology, University of Mysore, Manasagangotri, Mysore 570006, India. Email: niruraj@gmail.com*

Histochemical analysis of plant growth-promoting rhizobacteria PGPR mediated ISR in the pearl millet (*Pennisetum glaucum*) downy mildew system showed that induced resistance is associated with expression of the hypersensitive response (HR), enhanced lignification, callose deposition and hydrogen peroxide production in addition to increased expression of defence enzymes like glucanase, chitinase, phenylalanine ammonia lyase, peroxidase and polyphenol oxidase. There was rapid expression of HR in the resistant and induced-resistant (*Bacillus pumilus*-treated) seedlings after *Sclerospora graminicola* infection compared to the susceptible seedlings. Examination of inoculated tissues by microscopy showed that lignin, callose, and hydrogen peroxide accumulated earlier and to higher levels in resistant and induced-resistant seedlings. Tissue prints and light microscope coleoptile sections at various times after pathogen inoculation revealed a visual difference in the pattern of enzyme expression in resistant, induced-resistant and susceptible seedlings. In general, the enzymes were found to be localized in the vascular bundles of the seedlings, but the intensity varied among the resistant, susceptible and induced-resistant seedlings. Further, most of the enzymes were highly localized in the vascular regions. Differences in the speed, intensity, spatial pattern and range of expression of different histological and biochemical expression of the defence responses might reflect the resistant, induced-resistant and susceptible state of pearl millet seedlings.

32.27 L-ALANINE AUGMENTS RHIZOBACTERIA-INDUCED SYSTEMIC RESISTANCE IN CUCUMBER AS EVIDENCED THROUGH RT-PCR ANALYSIS. **K. Park, S. Bharathkumar, Y.K. Kim and S.S. Han.** *Plant Pathology Division, National Institute of Agricultural Science and Technology, RDA, Suwon. 441-707, Republic of Korea. Email: kspark@rda.go.kr*

Bacillus vallismortis strain EXTN-1 is an elicitor that induces systemic resistance in many crops against various pathogens. In an effort to boost the effect of this bacterium with a chemical inducer, L-alanine was tested in cucumber. L-alanine and EXTN-1 separately caused significant levels of disease suppression in cucumber against Anthracnose disease. When the plants were treated with EXTN-1 and L-alanine together there was augmented disease suppression. Treating PR-1a GUS-transgenic tobacco plants with different concentrations of L-alanine (10–250 ppm) gave strong GUS activity. In addition, the treatment also induced, in transgenic (PR-1a or PDF 1.2 over expressing) *Arabidopsis* plants, the expression of resistance genes PR-1a and PDF 1.2, as confirmed by RT-PCR analysis. The defence gene activation was higher with EXTN-1 than L-alanine and even higher with EXTN-1 and L-alanine together. The results indicated that there is a cumulative ISR effect when the bacterial and chemical elicitors are combined. Also since both PR-1a and PDF 1.2 were activated with both the elicitors, it was confirmed that both the salicylic acid-mediated and jasmonic acid-mediated defence pathways are involved in the system. The method could be developed at field level to augment rhizobacterial ISR in crops to bring about better disease suppression.

32.28 THAXTOMIN A PRODUCED BY *STREPTOMYCES*: POTENTIAL ELICITOR OF PHYTOALEXINS AND MODE OF ACTION IN SORGHUM SEEDLINGS. **S.F. Paschol ati, E.O. Garcia, M. Almeida and I.P. Bedendo.** *Plant Pathology Section - Esalq/USP, P.O. Box 09, 13418-900 Piracicaba, SP, Brazil. Email: sfpascho@esalq.usp.br*

Potato common scab occurs all over the world and is caused by bacteria of the genus *Streptomyces* that are able to produce the phytotoxin thaxtomin A (TA). The toxin potential as a resistance inducer and its mode of action are not well known. Thus, the objective of this work was to evaluate the phytoalexin elicitor potential and mechanism of action of TA on growth, membrane permeability, cell wall integrity and chlorophyll content in sorghum seedlings. TA was obtained from *Streptomyces scabies* isolate 79, and purified and quantified by HPLC. The seedlings were placed in test tubes and treated with 0, 25, 50, 100, 150 or 200 µg TA/ mL and maintained at 25 °C under a 12 h photoperiod (fluorescent light). After seven days, the seedlings showed high phytoalexin (deoxyanthocyanidin) accumulation, reduction in growth and in the amount of chlorophylls A and B. There was an increase in electrolyte leakage, and abnormal cell wall formation, observed by electron microscopy. Thus, the results showed that TA acts by changing plasma membrane permeability, chlorophyll content and causing changes in the host cell wall. The phytoalexin accumulation indicates the elicitor potential of the toxin that could be explored to induce defence mechanisms in sorghum. Research fellowships funded by CNPq and FAPESP.

32.29 INDUCED RESISTANCE TO *RHYNCHOSPORIUM SECALIS* IN BARLEY. **L. Paterson, D.J. Walsh and D.R. Walters.** *Crop & Soil Systems Research Group, Scottish Agricultural College, West Mains Road, Edinburgh, EH9 3JG, UK. Email: dale.walters@sac.ac.uk*

The resistance elicitors *cis-jasmone,* β*-aminobutyric acid* (BABA) and Bion® were examined for their ability to induce resistance in barley to the necrotrophic pathogen *Rhynchosporium secalis*. All elicitor treatments examined reduced infection of barley by *R. secalis*. Used singly, cis-jasmone and Bion® reduced infection by 36 % and 34 % respectively. Better control was achieved using elicitor combinations, with all combinations used reducing infection by 55-59%. Elicitor effectiveness was greatly influenced by genotype. Best disease control was observed with the variety Westminster, with a high resistance rating (RR8) against *R. secalis*, where, although infection intensity in the control was low, elicitor treatment reduced infection by 94%. Reductions in infection of between 58% and 65% were obtained with Oxbridge (RR7), Decanter (RR6) and Cellar (RR4), while little effect was observed with Chalice (RR5) and Troon (RR4).

32.30* COSTS ASSOCIATED WITH USE OF THE RESISTANCE ELICITOR SACCHARIN. **L. Paterson, D.J. Walsh and D.R. Walters.** *Crop & Soil Systems Research Group, Scottish Agricultural College, West Mains Road, Edinburgh, EH9 3JG, UK. Email: dale.walters@sac.ac.uk*

Saccharin was found to induce resistance in barley to the necrotroph *Rhynchosporium secalis*, reducing infection intensity by the same amount (40%) under both low and high inoculum pressure. In plants treated with saccharin, numbers of ears per plant, numbers of grains per ear and grain weight per plant were increased compared to controls, although only the increase in grain weight per plant was significant. If plants were inoculated with a low concentration of *R. secalis* spores following treatment with saccharin, there was a non-significant increase in ear number per plant and a significant decrease in the number of grains per ear, although this was not reflected in grain weight per plant, which was unchanged compared to inoculated control plants. When plants were inoculated with a high spore concentration of *R. secalis* following saccharin treatment, although numbers of ears per plant and grain weight per plant were unchanged, there was a small, but non-significant increase in grain weight per plant. These data suggest that under pathogen-free conditions, saccharin does not incur costs and indeed, the increases in yield components compared to controls suggests a beneficial effect on host physiology. Although there are changes in some of the yield components when saccharin-treated plants are exposed to low and high pathogen pressure, yield is not compromised in either situation.

32.31 BIOCHEMICAL CHANGES INDUCED BY *CUCUMBER MOSAIC VIRUS* IN CHILLI (*CAPSICUM ANNUM* L.). **M.S. Patil and N. Kotreshe.** *Department of Plant Pathology, University of Agricultural Sciences, Dharwad 580005, Karnatak, India. Email: chinmayi3@rediffmail.com*

The chilli crop suffers heavily from mosaic disease caused by *Cucumber mosaic virus* (CMV). Infected leaves can exhibit different kinds of symptoms like chlorosis, mottling, curling and necrosis. The present investigation was made to find out the biochemical changes taking place after CMV infection in leaves. Healthy and CMV-infected leaves of the cultivar 'Byadgi Kaddi' were taken for biochemical analysis at 40 and 80 days after transplanting. Total sugars and reducing sugars were estimated following Nelson's modification of Somogyi's method (Nelson, 1944) and total

phenols of plant samples were estimated by the Folin-Ciocalteau Reagent (FCR) method (Beihn *et al.,* 1968). The results indicated that levels (mg) of total sugar, reducing sugar and non-reducing sugars in diseased leaves at 40 days after transplant were 23.3, 2.5, 8.4, and 7.9 and 14.9, 12.6 mg after 80 days compared to 32.8, 38.6, 14.3 mg (40 days) and 16.6 and 18.5, 21.3 mg (80 days) in healthy leaves. There was a gradual reduction in total sugars, reducing sugars and non-reducing sugars in infected leaves at 40 and 80 days after transplanting compared to healthy leaves. This indicates that viral infection reduced sugar content in the host. At 40 days after transplanting, total phenol content was greater (2.6 mg/5 g fresh leaves) in the CMV-infected plants as compared to healthy samples (1.7 mg/5 g fresh leaves). This indicates that there was an increase in phenol content upon virus infection, which helps in controlling spread of the disease in plants.

32.32 INDUCTION OF SYSTEMIC ACQUIRED RESISTANCE IN CUCUMBER AGAINST *FUSARIUM* WILT BY SEED TREATMENT WITH BENZOTHIADIAZOLE. **A. Ramasamy, R. McConchie and D. Nehl.** *Faculty of Food Agriculture and Natural Resources, University of Sydney, NSW 2006, Australia. Email: aram7272@usyd.edu.au*

Fusarium wilt of cucumber (*Cucumis sativus*) is caused by *Fusarium oxysporum* f.sp. *radicis-cucumerinum*, and is one of the destructive diseases in cucumber cultivation around the world. The pathogen will cause yellowing, wilting of plants and stem cracking. Severely affected plants have a visible fungal growth on the outside of the stem; eventually plants turn brown and die. Chemical fungicides, biologicals, adoption of proper sanitation and cultural methods are the options currently available to reduce or delay the *Fusarium* wilt, but there is no effective control method available to date. Benzothiadiazole (BION® Syngenta Crop Protection Private Ltd, NSW, Australia) is a prophylactic compound, a strong defence activator used to suppress many foliar and *Fusarium* diseases in different crops. The aim of the current study was to check the effect of BTH concentrations and times of soaking seed on cucumber seedlings, and also to determine its efficacy against *Fusarium* in inducing resistance and controlling the disease. Seeds of cucumber cv Crystal salad were soaked in 0, 25, 50, 75 or 100 µg a.i. ml^{-1} BTH for 6, 12 and 24 hours. Germination percentage and seedling vigour index were recorded. Statistical analysis indicated that BTH concentration had a significant effect on germination percentage, where as soaking time did not, although soaking time significantly affected the seedling vigour index. Initial studies indicated that higher BTH concentration delays the germination and longer soaking time negatively affects the seedling vigour index. We are investigating the effect of BTH in inducing SAR activity against *Fusarium* and hence its control of the fungus.

32.33 PROTEIN HYTRA1 SECRETED BY *TRICHODERMA* PLAYS A KEY ROLE IN INDUCED SYSTEMIC RESISTANCE. **M. Ruocco, S. Lanzuise, D. Turrà, M. Reverberi, M. Tucci, L. De Masi, F. Vinale, R. Marra, V. Aloy, S. Woo and M. Lorito.** *Dipartimento d'Arboricoltura, Botanica e Patologia Vegetale sez. Patologia Vegetale, University of Naples, Via Università 100, 80055 Portici (NA), Italy. Email: lorito@unina.it*

Trichoderma harzianum T22 is one of the *Trichoderma* isolates most used as active ingredient in commercial bio-fungicides and bio-fertilizers. In addition to their mycoparasitic abilities, many *Trichoderma* strains can colonize and grow in association with plant roots and can significantly increase plant growth, development and systemic resistance to pathogen attack. During *Trichoderma*–plant interaction, a plant response has been confirmed as responsible for at least part of the protection effect, but the molecular mechanisms involved are still largely unknown. The protein Hytra1 secreted by *Trichoderma* was tested for its ability to induce a hypersensitive reaction (HR) and induced systemic resistance in tomato plants. Hytra1 infiltration elicited a strong HR on tomato leaves and could trigger plant defence reactions subsequent to pathogen infections both locally and systemically. Physiological analyses of tomato leaves treated with Hytra1 showed that this hydrophobin can induce an oxidative burst in plant cells. Low Hytra1 concentrations also triggered activation of the antioxidant system that controls accumulation of reactive oxygen species (superoxide anions and peroxides). This modulation plays a role in transduction of the oxidative signal, leading to formation of signal molecules (lipoperoxides) that trigger the accumulation of defence molecules (e.g. riscitin) and PR proteins. Real-time PCR analysis of RNA extracted from tomato plants 24 hours after treatment with 0.31 µM Hytra1 showed strong activation of PR4 transcription, while PR1 expression was induced to a lesser extent in the same conditions. Steady-state mRNA levels of PR4 and PR1 were about 13-fold and 5-fold higher, respectively, than in controls.

32.34 THE USE OF INDUCED RESISTANCE TO CONTROL MAIZE EAR ROT CAUSED BY *FUSARIUM VERTICILLIOIDES*. **K.K. Sabet, E.M. El-Assiuty, A.M.A. Ashour and E.M. Shabrawi.** *Plant Pathology Dept., Faculty of Agriculture, Cairo University, Giza 12613, Egypt. Email: kamel.sabet@gmail.com*

A new approach towards management of ear rot caused by *Fusarium verticillioides* (which produces the mycotoxin fumonisin) was made in field trials applying some elicitors (known to induce plant resistance in some cases) such as salicylic acid, acetyl salicylic acid, cobalt nitrate, magnesium sulphate, potassium, ethephon and ascorbic acid. Aqueous solutions of these elicitors, in two concentrations were sprayed on two maize cultivars (cv. TWC-130 and cv. Balady) susceptible to the toxigenic *Fusarium verticillioides*. Spraying was done to 15 and 30 day old seedlings as well as at flowering stage (65 days post-planting). All substances tested significantly reduced ear rot. Acetyl salicylic acid at 960 mg/l reduced infection by 83.6%, Ethephon at 600 ppm reduced infection by 81%, and ascorbic acid at 2000 ppm reduced infection by 80% in the case of cv. TWC-130. The above treatments did not cause phytotoxicity. The reductions of infection in cv. Balady were less than that of cv. TWC-130. When the treatments were combined with the bio-insecticide Dipel 2x (*Bacillus thuringiensis*) they were found to be effective against stem borers and this in turn reduced the spread of ear rot caused by *F. verticillioides*. Hence the seeds had reduced content of the mycotoxin fumonisin.

32.35 NEW INSIGHTS INTO PLANT DEFENCE RESPONSES TO PHLOEM-FEEDING INSECTS. **H. Samaha, C. Rusterucci, A. Cherqui, F. Baillieul and P. Giordanengo.** *Université de Picardie Jules Verne, Biologie des Plantes et Contrôle des Insectes Ravageurs (BPCIR-EA3900), 33 Rue St Leu, 80039 Amiens Cedex 1, France. Email: hala.samaha@u-picardie.fr*

Despite the important yield losses caused by aphid attacks on numerous crops, aphid-plant interactions are not well studied. In

the aim to develop integrated pest management programs, the use of host plant physiological responses is one of the most promising strategies. As aphids probe phloem sap, they insert their stylets between the cell wall layers to reach sieve elements. During this progress, aphids periodically inject saliva that may prevent plant defence responses and ensure feeding. We characterized potato responses against *Macrosiphum euphorbiae* at macroscopic, microscopic and molecular levels. Our results revealed a set of local plant responses induced by aphid infestation. Brown spots associated with the salivary sheath appeared at the infection site within 72 hours after infestation. The corresponding deposit is under investigation. Following massive infestation, localised cell death was observed. Callose was deposited after plant cells were damaged, in order to seal punctured cells, but the response was highly reduced during aphid presence and salivation. All these plant responses are dependent on both duration of infestation and aphid population density. The characterization of elicitors or plant defence inhibitors produced by these phloem-feeding insects opens promising perspectives.

32.36 DEVELOPMENT OF QUALITY PLANTING MATERIAL BY USING PLANT GROWTH-PROMOTING RHIZOBACTERIA AND PHYTOPROTEIN. N. Sharma, D. Shrivastava, D. Jain, A. Singh and H.N. Verma. *Institute of Biotechnology and Allied Sciences, Seedling Academy of Design, Technology and Management Jagatpura, Jaipur 302019, India. Email: divya_shrivastava@hotmail.com*

An indigenous plant growth-promoting rhizobacterium able to solubilize phosphate and produce auxin was isolated from the rhizosphere of a grass root. The isolate was identified as *Pantoea agglomerans* by Biolog and 16S r-DNA analysis. The sequence has been deposited in NCBI nucleotide repository under the accession number EF543162, *Pantoea* spp AR2,16S ribosomal gene, partial sequence. Two phytoproteins isolated from *Boerhaavia diffusa* (BD) and *Clerodendron aculeatum* (CS) are known to induce systemic resistance in plants against viral infections. Pot studies were carried out to study the effect of *Pantoea* spp. alone and in combination with BD or CS. Seeds treated with *Pantoea* spp. and CS had 75% germination whereas seeds treated with the combination of *Pantoea* spp. and BD showed 48% germination. Germination for seeds treated with BD or CS alone was 50%. However, only 40% seed germination was observed in untreated control seeds. A significant difference in growth was observed between the untreated control plants and the plants treated with *Pantoea* spp. in combination with the phytoprotein. The ability of *Pantoea* spp. to inhabit roots and the combined effect of the two on plant growth promotion is being studied.

32.37 CONTROL EFFECTS AND MECHANISM OF CHITO-OLIGOSACCHARIDE ON TOBACCO BLACK SHANK. Y. Shen, Z. Gao, G. Wang and Y. Pan. *College of Plant Protection of Anhui Agricultural University, Hefei 230036, P. R. China. Email: gzm@ahau.edu.cn*

The inhibitory activity of chito-oligosaccharide (CO) on the oomycete *Phytophthora nicotianae* var. *nicotianae* and the effectiveness of control by this compound on tobacco black shank caused by the fungus were studied. The activities of disease-resistance enzymes such as superoxide dismutase in tobacco plants treated by CO by artificial inoculation were measured to explore the bio-control effects and mechanism of CO on the diesase. The results showed that *in vitro*, CO did not inhibit the mycelium growth of *P. nicotianae* var. *nicotianae* on lima bean agar. However, with artificial inoculation and pot cultivation in a growth room, the black shank lesion area on tobacco stems was 42% of that in the control, so effectiveness was 58% in the treatment with CO alone. Next was the effectiveness of CO + *Trichodema* in which the lesion area of tobacco stems was 48% of the control, and the effectiveness was 52%. The activities of PAL, PPO, POD, SOD and chitinase in the tobacco plants treated with CO and artificially inoculated were analyzed after 10 days treatment in the growth room. The results showed that CO enhanced activities of POD, SOD, PPO and chitinase in tobacco plants, which was consistent with the result of the control test. Our results suggest that CO had biological activity which induced and improved resistance of tobacco plants to black shank, and has considerable potential in control of this destructive tobacco disease.

32.38 BETA-AMINOBUTYRIC ACID-INDUCED RESISTANCE IN GRAPEVINE AGAINST DOWNY MILDEW. A. Slaughter, J.M. Neuhaus and B. Mauch-Mani. *University of Neuchâtel, Laboratory of Molecular and Cellular Biology, Rue Emile-Argand 11, C.P. 158, CH-2009 Neuchâtel, Switzerland. Email: ana.slaughter@unine.ch*

Grapevine (*Vitis vinifera* L.) is a major fruit crop worldwide, affected by many diseases. Downy mildew, caused by the oomycete *Plasmopara viticola* is one of the most serious diseases in vineyards. Both susceptible and resistant cultivars can be colonised by *P. viticola* zoospores, but in resistant ones, development of the parasite is rapidly inhibited. Most traditionally grown cultivars are susceptible to this disease, and require intensive use of chemicals to limit the damage. One possible solution would be activation of the plant's own defence system, known as induced resistance (IR). β-aminobutyric acid (BABA), a non-protein amino acid, has previously been shown to induce resistance against many oomycetes and to be effective in inducing resistance against various downy mildews. It was observed that the protective effect of BABA in *Arabidopsis* was due to potentiation of natural defence mechanisms, a phenomenon referred to as priming. Priming is the capacity of a plant to express a faster and stronger basal defence response upon pathogen infection. Recently, in grapevine it has been shown that callose deposition as well as defence mechanisms depending on the phenylpropanoid and jasmonic acid pathways all contributed to BABA-IR in the susceptible cultivar Chasselas. Microarray analysis of infected Chasselas treated with BABA (or water controls) will be discussed.

32.39 POSSIBLE EFFECTS OF SYSTEMIC RESISTANCE IN TOMATO, INDUCED BY SALICYLIC ACID AGAINST *ALTERNARIA* LEAF SPOT. M.J. Soleimani and M. Esmailzadeh. *Dept. of Plant Protection, College of Agriculture, Bu-Ali Sina University, Hamadan, Iran. Email: j_soleimaniuk@yahoo.co.uk*

Resistance to disease can be induced systemically in a number of plant species by biological and chemical means. One of the most commonly used chemicals is salicylic acid (SA) which appears to mimic the systemic effects of localized infection. *Alternaria* leaf spot or early blight is a common foliar disease of tomato in most regions of the world, including Iran. *Alternaria alternata* is one of the causal agents of early blight and is responsible for significant losses each year. Since SA is an important signal molecule that plays a critical role in plant defence against pathogen invasion, we investigated if exogenous application of SA would activate systemic acquired resistance (SAR) against *A.*

alternata in tomato leaves. Foliar application of 400 µM SA significantly increased the endogenous SA content of leaves. Challenge inoculation of SA-treated tomato plants using conidia of *A. alternata* resulted in 54.7% fewer lesions per leaf and an 87.5% reduction in blighted leaf area as compared with control plants not receiving SA. The results obtained in greenhouse experiments indicated that application of SA to tomato plants can activate SAR that is effective against early blight.

32.40 RESISTANCE INDUCED BY ULVAN TO ANTHRACNOSE (*COLLETOTRICHUM LINDEMUTHIANUM*) IN COMMON BEAN (*PHASEOLUS VULGARIS*). M.J. Stadnik and W.S. Fernandes. *Departamento de Fitotecnia, CCA, Universidade Federal de Santa Catarina, C.P. 476, 88040-900 Florianópolis, Brazil. Email: stadnik@cca.ufsc.br*

The effect of the algal polysaccharide ulvan was studied on the resistance of leaf veins to anthracnose. Three bean (*Phaseolus vulgaris* L.) cultivars with different resistance levels were used, i.e. 'Uirapuru' (susceptible), 'Mouro Graúdo' (with adult plant resistance) and 'Valente' (resistant). Plants at the V3-growth stage were sprayed with ulvan (10 mg/ml) or water (control) six and three days before inoculation with *C. lindemuthianum*. The central leaflet of the 2nd leaf was covered with a plastic bag at treatment time, to evaluate the systemic effect, whereas the two lateral leaflets remained uncovered. Disease severity was assessed by measuring the number and size of necrotic lesions on each leaflet vein. At 48 h after inoculation, 8-mm diameter leaf discs from lateral leaflets were collected, bleached, stored in lactoglycerol and examined by light microscopy (480×) to determine conidial germination, appressorium formation and frequency of hypersentive cells. Ulvan locally and systemically reduced the development rate of both lesion number and length from the 8th to 14th day in both susceptible cultivars. The primary vein was more susceptible to anthracnose than others. Lesion formation rate was systemically reduced by ulvan only at the secondary leaf. Conidia germinated on all cultivars equally well, but ulvan reduced their germination on 'Uirapuru' and 'Mouro Graúdo'. The highest appressorium formation (42%) was recorded on 'Valente'. Ulvan did not influence appressorium formation. The frequency of hypersensitive cells was similar among cultivars, but significantly higher in ulvan-treated 'Uirapuru' than in 'Mouro Graúdo' control plants.

32.41 EVALUATION OF FERTILIZERS, ALGAL EXTRACTS, FUNGICIDES AND RESISTANCE-INDUCERS TO CONTROL ONION DISEASES. M.J. Stadnik and J.A. Wordell Filho. *Departamento de Fitotecnia, CCA, Universidade Federal de Santa Catarina, C.P. 476, 88040-900 Florianópolis, Brazil. Email: stadnik@cca.ufsc.br*

Field experiments were done to evaluate the effect of foliar sprays with the following treatments on downy mildew (*Peronospora destructor*) and stored bulb rot (*Burkholderia cepacia*) in onion (*Allium cepa*): a) untreated control; b) the fungicides chlorotalonil or metalaxyl+chlorotalonil; c) potassium phosphite; d) foliar fertilizer (03-00-16, N-P-K); e) bordeaux mixture; f) bordeaux mixture/potassium phosphite; g) acibenzolar-S-methyl; h) extract of the alga *Ulva fasciata;* i) ulvan (applied weekly); j) alga extract; k) ulvan (at 14-day spray intervals); l) alga extract; m) ulvan (at 21-day intervals). The weekly spray with fungicides and fertilizer (03-00-16, 400 ml /100 l) significantly reduced mildew severity by 60% and 23%, respectively, but did not increase bulb yield. Potassium-rich fertilizers resulted in a higher incidence of rotten bulbs after 5 months in storage. Soluble sugar content and bulb rot incidence were significantly correlated.

32.42 FITNESS COSTS OF TOMATO PLANTS INDUCED BY NATURAL AND CHEMICAL AGENTS. J.R. Stangarlin, O.J. Kuhn, M. Baldo, L. Iurkiv, C. Meinerz, G. Franzener and K.R.F. Schwan-Estrada. *Centro de Ciências Agrárias, Universidade Estadual do Oeste do Paraná, C.P. 91, CEP 85960-000, Marechal Cândido Rondon, Paraná, Brazil. Email: jrstangarlin@unioeste.br*

Plants that invest resources in defence against pathogens may pay off the costs in increased productivity. The objective of this work was to test the fitness costs related to resistance induction in tomato against *Xanthomonas vesicatoria* using acibenzolar-S-methyl (ASM) at 50 mg/l, *Bacillus cereus* (10^8 ufc/ml), turmeric, and *Rosmarinus officinalis* aqueous extracts at concentration of 10%, copper oxychloride (4 g/l) and water. Eight sprays were made at weekly intervals and the pathogen was inoculated seven times, four days after each spraying. The results showed that plant height was not affected by the inducers, except by *B. cereus* in the presence of pathogen. The dry weight of roots treated with *Rosmarinus* was superior than that of roots treated with ASM or water in the absence of the pathogen, while in its presence, plants treated with *Rosmarinus* showed a similar trend. The aerial parts, leaf and total dry weight were increased by turmeric and *Rosmarinus* in the absence of pathogen, but in its presence, only the plants treated with fungicide showed increase. The fresh weight of fruits in absence of the pathogen was not changed while in presence of the pathogen plants treated with turmeric were superior to plants treated with ASM, while the dry weight and the number of fruits per plant did not differ. These inducers did not cause allocation of significant resources in tomato plants in the absence of pathogen, and turmeric and *Rosmarinus* extracts reduced disease severity by 65% and had a tonic effect on tomato plants.

32.43 RESISTANCE AGAINST *PSEUDOCERCOSPORA GRISEOLA* INDUCED IN BEAN BY *PYCNOPORUS SANGUINEUS* EXTRACTS. J.R. Stangarlin, C.A. Viecelli, O.J. Kuhn, G. Franzener and K.R.F. Schwan-Estrada. *Centro de Ciências Agrárias, Universidade Estadual do Oeste do Paraná, C.P. 91, CEP 85960-000, Marechal Cândido Rondon, Paraná, Brazil. Email: jrstangarlin@unioeste.br*

Aqueous extracts from basidiocarps, mycelium and liquid culture filtrate of *Pycnoporus sanguineus* were used to test induction of resistance in bean plants against *Pseudocercospora griseola* (syn. *Phaeoisariopsis griseola*), cause of angular leaf spot. Water, acibenzolar-S-methyl (ASM, 150 mg/l) and fungicide (Azoxystrobin 0.08 g/l) were used as control treatments. *In vitro*, 5% mycelial extract, when sterilized by filtration, but not by autoclaving (1 atm and 120 °C for 20 min), significantly inhibited growth of *P. griseola* mycelium, sporulation and conidial germination, indicating the presence of thermo-labile compounds. In greenhouse, bean plants were treated three days before inoculation with the pathogen and were evaluated for symptom severity and the activity of the plant defence enzymes peroxidase, β-1,3-glucanase and polyphenol oxidase in 3rd leaves, treated with the inducers, as well as in 4th leaves, not treated, to test for induction of local or systemic resistance. The pathogen (1×10^4 conidia/ml) was inoculated in both leaves. Severity was reduced by 48% and 59% in 3rd leaves and by 35% and 85% in 4th leaves by *P. sanguineus* extracts at concentrations of 10% and 20%, respectively, while treatment with fungicide or ASM gave averaged reduction

of 82% and 55%, respectively. Only peroxidase and polyphenol oxidase activities increased in plants treated with extracts, and there was a reduction of severity. These results indicate the potential of *P. sanguineus* for alternative control of angular leaf spot in bean plants.

32.44 REDUCED SENSITIVITY OF *MONILINIA LAXA* TO BENOMYL IN SERBIA. **M. Stevic, E. Rekanovic and P. Vuksa.** *University of Belgrade, Faculty of Agriculture, Serbia. Email: stevicm@agrifaculty.bg.ac.yu*

Brown rot, caused by *Monilinia laxa* (Ader. & Ruhl.) is a major disease of all commercialy grown *Prunus* species in Serbia. Sensitivity of *M. laxa* to benomyl and tebuconazole was tested in vitro. Strains were isolated from mummified plum fruits taken from orhards where benzimidazoles and DMI fungicides had been used for ten years. Micelial growth inhibition on PDA medium was monitored and sensitivity parameters were determined using probit analysis. EC_{50} values for benomyl were in the range 123.1 to 901.5 µg/kg, and 15.8 to 41.8 µg/kg for tebuconazole. According to resistance factor (RF) values, two of five isolates tested had low resistance to benomyl (3 < RF <20). All isolates tested were sensitive to tebucnazole (RF < 3). Dependence between benomyl use and sensitivity of *M. laxa* was established.

32.45* NOVEL ELICITIN-LIKE CELL WALL PROTEINS OF THE BIOCONTROL AGENT *PYTHIUM OLIGANDRUM* INDUCE MULTIPLE DEFENCE RESPONSES IN TOMATO AND SUGAR BEET. **S. Takenaka, H. Sekiguchi, A. Masunaka, S. Hase and H. Takahashi.** *Memuro Research Station, National Agricultural Research Center for Hokkaido Region, Shinsei, Memuro-cho, Kasai-gun, Hokkaido 082-0071, Japan. Email: stake@affrc.go.jp*

The cell wall protein fraction (CWP) of the biocontrol agent *Pythium oligandrum* contains two major proteins (POD-1 and POD-2), and has elicitor activity in tomato and sugar beet. Characterization of POD-1 and -2 indicated that their amino acid sequences were 82.9 % identical, and they have an elicitin domain followed by a C-terminal glycosylated domain, which is structurally similar to class III elicitins. However, phylogenetic comparison with representative elicitins and elicitin-like proteins showed that POD-1 and -2 are novel elicitin-like proteins. CWP pretreatments of roots in tomato and sugar beet resulted in reduction in disease severities caused by *Ralstonia solanacearum* and *Aphanomyces cochlioides*, respectively. The effect of CWP-treatment on the induction of defence-related genes in tomato and sugar beet was examined. CWP activated genes mediated by JA and ET-dependent signaling pathways in tomato, and triggered multiple genes related to the oxidative burst, phenylpropanoid synthesis and amino acid metabolism in sugar beet. POD-1 and -2 were purified by chromatography from CWP, and recombinant POD-1 and its elicitin domain were produced in *E. coli.* Furthermore, 16-25 amino acid-long peptides in elicitin domains of POD-1 were synthesized chemically. Transcript accumulation of defence-related genes was analyzed in tomato and sugar beet treated with equal concentrations of these preparations. In tomato, the synthetic peptides elicited defence gene expression, but their elicitor activity was significantly lower than that of CWP. In contrast, the synthetic peptides were able to induce defence gene expression comparable to CWP in sugar beet.

32.46 RHIZOBACTERIA-MEDIATED INDUCED SYSTEMIC RESISTANCE AGAINST *BEAN COMMON MOSAIC VIRUS* STRAIN BLACKEYE COWPEA MOSAIC IN COWPEA. **A.C. Uday Shankar, S. Chandra Nayaka, H. Bhuvanendra Kumar and H.S. Prakash.** *Department of Studies in Applied Botany, Seed Pathology and Biotechnology, University of Mysore, Manasagangotri, Mysore 570- 006, India. Email: ac.uday@gmail.com*

Plant protection by plant growth-promoting rhizobacteria is a hot topic of agricultural research. Plant viruses seem nearly impossible to control; instead, practical attempts are made to keep them in check, to reduce loss, basically to manage their existence within a crop. The use of bio-resources to replace chemicals is growing. In this context, plant growth-promoting rhizobacteria are novel and tools to provide substantial benefits to agriculture and show potential and promise as substitutes for chemicals. Seed treatment with PGPR has been used to enhance growth of several crops and to suppress the growth of seed-borne pathogens. PGPR strains viz., *Bacillus pumilus*, *B. amyloliquefaciens*, *B. subtilis*, and *Brevibacillus brevis* were employed to induce resistance against *Bean common mosaic virus* strain blackeye cowpea mosaic (BCMV-BlCM). In general, strains of PGPR promoted the vegetative and reproductive growth of cowpea plants. *B. subtilis* offered 56% protection against BCMV-BlCM in screenhouse conditions. Similar to the trend observed in screenhouse experiments, the best strains in reducing BCMV-BlCM disease incidence under field conditions were *B. subtilis* and *B. pumilus*, which recorded 52% and 54% protection against BCMV-BlCM. The protection offered by strains in combination against BCMV-BlCM was significantly greater than protection offered individually. A highest protection of 69% was recorded for strains *B. subtilis* + *B. pumilus* when applied to cowpea seeds under screenhouse conditions.

32.47 LIGHT-ENHANCED RESISTANCE IN LESION MIMIC MUTANT OF RICE INFECTED WITH *MAGNAPORTHE GRISEA.* **M. Ueno, A. Imaoka, J. Kihara and S. Arase.** *Faculty of Life and Environmental Science, Shimane University, Matsue 690-8504, Japan. Email: arase@life.shimane-u.ac.jp*

Sekiguchi lesion (sl)-mutant rice (cv. Sekiguchi-asahi) showed light-enhanced resistance to *Magnaporthe grisea* infection, inducing Sekiguchi lesion formation and tryptamine (Try) accumulation. Try is biosynthesized from tryptophan by tryptohan decarboxylase (TDC) and oxidized by monoamine oxidase (MAO). In *M. grisea*-infected leaves, TDC and MAO activities increased under light and maintained high levels even after Sekiguchi lesion formation, but not in darkness. Sekiguchi lesion formation and Try accumulation in leaves infected with *M. grisea* were significantly inhibited even under light by pre-treatment with cycloheximide (Cy) and heat. DNA fragmentation was significantly induced in *M. grisea*-infected leaves under light, but not in Cy- and heat-pre-treated leaves. When *M. grisea*-infected leaves were kept under different visible light conditions (400-700nm), the longer wavelengths induced greater Try accumulation, and TDC and MAO activities, and the H_2O_2 generation were enhanced. The effective wavelength for Try pathway activation agrees with that for induction of DNA fragmentation in leaves infected with *M. grisea*. Glyphosate [N-(phosphonomethyl) glycine] suppressed Sekiguchi lesion formation and Try accumulation in the sl mutant after *M. grisea* infection even under light. Such glyphosate inhibitions were blocked by supply of exogenous tryptophan, but not by exogenous phenylalanine. Under tryptophan starvation, catalase activity was maintained at a high level even under light, leading to the suppression of H_2O_2 generation and DNA fragmentation. We

conclude that Try pathway-mediated DNA fragmentation is involved in Sekiguchi lesion formation for light-enhanced resistance to *M. grisea* infection in the lesion mimic mutant of rice.

32.48 INDUCED RESISTANCE TO POWDERY MILDEW (*BLUMERIA GRAMINIS* F.SP. *TRITICI*) IN WINTER WHEAT (*TRITICUM AESTIVUM*). L. Vechet and L. Burketova. *Crop Division of Plant Genetics, Breeding and Product Quality, Crop Research Institute, Drnovska 507, Prague, Czech Republic. Email: vechet@vurv.cz*

Inducers of both synthetic origin: benzothiadiazole (BTH), salicylic acid (SA), and biological origin: glycine betain (GB), extracts prepared from oak bark (OB), *Reynoutria sacchaliensis* L. (RS), curcuma (CU) and ginger (GI) were effective against powdery mildew (*Blumeria graminis* f.sp. *tritici*) on the winter wheat cultivar Kanzler (susceptible standard to powdery mildew) in four-year small-field experiments. Differences between disease severity after treatment by individual inducers and untreated control evaluated by Pearson correlation were highly positive. Powdery mildew disease severity was strongly influenced (negative correlation) by average daily temperature in May and June. The biggest differences between control and inducer-treated plants were found in 2004 when the lowest temperature and the highest disease severity were reported. In contrast, development of the disease was lowest and the effect of the inducers was minimal in 2007, when temperatures were high. Inducers of biological origin, mainly extracts of OB, RS, CU and GB, were highly effective. Application of BTH produced a number of chlorotic blotches on leaves. Action of all inducers was long-lasting.

32.49 IS PROLINE INVOLVED IN DROUGHT-INDUCED SUSCEPTIBILITY OF AUSTRIAN PINE TO *DIPLODIA PINEA* SHOOT BLIGHT? C. Villari, P. Capretti and P. Bonello. *Dept. of Plant Pathology, The Ohio State University, Columbus, OH 43214, USA. Email: cate.vill@gmail.com*

Drought is known to predispose Austrian Pine (*Pinus nigra* Arnold) to *Diplodia pinea* (Desmaz.) J. Kickx [syn. *Sphaeropsis sapinea* (Fr.:Fr) Dyko and Sutton] shoot blight. It is also known that water stress induces accumulation of the amino acid proline in plant tissues, in which it is generally assumed to function as an osmoregulator. However, recent evidence suggests that proline accumulation may also interfere with host defense responses to fungal attack, perhaps by acting as a scavenger of reactive oxygen species (ROS), which are involved in the metabolic cascade leading to resistance in many pathosystems and can also be directly toxic to a pathogen. The aim of this study was to investigate if drought-induced proline accumulation may be one of the mechanisms for induced susceptibility of Austrian pine to *D. pinea*. Three-year-old potted Austrian pine seedlings were subjected to water stress and inoculated with the pathogen in a greenhouse experiment. Changes in susceptibility were monitored by measuring the resulting lesion lengths and were correlated with variation in *in planta* concentrations of proline and ROS, using H_2O_2 as a proxy. Potential direct effects of proline and H_2O_2 on fungal viability and growth were measured *in vitro*. Here we show that: (1) proline and H_2O_2 accumulate in shoots of drought-stressed seedlings and (2) proline protects the fungus from H_2O_2 toxicity *in vitro*. These results are discussed in relation to other hypotheses addressing the mechanisms of induced susceptibility of drought-stressed plants to fungal infection.

32.50 DEVELOPMENT OF STRATEGY FOR PATHOGEN-DERIVED RESISTANCE IN *NICOTIANA BENTHAMIANA* TO *BEET YELLOWS VIRUS* AND *BEET NECROTIC YELLOW VEIN VIRUS*. S. Vinogradova, A. Rakitin, A. Kamionskaya, A. Agranovsky and N. Ravin. *Bioengineering Centre, Russian Academy of Sciences, 117312 Moscow, 60-letia Octyabrya prospect 7, Bldg 1, Russia. Email: sveta2506@bk.ru*

To obtain transgenic plants resistant to *Beet yellows virus* (*Closterovirus*, BYV) and *Beet necrotic yellow vein* (*Benyvirus*, BNYVV, cause of rhizomania), we employed a strategy based on post-transcriptional gene silencing (PTGS). The 3'-terminal untranslated regions (3'-UTR) of the BYV or BNYVV genomes were used as the PTGS targets. The cDNA inserts BYVsil and BNYVVsil contained the respective 3'-UTRs as sense and antisense, separated by maize intron *ubi1*. The efficiency of these inserts as potential PTGS inducers was confirmed by a newly developed method of 35S-promoter-driven transient co-expression of the inductor RNA (BYVsil or BNYVVsil) and a target (GFP mRNA with a viral 3'-UTR) in *Nicotiana benthamiana*. As detected by Western blotting, plants agroinoculated with the target and the inducer expressed 10 times less GFP compared to the controls inoculated with the target only. *N. benthamiana* was also used as model plant for agrobacterial transformation with vectors containing the inserts of *bar* marker (phosphinotricinacetyltransferase) and BYVsil or BNYVVsil cDNAs, each under the control of separate 35S promoters. PCR analysis of regenerated *N. benthamiana* confirmed the presence of both the virus-specific and *bar* inserts in some lines. Additionally, *bar* expression was detected serologically in these lines. Transgenic *N. benthamiana* plants were multiplied *in vitro* and adapted to soil growing for further testing of resistance to artificial inoculation with the viruses.

32.51 RESEARCH ON *PENICILLIUM OXALICUM* ISOLATES. T. Wenhua, A. Turok, W. Qi and X. Yang. *Depart. of Plant Pathology, China Agricultural University, Haidian, Beijing100094, P. R. China. Email: wenhuatang@vip.sohu.com*

Hundreds of isolates of *Penicillium oxalicum* were obtained from soil and roots of wheat on selected medium for phosphate-dissolving micro-organisms. Several isolates showed ability to promote wheat seedling growth in pot experiments. The reasons for this were examined. Content of active phosphate increased in soil for some isolates, particularly in soil amended with phosphorite powder. Some isolates, while promoting plant growth, did not increase active phosphate content significantly. However, active phosphate content in the fungus body was significantly increased. Analysis of phytohormone concentrations in fermented cultures of the isolates showed that IAA content was higher in acidic conditions. Resistance induced by fermented cultures of *P. oxalicum* (P-o-41) was determined on *Xanthi*^{N} tobacco with the method of separating treated leaves and leaves inoculated with TMV. Results showed that reduction rate of spots comparing with CK (water treatment) was significant. No significant difference was found comparing with treatment by 38 ppm BTH. In experiments on control of wheat powdery mildew conducted in the greenhouse, disease incidence reduction was significant, but not for control of common root rot caused by *Bipolaris sorokiniana*. Controlling take-all with *P. oxalicum* (PG-P- 01) culture filtrate was tested in field trails. The results showed that disease incidence was reduced and yield increased. This project was supported by national 863 project 2006AA10A211.

32.52 DEFENCE RESPONSES IN SILICON TREATED COTTON INFECTED WITH *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM*. **J. Whan, L. Smith, E.K. Dann and E.A. Aitken.** *Department of Integrative Biology, University of Queensland, St Lucia, QLD, Australia. Email: j.whan@uq.edu.au*

Silicon has successfully ameliorated the impacts of fungal or bacterial pathogens in numerous plant-pathogen interactions. Research with several important crop and horticultural species infected by root or foliar pathogens has identified histological defence responses including the formation of fungitoxic phenolic aggregations in cells of infected plants, which appear to reduce pathogen fitness. Other biochemical responses identified include the production of phytoalexins and pathogenesis-related proteins, and also gene expression changes. *Fusarium* wilt of cotton caused by *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) is a major threat to cotton production in several regions in Australia. Control is currently based on use of resistant cultivars, on enforcing farm hygiene, and on crop rotation and stubble management. Silicon treatment has been shown to reduce *Fusarium* wilt incidence and severity in selected cotton cultivars in glasshouse trials. Following on from this, we are investigating histological and biochemical defence responses elicited by silicon treatment in cotton infected with Fov. Increased phenolics production in Fov-infected cotton treated with silicon has been observed, as have cellular accumulations of dense, osmiophillic material impeding fungal structures. Similar histological responses have also been observed in infected cotton not treated with silicon. Using real-time PCR, the expression of numerous defence-related genes has been investigated and results have shown that silicon treatment has affected the expression of some key pathogenesis related genes, such as thaumatin and osmotin, and also the expression of several other genes associated with lignin production and the inhibition of fungal wall degrading enzymes.

32.53 RESISTANCE INDUCED AGAINST *SCLEROTINIA SCLEROTIORUM* IN *BRASSICA NAPUS* TREATED WITH OLIGOCHITOSAN. **H. Yin, X. Zhao and Y. Du.** *1805 Group, Biotechnology Department, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, P. R. China. Email: yrafaelh@gmail.com*

Oligochitosan is considered to be a potent elicitor of plant defence; in this work induction of resistance by oligochitosan to *Sclerotinia sclerotiorum* on *Brassica napus* was studied. Although oligochitosan did not affect radial growth of *S. sclerotiorum* colonies on potato dextrose agar plates, it reduced the frequency and size of rot compared to untreated controls when applied to oilseed rape before inoculation with *S. sclerotiorum*. The best resistance-inducing concentration was 50 µg/ml and the best pretreatment time was 3 days before inoculation with *S. sclerotiorum*. The activities of plant resistance-correlated enzymes including superoxide dismutase, lipoxygenase and catalase were induced by oligochitosan on both the treated leaves and leaves on the same plants, not directly treated. Furthermore, mRNA levels of PDF1.2 and BnMPK4 were also induced by oligochitosan. Our results suggest that the oligochitosan treatments induced host resistance to *S. sclerotiorum*.

32.54 INDUCTION OF SYSTEMIC ACQUIRED RESISTANCE IN TEA (*CAMELLIA SINENSIS*) BY PLANT ACTIVATORS. **K. Yoshida, K. Yamada and R. Sonoda.** *Tea Pest Management Research Team, National Institute of Vegetable & Tea Science, Kagoshima 898-0087, Japan. Email: gohteny@affrc.go.jp*

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the most important cash crops in the warm southwestern areas of Japan. About 75% of the tea fields in Japan grow 'Yabukita' which is susceptible to many diseases such as anthracnose, gray blight and bacterial shoot blight. Therefore, broad range effective and durable disease control methods are required. Plant activator (PA) is a compound that induces systemic acquired resistance (SAR) in plants. In the present study, we tried to use PA for disease control in tea. The different type plant activators, tiadinil (TDL) and validamycin A (VMA) were used in this study. Aqueous solutions of TDL or VMA were sprayed on field-grown 'Yabukita'. Tea leaves were harvested by time course and inoculated with conidial suspensions of grey blight fungus, *Pestalotiopsis longiseta* (PL). PL lesion development was suppressed by pretreatment with each PA and the induced resistance was present at least 30 days after PA treatment. Both TDL and VMA induced disease resistance in tea not only on treated leaves but also untreated-upper leaves. In field trials, anthracnose, grey blight and bacterial shoot blight were suppressed with TDL and VMA. Based on these experiments, we suggest that TDL and VMA induces SAR to some diseases on tea.

32.55 A YEAST EXTRACT PREPARATION 'AGREVO EX' INDUCES DISEASE RESISTANCE IN TEA (*CAMELLIA* SINENSIS). **K. Yoshida, A. Ogino and Y. Matsuura.** *Tea Pest Management Research Team, National Institute of Vegetable & Tea Science, Kagoshima 898-0087, Japan. Email: gohteny@affrc.go.jp*

It is well known that yeast extract induces non-specific disease resistance in plants in laboratory experiments. A yeast extract preparation 'AGREVO EX' (AGEX) induces accumulation of basic PR proteins and ethylene biosynthesis in tobacco (Obara *et al.* Jpn. J. Phytopathol. 73: 94-101, 2007). Therefore, the mode of action of AGEX is different from plant activators that induce systemic acquired resistance (SAR) in plants. In the present study, we investigated the induced defense activity of AGEX in field-grown tea (*Camellia sinensis* (L.) O. Kuntze cultivar 'Yabukita' by laboratory and field experiments. Aqueous solutions of AGEX were sprayed on tea plants and leaves were harvested by time course and inoculated with conidial suspensions of *Colletotrichum theae-sinensis* (CT) and *Pestalotiopsis longiseta* (PL). Lesion development of both fungi was suppressed by pre-treatment with AGEX. No remarkable activity of AGEX was observed on mycelial growth or conidial germination of CT and PL. AGEX induced disease resistance not only in treated leaves but also untreated-upper leaves. Field inoculation tests showed that AGEX spraying reduced CT and PL infection in leaves. Application of AGEX on tea did not influence the yields of tea shoots, or the chemical constitutions and quality of green tea. These results suggest that AGEX induces systemic disease resistance on tea like plant activators. AGEX will be a useful tool for disease control on tea especially in low-input sustainable agriculture.

32.56 RIDOMYL-INDUCED RESISTANCE AGAINST PINK ROT DISEASE OF POTATO. **M. Zaker.** *Agriculture Research Centre, P.O. Box: 36155-313, Shahrood, Iran. Email: mzakerus@yahoo.com*

Potato pink rot in Semnan province of Iran is mainly caused by *Phytophthora cryptogea*. During 2005-2006 a study was conducted to determine the effect of Ridomyl in producing induced resistance in three potato varieties agaist *P. cryptogea* in a split plot design, with three varieties as main plots and 5 treatments as

sub-plots. Treatments were: 1- Ridomyl applied at sowing time @ 2.5 kg/ha, 2- one time Ridomyl applied at flowering @ 2.5 kg/ha, 3- Ridomyl applied at flowering and again after 28 days, 4- maneb applied three times, at flowering and then at 10 days intervals, 5- Control. After 1, 2 and 3 months of storage, healthy tubers were artificially inoculated with the pathogen and incubated at 27°C for 6 days. Tubers were then cut and after the appearance of pink colour, fungal progress was measured. 20 tubers were used in each test. Statistical analysis of data showed significant differences between treatments in all tests (p=0.1%). Two applications of Ridomyl at a 28-day interval after one month of storage gave best results in inhibiting pathogen progress. One Ridomyl application was less effective. Treatments 4 and 5 had no effect in inhibiting the pathogen and fell in the same group in all tests. It seems that induced resistance decreases in time, as the mean difference between pathogen progress of treatments 3 and 5 after 1, 2 and 3 months of storage were 2.35, 1.02 and 0.64 cm respectively. There were no significant differences between varieties.

Innovative disease control strategies

34.1 INHIBITORY EFFECT OF ALUMINIUM CHLORIDE AND SODIUM METABISULFITE ON *FUSARIUM SAMBUCINUM*.

T.J. Avis, M. Simard, M. Michaud, D. Rioux and R.J. Tweddell. *Centre de Recherche en Horticulture, Pavillon de l'Envirotron, Université Laval, Québec, QC, Canada. Email: russell.tweddell@crh.ulaval.ca*

Aluminium chloride and sodium metabisulfite have been shown to possess antimicrobial activity against several plant pathogens. In an effort to develop new methods to control the fungus *Heterobasidion annosum*, cause of a major root rot of conifers in temperate regions, the objectives of this study were (1) to evaluate the inhibitory effect of aluminium chloride and sodium metabisulfite on conidial viability of the pathogen and (2) to visualise the effect of these salts on fungal membrane integrity using the SYTOX Green fluorochrome and electron microscopy. Results demonstrated that as little as 1 mM of sodium metabisulfite and 10 mM of aluminium chloride were sufficient to kill *H. annosum* conidia. Fluorescence analysis with SYTOX Green indicated that both salts affect fungal cells apparently through the loss of cellular membrane integrity. Ultrastructural observations confirmed that these salts cause plasmalemma invagination and/or rupture and cytoplasmic aggregation and/or leakage. This study points to the possibility of exploiting these salts as antifungal agents for controlling annosum root rot through their application to cut tree stumps.

34.2 NUTRIENTS TO ENHANCE THE BIOCONTROL ACTIVITY OF YEASTS AGAINST BLUE MOULD OF APPLES – TOWARDS RESTORATIVE BIOLOGICAL CONTROL.

K.S.H. Boyd-Wilson and M. Walter. *The Horticulture and Food Research Institute, P.O. Box 51, Lincoln, New Zealand. Email: kboyd-wilson@hortresearch.co.nz*

A number of studies have shown that nutrient supplements enhance the biocontrol activity of yeasts against a range of pathogens, including *Penicillium expansum,* one of the causal organisms of blue mould of apples. The aim of this research was to identify in the laboratory, nutrients that improve the performance of yeasts. From the results of previous laboratory studies, using an apple wound assay, calcium chloride ($CaCl_2$, 20 mg/ml) and calcium carbonate ($CaCO_3$, 20 mg/ml) were chosen and evaluated against *P. expansum* at 10^5 spores/ml using three yeasts known to inhibit lesion development. Cell suspensions were prepared in sterile distilled water (SDW) and in the nutrient at 10^5, 10^6 and 10^7 CFU/ml. The nutrient-alone treatment was also tested against the pathogen. When yeasts were prepared in SDW, a yeast cell concentration of 10^7 CFU/ml reduced disease incidence by an average of 85% compared with the pathogen control. Disease incidence was not reduced at 10^5 or 10^6 CFU/ml. Calcium chloride alone reduced disease incidence by 36%. When yeast cell suspensions were prepared in $CaCl_2$, disease incidence was reduced by 80% and 85% at 10^5 or 10^6 CFU/ml, respectively. Calcium carbonate alone reduced disease incidence by 82% and addition of yeasts did not improve this. As a combination of the yeast and the nutrient supplement $CaCl_2$ improved performance of both the yeast alone and the nutrient alone; this nutrient will be trialled in the field as a step towards a new disease management strategy termed restorative biological control.

34.3* GENOTYPE ROTATION FOR MANAGEMENT OF RESISTANCE TO THE SORGHUM ANTHRACNOSE PATHOGEN *COLLETOTRICHUM SUBLINEOLUM*.

C.R. Casela, A. da Silva Ferreira and F. Giacomini dos Santos. *Embrapa, Maize and Sorghum Research Center, Rod. MG424, km 65, C.P. 151, 35701-970 Sete Lagoas, MG, Brazil. Email: casela@cnpms.embrapa.br*

The high variability of *Colletotrichum sublineolum* in Brazilian conditions poses a continuous threat to the control of anthracnose through genetic resistance. Several alternatives such as dilatory resistance, cultivar mixtures, and gene pyramiding have been evaluated as ways to stabilize pathogen populations in the different sorghum-growing areas of the country. This work reports results on the rotation of sorghum genotypes in time as a strategy to increase the durability of genetic resistance and to stabilize populations of *C. sublineolum*. Anthracnose progress and the pathogen population virulence structure were evaluated, for three consecutive years, on sorghum genotypes BR008, BR005, BR009, and CMSXS210 both in continuous and alternating plantings in a no-tillage system, totaling 14 treatments. Specific responses of the pathogen and the predominance of less complex races (as indicated by the number of virulence genes) were observed in response to each sorghum genotype. Disease progress in each treatment was influenced by the virulence structure of the pathogen population developed in response to the genotype cultivated in the previous year. Higher disease severities were observed in treatments in which the same genotypes were cultivated continuously in the same area, as compared to the genotype rotation treatments. These results indicate that this can be a viable alternative to manage resistance to *C. sublineolum* in Brazil.

34.4* WHAT CAUSED THE RECENT EMERGENCE OF ANTHRACNOSE DISEASE ON GOLF COURSE GREENS IN NORTH AMERICA?

J.A. Crouch, B.B. Clarke and B.I. Hillman. *Rutgers University, 59 Dudley Road, New Brunswick, NJ, 08901, USA. Email: crouch@aesop.rutgers.edu*

Grasses cultivated as turf make up a major component of the North American landscape. Beginning in the 1990s, anthracnose caused by the fungus *Colletotrichum cereale* emerged as one of the most destructive diseases of golf course turf, with its incidence, severity and geographic range greatly expanded. The sudden emergence of this disease on greens is puzzling. Was the spread of turfgrass anthracnose due to the introduction of novel genotypes or did recent environmental/cultural change provide an opportunity

for adaptation by endemic genotypes? Here we present an investigation of the origin of North American turfgrass anthracnose epidemics. Genotypic signatures from four genes and 22 microsatellite markers were analyzed from an extensive sample of pathogenic turfgrass isolates and non-pathogenic prairie and cereal crop isolates from the US, Canada, Japan, New Zealand, Germany and the Netherlands. Eleven genetic populations were identified: three turfgrass pathogen groups, six prairie/cereal-derived groups and one diverse group comprised of both turf and non-turf isolates. How populations were defined significantly affected how variation was partitioned: 72% was attributable to genetic populations, 40% was attributable to ecosystem, and 25% was attributable to lifestyle (pathogen vs. non-pathogen). High levels of genetic diversity, ecosystem specificity, turf host specificity and recombining populations provided evidence of endemic populations assuming a pathogenic lifestyle in response to changing environmental conditions on golf courses. Endemism is also consistent with the observation that North American turfgrass populations and genotypes of *C. cereale* are more closely related to one another than to any international or non-turf isolates.

34.5 EFFECTS OF FOSETYL AL TREATMENTS ON GRAPEVINE PLANTS IN ESCA MANAGEMENT. **S. Di Marco, R. Roberti, F. Calzarano, C. Amalfitano, A. Veronesi and F. Osti.** *Istituto di Biometeorologia, CNR, Via Gobetti 101, 40129, Bologna, Italy. Email: s.dimarco@ibimet.cnr.it*

Esca is the most severe and widespread wood disease of grapevines. Although foliar symptom expression is not correlated with vine wood deterioration because of the intermittent nature of the leaf symptom, production losses are associated with foliar symptom expression, because diseased but symptomless vines can produce grapes with similar characteristics to grapes from healthy vines. Foliar symptoms seem to be associated with transport of toxins from infected wood to leaves. A reduction in esca symptom severity was observed in many-year trials applying commercial formulations of fosetyl Al either before or soon after the appearance of esca in vineyards properly managed and following the plan for downy mildew control. Moreover, in potted vines, there was a reduction of the average length of internal necrosis following inoculations with vascular esca pathogens and treated with fosetyl Al. Investigations were carried out in order to explain the activity of fosetyl Al towards esca and associated pathogens. Physiological parameters of potted plants were measured: net photosynthetic rates of infected treated plants were depressed by 20-50% for 24-60 hours after treatment compared to infected untreated ones. Total leaf proteins were extracted and analysed spectrophotometrically. In infected treated leaves chitobiosidase and glucosaminidase increased from 3 to 9 days after treatment, glucanase was enhanced at the third day and lypoxigenase increased over time at pH 6.5, compared to untreated leaves. No differences in trans-resveratrol and ε-viniferin contents were found in the wood of treated and untreated vines.

34.6 *IN VITRO* PRODUCTION OF ORGANIC ACIDS BY *PENICILLIUM EXPANSUM*. **I. Donati, D. Mazzoni, M. Mari and P. Bertolini.** *CRIOF-DIPROVAL, Alma Mater Studiorum, University of Bologna, Italy. Email: idonati@agrsci.unibo.it*

Twenty isolates of *Penicillium expansum,* recovered from rotten pome fruits, were tested in vitro for ability to produce acid. To measure acid production, isolates were grown on creatine sucrose agar for 7 days at 20°C, the halo round the colony revealing a change of pH. Three isolates out of 20 did not produce the halo, while the others showed wide halos, revealing abundant acid production. The same isolates were also grown in yeast sucrose liquid medium (YSM) at different pH levels (7.0, 5.0, 3.0) for 3 days at 20°C. In general, the greatest reduction of pH was observed in isolates grown at pH 7. Four isolates maintained the pH of the medium close to 7, but the others significantly decreased the pH, raging from 5.5 to 4.1. To determine the prevalent organic acids produced, isolates were grown on liquid YSM for 7 days at 20°C, and the medium was analyzed by HPLC. Results showed abundant production of galacturonic (GA), malic, and citric acids and some unknown organic acids in smaller amounts. A high concentration of GA was recorded in the isolates that most reduced the pH of the medium. These isolates produced GA within 48 h of inoculation, while the concentration of citric and malic acid initially increased, remained constant up to 72 h and then increased again. More work is needed to understand whether organic acid production and the aggressiveness of *P. expansum* isolates are correlated.

34.7 EUROPEAN NETWORK FOR THE DURABLE EXPLOITATION OF CROP PROTECTION STRATEGIES (ENDURE). **N. Evans, I. Denholm, M.Barzman and P. Ricci.** *Rothamsted Research, Harpenden, Herts., AL5 2JQ, UK. Email: neal.evans@bbsrc.ac.uk*

The overall objective of this Network (acronym ENDURE) is to reshape European research and development on pesticide use in crops and establish Europe as a leader in the development and implementation of sustainable pest management strategies. We will create a coordinated structure that takes advantage of alternative technologies, builds on advances and complementary expertise in agricultural sciences, ecology, behaviour, genetics, economics and social sciences and connects researchers to other stakeholders in extension, industry, policy-making and civil society. This multi-disciplinary and cross-sector approach is designed to foster the development and implementation of strategies rationalising and reducing pesticide inputs as well as reducing risks. Our specific goals are to integrate research capacity and resources currently fragmented across Europe, to enhance the research-to-R&D innovation process by creating working relationships between researchers and practitioners in extension and farming, to engage with industry, policy-makers and civil society to help define the research agenda, and to pass on knowledge, know-how and resources through training, education, and dissemination.

34.8 CONTROL OF FAIRY RING SYMPTOMS IN TURFGRASS WITH FUNGICIDES AND SOIL SURFACTANTS. **M. Fidanza, S. McDonald, B. Martin, F. Wong, S. Kostka and D. Settle.** *Pennsylvania State University, Berks Campus, 2080 Tulpehocken Road, Reading, PA, 19610-6009, USA. Email: maf100@psu.edu*

Fairy ring is a common disease of turfgrasses worldwide, and is caused by soil-borne basidiomycete fungi. Visual symptoms in turfgrasses are categorized into three distinct "types". Type I fairy ring symptoms are the most severe, with wilted, necrotic, and dead turf appearing in rings or arcs. These sites are associated with hydrophobic or water repellent soil conditions, extremely high concentrations of ammonium nitrogen in the soil root zone, and low soil moisture content. Type II symptoms are the appearance of circles or arcs of dark green, luxuriant turf. The turf responds as if receiving a dose of nitrate nitrogen, which is provid-

ed by the breakdown of organic matter by the fairy ring fungi. Type III symptoms are mushrooms or basidiocarps growing in rings or semicircles, and these mushrooms can appear alone or in groups or clusters. Also, mushrooms are often observed growing along the edges of type I or type II rings. Successful management or control of fairy ring symptoms in turfgrasses on golf courses, especially type I symptoms, has been inconsistent. Recent field studies in the USA, however, have shown the benefit and effectiveness of using fungicides (i.e., azoxystrobin, flutolanil, polyoxin-D, pyraclostrobin, and others) in combination with soil surfactants (a modified alkylated polyol, and others) to significantly reduce symptoms. Overall, fairy ring symptoms were reduced in test plots treated with fungicides plus soil surfactants compared to plots treated with fungicides alone or untreated plots.

34.9 ANTIFUNGAL EFFECT OF PLANT METABOLITES AGAINST *PHYTOPHTHORA CAPSICI, IN VITRO* AND *IN VIVO*. **H.E. Flores-Moctezuma.** *Centro Desarrollo de Productos Bióticos IPN, Carr. Yautepec-Jojutla km.8.5, San Isidro, Yautepec, Morelos, C.P. 62731, Mexico. Email: hfloresm@ipn.mx*

An alternative for the ecological management of *Phytophthora capsici* Leo. in soil is the application of secondary antifungal plants metabolites. Thymol, carvacrol, citral, citronellol, geraniol, linalool, cineolle, borneol, menthol, eugenol, and naringinin were tested *in vitro* starting at 5% concentration down to 0.005% in Petri plates with PDA medium, where a mycelial disk of the pathogen was placed. Metabolites which inhibited mycelial growth were used in various mixtures at minimum inhibitory rates (eugenol at 2500 ppm, carvacrol at 500 ppm, geraniol at 500 ppm, and thymol at 1200 ppm). The best combinations were then evaluated in the greenhouse. For *in situ* evaluation, 200 g per pot of a mixture of sorghum grain and the pathogen were mixed with the soil, making up the following treatments geraniol-eugenol, eugenol-carvacrol, geraniol-eugenol-thymol, metalaxyl, and the inoculated and uninoculated checks. The soil was covered with plastic. After 5 days, five seedlings of "criollo" pepper were transplanted to each pot; there were four replications per treatment consisting of 10 pots each. After 40 days, we counted the number of plants alive, and measured height, and fresh and dry weight. The results indicated that metalaxyl and the inoculated check had 70 and 60% plants survival, whereas eugenol-carvacrol (2500 and 500 ppm) and geraniol-eugenol-thymol (500, 500, and 1200 ppm) had 91 and 84%, respectively; in addition, plants showed greater height, fresh and dry weight in comparison with the rest of the treatments.

34.10 IMPACT OF PLANT EXTRACTS AND ORGANIC AMENDMENTS ON THE GROWTH OF *RALSTONIA SOLANACEARUM* AND SEVERITY OF POTATO BACTERIAL WILT. **D.A. Fontem and B.M.J. N'tchorere.** *Faculty of Agriculture, University of Dschang, Box 208, Dschang, Cameroon. Email: dfontem@yahoo.com*

Continuous potato (*Solanum tuberosum)* production in the tropics and subtropics is usually handicapped by bacterial wilt incited by *Ralstonia solanacearum*. Laboratory and screenhouse experiments were conducted to assess the bactericidal activities of plant extracts and organic amendments respectively on pathogen growth and bacterial wilt severity. The antibacterial activity of the extracts was determined by colony growth on potato sucrose agar medium amended with various concentrations of the extracts. Potato tubers (cv. Cipira) were planted at Dschang and Foumbot in 10% organic amended soils and inoculated 30 days later with 10 ml of 10^7 cfu/ml of bacterial suspension. Data on wilt severity and tuber production were recorded. Methanol leaf extracts of *Crotalaria falcata* and *Tephrosia vogelii* were more active (ED_{90} = 1.40–5.94 mg/ml) than the corresponding acetone extracts (ED_{90} = 47.60-77.14 mg/ml), while the reverse was observed for leaf extracts of *Brassica integrifolia* and *Cissus aralioides*. All organic amendments significantly reduced bacterial wilt severity at both sites. Significant (P = 0.001) negative correlations were observed between bacterial wilt severity and tuber yields. *Crotalaria* and *Tephrosia* amendments were more effective in the reducing wilt severity and increasing tuber yield than *Brassica* or *Cissus* amendments. Results indicate a potential of plant extracts in bacterial wilt management and a necessity for an adoption of integrated pest management strategies, through a judicious use of organic amendments in potato production.

34.11 INNOVATIVE APPROACH FOR MANAGEMENT OF TOMATO LEAF CURL. **P.A. Fugro, V.B. Mehta and D.R. Pawar.** *Dr. B.S.Konkan Krishi Vidyapeeth, Dapoli 415 712, MS, India. Email: sfugro@yahoo.co.in*

Tomato crop is affected by a number of fungal, bacterial and viral diseases in India. Viral leaf curl is a quite serious disease of tomato in Konkan region of Maharashtra (India) and has become a major set-back in commercial cultivation of this crop. So far, there are no direct measures recommended for the control of leaf curl in any part of the world. Most of the procedures that can be used to some effect, involve evasive measures designed to reduce the spread by vectors and to minimize the effect of infection on yield. Such measures offer no permanent solution to a virus disease problem in a particular area. Resistant or field tolerant varieties may also not offer permanent protection because new strains of the virus arise from time to time. An experiment was laid out in randomized block design with 3 replications and 12 treatment combinations comprising herbal antiviral product (Viroasan), Neem (*Azadirachta indica*) based pesticide (Econeem plus), bioplant growth stimulant-cum-regulator (Bio-force) and micronutrients (Multiplex). Among individual treatments, maximum disease reduction (40.96%) was obtained in treatment comprising Econeem plus followed by Virosan (40.46%). Bioforce and Multiplex were not effective as individual treatments. However, among all the treatments, the combination of Virosan + Econeem plus + Bioforce proved to be most effective and recorded 57.7 % disease reduction over control. The same treatment also recorded significantly higher yield (13.44 t^{-ha}) over control (5.33 t^{-ha}).

34.12* TRIPLE RNAI-CONSTRUCTS FOR BROARD-RANGE RESISTANCE AGAINST FUNGAL LEAF DISEASES. **A. Gay and P. Schweizer.** *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Dept. of Cytogenetics and Genome Analysis, Research Group Transcriptome Analysis, Corrensstrasse 3, D-06466 Gatersleben, Germany. Email: gayalex@ipk-gatersleben.de*

Fungal diseases are usually controlled by different agronomic techniques, applications of natural or chemical substances and/or resistance breeding strategies. Recently technologies involving small interfering RNA (siRNA) have been proposed to protect plants against root nematodes (Bakhetia et al. 2005, Trends in Plant Science). Here, we tested RNAi as a novel plant protection strategy against fungal diseases. RNAi constructs targeting conserved fungal genes, which may be active against several pathogens, were built in combination with three plant promoters.

Two of these promoters are pathogen induced and/or epidemic-specific. In addition to single-target RNAi constructs, triple-target constructs, that should allow the expression of three different fungal genes, were made to enhance the fungus-inhibitory effect. Single- and triple-target RNAi constructs were tested in transient experiments in barley leaves, which were inoculated after bombardment with *Blumeria graminis* f.sp. *hordei* spores. The haustorial index (haustoria per transformed GUS-expressing cells) was used as a measure of silencing effect on the fungus. Significant effects of most of the single and triple RNAi constructs were found. The main objective of this project is the generation of transgenic plants, which possess high resistance levels against many fungal plant pathogens.

34.13 EFFICACY OF *BACILLUS SUBTILIS* AND *AMPELOMYCES QUISQUALIS* ALONE AND IN COMBINATION WITH FUNGICIDES AGAINST *PODOSPHAERA XANTHII* OF ZUCCHINI. G. Gilardi, D.C. Manker, M. Benuzzi, A. Garibaldi and M.L. Gullino. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: giovanna.gilardi@unito.it*

Powdery mildew, incited by *Podosphaera xanthii*, causes severe losses on zucchini crops grown in the open as well as in greenhouses in Mediterranean countries. Intensive use of chemicals against this pathogen has often resulted in development of pathogen resistance. Biological control agents used alone and in combination with fungicides are one possible disease management strategy for cucurbit powdery mildew control. Five trials were carried out under greenhouse conditions through experimental inoculation of *P. xanthii* at levels able to cause slight infection on zucchini plants treated with the field dosages of azoxystrobin (0.186 ml/l). *Bacillus subtilis* QST 713 (Serenade WP) at 0.2 and 0.4 g/ml, *Ampelomyces quisqualis* (AQ 10) at 0.029 g/l, azoxystrobin (Ortiva) at 0.186 ml/l, mychlobutanil (Thiocur forte) at 0.071 ml/hl were applied alone or mixed together before spraying. Penconazole (Topas 10 EC) at 0.041 ml/l and quinoxifen (Arius) at 0.056 ml/l, were applied alone as chemical control. Each treatment was repeated at 6-8 day intervals. In the presence of medium-high disease severity, *B. subtilis* when combined with azoxystrobin provided better powdery mildew control than azoxystrobin used alone. A similar synergistic effect may be exploited by the combination of *A. quisqualis* with mychlobutanil. Quinoxifen was very effective in controlling *P. xanthii*, while penconazole and mychlobutanil were only partially effective. Our results support the possibility of a synergistic effect among the biocontrol agent *B. subtilis* and QoI fungicides. This interaction is very interesting because of the presence of resistance towards Qo inhibitors (QoI) in populations of *P. xanthii*.

34.14 INTEGRATION OF SOIL SOLARIZATION WITH VESICULAR-ARBUSCULAR MYCORRHIZAS AND *AZOTOBACTER CHROCOCCUM* FOR MANAGEMENT OF SAPLING WILT OF MANGO. G. Harender Raj and S.D. Sharma. *Department of Mycology and Plant Pathology,Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan 173230, Himachal Pradesh, India. Email: hrg_mpp@yahoo.com*

Sapling wilt of mango (*Mangifera indica*) in nurseries caused by *Fusarium solani* is an important disease in India resulting in around 30% mortality. In this study, soil solarization was integrated with four indigenous isolates of vesicular-arbuscular (VA) mycorrhiza (AMUHF1, AMUHF2, AMUHF3 and AMUHF4) and two isolates of *Azotobacter chrococcum* (AZUHF1 and AZUHF2) in different combinations to observe their effect on sapling wilt and also on sapling shoot and root length. Soil was solarized with transparent polyethylene mulch (25 µm thick) for 40 days during May and June. Plots were also sterilized with formaldehyde in another treatment. Plots were then inoculated with different isolates of VA-mycorrhiza and *A. chrococcum* in different combinations. Soil solarization resulted in 11.6 ºC increase in average maximum soil temperature in comparison to unsolarized soil. Soil solarization in combination with different isolates of VA-mycorriza and *A. chrococcum* resulted in 0 to 2.9% of sapling mortality in comparison to 32.8% in control plots. However, soil solarization in combination with AMUHF4 isolate of VA-mycorrhiza and AZUHF1 isolate of *A. chrococcum* was found most effective, with zero disease incidence and also 123.1 and 85.4% increases in shoot and root length respectively in comparison to control. However, different combinations of VA-mycorrhiza isolates and *A. chrococcum* inoculated in formaldehyde-treated and untreated plots proved less effective with 0 to 4.1 and 6.9 to 17.4% sapling mortality, respectively and also resulted in less increase in shoot and root length.

34.15 POST-TRANSCRIPTIONAL GENE SILENCING AND *PAPAYA RINGSPOT VIRUS* RESISTANCE. P. Itthisoponpisarn, R. Yapom and Y.-K. Yap. *Institute of Molecular Biology and Genetics, Mahidol University, Salaya, Nakhon Pathom 73170, Thailand. Email: fryky@mahidol.ac.th*

Increasing evidence has indicated that post-transcriptional gene silencing (PTGS) is a natural antiviral mechanism in plants. Virus-derived transgenes have long been used to construct virus-resistant transgenic plants. Lately, various transgenic plants expressing virus-derived transgenes which produce double-stranded RNA (dsRNA) have been reported to be highly resistant to the homologous virus. In this study, we are developing a transient system to investigate *Papaya ringspot virus* (PRSV) resistance induced by exogenous application of dsRNA into zucchini plants as a crop model. The objectives of our study are (i) to examine and compare different gene regions in conferring viral resistance (ii) to investigate the viral resistance mechanism and explore the potential of dsRNA inoculation as a way of controlling viral disease in addition to the transgenic approach. In this system, we used either PRSV virions or a PRSV cDNA clone as inoculum source. Double-stranded RNAs were produced by expressing hairpin RNA in *Escherichia coli*. PRSV virions and dsRNA were introduced by mechanical inoculation whereas the PRSV cDNA clone was introduced by particle bombardment. We found that dsRNA derived from two gene regions tested, namely the coat protein and Hc-Pro genes conferred PRSV resistance. However, our result indicated that both the dsRNA (inducer of PTGS) and virus source (target) had to be present in the same cell at the same time in order to confer PRSV resistance in the test plants.

34.16 EARLY SEASON SUPPRESSION OF *SCLEROTINIA HOMOEOCARPA* INOCULUM IN TURF. J.E. Kaminski and A.I. Putman. *University of Connecticut, Department of Plant Science, 1376 Storrs Road Unit 4067, Storrs, CT 06269, USA. Email: john.kaminski@uconn.edu*

Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) is a major disease of turfgrasses in the United States. The pathogen is believed to overwinter as mycelium or stromata in previously infect-

ed tissues or in dead or decaying plant material in the thatch. The ability to reduce *S. homoeocarpa* inoculum with fungicides applied early in the season was assessed on a Connecticut golf course fairway in 2006 and 2007. Various fungicides were applied on 13 April 2006 and 24 April 2007. Treatments were initiated after the second spring mowing and approximately 4 to 6 weeks prior to the appearance of dollar spot symptoms. In 2007, the influence of post-application irrigation was also assessed. Disease suppression was determined by visually assessing the percent dollar spot per plot (1.6 or 2.3 m^2). Disease pressure was considered high in both years and untreated plots had an average of 33% and 21% dollar spot on the final rating dates in 2006 and 2007, respectively. When compared to the untreated control, dollar spot was reduced 48-67% for up to 5 weeks after the appearance of initial symptoms in 2006. In 2007, percent dollar spot was reduced between 68-87% for approximately 6 weeks after initial symptoms appeared. Post-application irrigation did not positively or negatively influence the fungicides' ability to suppress disease. Results of these field experiments suggest that *S. homoeocarpa* may be active in the thatch, soil, or living plant tissues for up to 1 month prior to the appearance of dollar spot symptoms.

34.17 ETIOLOGY AND EPIDEMIOLOGY OF *PYTHIUM* ROOT DYSFUNCTION OF CREEPING BENTGRASS IN NORTH CAROLINA, USA. J.P. Kerns and L.P. Tredway. *Department of Plant Pathology, North Carolina State University, Raleigh, NC, USA. Email: lane_tredway@ncsu.edu*

Pythium root dysfunction (PRD) has become a widespread problem in golf course putting greens established with creeping bentgrass (*Agrostis* sp) in the southeastern United States. Symptoms appear in distinct circular patches during summer and other times of stress, but the majority of pathogen activity is observed in the fall and spring. Of 80 *Pythium* isolates collected from roots showing symptoms of PRD, 58 (73%) were identified as *P. volutum* and 16 (20%) were identified as *P. torulosum*. A variety of saprophytes and weak pathogens comprised the remaining isolates. Pathogenicity tests were conducted by inoculating roots with isolates of *P. volutum* or *P. torulosum*, incubating at 24ºC/16ºC to permit root infection, then exposing to heat at 32ºC/26ºC. Neither species induced foliar symptoms nor impacted creeping bentgrass root growth at 24ºC/16ºC. Symptoms of foliar dieback appeared in creeping bentgrass inoculated with *P. volutum* after 12 days of heat exposure, whereas *P. torulosum* induced no significant symptoms after 4 weeks of heat exposure. *P. volutum* infections significantly reduced the growth and survival of creeping bentgrass roots at 32ºC/26ºC, whereas roots inoculated with *P. torulosum* continued to increase in depth and mass at this temperature. In growth chamber experiments to determine the effect of temperature on infection of creeping bentgrass roots by *P. volutum*, this species was most aggressive at 16ºC to 24ºC, but significant activity also occurred at 12ºC. Preliminary investigations indicate that *P. volutum* infections initially increase water and nitrate uptake at 32ºC, followed by a sharp decline after 14 days of heat exposure.

34.18 *SIMILIA SIMILIBUS CURENTUR* STRATEGY (SET A THIEF TO CATCH A THIEF) FOR BIOCONTROL OF WHEAT BUNT USING ENDOPHYTIC ANTAGONISTIC STRAINS OF *BACILLUS SUBTILIS*. R.M. Khairullin, A.A. Egorshina, M.A. Luk'yantzev, R.R. Kamaletdinov. *BIOFORT Ltd, Prospekt Oktyabrya, 15, Ufa, 450001, Russia. Email: khram@ufanet.ru*

Common bunt and wheat smut are controlled mainly using chemical fungicides. The problem of the biocontrol of these diseases remains unsolved. The endophytic properties of the fungi *Tilletia* and *Ustilago* allow us to compare these pathogens with specific endophytes, microorganisms that can pass most of their life cycle inside plants. Some bacterial biocontrol agents are endophytes, for example, *Bacillus subtilis* strain 26D, which is the basis of the fungicide phytosporin. This can penetrate inside plants and stimulate growth and disease resistance. On this basis, one innovative way to control *Tilletia* and *Ustilago* pathogens can be the principle *Similia similibus curentur*, that is, "Set a thief to catch a thief": endophytic antagonistic bacteria or other microorganisms may inhibit or stop growth of endophytic plant pathogens. Using technology developed by us we have found in wheat plant tissue endophytic strains of *B. subtilis* able to reduce circulation of common bunt. From more than 50 strains we have selected some that have reduced bunt circulation as effectively as chemical fungicides in field conditions. We have also developed the technology of culturing these bacteria in a new economic universal gas-vortex gradientless bioreactor. Preliminary results show that culture for 16-18 hours can give concentration up to 30 bln./ml with the contents up to 99%. This approach is therefore quite realistic to research and develop the technology for biofungicide control of cereal smut disease. The research was supported by the Russian Foundation for Assistance to Small Innovative Enterprises (FASIE, project 4822r/7290).

34.19 DO WE NEED SYNTHETIC PESTICIDES ANY MORE? V. Kurucheve, D. Saravanan and J. Priya. *Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India. Email: vkurucheve@yahoo.co.in*

Groundnut (*Arachis hypogaea* L.) is a major world food legume crop. The leading groundnut-producing countries are India and China. The most important diseases are tikka (*Cercospora personata)* and rust (*Puccinia arachidis*) causing >50% losses. The excrements of cow, buffalo and sheep were tested against these pathogens. Cow urine (20%), cow dung (10%) sheep urine and sheep dung (5% each) totally inhibited the germination of *Cercospora* conidia. With a detached-leaf technique, higher concentrations gave complete inhibition of tikka and rust pustule formation. The sheep urine followed by sheep dung treatment gave maximum seed germination and growth parameters for VRI-2 groundnut. In two pot trials, plants sprayed four times at fortnightly intervals with sheep urine and cow dung plus sheep dung combination (1:1v/v) suffered less disease incidence, and maximum growth and yield, nodule formation and dry matter production were recorded. In another pot and field trial conducted using cow excrement, spraying with cow urine plus cow dung combination (1:1) gave significantly less disease incidence and increased the growth, yield parameters and oil content. No aphid colonies were observed on such treated plants. Sugar content was reduced and there was an increase in phenol, starch, total N and crude protein. Thus, cow and sheep excrements either alone or in combination may effectively be used in managing these two foliar diseases and aphids in groundnut; in this way pesticides may be totally avoided in future.

34.20 A NEW PEST CONTROL STRATEGY IN PLANT VIROLOGY. V. Kurucheve, C. Reena, P. Balabaskar and S. Arivudai Nambi. *Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalainagar 608 002, Tamil Nadu, India. Email: vkurucheve@yahoo.co.in*

India is the major pulses-growing country in the world contributing 28% of the global pulses basket. In recent years, the total production of blackgram (*Vigna mungo* L.) has declined due to high susceptibility to leaf crinkle and yellow mosaic diseases. The over-reliance and injudicious use of synthetic pesticides has led to several problems. The effects of natural products were tested against these viruses and their vectors, aphids and whitefly respectively, and are reported here. Higher concentrations of leaf extract of *Prosopis juliflora*, *Acacia nilotica* and animal excreta (cow and sheep) caused complete mortality of both the insect vectors. When the natural products were mixed with the viral inoculum or in a pre-inoculation experiment, combination treatments recorded 100 % reduction in infection. Mere seed soaking alone with combination products did not eliminate the viruses totally, whereas seeds soaked overnight followed by foliar spray, biweekly, i.e. 10 times in all, eradicated the two viruses and there was no vector colony found. The combination treatments gave maximum growth and yield. In such treatments there was an increase in phenol, and reduction in sugar content. Phenolics in *Prosopis* and ammoniacal compound or silica in livestock excreta might have caused a synergistic effect and thereby eliminated the seed-transmissible viruses. Such a dual role of natural products in eradicating the viruses and protecting the crop from the vectors should be of immense use as there is no such viricide available so far. Hence, it was named "Annamalai mixture".

34.21 BACTERIOPHAGE AS A NATURAL BIOCONTROL AGENT AGAINST TOMATO BACTERIAL SPOT CAUSED BY *XANTHOMONAS VESICATORIA*. G. Kvesitadze, N. Ghudumidze, T. Sadunishvili, N. Shapovalova and A.M. Kropinski. *Durmishidze Institute of Biochemistry and Biotechnology, David Agmasheneblis Kheivani 10 km,Tbilisi 0159, Georgia. Email: gkvesitadze@yahoo.com*

We have studied the possibilities of applying phage as a natural biocontrol agent against bacterial spot of tomato. Washings from 24 h cultures of four pathogenic strains of *Xanthomonas vesicatoria* (three from damaged tomato foliage and one from damaged tomato fruit) were used for artificial infection of tomato plants, grown in separate pots in greenhouse. 7×10^6 p.f.u. of a mixture of pure lines of phages isolated from sewage and damaged tomato materials was sprayed on the leaves. Disease development was expressed in yellowing and finally falling of the leaves. No signs of disease were observed on the leaves of control plants. The disease was eliminated by phage spray; light spots remained at the infected sites, but the disease did not develop and none of the leaves of any plant became yellow or fell. Phage hindered disease onset when it was sprayed just at the moment of bacterial infection, or after 24 h and even after 7 days. Infected plants not phage-treated remained diseased. Remarkably, phage was only sprayed once. Evidence of disease was not observed over the next 3 weeks. Thus, bacteriophage application succeeded in dealing with bacterial spotting developed on the tomato foliage infected with *X. vesicatoria*.

34.22 PRE-INFECTIONAL STRUCTURAL BASIS OF RESISTANCE AGAINST DOWNY MILDEW IN MUSKMELON. M. Mahajan and T.S. Thind. *Department of Biotechnology, Doaba College, Tanda Road, Jalandhar 144 004, Punjab, India. Email: dr_mcajan@yahoo.com*

Downy mildew caused by *Pseudoperonospora cubensis* (Berk. & Curt.) Rostovzev is one of the most destructive fungal diseases of muskmelon (*Cucumis melo* L.) posing a serious problem in the cultivation of this crop. Muskmelon genotypes differ widely in their resistance to attack by this pathogen. The pathogen penetrates the leaf through stomata, and leaf characteristics may greatly affect the ability of the pathogen to invade. Besides many other attributes, differences in resistance have often been attributed to structural differences in leaf anatomy. With this in view, the leaf anatomy of 42 muskmelon genotypes (19 resistant and 23 susceptible) was examined in relation to their resistance/susceptibility to downy mildew. The genotypes were obtained from Punjab Agricultural University, Ludhiana, India. Their disease score was rated using a 0–5 scale. The results showed that stomatal size, frequency and index were significantly higher in susceptible genotypes, whereas resistant genotypes had higher frequencies of large trichomes. Thickness of cuticle, palisade tissue, proportion of palisade and palisade index values were significantly higher in resistant genotypes indicating compact arrangement of palisade cells thus preventing further spread of the pathogen. The results indicated that leaf epidermal and other anatomical characteristics may act as structural barriers against penetration by the downy mildew pathogen. Evaluation for these characteristics may prove useful for early and preliminary screening of newly evolved muskmelon genotypes when assessing their resistance/susceptibility to downy mildew infection in large breeding populations.

34.23 OZONATION AS A TOOL FOR DECONTAMINATION IN NURSERIES AGAINST GRAPEVINE TRUNK DISEASE FUNGAL PATHOGENS. N. Mailhac, M. Lummerzheim and F. Violleau. *Laboratoire d'Agrophysiologie, Ecole d'Ingénieurs de Purpan, 75 Voie du TOEC, 31076 Toulouse Cedex 3, France. Email: nathalie.mailhac@purpan.fr*

Fungi responsible of grapevine trunk diseases can produce latent infections in the nursery and cause Petri disease. These pathogens can be transmitted from infected mother vines via contamination on the external bark or internal vascular tissues. Water baths, grafting and callusing boxes are the main steps in the process of grapevine propagation. Contamination at these stages is likely to be a contributing factor in poor performance of grapevine planting material, so the development of procedures to prevent and/or reduce the infection is crucial. Hot water treatment (HWT) has some beneficial effects, but 100% efficiency is out of reach with this method. Ozone is a known and potent oxidative agent able to decontaminate water from fungal pathogens. More recently, this method has proved successful for decontaminating cereals infected by pathogenic fungi. Moreover, ozone is known to stimulate systemic acquired resistance in plants. In this study, we did preliminary experiments on ozonation as a tool for grapevine stocks decontamination. A gradient of ozone concentrations combined with various treatment times were applied with aqueous solutions containing fungal cultures and grapevine wood chips contaminated or not. Fungal survival rates were scored through their capacity to grow on solid medium. Preliminary results are presented. If an efficient decontamination process is found through ozonation, without harming the plant material, it will be a significant step forward in the battle against grapevine trunk diseases.

34.24 IDENTIFICATION AND DESIGN OF ANTIMICROBIAL PEPTIDES TO CONTROL FUNGAL PLANT PATHOGENS. J.F. Marcos, A. Muñoz and B. López-García. *Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), P.O. Box 73, Burjassot, 46100 Valencia, Spain. Email: jmarcos@iata.csic.es*

Peptides and small proteins with antimicrobial activity have

been characterized from a vast number of organisms from bacteria to humans, including plants. The use of antimicrobial peptides (AMPs) in plant protection has been proposed by several authors, and there are examples of expression of bioactive AMPs in transgenic plants. However, some natural AMPs have undesired properties such as non-specific toxicity and low stability that compromise their application in agriculture. The short sequence length of this class of peptides favours structure/activity studies in a holistic approach to increase their stability and activity towards specific pathogens, while also lowering toxicity. We summarize our group's contributions to the identification of AMPs from natural sources or by combinatorial chemistry methods, as well as to the rational design of improved sequences based on previous knowledge, to control fungal plant pathogens. These studies are aided by advances in peptide synthesis and high-throughput activity screening, which have made possible the *de novo* design of novel AMPs with enhanced properties. Using as a working model the interaction between *Penicillium digitatum* and Citrus fruit that causes green mould postharvest disease, we highlight the differences between in vitro peptide activity and protection against disease achieved in laboratory bioassays. Differences in peptide protective effect are likely related to distinct modes of antifungal action. Unveiling the mechanism of antifungal action of selected model peptides as well as the corresponding cell targets in fungi is required to further advance towards the safe use of AMPs in plant protection.

34.25 PHYSICAL CONTROL OF TOMATO POWDERY MILDEW *OIDIUM NEOLYCOPERSICI* WITH AN ELECTROSTATIC DIPOLAR SCREEN. **Y. Matsuda, T. Nonomura and H. Toyoda.** *Department of Agricultural Science and Technology, Kinki University, Nara 631-8505, Japan. Email: ymatsuda@nara.kindai.ac.jp*

In an attempt to physically protect greenhouse tomatoes from the powdery mildew fungus *Oidium neolycopersici*, we developed a new electrostatic spore precipitator (electrostatic dipolar screen), in which a copper wire conductor is linked to an electrostatic generator and covered with a transparent acrylic cylinder (insulator). The conductor was negatively charged by the generator, and the electrostatic field created by the conductor was used to dielectrically polarize the insulator cylinder. The dielectrically polarized cylinder also produced an electrostatic force without a spark discharge. This force was directly proportional to the potential applied to the conductor and was used to attract conidia of the pathogen. The efficacy of this spore precipitator in protecting hydroponically cultured tomato plants from powdery mildew was evaluated in the greenhouse. The hydroponic culture troughs were covered with a cubic frame installed with the spore precipitator, and the disease progress on precipitator-guarded and unguarded seedlings was traced after the conidia were mechanically disseminated from inoculum on tomato plants. Seedlings in the guarded troughs remained uninfected during the entire experiment, in spite of rapid spread of the disease to all leaves of the unguarded seedlings. The use of this spore precipitator effectively prevents susceptible plants from becoming exposed to inoculum, and is likely to lead to a reduction in the use of chemical fungicides. This should be more acceptable to the public as a physical protection for high value crop plants.

34.26 WILLOW GENOTYPE MIXTURES AS A DISEASE CONTROL STRATEGY. **A.R. McCracken and D. Begley.** *Applied Plant Science Division, Agri-Food & Biosciences Institute, Newforge Lane, Belfast BT9 5PX, N. Ireland, UK. Email: alistair.mccracken@afbini.gov.uk*

Rust disease caused by *Melampsora epitea* is a potential major limiting factor in growing short rotation coppice (SRC) willow for biomass in renewable energy production. The disease control strategy for managing rust is to plant mixtures of willows of different genotype. To determine the importance of the diversity within such mixtures a trial was planted in 2001 which comprised only straight *Salix viminalis* genotypes. Disease incidence was recorded every year 2001-2006 and the trial was harvested in 2003/04 and 2006/07. At the first three-year harvest in 2003 there were small, but significantly higher yields from the mixtures compared to the mono-plots. These differences were not evident at the second harvest, three years later. There were no consistent effects of mixtures in reducing the level of rust on the most susceptible genotype, *S. viminalis* '77082'. The diversity of *M. epitea* pathotypes within the rust population was also determined in a number of seasons throughout the course of the trial. There were no major differences in the number or diversity of pathotypes found on genotypes growing in 3-way or 7-way mixtures compared to when they were grown as mono-plots. This is in contrast to previous studies using diverse inter- and intra-species mixtures of willow, when there was significantly greater pathotype diversity on plants in mixtures compared to mono-plots. These data fully support the commercial planting recommendations of incorporating at least six willow genotypes within a mixture with at least three coming from the European Breeding Programme and three from the Swedish Breeding Programme.

34.27 INTEGRATED MANAGEMENT OF SUNFLOWER NECROSIS DISEASE. **R.K. Mesta, P. Katti, H.T. Chandranath, I. Shankergoud, V.I. Benagi and M.K. Naik.** *Agricultural Research Station, Devihosur, 581 110, Haveri, Karnataka, India. Email: rkmesta@yahoo.com*

Sunflower (*Helianthus annuus* L.) is an important oilseed crop in India. Sunflower necrosis disease (SND) caused by *Tobacco streak virus*, genus *Ilarvirus*, is a major threat to the sunflower crop. Since the disease is transmitted by thrips and *Parthenium* pollen, We studied how to manage this disease by vector control and modification of cultural practices, involving methods like use of a barrier crop, use of insecticides and alteration of sowing time. The experiment was conducted for two seasons, with a split plot design in which main plot treatment consisted of different barrier crops and sub-plot with different combinations of insecticides. To study the effect of sowing date, two cultivars, KBSH-1 and Morden were planted at 15 day intervals in 20 different sowings. In both the above experiments, data on % necrosis, thrips population and yield were recorded. The border crop of 4 rows surrounding the sunflowers, either of sorghum or bajra, sown 15 days earlier, gave the best protection against thrips and necrosis. Seed treatment with imidacloprid at 5 g/kg followed by spraying of at 0.5 ml/l was found to reduce disease incidence by 50%. The best date for sowing sunflower, which gave lowest disease incidence, was after the first fortnight in September. Crop sown in June and July recorded highest disease incidence.

34.28* SMALL SYNTHETIC ANTIMICROBIAL PEPTIDES AS NEW BACTERICIDES FOR THE CONTROL OF PLANT PATHOGENIC BACTERIA. **E. Montesinos, E. Badosa, R. Ferré, S. Monrroc, L. Feliu, M. Planas, J. Cabrefiga and E. Bardají.** *Institute of Food and Agricultural Technology-CIDSAV-CeRTA*

and Department of Chemistry, University of Girona, Campus Montilivi, 17071 Girona, Spain. Email: emonte@intea.udg.edu

Bacterial plant diseases cause important crop losses worldwide, and control is based on copper compounds and antibiotics. However, antibiotic use is restricted in several countries and resistance has been reported. Therefore, there is a need for new bactericides in plant protection. Our research is focused on the design, evaluation and improvement of small synthetic antimicrobial peptides against plant pathogenic bacteria, specifically *Erwinia amylovora, Pseudomonas syringae* pv. *syringae* and *Xanthomonas axonopodis* pv. *vesicatoria*. A 125-member library of synthetic cationic undecapeptides based on melittin-cecropin hybrids was prepared using a combinatorial chemistry approach. Also, head-to-tail cyclic peptides of 4 to 10 residues were developed and a library of 56 cyclic decapeptides was prepared. Both libraries were screened in vitro against the three bacteria, and several compounds were found to inhibit growth at micromolar concentrations (1-7 micromolar). The best linear undecapeptide H-KKLFKKILKYL-NH_2 and the best cyclic decapeptide c(KKLKKFKKLQ) showed an optimal balance between antibacterial and haemolytic activity, was strongly bactericidal, and was highly resistant to protease degradation. The most promising peptides were tested in vivo by assessing their capacity to inhibit *E. amylovora* infection on apple and pear flowers. The efficacy observed ranged from 63 to 76% at 100 micromolar concentration; this was similar to the antibiotic streptomycin, currently used for fire-blight control in several countries.

34.29 ENDOPHYTIC FUNGI AND PLANT EXTRACTS AS ALTERNATIVES IN BLACK SIGATOKA DISEASE MANAGEMENT IN BANANA. G.P. Osorio and A.S. Riveros. *Sciences of Faculty, Biology of Department, Tolima University, Ibague, Tolima, Colombia-Agreement CATIE-UTOLIMA c/o 7170 Turrialba, Costa Rica. Email: asrivero@catie.ac.cr*

Mycosphaerella fijiensis causes black Sigatoka disease, an important threat to banana production. Use of fungicides has been the most efficient control. The objective of this research was to evaluate endophytic fungi and extracts from the plants *Momordica charantia* (B1) and *Senna reticulata* (B2) to control black Sigatoka. Banana seeds were inoculated with *Trichoderma atroviride* (E1/E2) strains and planted in the field under a complete random block design in split plots. Applications of plant extracts started 6 months after planting. Black Sigatoka severity and incidence were evaluated. ANOVA and Duncan tests were run using the SAS system. The area under the disease progress curve (AUDPC) expressing severity of disease (SD) and incidence percentage (IND) was calculated. Results at root level revealed statistically significant differences ($p<0.05$) for SD and IND, especially for E2 after chemical (Q) treatment, with low values showing the best behavior for disease protection, compared with nematicide and fungicide interaction. At foliar level, there were statistically significant differences ($p<0.05$) favoring B2 with a lower SD and IND. Nevertheless, the Q treatment was the most effective control. Phytochemical analysis indicated a variety of secondary metabolites. This research allowed to select B2 as a "highly promising" candidate for integrated management due to its protectant action. However, it is not known which molecule exerts the disease control effect and if we can continue this investigation, it is recommended to isolate the active compound.

34.30 IMPROVING SUPPRESSIVENESS TOWARDS *PHYTOPHTHORA NICOTIANAE* USING BONE CHARCOAL. M. Pugliese, J. Liu, G. Gilardi, E. Someus, M.L. Gullino and A. Garibaldi. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: liujianbinchina@hotmail.com*

During 2007, a high phosphorus containing non-SRM organic waste was enriched with suppressive microbial strains and assessed as a product to protect tomato against *Phytophthora nicotianae* and as fertilizer under greenhouse conditions. Bone charcoal was inoculated with seven microorganisms (3 *Trichoderma* and 4 bacteria) and applied at 2% v/v mixed with sterilized peat before sowing tomato cv. 'Cuor di Bue'. A commercial formulation of *Trichoderma harzianum* T22 (Rootshield) and of *Streptomyces griseoviridis* (Mycostop) were used as controls at recommended dosages. After 15 days, tomato plants were transplanted into white boxes (45×38 cm = 0.17m^2) and inocula of *P. nicotianae*, propagated in flasks on wheat plus hemp, was distributed at 30g/m^2 on the soil surface. Fifteen tomato seedlings were transplanted into each box, for a total of 45 seedlings in each treatment (3 replicates). The effect of adding the micro-organisms to the compost on disease incidence was assessed. Bone charcoal at 2% suppressed disease up to 32% compared to peat, when inoculated with a strain of *Pseudomonas chlororaphis*. In no case did addition of bone charcoal increase disease incidence, and the results were comparable to commercial formulations of antagonists.

34.31 EFFECTS OF HEAT TREATMENT ON THE INCIDENCE OF *FUSARIUM* SPP. IN MAIZE SEEDS, AND COMBINATION WITH BIOLOGICAL CONTROL ON THE EMERGENCE AND TRANSMISSION OF THE FUNGUS TO SEEDLINGS. P.J. Querales, M.H.D. Moraes, V.C. Frare and J.O.M. Menten. *UCLA, Estado Lara, Venezuela. Email: pastoraq@ucla.edu.ve*

Fusarium spp. associated with maize seeds can be introduced or spread in the field, causing disease or reducing grain quality through the accumulation of mycotoxins. Levels of field inoculum can be controlled by using seeds that are healthy or carry low infection. The aim of this study was to evaluate the effects of heat treatment on the incidence of *Fusarium* spp. in seeds of two maize hybrids and the combination of heat treatment and biological control on the emergence and transmission of the fungus to seedlings. Incidence was evaluated by modified blotter test, emergence by sterile substratum in greenhouse, and transmission by use of BSA culture medium, in completely random design, with four repetitions. In the hybrid SG-6418, without treatment, lower incidence of the fungus was observed, and higher percentage of seedling emergence was obtained. In contradict, the hybrid SG-150 showed higher percentage of emergence from seeds treated with heat and biological control agents. Transmission of *Fusarium* spp. to seedlings was reduced in the hybrid SG-6418 with biological control and without heat treatment, but the same hybrid showed major transmission to seedlings from seeds with heat treatment and without biological control. There was no interaction between heat treatment and biological control on fungal transmission to the seedling.

34.32 USE OF ANIMAL URINE FOR CONTROL OF SHEATH BLIGHT OF RICE. J. Raja, C. Suganthi and V. Kurucheve. *Department of Plant Pathology, Annamalai University, Annamalainagar 608 002, India. Email: rajaj_au@yahoo.co.in*

The aim of this study was to determine the effect of animal urine (buffalo, cow, goat, sheep and horse) on sheath blight disease of rice. Adult stage of animal urine is more effective on *Rhizoctonia solani* than young stage of animal urine. The viability of sclerotia of *R. solani* was completely lost, after 72 h of soaking in urine. Reinoculating the inhibited sclerotia on regular agar medium did not retrieve viability. The antifungal activity of urine was not affected by high temperature (90°C) and storage at room temperature (25±1°C) for one year. Urine rate 50 l ha^{-1} sprayed three times (at panicle differentiation, boot, and heading growth stages) was found significantly superior to propiconazole fungicide in respect of reduced disease severity and increased grain yield. Soils treated with animal urine contained maximum numbers of bacterial, fungal and actinomycete colonies when compared to controls. Species of *Trichoderma, Penicillium, Aspergillus, Bacillus*, *Cellulomonas*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Rhodococcus*, *Serratia* and *Streptomyces* were isolated from urine-treated soils. The total and *ortho*-dihydroxy phenol content was profoundly influenced by urine applied to plants and also significantly increased peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, chitinase and β-1,3-glucanse activities. Earlier and higher accumulation of enzymes involved in phenylpropanoid and PR-protein metabolism were found in urine-treated plants in response to invasion by *R. solani*.

34.33 SEVERE PRUNING TO MANAGE PISTACHIO TREES WITH BACTERIAL DIEBACK. C.E. Taylor, E. Facelli, D. Giblot Ducray, R.W. Emmett, M. Sedgley and E.S. Scott. *The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, 5064 SA, Australia. Email: daniele.giblotducray@adelaide.edu.au*

Pistachio dieback caused by *Xanthomonas transluscens* (Xtp) affects trees in the main pistachio growing regions of Australia. Symptoms include internal staining, trunk and limb lesions, stunted growth, dieback, reduced yield and tree death. Studies of the pathogen distribution in trees showed that Xtp was mostly confined to older xylem tissue. When one-year-old twigs on trees were inoculated annually, Xtp appeared to be confined to the xylem tissue present at the time of inoculation. The aim of this study was to determine if a combination of severe pruning and the disinfection of pruning cuts could be used to promote new, uninfected growth and rejuvenate diseased trees. Pairs of trees with similar disease severity were selected. One tree of each pair was severely pruned, leaving only secondary or tertiary branches and the other tree left as an unpruned control. Cuts on pruned trees were flooded with disinfectant. New shoots on pruned trees were tested for the presence of Xtp in each of the following three years. Incidence of Xtp in 1, 2 and 3-year-old twigs was 2-5%, 9-24% and 32%, respectively, so as the twigs aged, incidence of Xtp increased. Severe pruning produced strong, healthy regrowth but did not prevent the gradual re-establishment of Xtp in the new structure of the trees. The growth and yields of pruned and control trees will be compared during the next two years. To date these results indicate that severe pruning may be a partial solution to managing trees infected with Xtp.

34.34 AN OZONE GENERATIVE DIPOLAR SCREEN PROTECTS HYDROPONIC TOMATOES FROM RHIZOSPHERE AND AERIAL PATHOGENS. H. Toyoda, Y. Matsuda and T. Nonomura. *Department of Agricultural Science and Technology, Kinki University, Nara 631-8505, Japan. Email: toyoda@nara.kindai.ac.jp*

An ozone-generating electrostatic spore precipitator was developed to protect nursery-stage tomato seedlings from both airborne conidia of powdery mildew (*Oidium neolycopersici*) and root-infecting pathogen propagules of bacterial wilt and *Fusarium* crown and root rot. The device is a cylindrical electrostatic spore precipitator in which a positively charged straight conductor wire is insulated with a transparent acrylic cylinder. The cylinder consists of two sites, for conidial attraction and ozone production. The site for ozone production is located at the end of the cylinder, where an earthed copper conductor ring (as cathode) was attached, close to the anodal tip of the positively charged central conductor wire. Discharge between the two electrodes depended on the voltage applied to the wire and the distance between the electrodes. Highest ozone production was observed through streamer discharge. The remaining portion of the cylinder, which was dielectrically polarised by the positively charged wire, created a non-uniform electric field outside the cylinder to attract conidia that came within the field. In the present study, hydroponic culture troughs to raise tomato seedlings in a nursery greenhouse were arranged paralleled with the cylinders. The aim was to control rhizosphere pathogens and to prevent them entering the hydroponic system during cultivation, while at the same time trapping *O. neolycopersici* conidia from entering spaces between the cylinders. The results indicated that susceptible tomato plants in the culture troughs equipped with cylinders remained uninfected by both rhizosphere and aerial pathogens throughout the experimental period.

34.35 HETEROLOGOUS PRODUCTION OF PLANT FLAVONOIDS FROM YEAST AND THEIR IMPACT AS ANTIBIOTICS. E. Trantas, N. Panopoulos and F. Ververidis. *Department of Plant Sciences, Technological Educational Institute of Crete, P.O. Box 1939, 710 04 Heraklion, Greece. Email: ververidis@teicrete.gr*

Plants have evolved defensive mechanisms such as the production of induced or non-induced protective compounds (phytoalexins and phytoanticipins respectively). Among these, flavonoids and stilbenoids are of exceptional interest not only because they can act as antimicrobial agents but also because they possess beneficial properties in respect to humans. For this reason in sustainable and organic agriculture, farmers use plants as factories for the extraction of compounds that are used as phytoprotectants e.g. spraying with tobacco extracts. Metabolic engineering gives us the ability to produce such compounds more efficiently out of the plant body in heterologous organisms. In such an approach, yeast, which does not naturally produce flavonoids, is genetically modified by reconstituting its biosynthetic pathway to direct the initial carbon sources into more valuable products such as plant-derived stilbenoids and flavonoids. The genes leading to production of the stilbene resveratrol, the isoflavone genistein and the flavonols kaempferol and quercetin have been cloned and sequenced for determining their homology. The four pESC dual-promoter galactose-induced expression vectors were used to subclone the implicated genes in pairs. The pathway that leads to p-coumaric acid and resveratrol production has been efficiently introduced into yeast cells that are now able to produce sufficient amounts of the respective compounds. These compounds, synthesized regularly in yeast are tested against known plant pathogens. The results of these tests are discussed. This project is funded by the research grant PENED (03ED776, GSRT) awarded to F.V.

34.36 ETIOLOGY AND MANAGEMENT OF SPRING DEAD SPOT OF HYBRID BERMUDAGRASS IN NORTH CAROLINA, USA. **L.P. Tredway, E.L. Butler and M.D. Soika.** *Department of Plant Pathology, North Carolina State University, Raleigh, NC, USA. Email: lane_tredway@ncsu.edu*

Spring dead spot is a destructive fungal disease of hybrid bermudagrass (*Cynodon dactylon* × *transvaalensis*) in temperate and subtropical climates where the turf is exposed to freezing during winter dormancy. The disease is caused by three species of *Ophiosphaerella*, *O. korrae*, *O. herpotricha*, and *O. narmari*, which differ in their distribution and aggressiveness. The distribution of spring dead spot pathogens in North Carolina was determined. *O. korrae* was dominant in 17 locations and comprised 82% of the isolates collected, whereas *O. herpotricha* was only present in 3 locations and dominant in 2 locations. Two years after inoculation of field plots with each species, patches induced by *O. korrae* were an average of 9.4 cm in diameter, with the largest number of patches falling in the 5 to 15 cm range. Patches induced by *O. herpotricha* were an average of 22.0 cm in diameter, and most patches were 20 to 30 cm in diameter. In fields naturally infested with *O. korrae*, preventative applications of fenarimol provided the greatest and most consistent reductions in spring dead spot incidence. Azoxystrobin, propiconazole, and tebuconazole gave control in certain experiments, but myclobutanil did not reduce disease incidence. Preventative applications were equally effective in the fall when soil temperatures were between 15°C and 27°C. The method of fungicide application did not significantly influence fungicide performance. In field plots that were artificially inoculated with each species, *O. korrae* and *O. herpotricha* responded similarly to preventative fungicide applications and both species were effectively controlled by fenarimol.

34.37 MANAGEMENT OF *FUSARIUM* CORM ROT AND YELLOWS OF GLADIOLUS USING AN ECOFRIENDLY INTEGRATED APPROACH OF HOT WATER, UV-C IRRADIATION AND *HYPTIS SUAVEOLENS* ESSENTIAL OIL. **A. Tripathi and N. Sharma.** *Department of Bioscience and Biotechnology, Banasthali University, P.O. Banasthali Vidyapith, 304 022, Rajasthan, India. Email: abhitri77@yahoo.com*

Fusarium corm rot and yellows is one of the most important diseases of gladiolus responsible for major commercial losses. The pathogen is responsible is *Fusarium* f. sp. *gladioli*. In the present study various ecofriendly integrated approaches were assessed for the management of gladiolus corm rot and yellows. Experimental plots artificially infested with the pathogen were used. These plots were soil solarized (SS) or fumigated with the essential oil (EO) of *Hyptis suaveolens* L. Poit. Corms were given hot water treatement (HWT), UV-C irradiation (UV-C) or essential oil (EO) separately or in an integrated way. For integrated treatment, SS and EO soil treatment was given for 45 days. Corms were treated with hot water at 55 ºC for 30 min, with a UV-C dose of 4.98 kJm^{-2} for 387 sec and/or with EO treatment with 800 ppm for two weeks. Percent disease incidence was recorded and the best results were seen in solarized and EO fumigated plots having corms treated with HWT + UV-C + EO. In this integrated treatment very low disease incidence (7.44%) was recorded, compared to controls having disease incidence of 38.74%.

34.38 VARIATIONS IN PHENOLICS AND OXIDATIVE ENZYME ACTIVITIES IN TOMATO CALLI SELECTED FOR RESISTANCE TO *ALTERNARIA SOLANI*. **C. Venkatesh and S.K. Gandhi.** *Department of Plant Pathology, CCS Haryana Agricultural University, Hisar 125004, India. Email: gandhisk@hau.ernet.in*

Tissue culture techniques offer promise of early selections wherein explant cultures of host plants are treated with selective agents such as culture filtrate, toxins or elicitors to obtain resistant breeding lines. In the present study tomato genotypes differing in susceptibility to *Alternaria solani* (Ellis & Martin) Jones & Grout showed reduction in callus formation in MS medium supplemented with 0.5 mg/l NAA and 3.0 mg/l BAP containing different concentrations of *A. solani* culture filtrate. Since 20% concentrations of culture filtrate in callus induction medium proved to be lethal, so a sublethal concentration of 15% was used for *in vitro* screening for resistance and to check further stability for resistance upon subculturing. Increase in the level of culture filtrate in the callus induction medium enhanced both total phenol and flavonol contents in resistant calli of all the genotypes. The maximum level of phenols and flavonols i.e., 4.43 mg/g of dry weight and 0.75 mg/g of dry weight respectively, were observed in genotype H-121. Levels of these constituents in calli of highly susceptible genotypes DT-1 and BL-982 after selection were at par with constitutive levels observed in the moderately resistant genotypes H- 121 and Sel-7. Activity of the oxidative enzymes polyphenol oxidase, peroxidase and catalase increased in calli of all genotypes with increase in the level of culture filtrate. Oxidative enzyme activities in calli of highly susceptible genotypes after selection were comparable with the level of activities constitutively expressed in calli of resistant genotypes.

34.39 CONTROL OF GARLIC LEAF BLIGHT IN CHINA. **L. Zheng, J.B. Huang and T. Hsiang.** *The Key Lab of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan, Hubei, 430070, P.R. China. Email: junbinhuang@mail.hzau.edu.cn*

Leaf blight caused by *Stemphylium solani* is one of the most significant constraints to garlic production in Dangyang county, Hubei province, China. It is essential to develop suitable crop management practices. The objective of this research was to study the effects of six different seed treatments (including water as a check), eight fungicide sprays and 11 garlic varieties on disease incidence and production. Field trials were conducted in 2006 in Lianghe of Dangyang county. Throughout the study, all seed treatments promoted seedling emergence, but the most significant effect ($p<0.01$) was observed with 2.5% Celest SD (5 ml fludioxonil/100 kg seed) and 50% Thiram WP (125 g thiram/100 kg seed). Application of fungicide sprays was effective in controlling leaf blight, and 40% Nustar EC (0.5 ml flusilazole/100 m^2) and 70% Mancozeb WP (3.5 g mancozeb/100 m^2) had the highest efficicacy at 63.5% and 56.2% disease suppression, respectively. The disease index of cultivars Chunganruanye and Ruanruanye were the lowest at 9.1 and 9.4 (0=lowest, 100=highest). These cultivars were much more resistant to the disease than the others, but did not have the desired bolting characteristics. 'Chunganruanye' showed highest leaf yield (428.2 t/ha) and bulb yield (39.4 t/ha) at harvest compared to the other varieties tested. The field trial will be repeated again in 2007 to 2008.

KNOWLEDGE TRANSFER FOR PLANT PATHOLOGY

25.1 OCCURRENCE OF CHARCOAL ROT DISEASE (*MACROPHOMINA PHASEOLINA*) ON SESAME IN MAZANDARAN PROVINCE, IRAN. **S.A. Alian and V. Khosravi.** *Department of Plant Protection, Deputy of Rice Research Institute, Amol, Iran. Email: aminalian@gmail.com*

Sesame (*Sesamum indicum* L.) is not considered an important crop in Mazandaran province, and its cultivation plays hardly any role in cash products, but here it is one of the daily foodstuffs. From July to October 2007, sesame plants showing symptoms of charcoal rot were observed. Symptoms included wilting, premature senescence, and death of plants. Plants in the early stages of the disease, although often without any characteristic top symptoms, showed a light brown discoloration of the sub-epidermal root tissue and the basal portion of the stem. Plants in a more advanced stage of the disease, with yellowing leaves and general wilting, had a brown superficial lesion extending from ground level up the stem. Light brown discoloration of the sub-epidermal tissue extended up to 10 cm beyond the lesion. When the epidermis was pulled back at the lesion, small black microsclerotia, just visible to the naked eye, were observed. Average disease incidence at sampling points was 27% (range 21-34%). Stem tissues were surface sterilized and placed on PDA, yielding fungal colonies identified as *Macrophomina phaseolina* (Tassi) Goidanich based on grey color, colony morphology, and the presence of microsclerotia 60 to 112 µm in diameter. This is the first report of the occurrence of charcoal rot disease on Sesame in Mazandaran province. More studies are needed to determine the distribution and host specialization of this fungus in Mazandaran.

25.2 CONSENSUS PROTOCOL FOR *PLUM POX VIRUS* DIAGNOSIS. **M. Cambra, N. Capote, A. Olmos, M.T. Gorris, N.L. Africander, L. Levy, S.L. Lenardon, G. Clover and D. Wright.** *Instituto Valenciano de Investigaciones Agrarias (IVIA), Carretera Moncada-Náquera km 5, 46113 Moncada, Valencia, Spain. Email: mcambra@ivia.es*

Sharka is one of the most serious diseases of stone fruit trees in terms of agronomic impact and economic importance. The disease, caused by *Plum pox virus* (PPV), affects species of the genus *Prunus*, being particularly detrimental in apricot (*P. armeniaca*), European plum (*P. domestica*), Japanese plum (*P. salicina*) and peach (*P. persica*). Estimated costs associated with sharka management worldwide since the 1970's exceed 10,000 million euros. An international protocol for the specific diagnostic of PPV was updated by a working group from different organizations. The protocol was developed for the International Plant Protection Convention (IPPC) governed by the Interim Commission on Phytosanitary Measures hosted by FAO. The consensus protocol includes detection and identification techniques. The selected detection techniques were: 1) Biological indexing or inoculation of woody indicator plants (GF305, Nemaguard peach seedlings, or *Prunus tomentosa*), 2) Serological tests including DASI-ELISA with 5B-IVIA universal monoclonal antibody or DAS-ELISA with 5B-IVIA or polyclonal antibodies, and 3) Molecular tests using RT-PCR or IC-RT-PCR (using P1/P2 or 3'NCR primers), Co-PCR (using P10/P20/P1/P2 primers and hybridization with universal probes) or real-time RT-PCR (using either SYBR Green or TaqMan chemistries). The combination of two different screening methods using the validated and recommended protocols and reagents, is required to officially support a positive PPV detection. The identification protocol includes serological and molecular tests for typing PPV isolates into D, M, EA, C, W and Rec strains. Sequencing all or part of the coat protein gene (and 3' end of the replicase gene in the case of PPV-Rec) for first PPV findings is recommended.

25.3* LOCAL PLANT CLINICS PAVE THE WAY FOR A NATION-WIDE HEALTH CARE SYSTEM FOR PLANTS IN NICARAGUA. **S. Danielsen.** *Danida Agricultural Sector Program Support, Royal Danish Embassy, Apdo 4942 Managua, Nicaragua. Email: soldanielsen@gmail.com*

Nicaragua and many other developing countries struggle against inherent failures and weaknesses in the agricultural advisory systems. Plant health services are almost non-existent and most efforts to reach small-scale farmers with training and advice are channelled through short-term IPM projects and farmer field schools aimed at transferring specific technologies. Such technology-driven projects may be valuable for on-farm validations or in-depth training on certain themes, but are not suitable to provide general plant health advice to a broader public. The establishment of a few local plant health clinics in Nicaragua in 2005 in collaboration between FUNICA, a Danida-supported agricultural technology foundation, and the Global Plant Clinic, became the starting point for a new perspective on the roles of existing institutions engaged in crop protection. The clinics are run by local extension workers from farmer organizations, NGOs or government institutions. They typically operate once a week at a public place where they receive queries and 'patients', diagnose problems and give practical advice on disease management. What started as a grassroots pilot soon developed into a nation-wide initiative where universities, research institutions, diagnostic laboratories and regulatory services created a network to provide technical backstop support to the plant clinics. The clinics and the network share a common goal: the provision of timely quality advice to farmers. Such a national 'health care system for plants' is unique. This experience shows that it is possible to overcome some of the historical obstacles in service provision by creating new models of collaboration within the existing system.

25.4* FIVE YEARS OF COOPERATION ON SUSTAINABLE CROP PROTECTION BETWEEN CHINA AND ITALY. **M.L. Gullino, A. Garibaldi, V. Rizzo and C. Clini.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco, Italy. Email: marialodovica.gullino@unito.it*

In the year 2000 the Italian Ministry for Environment, Land and Sea and the State Environment Protection Administration of China jointly launched the Sino-Italian Cooperation Programme for Environment Protection. In this framework several cooperation and technology transfer projects in different rural areas of China have been carried out with special attention to sustainable crop protection. The projects, located in Inner Mongolia, Xinjiang, Shandong, Hebei and Chongming Island, were aimed at increasing yields and quality of vegetable crops (e.g. tomato, eggplant, pumpkin, and cabbage), fruits (e.g grape.) and cereals. The implementation of innovative technologies characterized by low cost of application, simple set up and management and economic suitability to the local conditions provided successful results. Obsolete, polluting pesticides were replaced with chemicals having a lower environmental impact. Drip irrigation systems in substitution of the old, water-consuming techniques of flood irrigation were used to distribute fertilizer at reduced dosages. Polyethylene mulching films were replaced by the use of starch-based biodegradable plastic films. In the demonstration areas, con-

sumption of water, pesticides and fertilizers were reduced by 5-6 times, while the new mulching films, being completely biodegraded a few month after the cropping cycle, solved the problem of white pollution. Academic institutions, public research centres and private companies have joined the Programme with the aim to ensure the replicability and long-term sustainability of the new technologies adopted. Workshops and seminars have also been organized in order to inform stakeholders on scientific, technical and economic feasibility of the new proposed techniques.

25.5 ENVIRONMENTAL MANAGEMENT AND SUSTAINABLE AGRICULTURE: AN ADVANCED TRAINING PROGRAMME. M.L. Gullino, I. Musu, A. Fornetti, I. Mannino, D. Spadaro and C. Clini. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco, Italy. Email: marialodovica.gullino@unito.it*

The Advanced Training Programme on Environmental Management and Sustainable Development is part of the "Sino-Italian Cooperation Program for Environmental Protection", a long-term cooperation project between the Italian Ministry for the Environment, Land and Sea and some major Chinese administrations. The Training Programme started in 2003 and is addressed to senior governmental officials, professors, and experts of the People's Republic of China, with the aim of updating and improving environmental knowledge of Chinese decision makers and experts who have a strategic role in planning, designing, regulating and promoting sustainable development in China. Sustainable agriculture is a significant topic in this programme. Sustainable crop protection, with emphasis on reducing pesticide use, global climate change, and disease management in organic farming are among the subjects explored. Training is given at Venice International University (Venice) and at AGROINNOVA (Turin) and is organized in such a way as to present real case studies in addition to the theoretical issues that form the environmental and economic frame of reference. Case studies are further complemented by a series of site visits to plants and companies of foremost importance in terms of advanced technologies and approach to sustainable development. The training programme fosters cooperation among Italian and Chinese enterprises and administrations. By the end of the fourth year of activity, the total number of participants in the training reached 2,000.

25.6 REPLACEMENT OF METHYL BROMIDE FOR SOIL DISINFESTATION IN ITALY. M.L. Gullino, G. Gasparrini, C. Clini and A. Garibaldi. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco, Italy. Email: marialodovica.gullino@unito.it*

In 1995, Italy, due to its intensive horticultural production, used more than 7,600 metric tons of methyl bromide (MB), ranking first in Europe and second in the world after the United States. Most MB was applied for pre-plant soil fumigation, in southern Italy, while structural fumigation represented only 6% of total use. Reducing the usage of MB without affecting its agriculture was a challenge that Italy did pursue with the involvement of all stakeholders. Research was aimed at: a) reducing the use of MB and its emission (short-term goal); b) developing alternative methods, based on available technologies (mid-term goal); c) developing new MB alternatives (mid- and long-term goal). Italian growers switched to other fumigants, applied at reduced dosages, lowering their environmental impact and risk of worker exposure. Moreover, the adoption of non-chemical alternatives slowly but steadily increased as a result of the many activities (demonstration trials, seminars, etc.) carried out in the country. The adoption of the different available alternatives permitted a strong decrease in MB consumption, from almost 9,000 metric tonnes in 1993 to 855 metric tonnes authorised by the European Commission for the 2006 critical use exemptions. The gradual shift to MB alternatives over a 10 year period, as well as the use of half rate of MB under virtually impermeable plastic films, has prevented the feared loss of competitiveness of Italian horticulture. The experience gained in Italy and how it can help developing countries to define strategies and training programmes needed to comply with the Montreal Protocol is discussed.

25.7* DECISION-SUPPORT SYSTEMS FOR CEREAL CROP DISEASE CONTROL IN RUSSIA. T.Z. Ibragimov and S.S. Sanin. *The All-Russia Scientific Research Institute of Phytopathology, Bolshie Vyazemy, Moscow region, 143050, Russia. Email: itz@vniif.rosmail.com*

Decision-support systems are being developed for management of disease in cereal crops in Russia. At the start of the program, users, with the help of a list of the various factors, are asked to choose the region, crops, weather conditions etc., for which the phytosanitary forecasts will be made. When the form is completed, the program produces tables and probability values, and makes a forecast of the phytosanitary situation, and recommendations. The program model is based on XML and .Net technologies. The entire data that is necessary for calculation is stored in an XML file. This format is compatible with the majority of existing data-base management systems; it also allows modifying the data in the auxiliary program. The structure of the data is the following: there is a basic table containing records on the name of disease and crop and auxiliary tables, each containing information on one parameter (for example weather conditions). For communication between tables, the primary key of the main table is an external key used for all other tables, that allows to define unequivocally an accessory of any subordinated record to this or that record from the main table. The program at the start opens a file of the data. The use of external storage of the data allows editing and adding data at any moment.

25.8 PROVIDING INDEPENDENT INFORMATION ON FUNGICIDE PERFORMANCE IN WHEAT. G.J. Jellis and V.J. Foster. *HGCA, Caledonia House, 223 Pentonville Road, London N1 9HY, UK. Email: graham.jellis@hgca.com*

The HGCA, a public body funded through a levy on growers, processors and the trade, provides growers and agronomists with independent information on the performance of fungicides against the prevalent diseases of winter wheat. At a time when there have been significant shifts in fungicide performance due to resistance, HGCA research has enabled resistance to strobilurin and triazole fungicides to be detected, quantified and managed. Through collaboration with agrochemical companies, who provide new products for testing, the programme enables growers and agronomists to gain considerable insight into the performance of new products, enabling strategies to be developed based on knowledge of the spectrum of activity and effective dose rate, against a background of shifting activity due to changes in sensitivity in the target pathogens. The information is primarily delivered through activity rating 'star charts' and a fungicide dose tool which enables comparison of fungicide performance at various dose rates. Each year, major changes are also highlighted through workshops and the

press. The fungicide performance work links in to other important sources of information, providing effective integrated management of disease. These include Crop Monitor, which assesses disease incidence and risk based on regularly monitored field sites and provides updates via email and the web, the HGCA Recommended Lists which include up-to-date resistance ratings for wheat varieties, and the UK Pathogen Virulence Survey, which monitors the virulence of important pathogens on current varieties. All the information is available via the HGCA website www.hgca.com.

25.9 DEVELOPMENT AND SPREAD OF LATE BLIGHT EPIDEMICS IN MAINE. **S.B. Johnson.** *Extension crops specialist, P.O. Box 727, Presque Isle, Maine, 04769, USA. Email: sjohnson@umext.maine.edu*

Potato late blight epidemic risk is a function of primary inoculum, disease distribution, secondary spread, and the effect of host growth. Specific control targets, tactics, and keys can reduce the risk of potato losses from late blight. These change throughout the season to reflect change in the host/pathogen interaction over the season. Late blight epidemics develop differently depending on the distribution of the initial foci. If the foci are distributed widely across a region, many small epidemics soon coalesce into a large-scale epidemic across the region. If the same number of foci are narrowly distributed in an area of the region, that area will develop a localized late blight epidemic. This localized late blight epidemic must then spread to the rest of the region before a large-scale epidemic occurs. If this first spread is early in the season and extensive, severe epidemics are to be expected. Distance from the source of inoculum has a role along with the size of the foci in late blight risk. There is effectively a zero tolerance for late blight in the Maine potato production system so effort is directed at prevention of late blight epidemics as well as prediction and tracking of the epidemic. Initial late blight epidemics are affected by the levels of primary inoculum. Localized late blight epidemics are affected by the distribution of primary inoculum sources and early spread dynamics. The amount of spread is affected by host growth.

25.10 ADOPTION OF THE FARMERS ON USING FUNGAL BIOCONTROL FORMULATIONS FOR CONTROLLING CHILI FOOT ROT IN THAILAND. **T. Manond and J. Jomduang.** *Lampang Agricultural Research and Training Center, Rajamangala University of Technology Lanna, P.O. Box 89, Muang Lampang 52000, Thailand. Email: tmanond@hotmail.com*

Foot rot frequently occurs in several chili growing areas in Thailand. This study was aimed to assist farmers to adopt the use of granule-formulated *Trichoderma virens* to control foot rot of chili. Thirty farmers were selected using purposive random sampling technique from four villages in Muang district, Lampang province, north of Thailand. One farmer used *T. virens* to control foot rot in her chili plot as the demonstration plot under supervision of the researchers, while the other 29 farmers observed and learned by a study visit. Data collection was made by observation and using face to face interview questionnaires. The results revealed that the farmers responded with overall adoptability at 100%. Two motives were found to be crucial for adoptability: convenience of application and increasing yield. There were three secondary reasons, high effectiveness in controlling foot rot, better growth and higher quality fruits. Using *T. virens* provided more yield, hence, more benefit. Net profit was 11,300 Baht/Rai or 85.8% of total input cost. All farmers participating in the study expressed high satisfaction in the results and benefits. The findings implied that the process of observation and a study visit to exchange knowledge and experience among their peers was an effective strategy for successful adoption of *T. virens* formulations to control chili foot rot. In addition, using this non-harmful technology would enhance sustainable agriculture occupation in rural communities.

25.11 ROLE OF PLANT HEALTH CLINICS - AN INDIAN PERSPECTIVE. **N. Mehta.** *Department of Plant Pathology, CCS Haryana Agricultural University, Hisar 125 004, India. Email: nareshmehta@hau.ernet.in*

The basic objective of plant clinics is to provide comprehensive diagnostic and advisory services encompassing all possible causes for ill health whether biotic or abiotic. The plant clinic should be useful in providing all the valuable qualitative and quantitative local information which will help the farmers to improve their decision making capacity. By providing advice based on sound principles, plant clinics have important roles to play in reducing dependence on pesticides. The plant clinic in broad terms should be founded on human resources and physical infrastructures. Clinics should have laboratories with facilities for the whole range of plant diagnostic services integrated under one roof for the benefit of clients desiring simultaneously several diagnostic services. Plant pathologists working in plant clinics should be trained in the latest advances like Information Technology, and application of computer software for forecasting disease epidemics; laboratories should be equipped with audio, video and internet facilities for early dissemination of information to avoid losses. They should be located within the district or near to the campus of a University or Research Institute and easily approachable by the clients. There should be provision of electronic display with scrolling text so that important messages regarding plant health care can be displayed. A mobile plant clinic with modest diagnostic facilities and trained professionals can do on-the-spot diagnosis. Clinics have to play a greater role by organizing plant health camps periodically, issuing handouts, anticipating disease outbreaks and providing solutions or options tuned to farmers' needs with utmost clarity.

25.12 CITRUS BLACK SPOT, A MAJOR PHYTOSANITARY RISK IN INTERNATIONAL TRADE. **L. Meyer, R. Jacobs and L. Korsten.** *Dept. Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002, South Africa. Email: lise.korsten@up.ac.za*

Citrus black spot (CBS) remains one of the most important fruit diseases of citrus worldwide and has been considered a barrier to trade. The South African citrus industry has for the past six years focused on improving citrus production practices to reduce the impact of the disease on trade. Developing a predictive model to manage the disease has proven effective, as well as breaking the life cycle of the pathogen to reduce inoculum loads in orchards. In response to the European Union's (EU) new restrictive measures on CBS, a risk assessment (RA) approach has been followed and a research program initiated to address the questions raised by the EU. In this paper research activities to address the EU's response to the RA document from South Africa will be discussed. The development of a PCR-based diagnostic assay provided an effective means of detecting the pathogen on symptomatic fruit rejected for export, and of confirming the presence or absence of the pathogen in export consignments. In addition the test has been extended to sample other materials such as nursery symptomless leaves, symptomatic leaves collected from orchards, litter, branches and soil. This ISO 17025: 2005 accredited test method is cur-

rently commercially used by industry to declare production areas free of CBS and to gain market access.

25.13 TRANSFER TO FARMERS OF INTEGRATED DISEASE MANAGEMENT STRATEGY FOR FOLIAR DISEASES OF GROUNDNUT. **S. Pande, P.P. Rao, M. Sharma, P.L. Reddy, J.N. Rao and C.L.L. Gowda.** *International Crops Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, 502324 Andhra Pradesh, India. Email: s.pande@cgiar.org*

Groundnut or peanut (*Arachis hypogaea* L.) is an important oilseed crop in the rainfed environments of the semi-arid tropics (SAT) of Asia and Africa. It provides high quality oil and nuts for human consumption, and haulms as fodder for livestock. Two foliar diseases, late leaf spot (*Phaeoisariopsis personata*) and rust (*Puccinia arachidicola*) are particularly destructive and they together cause more than 70% losses in pod and haulm yield. These diseases also affect haulm quality. About 80% of the area under groundnut in Asia and Africa is covered by traditional cultivars which are highly susceptible to these diseases. Realizing the economic importance of foliar diseases in groundnut production in SAT regions of Asia and Africa, we at ICRISAT developed an integrated disease management (IDM) strategy. This comprised the ICRISAT-bred improved early-maturing cultivar ICGV 91114, fungicide seed treatment with bavistin + thiram @ 2.5 g/kg seed, and one foliar application of fungicide chlorothalonil (Kavach) at 65-70 days after sowing. The IDM package protected the crop from diseases and provided consistently higher pod and fodder yield under farm conditions in the Deccan Plateau of India where, by 2005, >10,000 farmers had adopted this practice. Several researchers and policy makers from Africa have seen the on-farm validation of IDM technology. The IDM of foliar diseases of groundnut is a successful example of knowledge transfer of plant pathology from scientists to end users – the farmers.

25.14 AN *IN VITRO* METHOD FOR RELIABLE INDUCTION OF MYCELIAL GROWTH AND PYCNIDIA OF *SEPTORIA TRITICI*. **J.B. Speakman, K. Hellmann and K. Perjak.** *BASF Aktiengesellschaft, 67117 Limburgerhof, Germany. Email: johnbryan.speakman@basf.com*

We describe a method of reliably inducing extensive mycelium development of *Septoria tritici* in agar cultures. Furthermore, after incubation of such cultures for about 14 days, viable pycnidia develop in which the typical pycnidiospores of *S. tritici* are produced. This method facilitates *in vitro* studies of the effects of fungicides on this important cereal pathogen in Northern Europe. Experiments are in progress aiming to induce production of pseudothecia of the teleomorph *Mycosphaerella graminicola*.

25.15 THE PLANT HEALTH CLINIC – A GLOBAL PERSPECTIVE. **M.P. Srivastava.** *Haryana Agricultural University, F-44 FF Tulip Garden, Sushant Lok-II, Gurgaon 122011, India. Email: mpsrivastava@indiatimes.com*

Unprecedented losses due to plant pests pose serious threats to food security. However, pests can be managed with the timely diagnostic support of plant clinics, which operate as a unit of Plant Pathology Departments, in India, the USA and Canada, and as global plant clinics run by CABI in the UK and Afro-Asian countries. Unfortunately we do not have organized plant clinics with independent identity like those for humans and animals. The plant clinic concept is likely to revolutionize plant protection but we need well-organized clinics/polyclinics for comprehensive diagnosis of all pests by skilled professionals equipped with diagnostic tools, such as monitor-aided microscope, audio, video, internet facility and toll-free telecommunication. Samples of diseases with colored blow-ups must be displayed and charging of diagnostic fees avoided. Clinics may have electronic displays with scrolling text for important message on plant health care. Mobile plant clinics with modest diagnostic tools and trained professionals must form an integral part of the system for providing on-the-spot diagnosis in the event of disease outbreak. Such clinics have helped to avert epiphytotics in India. Plant clinics need to organize workshops on plant health, biopesticides, judicious use of pesticides, resistance management and promoting IPM, besides issuing pest alerts like Plant Disease Warnings issued by the author in the past. Clinics must keep a vigil on bioterrorism and produce a fleet of Plant Doctors/ Pest-Control Advisers. These are much in demand for control of farm and domestic pests. Let us hope, the world will witness creation of organized plant clinic as a global phenomenon, meeting the aspiration of growers in assuring food security.

25.16 PLANT PROTECTION TOWARDS SUSTAINABLE AGRICULTURE – LESSONS FROM THE PAST. **M.P. Srivastava.** *Haryana Agricultural University, F-44 FF Tulip Garden, Sushant Lok-II, Gurgaon 122011, India. Email: mpsrivastava@indiatimes.com*

Plant diseases seriously affect sustainable agricultural production. With the availability of safer and more effective 4th generation fungicides including novel fungicides, biopesticides and IPM approaches, losses can be prevented to achieve sustainability. However, plant protection strategies need continuous monitoring, intervention and course correction. Certain glaring revelations have emerged from a study conducted on a group of vegetable growers and field staff of four villages which are an eye opener and from which lessons can be learned to make plant protection more effective. With regard to seeking advice, a whopping 62% of farmers approached pesticide dealers for pesticides to be applied, while only 12% called the Plant Protection inspector, 8% the Agriculture Science Centre, and another 8% applied their own wisdom. The remaining 10% went organic. Amongst pesticide users, 32% purchased pesticides on credit with least option of selecting a particular pesticide/brand. Cocktails of pesticides were used by 12% farmers on the insistence of dealers providing pesticide on credit without bothering to check for compatibility. No biopesticides were used. Guidelines on waiting period after pesticide application were ignored or flouted. A majority of 68% went for indiscriminate use of pesticides while the rest applied pesticides with caution on a small piece of land meant for their personal consumption. Most of the farmers were ignorant about resistance development. Field functionaries appeared abreast with ideas on resistance but lacked comprehensive guidelines and the FRAC Code. We conclude that farmers should not approach dealers, should avoid indiscriminate use of chemicals and cocktails, keep FRAC guidelines handy, encourage IPM, and use mid-term review for effective pest control.

MANAGEMENT OF FOREST DISEASES

16.1 THE PITCH CANKER FUNGUS, *FUSARIUM CIRCINATUM* IN CHILE. **R. Ahumada, E. Steenkamp, B. Wingfield and M. Wingfield.** *Bioforest S.A., Concepción, Chile. Email: rahumada @arauco.cl*

The pine pitch canker pathogen *Fusarium circinatum* (teleomorph *Gibberella circinata*) was first detected in *Pinus radiata* nurseries in Chile in 2001. In this environment the fungus is well-established, but outstanding efforts made to manage losses have greatly reduced its relevance. Despite ongoing surveys, *F. circinatum* has not been detected on established trees in plantations and there is no evidence of the pitch canker disease as it is known in plantations elsewhere in the world. Surveys have paid special attention to insects that are known to be associated with *F. circinatum*, for example the pine tip moth *Rhyacionia buoliana* and these have also not shown any association with the fungus. Population genetic studies on isolates of *F. circinatum* in Chile have shown that the pathogen has very low genetic diversity, suggesting limited introduction into the country. Significant efforts have been made to develop planting stock with high resistance to *F. circinatum*, which should minimise damage if pitch canker would become a problem in the *radiata* pine plantations in Chile.

16.2* AN EMERGING NEEDLE BLIGHT DISEASE OF *PINUS RADIATA* IN CHILE. **R. Ahumada, A. Duran, B. Slippers, M. Gryzenhout, B. Wingfield, A. Rotella, F. Flores and M. Wingfield.** *Bioforest S.A., Concepción, Chile. Email: rahumada@arauco.cl*

A new and serious needle blight disease has appeared and spread rapidly on *Pinus radiata* in the Arauco and Valdivia Provinces of Chile since 2003. Known in Chile as Daño Foliar del Pino (DFP), it now affects an area of about 60,000 ha with different levels of intensity. Infection is closely associated with the onset of rain at the end of summer. Needles from the previous season's growth are infected on the lamina and die rapidly, typically falling from the trees at the end of winter. Particularly on young trees, copious amounts of resin form at the needle bases, reflecting a strong reaction by the stems of trees to exclude infection. Although cankers can develop in the cambium at the bases of infected needles, these appear to be limited in extent and infections do not seem to enter the wood. Only *P. radiata* trees have been affected by DFP and other *Pinus* species, like *P. pinaster* in the affected area remain healthy. Isolations from infected needles on selective media have consistently yielded a *Phytophthora* sp. with non-papillate sporangia, which are caducous and typical of aerial *Phytophthora* spp. DNA sequence and morphological comparisons have shown that the fungus is an undescribed species, currently named *Phytophthora pinifolia* prov. nom. Research is underway to understand the life cycle of *P. pinifolia* and to develop appropriate management strategies to reduce its impact on Chilean forestry.

16.3 SPECTRAL STUDIES OF PLANTATION EUCALYPTS TO DEVELOP REMOTE SENSING TOOLS FOR FOREST HEALTH DETECTION, WITH REFERENCE TO FUNGAL PATHOGENS. **K.M. Barry, S. Ridge, R. Corkrey, C. Stone and C.L. Mohammed.** *CRC Forestry, Private Bag 12, Hobart, 7001 TAS, Australia. Email: karen.barry@utas.edu.au*

Foliar and crown symptoms of a range of stressors (abiotic and biotic) can be detected by spectral reflectance in the visible and near infrared wavelengths. A summary of three studies of *Eucalyptus globulus* will be presented which highlight detection of symptoms of eucalypt leaves commonly associated with fungal pathogens, including chlorosis, reddening, necrosis and leaf loss. Lesions due to *Mycosphaerella* leaf disease are initially detected as chlorotic spots, which then develop red regions and necrosis. Spectral data from a non-destructive study of artificially-inoculated plants was collected and vegetation indices were developed to discern lesions from healthy tissue. Sensitivity analysis and multivariate methods (e.g. penalized discriminant analysis) highlighted the importance of wavelengths in the red region of the spectrum (particularly the 680-690 nm range). The influence of each symptom type on spectral reflectance was further explored with a low-nutrient and -light experiment in which treatments variably resulted in healthy green, pale green or reddened leaves which were then either subjected to artificial lesions or not. Indices derived from reflectance spectra were correlated to pigments (chlorophyll, carotenoids and anthocyanins), other leaf properties and physiological variables. As well as foliar symptoms, leaf loss and reduction in crown size are important indicators of some diseases. Crown-level reflectance spectra of *E. globulus* showed that reductions in leaf area could be detected with a number of vegetation indices but some (e.g. NDVI) were highly influenced by background type while others (e.g. red edge indices) were not. In conclusion, a variety of symptoms are caused by different fungal diseases and these can be used to aid detection with remote sensing. Results gained here are vital to support development and interpretation of airborne or satellite imagery for forest health assessment in eucalypt plantations.

16.4 *KIRRAMYCES DESTRUCTANS* IN AUSTRALIA: BIOSECURITY THREAT OR ELUSIVE NATIVE PATHOGEN? **T.I. Burgess, V. Andjic, B. Dell, M.J. Wingfield and G.E.StJ. Hardy.** *Biological Sciences, Murdoch University, Murdoch, WA 6150, Australia. Email: tburgess@murdoch.edu.au*

Kirramyces destructans was first described in 1996 from north Sumatra, Indonesia, where it caused severe leaf and shoot blight on *Eucalyptus grandis* in nurseries and young plantations. Since then it has been reported in nurseries and plantations in Vietnam, Thailand and China, with its host range extending to include *E. camaldulensis* and *E. urophylla*. *K. destructans* has also been reported from native *E. urophylla* in East Timor and was considered a significant biosecurity threat to Australia's native eucalypt forests and plantations. A study on the population diversity of *K. destructans* isolates throughout south-east Asia in which 8 gene regions were sequenced (four nuclear genes, one mitochondrial gene and three microsatellite markers) detected very low nucleotide polymorphism. This genetic uniformity is indicative of an introduced population which has subsequently spread throughout Asia via human-mediated movement of germplasm. Surveys of sentinel plantings in northern Australia revealed a complex of *Kirramyces* spp. among which *K. destructans* was detected. The same gene regions and markers were sequenced as for the Asian study and diversity among the *K. destructans* isolates in Australia was found to be much greater than that in Asia. We believe that *K. destructans* is native to Australia where is resides symptomlessly within the native vegetation. The disease is only expressed when non-endemic eucalypts are planted. As such the pathogen is a major encumbrance to the establishment of commercial eucalypt plantations in Northern Australia. The disease has not been observed in native ecosystems, but the effect of inoculum build up within plantations on adjacent native eucalypt remnants is not known.

16.5 THE ETIOLOGY OF THE EUCALYPTUS AND NATIVE TREE DECLINE – MUNDULLA YELLOWS. **B. Czerniakowski, R. Crnov, I.W Smith and J.E Luck.** *Primary Industries Research Victoria, PMB 15 Ferntree Gully Delivery Centre, 3156 VIC, Australia. Email: barbara.czerniakowski@dpi.vic.gov.au*

Mundulla Yellows (MY) is a lethal dieback syndrome of Australian native species, affecting tens of thousands of trees Australia-wide. MY was first reported in the 1970s but despite being present for nearly 40 years, the etiology had not been resolved and no permanent recovery had been observed. MY was initially hypothesized to be caused by an infectious biotic agent however, no pathogen has been linked to symptoms. To investigate the association of both biotic and abiotic factors with MY symptoms, we conducted a systematic analysis of soil and foliage from sites with symptomatic trees and sites with asymptomatic trees. Pathogenic agents were not found to be associated with MY-affected trees at the investigated sites. Soil from sites with symptomatic trees had significantly higher levels of dissolved carbonates, EC, water content, organic matter content, and significantly lower available iron. Element deficiencies and/or toxicities were also identified in association with MY symptoms. To investigate whether physiological Fe deficiency was contributing to MY symptoms, as a result of the adverse soil conditions identified in this study, various treatments were tried. MY symptoms were most effectively corrected with the xylem delivery of acidifying Fe treatments, which confirmed that physiological Fe deficiency is the major cause of Mundulla Yellows. We report the first successful treatment of this tree decline since MY was first observed nearly 40 years ago.

16.6 EXPANSION OF EUCALYPT PLANTATIONS CONTRIBUTES TO THE GROWING IMPORTANCE OF *QUAMBALARIA* LEAF- AND SHOOT PATHOGENS. **Z.W. de Beer, G. Pegg, J. Roux, X.D. Zhou and M.J. Wingfield.** *Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Email: wilhelm.debeer@fabi.up.ac.za*

The smut genus *Quambalaria* accomodates four species, which were discovered as minor leaf and shoot pathogens of eucalypts between 1930 and 1993. They were not initially recognised as being closely related and were placed in different genera. The rapid expansion of commercial *Eucalyptus* and *Corymbia* plantations in Southern Africa, South America, Australia and China during the past two decades has resulted in a substantial increase in the importance of these little-known pathogens. Severe damage to seedlings and clonal hedges has been reported from four continents, leading to a renewed research interest in these fungi. In 2000, the new genus, *Quambalaria* was erected to accommodate the three species known at that time. In 2006, DNA sequence comparisons of these three species showed that they reside in a monophyletic lineage in the Microstromatales (Ustilaginomycetes) and this was the first clear evidence that they were Basidiomycetes. In the present study we combined and reanalysed all accessible DNA sequence data for *Quambalaria* spp., including those of *Q. coyrecup*, a species newly described from Australia. All available data on the distribution and host ranges of the species were also analysed. The results show that the host ranges and geographical distribution of especially *Q. pitereka* and *Q. eucalypti*, are expanding rapidly to all areas where eucalypts are grown commercially. Virtually nothing is known regarding the life cycles, general biology, and infection strategies of these pathogens. An appropriate understanding of these factors will be essential for the development of effective control measures.

16.7 IDENTIFICATION AND SPREAD OF *HETEROBASIDION ABIETINUM* IN *ABIES PINSAPO* FORESTS. **P. de Vita, J.E. Sánchez and M.E. Sánchez.** *Patología Agroforestal, Universidad de Córdoba, Spain. Email: ag1sahem@uco.es*

Abies pinsapo is an endemic fir species occurring only in a small area in southernmost Spain. Natural stands of this tree are frequently attacked by *Heterobasidion* root rot. To identify the *Heterobasidion* species causing the disease, pure cultures of the fungus were isolated from symptomatic trees in five localities in Sierra de las Nieves Natural Park and identified by pairing tests. In addition, genets of the fungus were identified in two pure stands of *A. pinsapo*. All the *Heterobasidion* specimens collected proved to be *Heterobasidion abietinum*. The largest genet found was 57 m long, and had colonized 10 trees. The large size of the main genets implies that *H. abietinum* might have spread via root contacts from old infections generated before the establishment of the Sierra de las Nieves Natural Park in 1989. Exceptionally dry summers during the last two decades may have weakened *A. pinsapo* and favoured the spread of the disease.

16.8 STEPS TOWARD INTEGRATED MANAGEMENT OF *PHYTOPHTHORA KERNOVIAE* AND *P. RAMORUM* ON MAGNOLIAS IN HERITAGE GARDENS, UK. **S. Denman, S.A. Kirk and A. Whybrow.** *Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK. Email: sandra.denman@forestry.gsi.gov.uk*

Phytophthora kernoviae (Pk) and *P. ramorum* (Pr) are introduced, invasive, highly aggressive pathogens of woodland trees and ornamental shrubs in the UK. The pathogens attack aerial parts of multiple hosts causing leaf necrosis; defoliation; bole cankers. Inoculum is generated on foliage hosts and spreads to tree stems. The key foliar host in UK woodlands is *Rhododendron ponticum*; European beech is the main stem host. Apart from woodlands exotic trees such as *Drimys*, *Magnolia* and *Michelia* may also be foliar hosts and are often foundation species in heritage gardens. The high value of these trees induced an integrated approach to managing disease on magnolias. Two essential parts of the management scheme are removal of infected rhododendron and sanitation burning of shed leaves. Epidemiological studies on Pk infected magnolias showed that Pk survived in leaf scars, buds and perules, and in leaf debris and soil. Chemical intervention could form part of the management strategy. Phosphonate (Agri-Fos 400, 45.8% a.i.) was applied to Pk infected saplings and to six Pr infected, mature, heritage garden magnolias in spring as stem injections (15 ml 20% a.i. per m canopy diameter). Phosphonate levels in foliage were monitored. Isolations were made from symptomatic foliage. Disappearance or reduction in symptoms occurred on treated trees and pathogen incidence was much reduced or negative compared with controls. Phytotoxicity effects could be seen but in most mature trees were transitory. The treatment has promise as part of integrated management if correct phosphonate levels in foliage can be achieved.

16.9 CONTAINMENT AND ERADICATION OF *PHYTOPHTHORA CINNAMOMI* IN NATIVE VEGETATION IN SOUTH-WESTERN AUSTRALIA AND TASMANIA. **W.A. Dunstan, T. Rudman, B.L. Shearer, G.E. StJ. Hardy, N.A. Moore, B. Dell, C. Crane and S. Barrett.** *School of Biological Sciences & Biotechnology, DSE, Murdoch University, Perth, WA 6150, Australia. Email: w.dunstan@murdoch.edu.au*

Phytophthora cinnamomi, and diseases caused by it, are listed

as one of five key threatening processes affecting biodiversity in Australia, particularly in south-western Australia where 40% of 5700 native plant species are susceptible. The aim of our experiments is to develop protocols to contain and eradicate small infestations of *P. cinnamomi* in native vegetation. In two separate experiments, in Tasmania (TAS) and south-western Australia (WA), a sequence of treatments was applied to plots within active disease centres. Treatments included two or more of the following: (1) removal of pathogen hosts, (2) installing root barriers and subsurface irrigation, for the application of fungicide at depth, (3) surface applications of Terrazole (triadiazole) and Ridomil (metalaxyl-M) fungicides, and (4) application of metham-sodium fumigant by injection, at depths of 0.2 m and 1 m. In contrast with control plots infested with *P. cinnamomi*, the pathogen was not recovered from plots treated by host removal + fungicide + fumigation in the WA experiment. The pathogen was recovered from a single soil sample in similarly treated plots in the TAS experiment. Treatment with Ridomil alone significantly reduced recoveries of *P. cinnamomi*, but did not eliminate it. The survival of *P. cinnamomi* will be further assessed to confirm whether it can be eradicated or only suppressed.

16.10 SPATIAL PATTERN OF A NEW AND SERIOUS NEEDLE BLIGHT DISEASE IN CHILE. **F. Flores, R. Ahumada, A. Rotella and M. Wingfield.** *Bioforest S.A., Concepción, Chile. Email: francisco.flores@arauco.cl*

During the summer of 2003-2004 and the winter of 2004 a new and severe defoliation was detected on radiata pine (*Pinus radiata*) plantations in the Arauco Province, in South Central Chile. The new disease, known in Chile as *daño foliar del pino* (DFP), has affected *P. radiata* stands every year since 2004 and spread to new areas mainly south of the original site. A new species of *Phytophthora* has consistently been isolated and associated with DFP. The DFP-affected area is along the coast and up to 40 km inland, characterized by cool moist winters and summers that are typically dry and with moderated temperature. There is a strong spatial pattern in damage severity, and at all scales the most severely affected needles are in areas with little sun and higher humidity and wetness. At branch level, needles on the lower part of the branch are first and most affected; at the tree level, lower branches are more severely affected; and at the landscape level, southern (i.e. shaded) slopes are more severely affected. A logistic model of probability of severe DFP damage as a function of environmental factors (elevation, solar radiation, temperature, and others) was fitted. The spatially explicit model output is in good agreement with independent estimates of severity of damage and is now being use by forestry companies to make decisions about species/ genotypes deployment, and chemical control strategies.

16.11 BASIDIOMYCETE ROOT ROTS OF PAPER-PULP TREE SPECIES IN INDONESIA – IDENTITY, BIOLOGY AND CONTROL. **A.A. Francis, C. Beadle, Mardai, H. Indrayadi, B. Tjahjono, A. Gafur, M. Glen, A. Widyatmoko, E. Hardyanto, Junarto, R.S.B. Irianto, D. Puspitasari, N. Hidayati, G. Pegg, A. Rimbawanto and C.L. Mohammed.** *University of Tasmania, TIAR, Private Bag 12, Hobart, TAS 7001, Australia. Email: anthony.francis@utas.edu.au*

We present the findings of the ACIAR project "Management of fungal root rot in plantation acacias in Indonesia". We outline the diversity of basidiomycetes associated with, and causing root rot in *Acacia* plantations, and *Eucalyptus* plantations on ex-*Acacia* sites, in Riau, South Sumatra and Kalimantan. We share insights into the biology of these fungi with implications for management of the various forms of root rot they cause. We present our findings regarding biological and silvicultural control options and a critique of available control strategies in light of the biology of the pathogens and the constraints facing the Indonesian pulp plantation industry. In keeping with the ACIAR aims of poverty reduction and sustainable development, we also present our progress in training, extension and adoption of the research findings in corporate and out-grower sectors of the Indonesian pulp plantation industry.

16.12 CONTROL OF *PASSALORA* LEAF AND SHOOT BLIGHT ON *ACACIA CRASSICARPA* THROUGH PLANT RESISTANCE. **A. Gafur, B. Tjahjono, C.Y. Wong and G.D. Golani.** *APRIL Forestry R&D, PT RAPP, Pangkalan Kerinci 28300, Indonesia. Email: gafur@uwalumni.com*

Since first noted in November 2002, *Passalora* leaf and shoot blight incited by *Passalora perplexa* has increased in distribution in lowland *Acacia crassicarpa* in Riau, Indonesia. Symptoms start to develop at early plant stages and include phyllode lesions which may lead to malformation and curling. Lesions are typically surrounded by a distinct chlorotic halo. Although its impact on growth has not been significant, the disease remains to be properly managed. Natural plant resistance was considered a key component in disease management. To study this, 49 clones vegetatively propagated from plus trees were tested for their field resistance. An Alpha design consisting of 5 replicates, 5 blocks, and 10 plots per block, was used. Experimental units were 5 trees of each clone planted in plots. The trees were exposed to natural and artificial inoculation. Resistance was assessed through a disease severity index. Results indicated that resistance, manifested by a hypersensitive reaction defense mechanism, was evident in most of the selected clones. Resistance was found to be under moderate/high genetic control ($h2 = 0.4$). Although further study is still needed, the results offer potential to using plant resistance as one component of *Passalora* leaf and shoot blight disease management on *A. crassicarpa*.

16.13 REAL-TIME PCR DETECTION OF *MYCOSPHAERELLA* SPP. INFECTING EUCALYPTS. **M. Glen and C.L. Mohammed.** *CSIRO Ensis Forest Biosecurity & Protection, Private Bag 12, Hobart, TAS 7001, Australia. Email: morag.glen@csiro.au*

Over 50 species of *Mycosphaerella* contribute to *Mycosphaerella* leaf disease (MLD) in eucalypts. Several species are often present in a single leaf, and up to five have been detected, using nested PCR, from a single lesion. This has raised questions about the role each of these species plays in disease development. In order to gain some insights into this, we extended our molecular detection of *Mycosphaerella* spp. to incorporate real-time PCR quantification of three species commonly associated with MLD in eucalypts. *M. cryptica* and *M. nubilosa* are currently regarded as the most damaging *Mycosphaerella* species in eucalypt plantations in southern Australia, and the most common in surveys using nested PCR detection. *M. parva* is considered to be endophytic rather than pathogenic, and was frequently detected in symptomless leaves. The relative biomass of *M. cryptica, M. nubilosa* and *M. parva* was determined in eucalypt leaves that were artificially inoculated in a glasshouse and in *E. globulus* leaves collected at fortnightly intervals from a genetics trial during a severe MLD

epidemic. This information is expected to contribute to an understanding of competition among *Mycosphaerella* species, host resistance and host-pathogen dynamics.

16.14 *CHERRY LEAF ROLL VIRUS*: A THREAT TO FINNISH *BETULA* SPP. E. Grubits, S. von Bargen, J. Langer, R. Jalkanen and C. Büttner. *Section Phytomedicine, Lentzeallee 55/57, Humboldt-Universität zu Berlin, 14195 Berlin, Germany. Email: phytomedizin@agrar.hu-berlin.de*

Virus-related symptoms such as vein banding, leaf roll, chlorosis and subsequent necrosis on birch leaves were increasingly recorded throughout Finland since 2002. Affected trees belonging to the species *Betula pendula* (silver birch), *B. nana* (dwarf birch), *B. pubescens* (downy birch), *B. pubescens* var. *czerepanovii* and var. *appressa* (mountain birch) often showed reduced growth and scattered defoliation. Trees exhibiting such symptoms are widespread in the country and have also been noted in northern Norway and Sweden. Leaves, buds and catkins of symptomatic shoots collected during the vegetation period in 2007 were subjected to an IC-RT-PCR specific for *Cherry leaf roll virus* (CLRV). Infection of several *Betula* species from different locations was confirmed, revealing a high virus incidence all over Finland. The virus was detected in *B. pubescens* as well as common, mountain and dwarf birch. This is the first time that the latter two *Betula* spp. have been confirmed as hosts of CLRV. CLRV has not previously been recorded in northern Finland. Sequence comparison of amplified fragments indicated a heterogeneous virus population in Finnish trees, which could be differentiated from other CLRV strains infecting *Betula* spp. in Germany and other European countries. Referring to the rate at which symptoms have spread within the last few years, CLRV might become a serious problem in northern birch forest ecosystems and for the local forest industry. Therefore rapid efforts are needed to conduct detailed studies on distribution, means of spread, and the biological and economic importance of CLRV among birch species and possible other hosts.

16.15 THE EFFECT OF VITALITY FERTILIZATION ON THE SEVERITY OF *SIROCOCCUS* SHOOT BLIGHT IN MATURE NORWAY SPRUCE. E. Halmschlager, K. Katzensteiner, H. Anglberger and H. Sterba. *Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Department of Forest and Soil Sciences, University of Natural Resources and Applied Life Sciences, Vienna (BOKU), Hasenauerstrasse 38, A-1190 Vienna, Austria. Email: erhard.halmschlager@boku.ac.at*

Since the early 1980s *Sirococcus* shoot blight has caused severe damage on Norway spruce, *Picea abies* (L.) Karst., in some parts of Austria. Symptoms are most prevalent and severe in secondary, pure spruce forests on poor soils with low cation exchange capacity and a low base saturation. Furthermore, our previous studies revealed insufficient Mg and Ca supply and enhanced N/Mg and N/Ca-ratios in the needles of severely diseased trees. Nutritional disorders are thus suggested to increase the susceptibility of Norway spruce to *Sirococcus* shoot blight. To test the hypothesis that vitality fertilization leads to a recovery of diseased trees, a single-tree fertilization experiment was established in a mature Norway spruce stand in Upper Austria. Two different fertilizer treatments (dolomitic liming and combined application of gypsum plus kieserite) and an unfertilized control variant were applied on a total of 144 dominant or co-dominant trees in a randomized block design. Half of the trees were severely affected by *Sirococcus* shoot blight, whereas the other trees were apparently healthy. Results indicate that application of vitality fertilizers mitigates disease severity of fertilized trees and promotes tree recovery. Best results were achieved by fertilizing spruce trees with a water soluble Ca- and Mg-fertilizer (gypsum plus kieserite-variant) resulting in an 18.9% decrease in disease severity from 2001 to 2006. Dolomitic liming also promoted tree recovery, but decrease in disease severity was slightly less pronounced (11.8%), whereas in the unfertilized control variant a 3.5% increase in disease severity was observed in the same period.

16.16 BENEFITS AND RISKS ASSOCIATED WITH BIOLOGICAL CONTROL OF SELECTED FOREST TREE DISEASES IN FINLAND AND THE UK. J. Hantula, J. Webber and K. Tubby. *Finnish Forest Research Institute, P.O. Box 18, 01301 Vantaa, Finland. Email: jarkko.hantula@metla.fi*

In the UK, the biological treatment of pine stumps against *Heterobasidion annosum* using *Phlebiopsis gigantea* has been practiced for more than 30 years. In Finland, the history goes back for a decade, and is now common in all summertime cuttings of both spruce and pine in the southern part of the country. Under Finnish conditions there is no risk of *Heterobasidion* spp. infection in wintertime. Due to economic and social reasons there is, however, a need for summertime cuttings, in which control of new spore infections is essential. Both biological and chemical methods are available, and their efficiencies are usually above 90%. The biological control of *Heterobasidion* spp. causes only minor risks for biodiversity and natural populations of *P. gigantea*. However, more serious risks are due to the lack of knowledge about the treatment among forest owners, and the possible failure of treatment under conditions of climatic change in the future. Therefore, research aiming to increase the efficiency of *P. gigantea* treatment, as well as attempts to increase the level of understanding among forest owners, are being conducted in Finland and the UK.

16.17 *NECTRIA FUCKELIANA* ASSOCIATED WITH FLUTE CANKERS IN *PINUS RADIATA* IN NEW ZEALAND. A.J.M Hopkins, P.E. Crane, M.A. Dick, L.S. Bulman and T.D. Ramsfield. *Forest Biosecurity and Protection, Ensis, Private Bag 3020, Rotorua, New Zealand. Email: anna.hopkins@ensisjv.com*

Stem cankers, often associated with pruned stubs, have become increasingly frequent in some *Pinus radiata* plantations in the South Island of New Zealand over the last 15 years. Although tree crowns generally remain healthy, stem cankers significantly reduce the commercial value of the timber, often leading to extensive deformation, stain and decay within the stem. *Nectria fuckeliana* is the fungus most commonly found in association with these stem cankers. Thought to be endemic to Northern Europe and Scandanavia, where it is a common saprophyte or weak pathogen of species of *Picea* and *Abies, N. fuckeliana* was first confirmed in New Zealand in 1996. This paper outlines early investigations into the ecology, epidemiology and management of *N. fuckeliana* in *P. radiata* in New Zealand. Of particular interest is an increased understanding of aspects of the basic biology of the fungus such as spore production and dispersal and their relationship to environmental conditions. This knowledge will enable forest managers to better manage their forests to limit the spread and infection of *N. fuckeliana* and thereby reduce the incidence of stem cankers.

16.18 ENVIRONMENTAL FACTORS INFLUENCING PINE DEATH CAUSED BY PINE WILT DISEASE IN KYOTO: THE VIEWPOINT OF LANDSCAPE CONSERVATION. **T. Ikeda and S. Naoe.** *Department of Forest Science, Kyoto Prefectural University, Shimogamohangi-cho, Sakyo, Kyoto 606-8522, Japan. Email: tikeda@kpu.ac.jp*

Kyoto is located in a basin surrounded by mountains to the west, north and east and has played a key role in traditional Japanese culture such as theatre, literature and painting for more than 1000 years. Pine forests surrounding Kyoto have often been part of this traditional scene. However, these forests have been severely damaged by pine wilt disease, and it is necessary to conserve such pines and pine forests in order to maintain the traditional culture. To understand environmental effects on pine death in the whole Kyoto area, the degree of damage in each forest stand was investigated by visual evaluation and measurement of every tree. The damage was estimated and the principles of saving the pine forest landscape are presented. Elevation was a more accurate index than the MB index to predict pine mortality. Mortality decreased with increasing elevation and it was lower on mountain ridges than on slopes. There was no difference in pine mortalities on mountains aligned in different orientations. At elevations above 500 m, mortality was reduced to 20%, and the forests were maintained because of regeneration, although even at 800 m elevation pine damage was observed. We did not find any places without pine damage. In conclusion, the control program must aim exclusively at limited areas.

16.19 ASSESSMENT OF CHEMICAL TREATMENTS TO INCREASE WOOD VOLUME IN *EUCALYPTUS GLOBULUS* PLANTATIONS IN WESTERN AUSTRALIA. **S. Jackson, H. Neumeist-Kemp, M. Calver, S. Collins, B. Dell and G. Hardy.** *Faculty of Sustainability, Environment and Life Sciences, Murdoch University, Murdoch, WA 6150, Australia. Email: sjackson@murdoch.edu.au*

A field trial was designed to determine whether the control of pests and pathogens by regular chemical treatments could increase volume in *E. globulus* plantations. There were four foliar treatments, fungicide, fungicide plus insecticide, insecticide and control. The treatment plots were separated by buffer zones. The trial involved two plantations (Bill's and Sixpenny) over four years. A total of 500 trees were used for each treatment (n= 2000). The incidences of pests and diseases were similar between plantations over the four years of the trial. Standardised tree volumes were higher at Bill's than at Sixpenny in each year and the rate of increase between years was also greater at Bill's. The differences in tree volume caused by treatment by 2004 were comparatively minor, but still significant. Post hoc LSD tests showed that at Bill's standardised tree volumes were greater when both insecticide and fungicide were added. At Sixpenny, the greatest improvements occurred with the use of insecticide alone. The improvements in volume as a result of insecticide or fungicide treatment ranged from up to 8.6% (Bill's) to up to 13% (Sixpenny). As the increase in yields was minimal, they represented very little benefit compared to the cost of applying the treatments on a regular basis. The differences between plantations were most likely due to soil type and climate. If disease and pest incidence had been higher, chemical treatments may have been cost effective.

16.20 MOLECULAR CHARACTERIZATION OF *FUSARIUM* SPP. AND BIOLOGICAL CONTROL OF *FUSARIUM* ROOT DISEASE IN FOREST TREE NURSERIES. **M.-S. Kim, J.E. Stewart, R.L. James, R.K. Dumroese and N.B. Klopfenstein.** *USDA Forest Service-RMRS, 1221 S., Main St., Moscow, ID 83843, USA. Email: mkim@fs.fed.us*

Root-rot disease caused by *Fusarium* spp. can cause severe losses in conifer nurseries. Isolates of *Fusarium* spp., morphologically indistinguishable from *F. oxysporum*, were collected from healthy and diseased conifer seedlings and nursery soils in the western USA. Over 350 isolates with *F. oxysporum*-like morphology were characterized using DNA sequences (mitochondrial small subunit and nuclear translation elongation factor 1 alpha). These isolates were characterized as non-pathogenic isolates of *F. oxysporum* and pathogenic *F. commune* using laboratory/greenhouse pathogenicity tests and phylogenetic analyses. Although the morphology of *F. commune* appears indistinguishable from *F. oxysporum*, these two species are quite distinct genetically. Greenhouse studies on Douglas fir seedlings were conducted to evaluate non-pathogenic *F. oxysporum* isolates for biological control of pathogenic *F. commune*. In a greenhouse study, inoculating Douglas fir seedlings with one isolate of *F. oxysporum* prevented expression of disease caused by a virulent isolate of *F. commune*. Moreover, seedling survival and growth was unaffected by the presence of *F. oxysporum*. These results demonstrated that isolates of non-pathogenic *F. oxysporum* can effectively reduce *Fusarium* root disease caused by *F. commune* of Douglas fir under nursery settings and have potential for further development.

16.21 DWARF MISTLETOE (*ARCEUTHOBIUM MINUTISSIMUM*) AND LEAFY MISTLETOE (*TAXILLUS KAEMPFERI*), IMPORTANT PATHOGENS OF BLUE PINE (*PINUS WALLICHIANA*) IN BHUTAN. **T. Kirisits, S. Dorji, E. Donaubauer, M.J. Wingfield and D.B. Chhetri.** *Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Vienna, Austria. Email: thomas.kirisits@boku.ac.at*

Blue pine, *Pinus wallichiana*, is an ecologically and economically important tree in temperate conifer forests at elevations between 2100 and 3100 m in Bhutan. Two parasitic plants, Himalayan dwarf mistletoe, *Arceuthobium minutissimum*, and the leafy mistletoe, *Taxillus kaempferi* are common on blue pine in this Himalayan country. *A. minutissimum* is widespread and very damaging in dry blue pine forests in the districts Paro, Ha and Thimphu in Western Bhutan. *T. kaempferi* occurs most frequently on blue pine, but also infests *Tsuga dumosa* and *Picea spinulosa*. It occurs in the districts Thimphu, Wangdi Phodrang, Trongsa, Bumthang and Mongar. A survey in a 156-hectare area of blue pine forests in Western Bhutan, conducted in 2004, has documented high infection levels of *A. minutissimum* and *T. kaempferi* on *P. wallichiana*. *A. minutissimum* occurred in 58% and *T. kaempferi* in 52% of the study area. Of the 2282 blue pine trees evaluated, 29% were infested with *A. minutissimum* and 5% with *T. kaempferi*. Incidence of both mistletoes increased with diameter of the host trees. However, *A minutissimum* was also prevalent on small trees, exemplified by the smallest diameter class (0 to 5 cm diameter at chest height), in which 25% of the trees were infected. Forest management practices in Bhutan have greatly favoured infestation of *P. wallichiana* with *A. minutissimum*. We recommend incorporating principles of disease management, particularly sanitation in the silvicultural system, to treat these blue pine forests affected by Himalayan dwarf mistletoe.

16.22* POTENTIAL INVASIVENESS OF *ARMILLARIA OSTOYAE*, A TREE-ROOT PATHOGEN OF CIRCUMBOREAL DISTRIBUTION. **N.B. Klopfenstein, M.S. Kim, J.W. Hanna and G.I. McDonald.** *USDA Forest Service-RMRS, 1221 S., Main St., Moscow, ID 83843, USA Email: nklopfenstein@fs.fed.us*

Armillaria ostoyae is a root pathogen that causes severe losses in growth and productivity of diverse forest trees throughout its circumboreal distribution. However, this species appears genetically variable with distinct ecological behavior in different regions. In North America, *A. ostoyae* in the Colorado Plateau exists in drier habitats and causes more disease on hardwoods in comparison with *A. ostoyae* in the northwestern USA. In China, previous reports indicate that *A. ostoyae* causes severe root disease on *Larix* sp. (Dai *et al*. 2007); whereas this pathogen only rarely impacts *Larix* sp. in North America. Surprisingly, *A. ostoyae* is apparently absent from south-central Idaho, USA, even though suitable habitat, susceptible hosts, and other *Armillaria* species are present. These examples indicate that intercontinental and interregional movement of *A. ostoyae* could represent an invasive species risk, especially under changing climate regimes. Furthermore, intraspecific and interspecific hybridization could create pathogens with novel ecological behavior, disease activity, and genetic adaptation. Studies are underway to assess the global phylogeography of *A. ostoyae* to examine genetic relationships within this species, and assess potential invasive risks associated with intercontinental and interregional movement.

16.23 FIRST OBSERVATION OF *PHYTOPHTHORA KATSURAE* ON CHESTNUT IN KOREA. **J.K. Lee, E.S. Oh, S.H. Lee and K.H. Kim.** *Division of Forest Insect Pests and Diseases, Department of Forest Environment, Korea Forest Research Institute, Seoul, 130-712, Republic of Korea. Email: jongklee@kangwon.ac.kr*

In the 19th century, chestnut trees (*Castanea* spp.) were devastated by *Cryphonectria parasitica* which is responsible for chestnut blight. In the meantime, *Phytophthora cambivora* and *P. cinnamomi* received less attention than in Europe and the United States. Chinese and Japanese chestnuts are relatively resistant to the two species of *Phytophthora* than the European and American chestnut. Additionally *P. katsurae* has been reported on Japanese chestnut in 1969 causing trunk rot and is also found in Taiwan, Australia, Papua New Guinea, and the islands of Hawaii, Kauai, Maui and Oahu. In the late 1990's, Phytophthoras became highly pathogenic and epidemic in chestnut stands. In Korea, most chestnuts, commercially cultivated are hybrids of *Castanea crenata* × *C. mollissima*. In November 2006, dead chestnut trees were found in three different locations showing inky ooze on necrotic trunks. In order to identify the pathogen and its pathogenicity on different cultivars of chestnut, eleven isolates were obtained using a *Phytophthora*-selective medium. The isolates were observed under a microscope and characterized by morphology and rDNA sequencing. Pathogenicity tests were performed in vitro and outdoors by inoculating isolates on branches and seedlings, respectively. On the culture medium, numerous homothallic oogonia with protuberances (34.0–46.2×21.9–26.7 μm) were produced. Also, papillate, ovoid to obpyriform sporangia (17.0–38.9×14.6–29.2 μm) were formed. The isolates showed 100% similarity with *P. katsurae* isolates from Japan and New Zealand and 99.6% similarity with other *P. katsurae* isolates indicating three base-pair differences. All isolates from 3 different locations were completely identical. The pathogenicity tests showed some variations in susceptibility/resistance among different cultivars of chestnut against the isolates.

16.24 CHEMICAL AND BIOLOGICAL CONTROL OF *PHYTOPHTHORA* DISEASE OF *ARALIA ELATA*. **S.H. Lee, K.H. Kim, S. Seo, E. Oh and J. Lee.** *Department of Forest Diseases and Pests, Korea Forest Research Institute, 130-712, Seoul, Republic of Korea. Email: saimonlee@empal.com*

Fresh and soft shoots of *Aralia elata* are a traditional vegetable in Korea due to their special flavor and health-promoting reputation. Recently, in nurseries, the roots of plants were severely infected and damaged by *Phytophthora cactorum*, a notorious soilborne pathogen. To select the most effective chemical control agent against this epidemic disease of *Aralia elata*, 5 different fungicides, i.e., oxadixyl+copper, metalaxyl, dimethomorph, copper oxadixyl, and phosphonic acid, were compared *in vitro*. Oxadixyl+copper was the most effective. In the field, *A. elata* plants treated with oxadixyl+copper showed 20% mortality, compared with 40-45% for dimethomorph. Treatment with phosphonic acid had no control effect with 70% mortality as compared to the untreated control showing 73.3% mortality. Among 1,342 microorganisms isolated from soil and screened for antagonism against the pathogen, two antagonists were finally selected, and identified as *Streptomyces sampsonii* and *Bacillus licheniformis* by 16s rDNAs sequencing.

16.25* DETERMINING THE SOURCE OF INOCULUM OF *FUSARIUM CIRCINATUM* IN THE WESTERN CAPE OUTBREAK OF PITCH CANKER IN SOUTH AFRICA. **O.M. Makhari, E.T. Steenkamp, T.A. Coutinho and M.J. Wingfield.** *Forestry and Agricultural Biotechnology Institute, Department of Plant Pathology and Microbiology, University of Pretoria, Pretoria 0002, South Africa. Email: olga.makhari@fabi.up.ac.za*

Fusarium circinatum causes the destructive pine pitch canker disease in many parts of the world. In South Africa, it first appeared in 1990 and has since spread to most forestry nurseries where it causes root and collar rot on various *Pinus* spp. Recently, pitch canker was reported for the first time to occur on *P. radiata* in established plantations in Tokai in the Western Cape Province of South Africa. Our aim was to test the hypothesis that inoculum for this outbreak originated in a nursery situated about 600 km from Tokai. For this purpose we determined vegetative compatibility groups (VCGs) for 70 *F. circinatum* isolates from the Tokai outbreak and 76 isolates collected from infected seedlings in the nursery under consideration. The resulting VCGs were also compared to those previously reported in South Africa. Our results showed that the Tokai and nursery isolates represent at least 8 VCGs, as well as 21 additional isolates that are incompatible with all the isolates examined. None of these isolates or VCGs was compatible to the known South African VCGs, although the nursery and Tokai populations shared 3 VCGs. Our findings therefore support the hypothesis that the nursery in question is the likely source of inoculum for the disease in Tokai. Furthermore, the relatively large number of unique Western Cape VCGs, suggests that the disease was introduced independently from those in other parts of the country, and that sexual reproduction potentially plays an important role in shaping the population biology of the pathogen.

16.26 MATING-TYPE SEGREGATION DISTORTION IN *FUSARIUM CIRCINATUM*. **O.M. Makhari, E.T. Steenkamp, T.A. Coutinho and M.J. Wingfield.** *Forestry and Agricultural Biotechnology Institute (FABI), Department of Plant Pathology and Microbiology, University of Pretoria, Pretoria 0002, South Africa. Email: olga.makhari@fabi.up.ac.za*

The causal agent of pine pitch canker, *Fusarium circinatum*, is a heterothallic ascomycete fungus. Sexual interactions between compatible isolates typically result in the formation of perithecia that ooze viable ascospores. Although mating-type of the progeny from these interactions is expected to segregate in a Mendelian fashion, we have observed a number of instances where mating-type distribution in the progeny appears to be distorted. The aim of this study was to determine the extent of this phenomenon in the South African population of *F. circinatum*. We crossed isolates of opposite mating-type representative of the known vegetative compatibility groups (VCGs) in the country. Progeny from fertile perithecia were subjected to VCG assays or genomic fingerprint analyses to ensure their recombinant nature, and mating-type was assayed using PCR-based methods. The perithecia from selected crosses were also examined microscopically. Our results showed that the mating-type segregation ratio was significantly distorted in a large number of crosses. However, the asci within such perithecia typically contained eight ascospores of normal appearance. This suggests that the observed distortion in mating-type segregation is caused by a deleterious factor that is active after ascospore formation. Nevertheless, preferential inheritance of a single mating-type after sexual reproduction may have a considerable impact on the population structure, especially since frequent occurrence of this phenomenon will significantly reduce the effective population size of the pathogen, thereby also lowering the genetic diversity in sexually produced offspring.

16.27 CLONAL POPULATIONS OF *CRYPHONECTRIA PARASITICA* IN SOUTHEASTERN EUROPE. M.G. Milgroom, K. Sotirovski, D. Spica, S.O. Cacciola, J.E. Davis, M.T. Brewer and P. Cortesi. *Institute of Plant Pathology, State University of Milan, Via Celoria 2, 20133 Milano, Italy. Email: paolo.cortesi@unimi.it*

Populations of the chestnut blight fungus, *Cryphonectria parasitica*, vary in genetic diversity and structure, with established populations typically more variable than expanding populations. Previous studies found that the diversity of vegetative compatibility (vc) types is lower in southeastern Europe and Turkey, where *C. parasitica* has been introduced recently, than in northern Italy, Switzerland and France, where populations were established earlier. To test whether vc types represent clones, we genotyped samples from southern Italy, Romania, Bulgaria, Macedonia, Greece and Turkey using 11 sequence-characterized amplified region (SCAR) markers and six vegetative incompatibility (*vic*) loci. These populations were clonal by all criteria tested: 1) among 375 isolates, we found only nine multilocus haplotypes, and the same haplotypes were found in multiple countries, sometimes separated in time by as much as 12 years; 2) few recombinant haplotypes were found; 3) populations were in linkage disequilibrium; 4) the two sets of independent markers, SCARs and vc types, were highly correlated; and 5) sexual structures of *C. parasitica* were found only in Romania. A haplotype network showed that the observed SCAR and *vic* haplotypes most likely arose from recombination, even though they have spread and persisted clonally. The spread of a few clones could be the result either of founder effect and restricted migrations, or these clones could be highly adapted to environmental conditions they encounter in southeastern Europe.

16.28 PRECISION HEALTH MANAGEMENT IN AUSTRALIAN EUCALYPT PLANTATIONS; A CASE STUDY WITH *MYCOSPHAERELLA* LEAF DISEASE. C. Mohammed, E.A. Pinkard, M. Battaglia and D. Culvenor. *Cooperative Research Centre Forestry, Private Bag 12, Hobart, TAS 7001, Australia. Email: caroline.mohammed@csiro.au*

Australia has adopted internationally recognized sustainable forest management certification. This has pushed the forest industry to move towards sustainable forest management in order to secure a sustainable supply of raw material and to ensure marketplace acceptance of Australian products. Detection in real time of an early stage of stress may permit intervention to either prevent or offset damage and assist in the selection of management strategies that will minimise any environmental footprint e.g. we can use fertilisation to offset damage of an early stage of biotic stress such as *Mycosphaerella* leaf disease (MLD) in eucalypt plantations. Satellite remote sensing is being explored in eucalypt plantations as a potentially automated, quantitative, and cost-effective mapping tool to identify extent and severity of a major change activity such as MLD. Integration of this digitised information with decision support systems such as the forest health module we have developed in the productivity model CABALA, can be used to guide decisions on intervention. Due to the low margins of profit involved in many eucalypt plantations, the high cost of intervention procedures such as aerial spraying and the paucity of other types of environmentally friendly control strategies available apart from fertilisation, it is likely that the decision will be to take no action. However, impact information is important in respect to planning; for example, the rotation length for trees can be extended to compensate in terms of growth for a reduction in volume associated with a previous biotic event such as MLD.

16.29 COMPARISON OF SUSCEPTIBILITY AMONG SIX FAGACEAE SPECIES AGAINST *RAFFAELEA QUERCIVORA*. M. Murata, Y. Matsuda, T. Yamada and S. Ito. *Laboratory of Forest Pathology and Mycology, Graduate School of Bioresources, Mie University, Tsu, Mie 514-8507, Japan. Email: ito-s@bio.mie-u.ac.jp*

Mass mortality of oak trees has been occurring in Japan since the late 1980s. It was proved by inoculation experiments that the fungus *Raffaelea quercivora* isolated from discolored sapwood in dead or wilting trees had pathogenicity to Fagaceae species. In this study we inoculated seedlings of six Fagaceae species with *R. quercivora* and, after 56 days, measured the vertical length of the discoloration and the areas of discolored and non-conducting sapwood on stem cross-sections. Xylem pressure potential (XPP) in inoculated seedlings of *Quercus crispula* decreased abruptly after inoculation. In contrast, the XPP of inoculated seedlings of the other four species remained almost the same as in control seedlings. These results indicate that *Q. crispula* is susceptible to *R. quercivora*. The sapwood discoloration and water non-conduction areas were also larger in *Q. crispula* and *Q. serrata* than in the other species.

16.30 SOURCES OF RESISTANT AGAINST RUST OF TEAK, PINK DISEASE OF *ACACIA* HYBRID AND TWIG BLIGHT OF *AZADIRACHTA INDICA*. S.T. Naik. *Dept. of Forest Protection, College of Forestry, Sirsi-581 401, Karnataka, India. Email: natpstn@sancharnet.in*

Rust in teak (*Tectona grandis*) caused by *Olivea tectonae*, Pink disease of *Acacia* hybrid caused by *Corticium salmonicolor*, and leaf spot and twig blight of Neem (*Azadirachta indica*) caused by *Colletotrichum gloeosporioides* are diseases of commercially important trees of south-western India. Some clones or seed sources were identified as resistant to these three diseases under field conditions. Out of 35 clones of *Acacia* hybrid screened for reactions against

Pink disease, the minimum disease severity index (DSI) was recorded in clones 91 and 87 (0.16), moderate DSI was recorded in clone 67 (0.56) while clones 2k and 15k showed the highest DSI. Out of 24 clones of *T. grandis* screened for reaction against rust disease, three, MYHV3 (clone No. 7), MYHV4 (Clone No.8) and MYHK1 (Clone No.32) were completely free from the disease while MYSS-2 (Clone No.16) showed heavy disease incidence. To assess the biochemical content in different clones, non-reducing sugars and total phenols were extracted. Clone 7 (resistant) contained a higher quantity of reducing sugars (0.323 mg/g), non-reducing sugars (2.155 mg/g) and total phenols (1.483 mg/g). Out of eight seed sources of *A. indica* screened for reaction to leaf spot and twig blight indicated that source No. 2 (Bailhongal) was completely free from the disease while three sources 3, 4 and 5 suffered from high DSI with 100 percent mortality.

16.31 BOTRYOSPHAERIACEAE INVOLVED IN DIE-BACK OF *OSTRYA CARPINIFOLIA* IN SLOVENIA AND ITALY FOLLOWING DROUGHT. **B. Piškur, D. Pavlic, B. Slippers, N. Ogris, G. Maresi, M.J. Wingfield and D. Jurc.** *Slovenian Forestry Institute, Department of Forest Protection, Večna pot 2, SI-1000 Ljubljana, Slovenia. Email: barbara.piskur@gozdis.si*

Extensive damage and mortality of European hop hornbeam (*Ostrya carpinifolia*) has been observed in western Slovenia and northern Italy in recent years. These symptoms have been associated with unusual weather conditions and extreme drought. Preliminary research revealed that *Botryosphaeria dothidea* was associated with *O. carpinifolia* die-back. However, further investigation showed that other Botryosphaeriaceae could also be involved. In this study, we considered that the disease on *O. carpinifolia* might be associated with a recently introduced invasive pathogen and alternatively, whether the problem is brought about by a native opportunistic pathogen and unfavorable environmental factors. Analysis based on anamorph morphology, ITS-rDNA PCR-RFLP and comparisons of sequence data for multiple gene regions revealed that the majority of isolates represented *B. dothidea* and few others *Dothiorella* spp. The pathogenicity of these species was tested using inoculations on naturally growing trees as well as in the laboratory. The *B. dothidea* isolates, obtained from different disease outbreaks and different host species and tissue types from Slovenia and Italy, were screened for population diversity using AFLP markers. The results indicated significant diversity among *B. dothidea* isolates, and no host- or geographic-specific lineages. These results, together with the knowledge that *B. dothidea* is common on various native trees in southern Europe suggest that *O. carpinifolia* die-back is associated with a native population of *B. dothidea.* Infections are most likely associated with stress relating to unusual weather in the affected area. This conclusion supports predictions that the impact of the Botryosphaeriaceae pathogens can be expected to increase due to climate change.

16.32 THE RELATIONSHIP BETWEEN PRUNING AND THE PRESENCE OF *NECTRIA FUCKELIANA* IN *PINUS RADIATA*. **T.D. Ramsfield and M.W.P. Power.** *Ensis Forest Biosecurity and Protection, Private Bag 3020, Rotorua, New Zealand. Email: tod.ramsfield@ensisjv.com*

Flute canker disease is an emerging problem of *Pinus radiata* in the South Island of New Zealand. The disease is characterized by elongated stem depressions that are centred on pruning wounds and *Nectria fuckeliana* is hypothesized to be the causal agent of the disease. To test the hypothesis that pruning wounds are necessary for infection by *N. fuckeliana*, the presence of the fungus was assessed in pruned and un-pruned trees using DNA- and culture-based methods. Ninety pruned and 90 un-pruned trees (all of the same cohort) were assessed in 2006 and 2007. A sterilized increment-core borer was used to sample from above pruning wounds, or as near to the whorl as possible on the un-pruned trees. A sub-sample from the core was placed on media and *N. fuckeliana* cultures were identified by morphology. DNA was extracted from the remaining core sample and a nested PCR protocol specific for *N. fuckeliana* was used to detect the presence of the fungus. The results from 2006 and 2007 were assessed independently, and pooled, using the Chi-square procedure. Chi-square values indicated that there was no significant relationship between the pruned status of the tree and the presence of *N. fuckeliana* based on the results from the PCR test and culturing. We therefore conclude that although *N. fuckeliana* can infect through pruning wounds, it is also able to infect the tree via other mechanisms, as the fungus was also present in unpruned trees.

16.33 *CERATOCYSTIS* SPECIES ON THE AFRICAN CONTINENT: CRYPTIC SPECIES AND HOST JUMPS. **J. Roux, G. Kamgan Nkuekam, R.N. Heath and M.J. Wingfield.** *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa. Email: jolanda.roux@fabi.up.ac.za*

Ceratocystis species include some of the world's most important plant pathogens, the majority of which occur on trees. They have long been confused with species of *Ophiostoma* and *Grosmannia*, which have a similar morphology and are also vectored by insects. Recent studies have suggested that *Ceratocystis* is polyphyletic and encompasses several generic groups. At the species level, there is room for considerable organisation as many phylogenetic lineages within, for example the *C. fimbriata sensu lato* species complex, most likely represent discrete taxa. In Africa, little attention has been given to *Ceratocystis* spp., despite the fact that they are responsible for diseases of important agricultural crops and forest trees. Many of the species reported from Africa werw identified only on morphology, and likely represent cryptic species. This was the case with *C. albifundus*, the cause of death of *Acacia mearnsii* trees in Africa, that was initially treated as *C. fimbriata*. A number of *Ceratocystis* spp. have recently been described from Southern Africa, some of which have wide host and geographic ranges. *C. albifundus* for example, occurs on native African trees and has undergone a host shift to introduced *A. mearnsii*. This capacity for host jumps is likely a result of the association of *Ceratocystis* spp. with insects, including Drosophilid flies and Nitidulid beetles that visit wounds on trees and consequently spread these fungi. This potential for host jumps and insect dispersal has serious implications for tree health, as has been seen with *C. albifundus. Ceratocystis* spp. in Africa require deeper study, which is likely to reveal new species, new pathogens and intriguing ecological patterns.

16.34 FIRST REPORT OF PARASITIC PLANT, *CYNOMORIUM COCCINEUM* ATTACKING *HALOXYLON* SP. IN SISTAN-IRAN. **M. Sarani, A. Ghasemi, A.R. Arjmandi Nezhad and S. Ramroodi.** *Agricultural and Natural Resources Research Center of Sistan Zabol, Iran. Email: arjmandy53@yahoo.com*

During surveys on the flora of Sistan, the parasitic plant *Cynomorium coccineum* was collected; it was found attacking roots of Taghi (*Haloxylon* sp.). The parasite survives on the root

of hosts 1.5 m below the soil surface. The morphological characters of this plant include: Annual, aerial stem 10-40cm high, almost leafless, dark red in color and cylindirical. The lower is portion covered by dense scales.Terminal inflorescence is club shape, thick and compact. The male flower is very small, 2-4 mm in length and 1.5 mm in width, linear and spoon-like. The style is single, thickened, the anther with two chambers. Female flowers are 1-3mm in length and 1.7mm in width, linear and clubbed. Ovaries are egg-shaped, with or without a short stalk. Ovules are single, disverse , style single, stigma thick. Fruits are spherical, 1-1.5 mm in diameter, and seeds near spherical. This species was collected from a locality at 502 meters above sea level.

16.35 PHOSPHITE APPLICATION AS AN EXPLORATIVE TOOL IN *EUCALYPTUS GOMPHOCEPHALA* DECLINE IN WESTERN AUSTRALIA. **P.M. Scott, H.T. Eslick, B.L. Shearer, P.A. Barber, M.C. Calver, I.J. Colquhoun and G.E. Hardy.** *Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Murdoch, WA 6150, Australia. Email: p.scott@murdoch.edu.au*

Eucalyptus gomphocephala is a mediterranean forest canopy species endemic to a narrow (5-10 km wide) coastal strip approximately 300 km in length in south-west Western Australia. *E. gomphocephala* is undergoing a significant decline that was first identified as a spot decline in 1994 and now occurs throughout large sections of its remnant distribution within Yalgorup National Park, in some areas resulting in 100% mortality. The reduction of this keystone species represents a significant modification to the associated ecosystem. Modifications to hydrology, fire regimes, entomological pressures, and fungal and Pythiaceous soil pathogens have been identified as possibly contributing to the decline syndrome. The potential of phosphite (phosphonate), nutrient and insecticide treatments to reverse the decline in tree health was assessed as (a) a method for controlling the decline and (b) a method for diagnosing possible causal agents. Phosphite has been successfully used to control *Phytophthora* and Pythiaceous soil pathogens by inducing a host defence response within the plant. Stem injection of declining *E. gomphocephala* in the present study has resulted in improved canopy health and vigor, indicating that *Phytophthora* and/or other Pythiaceous microorganisms may be playing a role in the decline. The impact of phosphite application on nutrient uptake and fine feeder root concentration was also assessed.

16.36 *CRYPHONECTRIA PARASITICA* IN SESSILE OAK IN HUNGARY. **I. Szabó and S. Varga.** *Institute of Silviculture and Forest Protection, P. O. Box 132, University of West Hungary, H-9400 Sopron, Hungary. Email: szaboi@emk.nyme.hu*

Since 1999 cankers caused by chestnut blight fungus (*Cryphonectria parasitica*) have been observed in sessile oak (*Quercus petraea*) in western and south-western regions of Hungary in young and middle-aged *Q. petraea* stands mixed with *Castanea sativa*. The work, started in 2003, aimed to survey the incidence and impact of the disease, study the diversity of vegetative compatibility (VC) types in the subpopulations of the pathogen, the occurrence of natural hypovirulence, and the possibility and means of control using hypovirulence. The average incidence of infection varied between 1.5 and 23.5% and tree mortality between 0 and 3.6 %. Although the mortality rate is lower in oak than in chestnut, perennial stem cankers gravely compromize timber quality. One or two VC types occurred in most of the sites, but up to 6 VC types were distinguished in a few plots mostly where sexual stromata developed. In the first years of investigation, only virulent isolates were recorded, but in 2006, 7.5 % of the isolates were hypovirulent. Field inoculations were performed in order to investigate the appearance and development in time of the symptoms in oak. Early appearance of the first symptoms (at two weeks in some trees) and progressive increase in the rate of symptomatic trees were recorded in the first year, although healing of the initial floem lesions was observed during the second year in more than the half the inoculated trees.

16.37 OCCURRENCE AND IMPACT OF *PHYTOPHTHORA* SPECIES IN FOREST TREES IN HUNGARY. **I. Szabó and F. Lakatos.** *Institute of Silviculture and Forest Protection, P.O. Box 132, University of West Hungary, H-9400 Sopron, Hungary. Email: szaboi@emk.nyme.hu*

Occurrence of *Phytophthora* species and their phytopathological role have been investigated in forest stands in Hungary since 1999. Decline symptoms, specific stem lesions and unspecific top-drying signs were surveyed in forest stands of different tree species in order to find the causal agents and to clarify the role of *Phytophthora* species in decline. *Phytophthora* was isolated from soil samples taken from around the diseased trees by baiting with *Prunus laurocerasus* leaves. The isolates were identified on morphology and by sequencing the ITS regions of rDNA and comparing with known sequences in the GenBank database. About 400 isolates were examined morphologically and more than 80 genetically. *Phytophthora* species were found in *Alnus glutinosa* with bleeding stem lesions and crown-drying symptoms, and in *Juglans nigra*, *Quercus petraea* and *Q. cerris* with crown-drying symptoms. We identified 8 *Phytophthora* species in *Alnus* (*P. alni*, *P. citricola*, *P. gonapodyides*, *P. inundata*, *P. megasperma*, *P.* sp.1, *P.* sp. 2, and *P.* sp. 3.), 4 in *Juglans* (*P. cactorum*, *P. citricola*, *P. hedraiandra*, and *P.* sp.1) and 2 in *Quercus* (*P. citricola*, *P. gonapodyides*). The *Phytophthora* species identified (except *P. cactorum*) were recorded for the first time in Hungary during this research. Experimental inoculations caused well-delimited bark necrosis in the stems of seedlings, the largest by *P. alni* in alder and *P. citricola* in black walnut. Roots infections resulted in lesions of the fine roots most pronounced by *P. citricola* in black walnut.

16.38 TESTING THE WOOD DECAY CAPACITY OF FUNGI COMMONLY OBSERVED ON *EUCALYPTUS GLOBULUS* COPPICE STUMPS. **F.J. Tovar, G. Hardy, R.M. Robinson and T. Burgess.** *School of Biological Sciences and Biotechnology, Murdoch University, South Street, Murdoch, Perth, WA 6150, Australia. Email: f.tovar@murdoch.edu.au*

In Western Australia, *Trametes versicolor*, *Pycnoporus coccineus*, *Stereum hirsutum*, *Stereum illudens* and two unidentified basidiomycetes (species A and species B), are all commonly observed fruiting and causing decay on *Eucalyptus globulus* coppice stumps. In some instances, fungal rot on colonised stump has been observed moving into the emerging coppice shoots. Depending on the severity of decay this could threaten the productivity of the coppice rotation by affecting wood quality or causing complete loss of trees due to wind-throw. A trial was set up in an 18-24 month old *E. globulus* coppice plantation to test the relative ability of each of the commonly observed fungi to decay wood of actively growing coppice shoots. Each of the six species was inoculated into coppice shoots using wooden dowels previously colonised in

the laboratory. Three inoculations were made at 5 cm, 30 cm and 1 m from the top of the stump surface along the coppice shoot. A hole slightly larger than the dowel was drilled at each inoculation point, the dowel introduced and then covered with Parafilm™. Control inoculations were performed by inoculating a separate coppice stem on the same stump with sterile dowels. All coppice shoots were harvested after 6 months. Decay was assessed by measuring and calculating the volume of lesions or discoloration at the point of inoculation. Differences in wood density between sound and discoloured wood were also measured. Results arising from this investigation will be presented and discussed.

16.39 EFFECT OF HARVEST TIME ON FUNGAL COMMUNITY DEVELOPMENT ON *EUCALYPTUS GLOBULUS* COPPICE STUMPS. **F.J. Tovar, T. Burgess, R.M. Robinson and G. Hardy.** *School of Biological Sciences and Biotechnology, Murdoch University, South Street, Murdoch, Perth, WA 6150, Australia. Email: f.tovar@murdoch.edu.au*

In Western Australia, reports of stump rot and wind-throw losses in second rotation *Eucalyptus globulus* coppice plantations led to an investigation of the possible negative impacts of fungi colonising stumps. Surveys showed that a variety of fungi colonise stumps, but no geographic trends were apparent. As plantations are harvested at different times of the year, it was hypothesized that differences between plantations may be due to harvest times coinciding with different fungal fruiting and dispersal times. This was tested by harvesting fifteen trees at each season (spring-Oct 2006, summer-Jan 2007, autumn-May 2007, and winter-July 2007). Five of the trees were assigned as controls and only had wood samples analysed for the presence of fungi at the start and end of the experiment. The remaining ten trees were sampled for fungi at the time of harvest and 1, 4, 8 and 12 months after harvest. The aim was to observe seasonal differences in fungal community development within the outer sapwood and the inner discoloured central core. Presence of fungi was determined using molecular and classical cultural techniques. In the classical approach wood samples extracted from the stumps were plated on four different agar media to select for as wide variety of fungi as possible. The molecular approach used RFLP analysis of genes encoding rRNA to detect the presence of any fungi in the samples. Results from this investigation will be presented and discussed.

16.40 DECLINE AND MASS MORTALITY OF YIKA SHING, (*AESANDRA BUTYRACEA*), A MULTIPURPOSE TREE IN BHUTAN. **K. Tshering, D.B. Chhetri, C. Stauffer and T. Kirisits.** *Institute of Forest Entomology, Forest Pathology and Forest Protection (IFFF), Department of Forest and Soil Sciences, University of Natural Resources and Applied Life Sciences, Vienna (BOKU), Hasenauerstrasse 38, A-1190 Vienna, Austria. Email: thomas.kirisits@boku.ac.at*

Yika shing, *Aesandra butyracea*, also known as Indian butter tree, is a deciduous multipurpose tree occurring in subtropical and warm temperate areas in the Himalayas. Since the early 1990s rapidly progressing decline and mass mortality of this species has been recorded in several parts of Bhutan. In order to document the extent and severity of Yika shing decline, five transects along foot paths were established in summer 2006 in Lhuntse district in North-Eastern Bhutan. In each transect the health status of all Yika shing trees was recorded. Of the 360 pole-sized, medium-sized and large trees registered, only 25% were evaluated as apparently healthy, while 21% were declining and 54% were dead. A variety of biotic and abiotic damaging agents were recorded on the dying and dead trees, with a bark beetle species, tentatively identified as a *Scolytomimus* sp. and a lepidopterous shoot borer occurring most frequently. It is, however, doubtful that these insects are the cause of mortality. A socio-economic study, based on interviews of 33 local people has emphasized that all parts of the tree and especially the fruits are used for an array of purposes. Thus, the life and economy of rural people in Lhuntse are affected by Yika shing decline. Yika shing is clearly threatened in Bhutan and we recommend conducting further studies on the extent and causes of its decline and mass mortality, as well as initiating *ex-situ* and *in-situ* measures to preserve this valuable tree.

16.41 CHANGES IN DIURNAL STEM DIAMETER VARIATIONS OF A DECLINING HINOKI CYPRESS DURING DROUGHT. **M. Ueda.** *Laboratory of Forest Ecology and Physiology, Kyoto Prefectural University, Sakyo-ku, Kyoto 606-8522, Japan. Email: uedam@kpu.ac.jp*

Many declining trees are water stressed. Continuous monitoring of the tree water status is therefore important for understanding of the decline process in trees. Stem diameters of trees shrink in the morning and swell in the afternoon. These diurnal diameter variations are consequent on the water status of the trees. Measurement of diurnal diameter changes therefore provides a lot of information about tree water status. Diurnal changes in stem diameter can be continuously measured at low coast using a strain gauge. To estimate the declining process of trees I measured diurnal changes in stem diameters of two 20-year-old Hinoki cypress (*Chamaecyparis obtusae)* trees during a month-long summer drought. One tree showed a greatly reduced water-transport area in its trunk cross-section and leaf specific hydraulic conductivity caused by a wound. At the start of the drought period, the diurnal changes in trunk diameters were similar in both trees reaching a maximum at dawn, decreasing gradually to a minimum in late afternoon. Their maxima and minima dropped gradually as the drought dragged on. However, the tree under reduced hydraulic conductance tended to decrease more rapidly and eventually did not show diurnal change in stem diameter. The diurnal patterns of stem diameter changes during drought conditions can be classified into five types (I to V). In declining trees, diurnal patterns of stem diameter variation would shift from the type I to V more rapidly than in healthy trees as dry conditions continued.

16.42 STATUS OF EUCALYPT DISEASES AND THEIR MANAGEMENT IN CHINA. **X.D. Zhou, Y.J. Xie and M.J. Wingfield.** *Forestry and Agricultural Biotechnology Institute (FABI), Univeristy of Pretoria, 0002, Pretoria, South Africa. Email: xu.zhou@fabi.up.ac.za*

Forests and forestry are important in China, especially in the Northeast and Southwest. Natural ecosystems have been strictly protected from logging since 2000, and this has resulted in the expansion of fast-growing Eucalypt plantations in South China to meet the needs of a rapidly growing local economy. More than 2 million ha of Eucalypt plantations have been established and over 50% of these represent clones of *Eucalyptus urophylla* × *E. grandis* hybrids. Very little work has been done on eucalypt pathology in China. Other than a few taxonomic studies on fungal pathogens, bacterial wilt caused by *Ralstonia solanacearum* is the only disease to have been studied extensively. In order to accelerate studies on Eucalypt diseases and their management, we have established the

programme known as the CFEPP (http://www.fabinet.up.ac.za/cfepp/index) focused on Eucalypt health problems in China. Surveys on Eucalypt diseases in major plantation areas have been conducted and will continue to be pursued. Results from these first extensive surveys of Eucalypt diseases in China have shown that the most obvious stem diseases are bacterial wilt associated with *R. solanacearum*, stem cankers caused by *Chrysoporthe cubensis* and *Kirramyces zuluensis*, as well as species of *Botryosphaeria* and a *Ceratocystis* sp. Leaf and shoot diseases are also important and pathogens including *K. destructans*, *K. epicoccoides*, *Quambalaria pitereka* and species of *Cylindrocladium*, *Mycosphaerella* and *Pilidiella* have also been encountered. Many of these fungi still need to be fully identified, and their biology including pathogenicity to key planting stock will then be considered.

Microbial endophytes

11.1* TOWARDS CELL-SPECIFIC GENE EXPRESSION PROFILES IN ARBUSCULAR MYCORRHIZAE (AM). R. Balestrini, H. Küster, S. Volpato, L. Lanfranco and P. Bonfante. *Istituto Protezione Piante, sezione di Torino – CNR and Dipartimento Biologia Vegetale – UniTO, Viale Mattioli, 25, 10125 Torino, Italy. Email: r.balestrini@ipp.cnr.it*

AM symbiosis is an essential feature of the biology and ecology of most terrestrial plants. The establishment of AM symbiosis requires both partners to undergo significant morphological and physiological changes leading to reciprocal beneficial effects. Extensive changes in gene expression profiles have been uncovered in recent global transcriptomic studies on the whole mycorrhizal root. However, root colonization by AM fungi occurs by sequential steps involving different cell types, and important spatial or temporal information on AM development can be masked by mixing symbiotic and non-symbiotic cell types. We have applied laser microdissection (LMD) technology to describe gene expression profiles associated with specific cell types in mycorrhizal roots, demonstrating that phosphate transporters are differentially expressed in three cortical cell populations (Balestrini *et al.*, 2007, MPMI, 20, 1055-1062). The aim of the present study is to identify the transcriptome profile of arbuscule-containing cells harvested by a Leica LMD system from paraffin sections of *Medicago truncatula* roots colonized by the AM fungus *Glomus mosseae*. We are setting up RNA amplification protocols that allow us to perform global cellular transcriptome profiling using Mt16kOLI1Plus 70mer oligonucleotide microarrays and *Medicago* GeneChips. A first round of experiments using three biological replicates detected some of the marker genes already described as arbuscule-induced and some novel genes. In a second step, real-time PCR and/or *in situ* hybridisation will be used to follow the expression of specific genes potentially involved in the interaction.

11.2 ENDOPHYTIC FUNGI IN COMMON BASIL (*OCIMUM BASILICUM*). D. Banerjee, S. Mahapatra and S. Manna. *Department of Microbiology, Vidyasagar University, Midnapore 721102, West Bengal, India. Email: debu33@yahoo.com, debu33@sify.com*

Plants serve as a reservoir for untold numbers of microbes, known as endophytes. Most of the isolated endophytes are fungi, which colonise the living internal tissues of their hosts and may protect them from pests, fungal pathogens or other microbial infections. Endophytic fungi are now used greatly as biocontrol agents. Production of new antibiotics, immunosuppressive compounds, taxol etc by fungal endophytes is already variously reported. A medicinal plant, *Ocimum basilicum* (L.), locally known as babui tulsi, member of mint family (Lamiaceae) was screened for fungal endophytes. Forty five plant samples from different places of Paschim Medinipur district, West Bengal, India, were screened for isolation of endophytic fungi. Eighty eight fungal endophytes from leaf, stem and root segments were isolated and belonged to six different genera. Among the isolates *Colletotrichum* sp. and *Hyalopus* sp. were the most common. *Aureobasidium* sp. and Mycelia sterilia were also isolated from different segments. *Hyalopus* sp. Was isolated in greatest numbers from leaf segments whereas *Colletotrichum* sp. and *Penicillium* sp. were most frequently isolated from stem and root segments respectively. The colonization frequency of fungal endophytes in different plant parts was leaf >stem>root. This study provides clear evidence of tissue specificity in endophytic fungi.

11.3 FUNGAL ENDOPHYTES OF COCOA FOR THE CONTROL OF DISEASE CAUSED BY *PHYTOPHTHORA PALMIVORA*. C. Blomley, E.C.Y. Liew and D.I. Guest. *Faculty of Agriculture Food and Natural Resources, The University of Sydney, NSW 2006, Australia. Email: c.blomley@usyd.edu.au*

The fungal endophyte community of *Theobroma cacao* (cocoa) is a diverse assemblage including pathogens and saprophytes. The interaction between these endophytes and their host is not yet understood, and some fungal endophytes may protect the plant against pathogens. We have sampled fungal endophytes from cocoa grown under different conditions in Papua New Guinea (PNG) and Australia. We then identified potential biological control agents for disease caused by *P. palmivora*, the major pathogen of cocoa globally. Fungal endophytes were sampled from the leaves of cocoa grown as monocultures (in Australia and PNG) or in a mixed farm environment (in PNG). Endophyte communities isolated from young (10–15 years old) monocultured cocoa in Australia and PNG were similar to each other, and were dominated by the latent pathogens *Colletotrichum gloeosporioides* and *Phomopsis* sp. The endophyte community isolated from old (>60 years old) cocoa grown in a mixed farm environment was very different, and was dominated by Xylariaceous taxa. Fungal endophytes that occurred at a frequency of at least 1% in any one community were tested for the ability to restrict the growth of *P. palmivora in vitro* and to control disease severity *in planta*. Four of the endophytes tested, including two Xylariaceous taxa, significantly reduced the growth of *P. palmivora in vitro*. Experiments are being conducted to determine whether these endophytes affect the severity of disease caused by *P. palmivora*, and to determine the mechanism of disease resistance.

11.4 ENDOPHYTES IN THE FERN *NEPHROLEPIS CORDIFOLIA*. G. Bruno, F. Tommasi, A. Dellorusso, L. Sparapano and L. d'Aquino. *Department of Biology and Plant Pathology, University of Bari, via Amendola, 165/A, 70126 Bari, Italy. Email: gbruno@agr.uniba.it*

Nephrolepis cordifolia (L.) C. Presl is a widely distributed fern in East Asia, Oceania, Japan and New Zealand, while in Italy it is commonly grown as an ornamental. It is the only species in the genus *Nephrolepis* which produces tuber-like structures on the roots. Microscopic observations were made using calcofluor and methyl green-red Congo staining. Endophytes were isolated from roots, tubers and rhizomes by growing them on several media (potato-dextrose-agar (PDA), malt-agar and rose bengala-streptomycin-agar). Transmission electron microscopy indicated the

presence of bacteria in the intercellular spaces throughout the plant. Bacteria were isolated on PDA and on nutrient broth-sucrose-agar by plating small slices of rachis and leaflets (pinnae). Among fungi, *Trichoderma viride* Pers. : Fr. was isolated from tubers and rhizomes, but it was never detected in the soil. The presence of starch in the tubers was proved by Lugol staining. Antioxidant compounds such as ascorbic acid and dehydroascorbic acid were detected in tubers, rhizomes and fronds. The association between the plant and endophytic bacteria and fungi could elicit resistance in *N. cordifolia* to plant pathogens and pests or its tolerance to air pollutants. The role of *T. viride* and its secondary metabolites in protecting the host plant are under investigation by using in vitro plants and seedlings.

11.5 ASSOCIATED FUNGI INVOLVED IN THE DEVELOPMENT OF ESCA OF GRAPEVINE IN APULIA (SOUTHERN ITALY). **C. Ciccarone.** *DiSACD, Facoltà di Agraria, Via Napoli 25, 71100 Foggia, Italy. Email: c.ciccarone@unifg.it*

The key role of *Phaeomoniella chlamydospora*, *Togninia minima* (anamorph *Phaeoacremonium aleophilum*) and *Fomitiporia mediterranea* in the development of esca disease of grapevine is clear. Usually, a number of endophytic fungi live in the same ecological environment but certain species prevail or are found only in the vine-growing area, the grapevine cultivar or vines of a certain age. Several such fungal species were recorded for adult vineyards and mother-plants in northern Apulia. In addition, some epidemiological studies were carried out for possible migration of esca fungi from bridge plants to *Vitis vinifera*. Various species of Fagaceae, tamarix, *Acacia*, *Prunus*, *Olea* and *Albizzia* were found to host wood-decaying fungi on living or dead wood, together with a similar court of accompanying fungi. In examining samples of Fomitiporia and *Phellinus* from various hosts, we observed the recurring presence of a particular fungal community. The relation of its components to the esca-associated fungi varied from mycophilia, competition for the substratum and mycoparasitism to reciprocal steric incompatibility or total indifference. Esca is often a chronic disease, affecting adult and old vines, and consequently physiological stresses and different overlapping diseases may complicate symptoms and make the damage worse. In recent years the Mediterranean climate has seen anomalous distributions of rain and temperature through the seasons. The influence of certain stressing conditions such as poor root oxygenation, low temperatures and *Fusarium* or black-foot infections on the mycological ensemble has also been considered.

11.6* A NEW ENDOPARASITIC MICROOGRANISM INFECTING PLANT PATHOGENIC FUNGI. **A.M.H. Esh and T.A. Evans.** *Agricultural Research Center, Sugar Crops Research Institute, 9 Cairo University Street, Giza, Egypt. Email: aymanesh@gmail.com*

We report a newly discovered endoparasite isolated from an Egyptian and some American isolates of *Rhizoctonia solani*. The endoparasite is cytoplasmically inherited, and highly infectious towards many plant-pathogenic fungi including *R. solani*, *Fusarium* spp., *Pythium* spp. and *Phytophthora infestans*; it inhibits their in vitro growth and also reduces their pathogenesis in replicated greenhouse studies. The organism was microscopically visualized with the oil-immersion lens (100×) using a modification to the microscope condenser and the light intensity or by observing it with phase contrast and confocal microscopy as slow-moving granules inside the infected mycelium in the early stages of infection, with the the speed increasing as the infection progressed. It was also clearly seen when the infected fungus was stained with 10 mg/ml Ethidium bromide or DPI. Dyes specific for mitochondria failed to stain the motile granules. The endoparasite is capable of spreading rapidly through infected fungal hosts, severely infecting the host fungi and killing the infected fungus. we have succeeded in infecting healthy fungi with the endoparasite artificially in the laboratory via anastomosis between healthy and endoparasite-infected isolates of *Rhizoctonia*. Light microscope studies showed that mycelia of infected fungi are empty in the final stage of parasitism and electron microscope and confocal microscope studies suggested that the endoparasite is mycoplasma-like. Infection of *R. solani* isolates by the endoparasite decreased their pathogenicity. Preliminary experiments suggested that the endoparasitism also affects the physiological characteristics of the infected fungus.

11.7 NEW ANTAGONISTIC ENDOPHYTIC STRAINS OF *BACILLUS SUBTILIS* AS POTENTIAL BIOCONTROL AGENTS. **R.M. Khairullin, V.D. Nedorezkov, N.A. Urazbachtina, R.Sh. Zakharova, T.S. Minina and M.A. Luk'yantzev.** *Bashkir State Agrarian University, Ul. 50 Let Oktyabrya, 34, Ufa, 450001, Russia. Email: khram@ufanet.ru*

Bacillus subtilis strains are basic agents of many commercial biofungicides (SUBTILEX, Kodiak, Phytosporin etc.). They are based on ability of bacterial strains to suppress the development of phytopathogenic fungi, to stimulate the growth of plants and to be harmless for humans. Widening the spectrum of useful properties of bacterial strains can raise the efficiency of biofungicides. Since 2005 we have looked for wheat endophytic strains of *B. subtilis* which can stimulate plant growth due to different mechanisms. We have collected more than 50 bacterial strains that are active antagonists against *Fusarium*, *Bipolaris*, *Alternaria* and other fungi. Some of the new strains stimulate plant growth, suppress development of phytopathogenic fungi and increase wheat productivity more actively than available biofungicides. Treatment of wheat seeds with preparations of some endophytes reduces circulation of bunt (*Tilletia tritici*) as effectively as chemical fungicides in field conditions. The selected strains of endophyte are not pathogenic, and not toxic or toxigenic for animals. These and other results will be presented.

11.8 THE ANTAGONISTIC ENDOPHYTE *BACILLUS SUBTILIS* 26D INCREASES PLANT TOLERANCE TO TOXIC METALS. **Z.M. Kuramshina, Y.V. Smirnova and I.G. Andreeva.** *Sterlitamak State Pedagogic Academy, Prospekt Lenina 49, Sterlitamak, 453103, Russia. Email: sspa@sspa.bashtel.ru*

Endophytic strain *Bacillus subtilis* 26D is an antagonistic bacterium that inhibits growth of phytopathogenic fungi. Cells of this strain are the basis of a biofungicide which increases disease resistance and productivity of agricultural plants. The protective effect of the endophyte is probably based on production of antibiotics, and also hydrolases that destroy fungal cell walls. At the same time, we have shown that the antagonist can induce systemic resistance of plants to pathogens. Thus, inoculation of seeds with bacteria resulted in increased resistance of wheat seedlings to salt stress and drought. Seedlings treated with endophyte cells were tolerant to toxic metal ions. The size and weight of inoculated seedlings of wheat, sunflower and maize were higher under stress from cadmium, copper, zinc and aluminium ions compared with control plants. It is possible that ability to reduce the level of plant growth inhibitors, such as, for example, ethyl-

ene and also to raise levels of growth factors IAA and cytokinins is one of the common mechanisms for increasing plant resistance to diseases and to abiotic stresses. Our further research will be devoted to studying hormone regulation in plants made resistant to stresses using bacterial endophytes.

11.9 ANTIMICROBIAL ACTIVITY OF BIOACTIVE COMPOUNDS FROM A GRASS ENDOPHYTE, *PERICONIA SIAMENSIS* CMUGE015. **S. Lumyong, W. Bhilabutra, T. Taeshowisan, J.F. Peberdy and P. Lumyong.** *Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand. Email:scboi009@chiangmai.ac.th*

Periconia siamensis (strain CMUGE015) was isolated from leaves of the grass, *Thysamoleana latifolia* (Poaceae). It was antagonistic against the human pathogens *Bacillus cereus*, *Listeria monocytogenes*, MRSA (methicillin-resistant *Staphylococcus aureus*) and *Pseudomonas aeruginosa*, causative agents of foodborne disease, listeriosis, skin infection and lung disease, respectively. Metabolites in both culture filtrates and crude extracts of the filtrates were inhibitory against the microbes tested. The two major active ingredients from the culture filtrate were purified by silica gel column chromatography and identified as modiolide A 5,8-dihydroxy ditetrahydro-2H-oxecin-2-one and 4-chromanon,6-hydroxy-2-methyl-(5CI) (Compound 2) by IR, NMR and mass spectroscopy (MS) data. Bioassays showed that both compounds had antibacterial activity against all the bacteria tested. Minimum inhibition concentrations were determined to be 50 µg ml^{-1} for compound 1 and 100 µg ml^{-1} for compound 2. Growth inhibition of some plant pathogenic fungi were also detectable. This is the first report of the production of these two antibacterial metabolites by a terrestrial endophytic fungus.

11.10 GROWTH RESPONSE AND ROOT COLONIZATION IN THREE VARITIES OF *RICINUS* DUE TO AM FUNGI. **R. Misra and A. Arya.** *Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, India. Email: aryaarunarya@rediffmail.com*

Castor (*Ricinus communis* L.) belongs to the family Euphorbiaceae. The oil produced from seeds is used in pharmaceuticals, industry, soaps, paints, lubricants and as an insecticide. Gujarat is the third state after Andhra Pradesh and Maharashtra in castor cultivation. Efforts are being made to develop hybrid varieties but sufficient increase in yield has not been achieved. An effort was made to assess the efficacy of native arbuscular mycorrhizal (AM) fungi in three different varieties of castor i.e GCH 4, Avani 41 and a local variety. AM fungi are known to enhance P and other mineral uptake as well as maintain water balance, which increases yield. *Glomus fasciculatum* (mixed inoculum) was multiplied on maize roots and was used in pot studies during June–August 2007. Results showed that shoot dry wt. was maximum (24.166 g) after 90 days in variety Avani 41. This was 3 times then the control values. In the other two varieties it was double the control. It was interesting to note that shoot and root fresh wt. increased in AM inoculated pots. Shoot length was also greater in all the 3 varieties. AM colonization was 92% in GCH4 after 90 days, and was 78 and 88% in Avani 41 and the local variety respectively. Use of AM fungi thus offers great potential to enhance castor yield.

11.11 INFLUENCE OF AM FUNGI ON GROWTH AND BIOMASS PRODUCTION OF BIO-DIESEL YIELDING PLANT SPECIES. **S.T. Naik and L. Venkatesh.** *Dept. of Forest Protection, College of Forestry, Sirsi-581 401, Karnataka, India. Email: natpstn@sancharnet.in*

A survey for occurrence of vesicular-arbuscular mycorrhizal (VAM) fungi based on soil types was made in 14 locations of three agro-climatic zones of Karnataka. *Jatropha curcas* on black soil and red soil of Khanapur taluk in Belgaum district of zone-9 contained the highest number of VAM spores (215.59 / 100 g and 190.81/100 g, respectively). *Pongamia pinnata* also on black soil and red/lateritic soil of Khanapur in Belgaum district of zone-9 contained highest number of VAM spores (187.91/100g and 169.93/100g, respectively). Spores of *Glomus* sp. were widely distributed in the rhizosphere of *Pongamia pinnata,* whereas spores of *Acaulospora* sp. were distributed in the rhizosphere of *J. curcas.* Root colonization in *J. curcas* varied from 18.26% to 65.18%. Similarly in *P. pinnata*, root colonization ranged from 18.29% to 70.91%. A polyhouse experiment was laid out to screen efficient VAM fungi for *J. curcas* and *P. pinnata.* Five VAM fungal genera with P-combination were used for screening and selecting the best symbiont for both species. *Acaulospora sp*+P for *J. curcas* and *Glomus sp* +P had significant influence on seedlings. At 120 days after inoculation of *Acaulospora sp* +P in *J. curcas* and *P. pinnata* inoculation with *Glomus sp*+ P showed highest percent increment in seedling height (44.18%, 55.78%), collar diameter (28.66%, 51.96%), root length (46.84%, 55.21%), shoot length (44.70%, 44.74%), total plant dry biomass (46.33g/plant, 8.03g/plant), mycorrhizal dependency (70.36%, 52.33%), total phosphorus content (0.78 mg/g, 0.40 mg/g) and mycorrhizal inoculation effect (57.32%, 50.00%) respectively.

11.13 VISUALIZATION OF GFP-TAGGED *PANTOEA* SPECIES, A COMPONENT OF A DISEASE-SUPPRESSIVE CONSORTIUM ON WHEAT ROOTS. **A.N. Pandey and D. Backhouse.** *School of Environmental & Rural Sciences, UNE, Armidale, NSW 2351, Australia. Email: apandey3@une.edu.au*

A study of root-associated bacteria and their antagonistic potential is important in understanding their role in disease suppression and interaction with agricultural crops and their pathogens. Three groups of beneficial bacteria, *Pantoea agglomerans, Exiguobacterium acetylicum* and *Microbacteria* were isolated from disease suppressive soil at Avon (South Australia), and shown to interact to suppress *Rhizoctonia* disease on wheat. The greatest reduction in *Rhizoctonia* disease suppression was noticed when a mixtures of all three bacteria were inoculated together in a bioassay. The detection of microorganisms in nature, particularly soil bacteria, has been severely limited due to inability to culture these organisms and lack of visualization methodologies. The application of green fluorescent protein (GFP) technology has enhanced the ability to monitor microbial populations in natural environments, such as in biofilms or on plant roots. In this study, we report the construction of plasmids which express the GFP from the jellyfish *Aequorea victoria* and which were stably maintained in *Pantoea* spp. The utility of these plasmids to detect the presence of individual cells in laboratory experiments was demonstrated. We also investigated the *in situ* localization of GFP labeled *Pantoea* on the wheat roots.

11.14* SUSCEPTIBILITY OF ENDOPHYTE-INFECTED TALL FESCUE TO *RHIZOCTONIA ZEAE* INFECTION. **D. Panka,**

C.P. West and C. Guerber. *University of Life Sciences, 20 Korderckiego St., 85-225 Bydgoszcz, Poland. Email: panka@utp.edu.pl*

Brown patch caused by *Rhizoctonia solani* is one of the most severe diseases in southern USA especially when occurring on tall fescue, St. Augustine grass, zoysiagrasses and creeping bentgrass. On tall fescue, *R. solani* causes foliar lesions with grey-silver to light brown colour surrounded by a reddish to dark brown border. Disease may develop in distinct patches or the pathogen may cause a general blight. Similar symptoms may be caused by *R. zeae*. There are few ways to protect tall fescue from these two pathogens; fungicides are the most effective but unfortunately, *R. zeae* is insensitive to some of them. For this reason, chemical control sometimes fails, especially when *R. zeae* is the causal agent. Another way to control this fungus gain more understanding of this situation. During disease evaluation of a tall fescue cultivar trial, we observed *R. zeae* occurring more often on plants not infected (E-) with *Neotyphodium coenophialum*. *R. zeae* was isolated almost exclusively from E- plants of some cultivars. In contrast, endophyte status did not matter for *R. solani*. More detailed pot experiments in controlled conditions were conducted to answer the following question: are tall fescue plants infected with *N. coenophialum* more resistant to sheath and leaf spot disease caused by *R. zeae*? Four tall fescue E+ and E- clones and 4 *R. zeae* isolates were tested. We found that endophyte presence in plants had a strong inhibiting effect on the development of the pathogen.

11.15 DIVERSITY OF ENDOPHYTIC FUNGI OF SOME MEDICINAL PLANTS IN KOREA AND THEIR ANTIFUNGAL AND PLANT GROWTH-PROMOTING ACTIVITY. N.C. Paul, M.S. Park and S.H. Yu. *Department of Applied Biology, Chungnam National University, Daejeon 305-764, Republic of Korea. Email: shunyu@cnu.ac.kr*

Endophytic fungi were isolated from roots of *Aralia elata*, *Aralia continentalis* and from leaf and root samples of *Taraxacum coreanum*. Based on ITS sequence analysis, 35 fungal genera were characterized from 431 cultures, belonging to 31 Ascomycota, 2 Basidiomycota, 1 Glomeromycota and 1 Oomycota. *Rhizopycnis* and *Strumella* in *Aralia*, and *Alternaria* and *Phoma* in *Taraxacum* were the most abundant taxa. Out of 35 genera, *Apodus, Ceriporia*, *Dothideales, Entrophosphora, Leptodontidium, Nemania, Neoplaconema, Paraconiothyrium, Phaeosphaeria*, *Plectosphaerella, Rhizopycnis, Strumella, Terfezia* and *Tumularia* were new to Korea. A total of 182 isolates were tested for antifungal and plant growth-promoting activities. Out of these, 49 isolates showed antifungal activity against at least one plant-pathogenic fungus and 8 isolates showed plant growth-promoting activity.

11.16 VARIATION OF ISOLATED ENDOPHYTIC FUNGI FROM *GINKGO BILOBA*. W. Thongsandee, Y. Matsuda and S. Ito. *Laboratory of Forest Pathology and Mycology, Graduate School of Bioresources, Mie University, Tsu, Mie 514-8507, Japan. Email: 507M109@m.mie-u.ac.jp*

To determine the dominant endophytic fungi of ginkgo (*Ginkgo biloba* L.) and to monitor their isolation frequency, fungi were isolated from living symptomless organs of ginkgo including leaves, petioles and current-year twigs from April to November 2004. After surface sterilization, 10 fungal taxa were isolated including species of *Alternaria*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Pestalotiopsis*, *Peyronellaea*, *Phoma*, *Phomopsis*, *Phyllosticta* and yeasts. Of the 10 fungal taxa obtained, 2 were most frequently isolated. *Phomopsis* sp. was isolated most frequently from twigs (71.5%) but less frequently from leaves (2.8%) and petioles (4.6%). *Phyllosticta* sp. was isolated frequently from leaves (12.6%) and petioles (12.3%) but was never isolated from twigs. The organ-specific distribution of these fungi was clear. *Phomopsis* sp. and *Phyllosticta* sp. were found to be the dominant endophytic fungi of ginkgo. To investigate the succession of endophytic fungi on leaf litter, fungi were isolated from leaves and petioles of freshly fallen and decomposing ginkgo leaves from November 2003 to September 2004. A total of 16 fungal taxa were isolated including *Phomopsis* spp. and *Phyllosticta* spp. Successional trends of these fungi were observed during leaf decomposition. The sum of frequencies of *Phomopsis* spp. was decreased temporarily on freshly fallen leaves and was increased on decomposing leaves while sum of frequencies of *Phyllosticta* spp. was initially high on freshly fallen leaves and later decreased.

11.17* ROLE OF MICROBIAL ENDOPHYTES ON THE BIOLOGICAL CONTROL OF *CLEMATIS VITALBA* IN NEW ZEALAND. N. Waipara, H. Harman, H. Kitchen and S. Dodd. *Manaaki Whenua Landcare Research, Private Bag 92179, Auckland, New Zealand. Email: waiparan@landcareresearch.co.nz*

A mycological survey of native and exotic *Clematis* (Ranunculaceae) species throughout New Zealand identified *Phoma clematidina* as a relatively common leaf endophyte as well as minor pathogen of some species. The fungus was also detected in the seeds of *C. vitalba* which may suggest seedborne transmission. An exotic and highly pathogenic strain of *P. clematidina* from the USA was previously introduced for the biocontrol of the invasive weed, *Clematis vitalba*, because native strains were mildly or not pathogenic on the target host. Diagnostic genetic techniques to differentiate the endemic strains from the exotic biocontrol strain were explored to help determine which strains were present on the different *Clematis* species surveyed. *In vitro* inoculation assays were undertaken to determine relative pathogenicity between isolates. Both pathogenic and non-pathogenic strains were identified and expression of symptoms ranged from symptomless endophytic colonisation through to necrotic leaf lesions. Synergistic and inhibitory interactions between the different strains of *P. clematidina,* as well as other endophytic, saprophytic and pathogenic fungi associated with the host, were observed. Such interactions are important in understanding the relatively low efficacy of *P. clematidina* since its introduction against this weed target.

11.18 ASSESSING THE BIODIVERSITY OF ROOT INHABITING FUNGI OF NATIVE GRASSES. N.R. Walker, S. Fuhlendorf, and S.M. Marek. *Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078-3033, USA. Email: nathan.walker@okstate.edu*

Plant-pathogenic fungi of agronomic crops often reside on wild hosts in natural landscapes. The objective of this research was to assess the diversity of fungi inhabiting the roots of various native prairie grasses. The roots of apparently heathly sideoats grama (*Bouteloua curtipendula*), blue grama (*Bouteloua gracilis*), hairy grama (*Bouteloua hirsuta*) and big bluestem (*Andropogon gerardii*) were collected from three prairie sites in Oklahoma that have never been tilled. The endophytic fungi inhabiting these roots were isolated from surface-sterilized roots, cultured, and stored. Fungi were tentatively identified by morphological characteristics and the ribosomal internal transcribed spacer regions

(ITS1-5.8S-ITS2) were amplified and sequenced from a representative subset of about 1000 isolates. Based on sequence similarities, the dominant genera of fungi cultured from grass roots were *Periconia*, *Fusarium*, *Gaeumannomyces*, *Magnaporthe*, and several groups of unidentified fungi from metagenomic surveys referred to as either 'uncultured' mycorrhizae or endophytes from a grass (*Stipa*), spruce (*Picea*), pine (*Pinus*) and an Australian ericaceous shrub (*Epacris*). Most fungi cultured from grass roots were members of the Ascomycota, but also included some Homobasidiomycetes, including members of the Ceratobasidiales and Agaricales. Other fungi of interest identified included *Monosporascus*, which is reported to occur only in cucurbit hosts, and members of the Xylariales, which are lignocellulytic inhabitants of woody plants. Fungal diversity trends were most dependent upon the location sites and were hypothesized to be influenced by the relative amount of rainfall and disturbance, such as fire, at each site.

11.19 ENDOPHYTIC FUNGI FROM RHIZOMES OF *PARIS POLYPHYLLA* VAR. *YUNNANENSIS* AND BIOASSAY-GUIDED FRACTIONATION OF THE ANTIMICROBIAL COMPOUNDS. J. Zhao. *College of Agronomy and Biotechnology, China Agricultural University, Beijing, P. R. China. Email: jianglinzhao2008@yahoo.com.cn*

Sixty-three fungal endophyte isolates were separated from rhizomes of *Paris polyphylla* var. *yunnanensis*, a traditional medicinal plant mainly distributed in China. Some representative isolates were identified as *Gliocladiopsis irregularis* (Ppf2), *Plectosphaerella cucumerina* (Ppf4), *Padospora* sp. (Ppf6), *Gliomastix murorum* var. *murorum* (Ppf7), *Aspergillus fumigatus* (Ppf9), *Pichia guilliermondii* (Ppf10), and *Neonectria radicicola* (anamorph: *Cylindrocarpon*) (Ppf12) separately based on their morphological and molecular features. Broad diversity and a wide taxonomic spectrum were exhibited by the endophytic fungi. Crude n-butanol extracts of the endophytic isolates were preliminarily screened for their antimicrobial activity. It was found that activity of the filtrate extract was stronger than that of mycelial extract. Bioassay-guided fractionation of the antimicrobial compounds from the endophytes is in progress.

11.20 FUNGAL ENDOPHYTES FROM *DIOSCOREA ZINGIBERENSIS* RHIZOMES AND BIOASSAY-GUIDED FRACTIONATION OF THE ANTIBACTERIAL COMPOUNDS. L. Zhou. *College of Agronomy and Biotechnology, China Agricultural University, Beijing, P. R. China. Email: lgzhou@cau.edu.cn*

Nine fungal endophytes were isolated from rhizomes of the traditional Chinese medicinal plant *Dioscorea zingiberensis.* The endophytes were classified by morphological traits and internal transcribed spacer (ITS) rRNA gene sequence analysis. Their ITS rDNA sequences were 99-100% identical to *Nectria*, *Fusarium*, *Rhizopycnis*, *Acremonium* and *Penicillium* spp. respectively. Of these, the most frequent genera were Fusarium and *Nectria*. Minimal inhibitory concentration (MIC) values of the n-butanol extracts of both mycelia and culture filtrates were between 31.25 µg/ml and 125 µg/ml. Beauvericin was separated by bioassay-guided isolation from endophytic *Fusarium redolens*. Its median effective inhibitory concentration (IC_{50}) values against the test bacteria were between 18.45 and 70.41 µg/ml. Bioassay-guided fractionation of other antibacterial compounds from other endophytes is in progress.

MOLECULAR DIAGNOSTICS FOR PLANT PATHOLOGY

7.1* ANTIBODY MICROARRAY FOR GRAPEVINE AND TREE FRUIT VIRAL DISEASE DIAGNOSTICS. I. Abdullahi and M. Rott. *Centre for Plant Health, Canadian Food Inspection Agency, 8801 East Saanich Road,Sidney, British Columbia, V8L 1H3 Canada. Email: abdullahii@inspection.gc.ca*

Antibody microarray methods and protocols were developed for the detection of plant viral diseases and compared to more traditional ELISA and PCR assays. Using the standard immunoassay ELISA as a benchmark, and employing either direct or indirect labelling approachs for the microarrays, both grapevine and tree fruit viruses could be detected. The direct labelling approach was used to detect *Arabis mosaic virus* (ArMV) and *Grapevine fanleaf virus* (GFLV) after incubating the antibody array with crude viral extract conjugated to alkaline phosphatase. Using either a chromogenic or fluorescence dye in a double or triple antibody sandwich format, good reaction signals were obtained to a number of viruses. In a multiplex system, four grapevine viruses including ArMV, GFLV; *Strawberry latent ringspot virus* SLRSV and *Raspberry ringspot virus* RpRSV were detected without compromising sensitivity and specificity. Compared to ELISA, the antibody microarray system is similar with respect to sensitivity and specificity, while using significantly less amount of the capture antibody. A high correlation (R^2 0.783) was observed in regression analysis of virus concentration measurement, but Bland-Altman bias plot show lack of agreement between the two methods. The Receiver operator characteristic (ROC) curve (AUC > 0.8) was used to demonstrate the high performance of the microarray system. We discuss the pros and cons of each experimental design, while work is still ongoing in the refinement of various aspects of the protocols to allow efficient multiplex detection of more viruses.

7.2 MOLECULAR DETECTION OF *PUCCINIA HORIANA* HENN. IN *CHRYSANTHEMUM* × *MORIFOLIUM* THROUGH CONVENTIONAL AND REAL-TIME PCR. H. Alaei, M. Maes, M. Höfte, and K. Heungens. *Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium. Email: kurt.heungens@ilvo.vlaanderen.be*

Puccinia horiana Henn., is a quarantine fungal pathogen within the EU and one of the most important pathogens of *Chrysanthemum* × *morifolium.* Molecular detection protocols were developed based on the specific PCR amplification of selected regions in the internal transcribed spacers of the nuclear ribosomal DNA (rDNA-ITS). The nucleotide sequence of the rDNA-ITS of 18 *P. horiana* isolates from 8 countries and 3 continents was determined. Based on the limited sequence variability between these isolates, several primer pairs were designed and tested for selectivity and sensitivity using target and non-target DNA as template. Highly selective primer pairs were identified for conventional, nested, and real-time PCR detection of *P. horiana*. Using these different PCR versions, the detection limits were 10 pg, 10 fg, and 5 fg genomic DNA or 5000, 50, and 5 target copies respectively. Due to the selectivity of the primers, SYBR Green I technology was sufficient for real time PCR signal detection. For detection in the plant, a CTAB extraction protocol or a selection of commercial DNA extraction methods allowed the use of 10 ng total (plant + pathogen) template DNA without interference of PCR inhibitors. The lowest proportion of infected plant material that could still be detected when mixed with healthy plant material was 0.001%. Using the nested and real-time PCR protocols, it was possible to detect the pathogen as soon as the infection started. The detection system proved to be accurate and sensitive and could help not only in pathogen detection and diagnosis but also in pathogen monitoring and disease forecasting systems.

7.3 IDENTIFICATION OF *CHRYSOMYXA* SPECIES WITH DNA BARCODING. M. Allaire, N. Feau and R.C. Hamelin. *Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Center, Canada. Email: richard.hamelin@ubc.ca*

Rust fungi of the genus *Chrysomyxa* are widespread in the northern hemisphere where they cause needle and cone diseases in conifer. Among the 14 species inventoried in Canada, many thrive in the same environment and on the same aecial conifer host (spruce). The precise identification in the aecial stage on spruce can be tedious due to similarity in spore morphology. For example, related species like *C. ledicola* and *C. empetri* are almost indistinguishable in their aceial state although they alternate on different telial hosts; similarly the aeciospores of *C. cassandrae* and *C. nagodhii* both present on spruce are difficult to distinguish morphologically. For some species such as *C. reticulata* and *C. chiogenis*, the extent of the aecial stage is still unclear and known only from inoculation experiments. To better understand the presence of each species on the conifer host and their role in needle infection, we used a DNA barcode approach with two markers: internal transcribed spacer of the nuclear ribosomal DNA (ITS), and the mitochondrial cytochrome c oxidase I (COI). This enabled us to clearly identify every species regardless of host or spore type. The complementary two gene approach provided enough information for diagnostic and clear differentiation of related and cryptic species of this rust genus.

7.4 QUANTITATIVE REAL-TIME PCR - AN EFFECTIVE TOOL FOR ASSESSMENT OF FUNGAL FLORA IN FIELD TRIALS. C. Almquist and C. Filipsson. *Analycen AB, P.O. Box 905, SE-531 19, Lidköping, Sweden. Email: charlotta.almquist@lantmannen.com*

Over 300 wheat samples were collected from field trials with fungicide and cultivar testing in southern and central Sweden during 2006 and 2007. Several different fungicides were used, both a strobilurin (pyraclostrobin) and triazoles (prothioconazole and propiconazole) and combinations of both. The cultivars tested were: SW Harnesk, Mon Olivin, PBIS Florett and LP Skalmeje. The dominating plant pathogens were recognized by visual inspection at sampling, and level of infection was recorded as percentage visibly infected leaf area. Most of the wheat samples were then analysed using quantitative real-time PCR (qPCR) assays to determine the amount of *Septoria tritici, Drechslera tritici-repentis* (DTR) and *Stagonospora nodorum.* The results showed that qPCR can be used to study the effect of fungicides on the fungal flora with very small variation between replications. There was a good correlation between visual grading and qPCR. However, in cases where several pathogens are present on the same leaf, visual grading can be misleading since the symptoms of the dominating fungus may conceal symptoms caused by others. Hence, qPCR could be a more reliable method for assessment of the fungal flora. In addition, it was shown that qPCR is a useful tool for quantifying the amount of the three wheat pathogens in field trials where different cultivars are evaluated.

7.5 MOLECULAR DETECTION OF *RALSTONIA SOLANACEARUM* FROM BANANA AGROECOSYSTEMS IN COLOMBIA. J. Alvarez, P. Rodríguez and M. Marín. *National University of Colombia – Medellín, Augura, Colombia. Email: mamarinm@unal.edu.co*

Moko disease of banana caused by *Ralstonia solanacearum* is one of the most limiting factors in the production of this crop worldwide. One way to reduce the incidence of this disease is early detection of affected plants and of soils with high levels of bacterial inoculum. This research evaluated different methods of nucleic acid extraction from plant material and soils, to be used in molecular diagnosis of *R. solanacearum* in the banana-growing region of Urabá, Colombia. Results showed that for diagnosis of plant material, DNA extraction should be done with commercial kits including silica gel columns or alternatively conventional methods using buffers containing PVPP. For detecting bacteria in soil samples the most appropriate method was microbial enrichment in SMSA broth before DNA extraction. Multiplex PCR analysis indicated that phylotype II, sequevar 4 was the causal agent of Moko disease of banana in the region of Urabá. Techniques applied in this research could be used in epidemiological studies as well as to support management of this disease in banana plantations.

7.6 PHENOTYPIC, GENETIC AND PATHOGENIC DIVERSITY OF THE SPANISH POPULATIONS OF *PHYTOPHTHORA CITROPHTHORA* CAUSING BRANCH CANKERS ON CITRUS TREES. L.A. Álvarez, M. León, A. Vicent, J. García-Jiménez and P. Abad-Campos. *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain. Email: luialber@eaf.upv.es*

Strains of *Phytophthora citrophthora* (n = 173) causing branch cankers on citrus trees were collected from 2003 to 2005 in 132 orchards across the most important citrus growing areas in Spain. To explain the occurrence of this new syndrome, the isolates were studied by means of phenotypic, genetic and pathogenic analyses to determine the population structure of the pathogen. The isolates presented broad variability in their morphological and physiological profiles. Mating type crosses showed that the 99.4% of the isolates were sterile. Analysis of random amplified microsatellite (RAMS) markers indicated that the *P. citrophthora* population was not homogeneous in citrus groves, consisting in fourteen genotypes. However, more than 80% of the strains were clustered in a major group of sterile isolates. Four of the genotypes have been previously described, and ten are described here for the first time. Among the 74 isolates tested for metalaxyl and mefenoxam sensitivity, 5.4 and 1.4%, respectively, were resistant *in vitro* to these fungicides. The aggressiveness of the two most frequent *P. citrophthora* genotypes were compared under field conditions, inoculating a selection of isolates on branches of the scion and on the rootstock of several *Citrus* species. All isolates tested proved to be virulent, but differences in aggressiveness between the two genotypes of *P. citrophthora* were not detected.

7.7 DESIGNING PCR PRIMERS TO DISCRIMINATE EIGHT SCLEROTIAL DISEASE PATHOGENS OF RICE. M. Arakawa, Y. Ohkawa, S. Urushizaki, M. Kato and K. Inagaki. *Faculty of Agriculture, Meijo University, 1-501 Shiogamaguchi, Tempaku, Nagoya, Japan. Email: aramasa@ccmfs.meijo-u.ac.jp*

The causal agents of rice sclerotial diseases mainly belong to genus *Rhizoctonia* and *Sclerotium*. As these diseases show very similar symptoms as well as morphologies of the causal fungi, diagnosis is difficult. In this study, in order to distinguish each of the rice sclerotial disease pahogens, containing 2 anastomosis groups of multinucleate *Rhizoctonia* (AG-1 IA and AG-2-2 IIIB), 2 of binucleate *Rhizoctonia* (AG-Ba and AG-Bb), 3 types of *R. circinata* (var. *agrostis*, *oryzae* and *zeae*) and *Sclerotium hydrophilum*, PCR primers were designed after sequencing the rDNA-ITS region.

The alignment with sequences of other related fungi in the database showed high variability in ITS1 regions among different groups. Forward primers for each of the 8 groups were designed to anneal at 55°C. A universal primer, ITS4 or ITS4-B, was used as the reverse primer. DNA templates extracted from cultured mycelia of Japanese isolates were used to confirm the specificity of primers. The primer designed for AG-1 (IA) showed DNA amplification to all of 104 isolates of rice sheath blight fungus, while no amplification was observed in isolates of the other groups. Similarly, the specificity of remaining 7 primers designed for the corresponding 7 groups were validated, using 13-94 isolates belonging to the respective groups. In addition, the specificity of these primers was verified by PCR using DNA templates extracted from lesions in inoculated rice. Now we will examine the utility of these primer sets for soil diagnosis, to detect rice sclerotial disease pathogens comprehensively.

7.8 REAL-TIME AND CONVENTIONAL PCR ASSAYS FOR DETECTION OF THE BANANA *XANTHOMONAS* WILT PATHOGEN AND RELATED *XANTHOMONAS VASICOLA* PATHOVARS. **V. Aritua, R. Thwaites, N. Parkinson, S.A. Weller, W. Tushemereirwe and J. Smith.** *National Agricultural Biotechnology Center, Kawanda Agricultural Research Institute, P. O. Box 7065, Kampala, Uganda. Email: arituavalentine@yahoo.com*

Banana *Xanthomonas* wilt (BXW) disease, originally described in Ethiopia on enset (*Ensete ventricosum*) and on banana (*Musa* spp.), is currently an emerging disease in East and Central Africa. Three *X. vasicola* species-specific conventional PCRs and a real-time TaqMan PCR were designed based on the sequence of a gene encoding the methylase chemotaxis methyl-accepting protein. These assays specifically detected all strains which cause BXW of banana as well as other members of *X. vasicola* which are pathogenic to sugarcane and sorghum. Primers XvasF1/XvasR1 and XvasF1/XvasR2 amplified single products of 358 bp and 272 bp respectively from 36 BXW and other *X. vasicola* DNA extracts but not from extracts from 46 other xanthomonads. All PCR assays detected as few as 100 bacteria per reaction, from pure bacterial cultures. Sigma Extract-N-Amp™ Plant PCR Kits (Sigma) were used for direct extraction of BXW DNA from banana tissues. The procedure was successfully used to detect BXW by all PCR assays, in DNA extracts from leaves, leaf sheaths, and pseudostem of artificially inoculated banana plants. The sensitivity of PCR for the specific detection of *X. vasicola* from banana leaves was determined to be 100 bacteria per amplification.

7.9 DISTINGUISHING VARIETIES WITHIN THE *PHOMA EXIGUA* COMPLEX USING DAF-BASED DNA BARCODES. **M.M. Aveskamp, E. Turco, J. de Gruyter, J.Z. Groenewald and P.W. Crous.** *CBS Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands. Email: m.aveskamp@cbs.knaw.nl*

Phoma exigua belongs to a group of anamorphic fungi comprising a range of destructive plant pathogens, opportunists as well as saprobes. *P. exigua* is considered to be an assemblage of at least nine varieties that are mainly distinguished on the basis of host specificity. However, these varieties can also act as opportunists on non-host tissue. In practice it is difficult to distinguish *P. exigua* from its close relatives, and correctly identify isolates to variety level, due to their low genetic variation and high morphological similarity. Organisations involved with plant quarantine inspections often not do have the time and experience to identify isolates to species or variety level. A rapid and robust DNA-based tool is therefore urgently required to aid discrimination between the harmless saprobic taxa and the pathogens in this species complex. The present study is therefore aimed at developing such a tool, based on DNA barcodes. Because poor results were obtained in discriminating taxa by means of multilocus sequence typing, a robust fingerprinting method was used. More than 60 strains of *P. exigua* and related species were subjected to DNA Amplified Fingerprinting (DAF) using short, arbitrarily mini-hairpin primers. Amplicons that varied in size over the different taxa, but not within the separate varieties were identified, purified and sequenced. Alignment of the sequence data and subsequent primer trials led to the identification of variety-specific DNA barcodes that can be used to identify these organisms during plant quarantine inspections.

7.10 INHERITANCE AND MOLECULAR MAPPING FOR BROWN SPOT DISEASE RESISTANCE IN RICE. **S.P. Banu, B. Meah, A. Ali, D.S. Brar, H. Leung and C.M. VeraCruz.** *Bangladesh Agricultural Research Institute, Gazipur, Bangladesh, Philippines. Email: salinapbanu@yahoo.com*

Brown spot (caused by *Bipolaris oryzae*) is a major fungal disease of rice, distributed worldwide. In this study a combination of genetic, molecular and pathological approaches was used to identify and map novel, brown spot-resistant genes. The traditional *japonica* cultivar Dinorado (resistant) was crossed with the semi-dwarf modern *indica* cultivar IR 36 (susceptible). Phenotypic segregation of 200 F_3 progenies suggested that resistance to brown spot is governed by two recessive genes with 1 (homozygous resistant) : 8 (heterozygote) : 7 (homozygous susceptible). In addition, corresponding F_2 progenies were used as mapping populations to identify DNA markers associated with resistance. Bulked segregant analysis was applied to analyze 186 F_2 lines with 160 SSR markers distributed equally over each of the 12 rice chromosomes. Marker analysis showed significant association (<0.0001) for 4 markers and explained 16.53-48.17% of the total phenotypic variation for brown spot resistance. Interval analysis suggested that genes for resistance to brown spot are located on chromosome 12. The two genes imparting resistance to brown spot in Dinorado are designated *bs1* and *bs2*. The present findings will provide guidelines to incorporate resistance from Dinorado to susceptible but otherwise high yielding cultivars of rice. The closely linked DNA markers would be suitable for use in marker-assisted selection in rice breeding programs.

7.11 HIGH GENETIC VARIABILITY IN THE MITOSPORIC SPECIES *ALTERNARIA DAUCI* AS REVEALED BY POLYMORPHISM AT MICROSATELLITE LOCI AND WITHIN THE INTERGENIC SPACER SEQUENCE. **S. Benichou, D. Peltier, B. Hamon and P. Simoneau.** *UMR PaVé A77-Faculté des Sciences-2 Bd Lavoisier-F49045 Angers cedex, France. Email: laliasb@yahoo.fr*

Alternaria dauci is the causal agent of leaf-blight of carrots and is responsible for significant losses in cultivated crops worldwide. Despite the economic importance of this disease, little is known concerning the pathogen. As for most *Alternaria* species, no sexual stage has been reported for *A. dauci* suggesting a low level of intra-specific variability. However, the observation of phenotypic variations (virulence, sensitivity towards xenobiotics) among field isolates prompted us to evaluate the genetic polymorphism with-

in this species. This was investigated using two types of molecular marker: variations in the number of repeats at different microsatellite loci and nucleotide sequence polymorphism in selected regions of the nuclear rDNA. Sequences carrying repeated motifs of various sizes were identified from microsatellite-enriched genomic libraries. Specific primers were designed to amplify these loci from 24 selected strains with various geographic and host origins. All the loci were found polymorphic, revealing from 2 to 16 allelic forms. Sequence polymorphism was also investigated in the intergenic spacer region (IGS). Comparison of complete IGS sequences from 2 different isolates revealed two domains: a conserved 3' domain and a 5' domain which was highly polymorphic even at the intra-specific level. Comparison of the sequences of these variable domains for the complete set of strains grouped *A. dauci* isolates into two main clusters. Taken together these analyses confirm the high level of genetic polymorphism in this mitosporic species but did not reveal any clustering by geographic origin, probably a consequence of long distance spread of the fungus through contaminated seeds.

7.12 APPLICATIONS OF A QUANTITATIVE ASSAY FOR THE POTATO CYST NEMATODES *GLOBODERA ROSTOCHIENSIS* AND *G. PALLIDA*. **V.C. Blok, A. Paterson, J. Heilbronn, A. Holt, L. Pylypenko and M.S. Phillips.** *Plant Pathology Programme, Scottish Crop Research Institute, Invergowrie, Dundee, Scotland, UK. Email: vblok@scri.ac.uk*

The potato cyst nematodes (PCNs) *Globodera rostochiensis* and *G. pallida* are economically important pests that occur in many potato-growing regions world-wide. In the EU the withdrawal of nematicides, the lack of varieties with resistance to *G. pallida* and the increasing prevalence of this species are of concern to growers. We have developed a quantitative PCR assay for PCNs which has been used to determine sensitivity of detection in soil and in statutory samples. We have used the assay to examine interspecific competition between the two species on potato lines with differing degrees of resistance and in different environmental conditions. This assay has the potential to provide assessments of species composition and quantities which will be valuable in enhancing the efficacy of decision support and improving our understanding of PCN ecology.

7.13 IMPROVED DETECTION OF GRAPEVINE PHYTOPLASMAS. **J. Boben, M. Hren, P. Nikoli, N. Mehle, M. Dermastia, K. Gruden and M. Ravnikar.** *National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia. Email: jana.boben@nib.si*

Phytoplasmas can cause many important vector-borne and graft-transmissible plant diseases. Different host plants can be affected including grapevine. Phytoplasmas associated with Bois noir (BN) and with Flavescence dorée (FD) are two major types of grapevine phytoplasma that cause disease in vineyards across Slovenia. The detection of phytoplasmas in general can be quite laborious – detection methods are based on detecting target DNA sequences (using PCR, nested PCR or PCR-RFLP) in combination with different DNA extraction methods (the CTAB method being widely used). There is a need for simpler and quicker diagnostic schemes that would give reliable results. We have optimized the detection procedure by developing a sensitive and specific real-time PCR method for BN and FD phytoplasmas and have tested an automated DNA extraction procedure that uses magnetic beads. In fruit tree samples, the automated procedure gave quick and effective DNA extraction and was for that reason also optimized for grapevine samples. The automated DNA extraction procedure was compared to the CTAB extraction procedure using over 140 samples that were then analyzed using real-time PCR. Results showed that combination of the two methods greatly decreases the time needed for analysis without the loss of sensitivity, which is of great importance for the inspection services and growers. The detection scheme developed for grapevine phytoplasma is simple, results can be obtained much quicker, and the reliability of the results is higher than with the previously widely used methods.

7.14 QUANTITATIVE MULTIPLEX DETECTION OF PLANT PATHOGENS USING PRI-LOCK PROBES AND UNIVERSAL, ULTRA-HIGH-THROUGHPUT REAL-TIME PCR ON OPENARRAYS. **P.J.M. Bonants, M. Szemes, R. van Doorn and C.D. Schoen.** *Plant Research International (PRI) B.V., Department of Biointeractions and Plant Health, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands. Email: peter.bonants@wur.nl*

Current technologies for multiplex, quantitative analyses frequently suffer from compromises between the level of multiplexing, throughput and accuracy of quantification. Microarrays provide very high level of multiplexing, but less accurate quantification and usually low throughput. At present, real-time PCR provides the most reliable means of target quantification, and is suitable for high numbers of samples. The achievable level of multiplexing is however low. Nano-scale technology provides high-density and low-volume microchambers, which could accommodate a very high number of standardized reactions. Many of these systems are under development and are not capable of monitoring the fluorescent signals in real-time for each microwell. Recently, a conceptually new, ultra-high-throughput platform has become available for real-time PCR, able to accommodate more than 3000 reactions per array. The OpenArray^{TM} system has 48 subarrays, each containing 64 microscopic through-holes of 33 nl volume. The primers are pre-loaded into the holes, while the sample along with the reagents are auto-loaded due to capillary action. We recently developed PRI-lock probes for multiplex detection which provide flexibility and bridge the gap between target-specific recognition and high-throughput amplification. PRI-lock probes are long oligonucleotides, containing artificially selected primer sites and a TaqMan probe region, flanked by target complementary regions. After ligation of the PRI-lock probes on target DNA the circular probes can be real-time-amplified using the specific primer pairs and a generic TaqMan probe. In this study, we have characterized the quantification power of circularizable ligation probes, and report the development of a high-throughput, quantitative multiplex diagnostic assay based on the principle described.

7.15 MOLECULAR DETECTION OF *SPONGOSPORA SUBTERRANEA* IN NATURALLY INFESTED SOILS USED FOR POTATO CULTIVATION. **V. Bravo, F. Moronta, F. Bittara, D. Rodríguez and I. Galindo.** *Universidad Simón Bolivar, Doctorado Interdisciplinario en Ciencias, Laboratorio de Genómica and Proteómica, Instituto de Estudios Avanzados, Caracas, Venezuela. Email: cristibu@cantv.net*

Potato is one of the most important crops, and in Venezuela it is considered strategic, occupying an important place in both consumption and production; however, yields as well as quality are affected by various diseases, primarily soil pathogens; pow-

dery scab, caused by *Spongospora subterranea* is one of the most serious today, in addition to being a vector of *Potato mop-top virus*. As the problem recent, current information is insufficient for management and control. In order to detect the pathogen in the states of Mérida, Trujillo, Lara and Tachira, DNA was extracted from soil by the method of Volossiouk *et al*. (1995) and PCR was used, with primers Sps1 and Sps2 designed from the ITS region of (rDNA) to amplify a specific product of 391 bp. *S. subterranea* was detected in 64, 53, 58.3 and 95% of the soils analyzed from the states of Mérida, Trujillo, Lara and Tachira respectively. These results suggest that the pathogen is present in most soils where potatoes are grown in the country, and show that this method is useful for rapid detection of the pathogen in soils even seemingly free of it.

7.16 VALIDATING PREDICTIVE DIAGNOSTICS FOR POTATO DISEASES. **J.L. Brierley, J.A. Stewart, S.J. Wale, A.J. Hilton, G. Budge, J. Elphinstone, B. Barrett, N. Boonham and A.K. Lees.** *Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland UK. Email: Jennie.Brierley@scri.ac.uk*

The use of molecular diagnostics for predicting disease in potato crops is being validated, making use of some of the specific real-time PCR assays now available for a wide range of potato pathogens. These assays have been used in conjunction with refined methods for the direct extraction of soil DNA to enable soil-borne inoculum of the pathogens to be detected and quantified. As part of a three year monitoring programme, the quantification of soil and seed-borne inoculum has been related to the incidence and severity of progeny tuber disease symptoms and levels of pathogen contamination as determined by real-time PCR. The work has focussed on three pathogens, *Colletotrichum coccodes* (black dot), *Rhizoctonia solani* (black scurf) and *Spongospora subterranea* (powdery scab). Results from this monitoring programme, describing the value of diagnostic assays in predicting disease for the management of potato crops, are discussed.

7.17 A NEW MOLECULAR APPROACH FOR INVESTIGATING *PHYTOPHTHORA* DIVERSITY IN DIFFERENT NATURAL ECOSYSTEMS. **S.O. Cacciola, A. Chimento, D.E.L. Cooke, C. Rizza, L. Schena and S. Scibetta.** *Università degli Studi di Palermo, Dipartimento Sen.Fi.Mi.Zo. Viale delle Scienze, 90128 Palermo, Italy. Email: cacciola@unipa.it*

A new molecular method has been developed, based on a nested-PCR assay, for the detection of *Phytophthora* species in soil and water samples. New *Phytophthora* genus-specific PCR primers based on the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) have been designed to discriminate different species. Clone libraries have been created in order to detect and identify all the possible species in each sample. Sequence analysis of a large number of clones showed a variety of *Phytophthora* species in soil and stream water. The procedure allows rapid analysis of many environmental samples, reducing the time needed for processing, and increasing the efficiency compared to traditional methods based on isolation. The protocol for processing water samples, based on in-field filtration and DNA extraction directly from the filters, can more efficiently capture *Phytophthora* propagules. The method was tested in Scottish natural ecosystems during surveys carried out in 2006, and then applied in Southern Italy to study *Phytophthora* diversity in beech, chestnut and oak stands, in natural reserves. The presence of 23 known *Phytophthora* phylotypes, from all main ITS-clades of the genus was detected. Furthermore, the phylogenetic analysis suggests the presence of yet undescribed species. This new approach is a powerful and practical tool for ecological studies of natural *Phytophthora* communities.

7.18 SIMPLE PREPARATION OF SAMPLES FOR DIRECT REAL-TIME AMPLIFICATION OF WOODY-PLANT VIRUSES. **N. Capote, E. Bertolini, A. Moreno, E. Vidal, A. Olmos, M.T. Gorris, M.C. Martínez and M. Cambra.** *Instituto Valenciano de Investigaciones Agrarias, Carretera Moncada-Náquera km 5, 46113 Moncada, Valencia, Spain. Email: ncapote@ivia.es*

Biological indexing and serological methods are widely used for plant virus diagnosis. Nevertheless, molecular techniques have revolutionized plant virus detection and identification. Real-time RT-PCR has proved to be the most sensitive and reliable molecular method for virus diagnosis. However, conventional real-time RT-PCR requires an RNA purification step that makes the procedure tedious, costly, time-consuming, and unsuitable for large-scale analysis. Fast and simple sample preparation procedures have been developed for real-time amplification of viruses of woody plants such as *Plum pox virus* (PPV), *Citrus tristeza virus* (CTV), *Citrus sudden death virus* (CSDV), *Apple chlorotic leaf spot virus* (ACLSV), *Prunus necrotic ring spot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Apple mosaic virus* (ApMV). Two of these methods avoid nucleic acid extraction: 1) Spotting of crude plant extracts on a nylon or Whatman membrane, and 2) Dilution of crude extracts in extraction buffer (for minimizing PCR inhibition). Another two methods even save the preparation of crude extracts: 3) Tissue-printing, that consists of pressing a fresh section of a plant tissue on a piece of membrane to make a print, and 4) Squash procedure, performed by squashing plant tissues or single aphids (or other vectors) on a piece of membrane. In these sample preparation methods, membranes carrying the immobilized targets can be stored frozen or at room temperature for long periods, without decreasing the sensitivity of detection. These fast and simple sample preparation procedures combined with real-time RT-PCR will allow inclusion of this molecular technique for high-throughput testing in routine analysis and certification programs.

7.19 IDENTIFICATION OF *PRATYLENCHUS THORNEI* USING A SATDNA SEQUENCE. **S. Carrasco-Ballesteros, B.J. Adams, P. Castillo and E. Pérez-Artés.** *Department of Microbiology and Molecular Biology, Brigham Young University, 775 WIDB, Provo, UT 84602, USA. Email: bjadams@byu.edu*

Pratylenchus spp. are economically important pathogens of many herbaceous and fruit crops. Identification at species level is important for quarantine inspection, implementation of integrated pest management strategies, and resistance breeding. Repetitive sequences known as satDNA have been characterized in a number of nematodes of agronomic interest. Here, we describe the use of a satDNA sequence as a probe for the specific identification of *P. thornei* populations. The satDNA sequence was amplified using an oligonucleotide primer pair designed from a previously identified RAPD fragment associated with *P. thornei*. Sequencing showed that this satDNA consisted of 17 consecutive repeats (31-32 bp long; [*TRAAGAAT*YGTCCYTYTYCKAACYGRWTYYC(C)]). Each repetition contained a core region composed of 8 nucleotides (TRAAGAAT). The flanking regions, located at the 5' (5 nt) and 3' (9 nt) ends of the core region, varied in 1 or 2 nts between repetitions. A selected fragment (32 nt: ACCGGGTTCCC*TAAAGAA*

*T*CGTCCCTTTCCGA) of the satDNA sequence was synthesized and labelled with DIG-11-dUTP. Purified total DNA of different isolates of *P. thornei*, other *Pratylenchus* spp., *Meloidogine* spp., *Heterodera mediterranea*, *Zigotylenchus guevarai*, *Ditylenchus dipsaci*, and *Radopholus similis*, was dot-blotted onto a nylon membrane and hybridized with the DIG labelled satDNA sequence. Hybridisation occurred with all of the *P. thornei* isolates but did not occur with any other nematode isolates in this study.

7.20 PREPARATION AND APPLICATION OF AN ANTISERUM AGAINST A PARTIAL NUCLEOCAPSID PROTEIN SEQUENCE OF *ORCHID FLECK VIRUS*. **C.A. Chang, Y.Y. Lin, M.J. Lin, H.C. Lu, Y.H. Cheng and C.C. Chen.** *Division of Plant Pathology, Agricultural Research Institute, Wufeng, Taichung, ROC. Email: cachang@wufeng.tari.gov.tw*

A cymbidium plant (LK2) showing systemic chlorotic flecks different from those induced by *Odontoglossum ringspot virus* and *Cymbidium mosaic virus* was found at Lu-Ku Township of Taiwan. LK2 was subsequently identified to be infected by a strain of *Orchid fleck virus* (OFV). Since this is the first encounter of OFV in Taiwan, infected and the neighboring orchids were immediately eradicated, followed with a 3-year surveillance program. Since then OFV has not been detected again. To preserve OFV antigen but not the live culture, we cloned its nucleocapsid protein (NP) gene in plasmid pET28b(+) (Novagen) and expressed the viral protein in *E. coli*. The bacteria-expressed NP can be used as positive control antigen and also for antiserum preparation. We have cloned the full length and also the C-terminal half of OFV NP gene encoding 52 K and 27 K NP proteins, respectively. The two NP proteins were separately expressed in bacteria and purified for antiserum preparation. The two antisera were tested in ELISA, SDS-immunodiffusion and Western blotting. Results showed that activity of antiserum against the 52 K NP protein was not satisfactory compared with antiserum to the 27 K NP. The latter could react strongly with OFV antigens in infected orchid tissue but not with healthy control ones. The reason for the unsatisfactory activity of antiserum against the full length NP is not clear. However, rapid degradation of the bacteria-expressed full-length NP was observed. This property may affect its immunogenecity during the immune response.

7.21 A MICROARRAY-BASED DIAGNOSTIC SYSTEM FOR DETECTING FUNGAL PATHOGENS IN FLOWER CROPS. **R.S. Chen and H.F. Ni.** *Department of Biochemical Science and Technology, National Chiayi University, Chiayi 600, ROC. Email: rschen@mail.ncyu.edu.tw*

The biochip (DNA microarray) is a powerful tool for reliable, accurate, and parallel analysis of large numbers of samples. The main objective of this study was to develop an oligonucleotide microarray-based diagnostic system for detecting fungal pathogens in flower crops. Internal transcribed spacer (ITS), and intergenic spacer (IGS) regions of ribosomal DNA (rDNA) from several fungal pathogens, including *Fusarium oxysporum*, *Phytophthora* spp., *Sclerotium rolfsii*, *Rhizotonia solani*, *Colletotrichum* spp. and *Botrytis* spp., were amplified, cloned, and sequenced. Based on these rDNA sequences, 60-nt-long oligonucleotide probes were designed and synthesized to fabricate the oligonucleotide microarray. For each fungal pathogen, 3-5 specific oligonucleotide probes were designed for making a diagnostic oligochip, followed by testing the specificity as hybridized to the dig-labeled rDNA fragments from tested pathogens and plant tissues infected by different fungal pathogens. Through this study, a single universal detection platform was developed to simultaneously detect different fungal pathogens of several main flower crops in Taiwan. Our results also showed that microarray-based assay could efficiently identify fungal pathogens in various crops, and was rapid and reliable for potential quarantine applications.

7.22 MOLECULAR TOOLS FOR IMPROVED CROP MANAGEMENT OF DOWNY MILDEW ON *BRASSICA*. **E. Clewes, C. Bilbao, B. Dez, E. Holub and R. Kennedy.** *University of Warwick, Warwick HRI, Wellesbourne, CV35 9EF, UK. Email: emily.j.clewes@warwick.ac.uk*

Downy mildew of *Brassica* (HpBo), caused by *Hyaloperonospora parasitica*, is a common disease on seedlings raised in glasshouses for transplanting in the field. Up to nine applications of fungicide are used to control the disease in the glasshouse and this has often resulted in the appearance of fungicide-insensitive variants in the pathogen. Although no major genes for resistance to HpBo are known in horticultural *Brassica* crops, alternative control strategies based on the use of resistant varieties, which will help to reduce the dependence on fungicides, are now being used. Such approaches rely on advice to growers involving rotation of resistant cultivars that is informed by monitoring of changes in virulence of the pathogen population. This project aims to derive better information regarding the population structure of the pathogen with a view to improved deployment of cultivars in field crops. Molecular techniques able to differentiate pathogen populations of HpBo have been developed using both recent information regarding the putative avirulence genes and neutral markers. The development and use of these markers will be discussed.

7.23 THE USE OF FLOW CYTOMETRY FOR THE DETECTION OF *XANTHOMONAS FRAGARIAE* AND *PSEUDOMONAS CICHORII*. **L. D'hondt, L. Leus, J. Van Vaerenbergh, J. Van Huylenbroeck, E. Van Bockstaele and M. Höfte.** *Caritasstraat 21, 9090 Melle, Belgium. Email: liesbet.dhondt@ilvo.vlaanderen.be*

Flow cytometry is gaining more and more importance in medical diagnostics. In plant science flow cytometry is known for ploidy analysis. Applications for plant pathology are still almost non existent. Nevertheless, flow cytometry is a promising tool for detection and viability assessment of plant pathogens, as it is rapid, sensitive and quantitative, does not rely on the ability of bacteria to grow on culture media and can discriminate living from dead cells. The principle is based on cells in a fluid stream passing one by one through a flow cell. A laser beam illuminates the cells and excites fluorescent compounds attached to or contained in the cells. These can be fluorescent dyes binding to cell compounds, autofluorescence of molecules, fluorescent-labelled antibodies and/or beads with specific capture probes. Within a few seconds thousands of cells can be analysed. We aim at the development of more applications for plant pathogens, using two examples. *Xanthomonas fragariae* is the causative agent of angular leafspot on strawberry. Detection of *X. fragariae* is possible by PCR, but viability assessment is difficult as no selective media exist. Flow cytometry as a tool for viability assessment of *X. fragariae* has been studied. *Pseudomonas cichorii* causes midrib rot of glasshouse lettuce causing severe losses and reduced market values. The infection mechanism and sources of *P. cichorii* are not yet well studied. It is thought that infected irrigation water is the

major infection source. We evaluated whether flow cytometry can be used to detect viable bacteria in irrigation water samples of infected glasshouse lettuce.

7.24 DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR THE DETECTION AND QUANTIFICATION OF *COLLETOTRICHUM ACUTATUM* IN STRAWBERRY LEAVES. J. Debode, S. Baeyen, K. Heungens and M. Maes. *Institute for Agricultural and Fisheries Research, Burg. van Gansberghelaan 96, 9820 Merelbeke, Belgium. Email: Jane.Debode@ilvo.vlaanderen.be*

A reliable, sensitive detection technique is needed to help understand the dynamics of *C. acutatum* in a strawberry field. Real-time PCR assays were developed using primers designed to the rDNA ITS1 region and the β-tubulin gene. Candidate primers were evaluated for sensitivity and selectivity, based on the amplification and melting curves using dilution series of target- and non-target DNA as template. The ITS-based assay was 10 times more sensitive than the β-tubulin-based assay. Using TaqMan technology, the ITS-based assay could reliably detect as little as 50 fg of genomic DNA or 100 copies of target DNA. The TaqMan-ITS assay recognized all *C. acutatum* isolates tested from different subspecific molecular groups (A2, A3 and A4) described as *C. acutatum*, while no amplification was observed with several other *Colletotrichum* species (e.g. *C. gloeosporioides*, *C. fragariae*), or with genomic DNA of other strawberry pathogens tested (e.g. Botrytis cinerea, *Verticillium dahliae*), indicating the specificity of this real-time PCR for *C. acutatum*. The assay was then used for the detection and quantification of *C. acutatum* in artificially and naturally infected strawberry leaves. Different mixes of infected plant material with healthy material were used to determine the detection limit of *C. acutatum* in 100 mg plant material. The TaqMan-ITS assay could detect *C. acutatum* where only 0.001% artificially infected plant material was present. In addition, the TaqMan-ITS assay was able to detect C. acutatum in symptomless strawberry leaves that were collected from strawberry fields where natural *C. acutatum* infections occurred.

7.25 ONE - STEP REAL TIME RT- QPCR FOR THE DETECTION OF *PEPINO MOSAIC VIRUS* IN DIFFERENT MATRIXES. D. Delic, N. Mehle, I. Gutiérrez-Aguirre, K. Gruden and M. Ravnikar. *National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia. Email: duska.delic@nib.si*

Pepino mosaic virus (PepMV) is a relatively stable and contagious virus, and a serious threat to tomato production. Despite strict control measures recommended by the EU, virus outbreaks are constantly being reported in many European countries. A reliable, sensitive and rapid detection method is of crucial importance for preventing the spread of this virus. We developed a one-step real-time RT-qPCR assay for the detection of PepMV targeted to different genes. We compared the sensitivity of the method with classical routine diagnostic tools such as ELISA, Pocket Diagnostic™ lateral flow devices (CSL, Great Britain) and electron microscopy. One-step RT-qPCR improved the detection of purified viral particles diluted in tap water and healthy tomato sap by several orders of magnitude. Infected seeds can be an important source for virus spread via imported/exported material. In order to test the ability of our method to detect PepMV in such a matrix, artificially infested tomato seeds were mixed with virus-free seeds in different ratios and qPCR detection was again compared with classic detection tools. Again the one-step real-time RT-qPCR showed the highest sensitivity and limit of detection. In summary, we present a new sensitive test for the continuous monitoring of PepMV in plant tissue and irrigation water samples, as well as in seeds.

7.26* REAL-TIME PCR IN PHYTOBACTERIOLOGY. T. Dreo, M. Pirc, K. Gruden and M. Ravnikar. *National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia. Email: tanja.dreo@nib.si*

Many real-time PCR tests have recently been described for detecting phytopathogenic bacteria. Case studies will be presented for tests designed and validated in our laboratory (targeting *Xylophilus ampelinus* and *Erwinia amylovora*) and tests adapted from the literature, with special regard to critical parameters. With its capacity for high-throughput analysis and low risk of cross-contamination, real-time PCR is most interesting for screening plant extracts. Specificity is highly important, and extensive knowledge of bacterial diversity, ecology and phylogeny is needed when choosing target sequences, material for subtractive hybridizations and/or strains for cross-reactivity testing. Real-time PCR is generally more sensitive than other widespread screening tests such as immunofluorescence and PCR. With these tests both viable and dead bacteria are detected. Only absence of bacteria in concentrations above the detection limit and not their complete absence can be determined. Although an advantage, high sensitivity can pose difficulties in diagnostics when a positive result cannot be confirmed by other methods because of their inferior sensitivity. An important advantage of real-time PCR is the possibility of assessing target viability through quantification of bacteria in samples before and after enrichment procedures even when isolation in pure culture is not possible. Quality control aspects such as crucial steps in method development, use of real-time PCR in evaluation of other methods, extent of validation and choice, preparation and use of suitable controls will be discussed, together with future prospects for bacterial diagnostics targeting mRNA.

7.27 DETECTION OF *PENICILLIUM EXPANSUM* ASSOCIATED WITH BLUE MOULD ON APPLES IN ONTARIO USING PCR-RFLP. D. Errampalli, V. Popovic and K. Ellens. *Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 4902 Victoria Ave. N., Vineland Station, ON L0R 2E0, Canada. Email: errampallid@agr.gc.ca*

Blue mould (caused by *Penicillium* spp.) is one of the most important postharvest diseases of apples in southern Ontario, Canada. Although *Penicillium expansum* is the most common and aggressive species, several others such as *P. brevicompactum*, *P. crustosum*, *P. aurantiogriseum*, *P. polonicum*, *P. viridicatum*, and *P. solitum* are also known to cause blue mould symptoms. The objective of this study was to identify species of *Penicillium* present among fifty six isolates in three apple packinghouses in southern Ontario. Genotypic species identification was performed by the restriction fragment length polymorphism (RFLP) analysis of a polymerase chain reaction (PCR) amplified product from the internal transcribed spacer (ITS4 and ITS5) region of rDNA. Digestion of the ~600 bp DNA fragment, amplified from the ITS region, using restriction enzymes *Hin*fI and *Taq*I revealed distinctive banding patterns: *Hin*fI produced three bands (300, 200, and 100 bp) for *P. expansum*, and two bands (325 and 275 bp) for *P. solitum*, while *Taq*I produced three bands (275, 160, and 150 bp) for *P. expansum*, and four bands (190, 180, 170, and 60 bp) for *P.*

solitum. In one exception, *Taq*I produced two bands (325 and 275 bp) for *P. brevicompactum*. The PCR-RFLP technique was successful in differentiating between all but two of the above mentioned *Penicillium* spp. Based on PCR-RFLP analysis, 53 isolates were identified as *P. expansum*, and 3 as *P. solitum*. Pathogenicity tests showed that *P. expansum* was more aggressive than *P. solitum* on apple 'McIntosh'.

7.28 VERY RAPID DETECTION OF *BROAD BEAN WILT VIRUS 1* BY FLOW-THROUGH HYBRIDIZATION OF TISSUE PRINTS. I. Ferriol, A.A. Olmos and L. Rubio. *Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain. Email: iferriol@ivia.es*

Broad bean wilt virus 1 (BBWV-1) is distributed worldwide and damages economically important crops such as pepper, bean, pea, broad bean, spinach, lettuce and tomato. Fast and reliable diagnostic tools are crucial to study incidence and epidemiology, and to control the disease. In this work, a procedure to detect BBWV-1 was developed based on molecular hybridization of RNA extracts and tissue prints with digoxigenin-labeled RNA probes. Hybridization was done using a Hybrimax device (Hybribio Limited) based on the principle of flow-through hybridization. Negative pressure under the airtight hybridization membrane was applied by a vacuum pump. Prehybridization, hybridization, washing, and developing solutions flowed through the membrane automatically, requiring small volumes, just enough to cover the membrane (0.5-2 ml). Vacuum pressure reduced each hybridization step to 30-60 seconds, providing results in just 15-30 minutes. Sensitivity was similar to that obtained by conventional hybridization, which is more expensive (larger amounts of probes and reagents are required) and time consuming (at least 7 hours). In addition, analysis of tissue prints saves time and labor necessary for extract preparation. Prints can be prepared in the field, stored at room temperature and mailed elsewhere. Thus, by saving sample processing and using flow-through hybridization, BBWV-1 diagnosis can be accomplished in record time.

7.29 COMBINED ANALYSIS OF GENETIC AND RESISTANCE DIVERSITIES IN WHEAT USING RGA MARKERS. A.R. Habibzade, M. Keshavarzi, S. Rashed, F. Afshari and M.R. Naghavi. *Ahvaz, Biotechnology Institute, Iran. Email: arhz2005@yahoo.com*

Genes cloned from diverse plants for resistance to different pathogens have sequence similarities in domains presumably involved in pathogen recognition and signal transduction in triggering the defence response. Based on conserved regions of resistance (RGA) of class NBS-LRR resistance genes, five pairs of degenerate primers were designed and used to detect genetic and resistance gene diversity in 30 local and foreign wheat cultivars. Results indicated sharp banding of PCR products in denaturing polyacrylamide gels electroforesed for 2-2.5 h at 1600 volts in a 40–50 cm apparatus. A total of 1486 bands with 33-42% polymorphism were detected. The local cultivars were grouped in a separate cluster, apart from foreign cultivars. In the cluster of local cultivars, the position of each cultivar was related to its pedigree. Also, cultivars with similar resistance gene formulations were clustered together. Within the dendrogram, the most susceptible cultivars Jupetco S and Bolani were clustered together. The results indicate the RGA approach can be useful to study genetic diversity combined with candidate resistance gene diversity.

7.30 THE RELATIONSHIP BETWEEN SOIL POPULATIONS OF *RHIZOCTONIA SOLANI* AG2.1 AND AG3 AND POTATO TUBER INFECTION. R.B. Harding, A. McKay, K. Ophelkeller and Herdina. *South Australian Research and Development Institute, GPO Box 397, Adelaide, SA, Australia. Email: harding.robin@saugov.sa.gov.au*

Rhizoctonia solani is a serious disease of potatoes which reduces yields and tuber quality. Recent research has shown potato plants can be infected with one or more anastomosis groups (AGs) of *R. solani*. In this study minitubers (cv. Shepody) were planted into soil containing varying levels of either AG2.1 or AG3. At 130 days post planting, levels of infection were assessed on tubers, stems and roots. It was observed that AG2.1 resulted in root necrosis and russetting of the tuber surface while AG3 induced sclerotia on both roots and tubers. There was a strong relationship between concentration of DNA for AG2.1 and AG3 in spiked soil and disease severity (percent of surface area infected) expressed on daughter tubers at harvest ($r^2 = 0.67$ and $r^2 = 0.82$ respectively), however significantly lower levels of AG3 were required to induce a similar disease severity as AG2.1. (25pg DNA/g soil and 1280pg DNA/g soil respectively). Only AG2.1 showed a correlation between the level in soil and severity on stems and roots ($r^2 = 0.64$ and $r^2 = 0.59$ respectively). Within each AG, there was no correlation between level in soil and yield of tubers at harvest, although yields within AG3 tended to be higher. Results confirm the potential for using quantitative molecular diagnostic assays based on real-time PCR to quantify different *R. solani* AG2.1 and AG3 levels in soil. They also show that initial inoculum levels may correlate with subsequent disease severity when using clean seed (cv. Shepody) under controlled environmental conditions.

7.31 THE USE AND VALIDATION OF REAL-TIME PCR AND MICROARRAY METHODS FOR PLANT PATHOGEN DIAGNOSTICS. L. Horvath, M. Hudecova and M. Feketova. *Department of Molecular Biology, Central Control and Testing Institute of Agriculture, Hanulova 9/A, 841 01 Bratislava, Slovakia. Email: l.horvath@uksup.sk*

The use of real-time PCR and microarray diagnostic techniques as alternatives to the EC and EPPO standard PCR procedures for diagnostics of quarantine plant bacteria *Ralstonia solanacearum* (RS), *Clavibacter michiganensis* ssp. *sepedonicus* (CMS), *Erwinia amylovora* (EA), ESFY phytoplasmas, the fungi *Monilinia fructicola* (MF), *Phytophthora ramorum* (PR) and *Cauliflower mosaic virus* (CaMV) were tested and compared. Real-time PCR analyses were done with standardized DNA extractions and primer pairs for the mentioned bacteria, phytoplasmas and fungi using DNA Sybr Green kit and LightCycler 2.0 or ABI 7900HT apparatus. DNA-microarray methods were used to detect CaMV with the DualChip GMO Eppendorf DNA-microarray system. Standard PCR procedures were based on apropriate Commission Directives and EPPO Standard diagnostic protocols for RS, CMS, EA, ESFYp, MF and PR. The standard PCR method for CaMV detection was based in GeneScan protocol and CaMV test kit. Real-time PCR and microarray comparative analyses were done according to EN ISO 16140 and IUPAC home validation protocols. Specific detection, melting curves and genotyping of individually tested pathogens gave results which fully conformed with standard PCR procedures. Computed relative accuracy, sensitivity and specificity of real-time PCR and microarray methods vs. standard PCR procedures, for all individual pathogens, were around 100%. The study shows that the alternative methods give the same results as the standard methods and could be used for

routine and complementary analyses of plant pathogens in phytosanitary diagnostics and crop biosecurity.

7.32 DEVELOPMENT OF MICROSATELLITE MARKERS FOR *PYTHIUM HELICOIDES*. **K. Kageyama, Yin-Ling, M. Senda, H. Fukui and H. Suga.** *River Basin Research Center, Gifu University, Gfiu 501-1193, Japan. Email: kageyama@green.gifu-u.ac.jp*

Diseases caused by *Pythium helicoides* have expanded on cuttings of rose, kalanchoe, chrysanthemum and strawberry in Japan, since the species was reported to cause root rot of miniature roses in 1996. In such epidemic diseases, it is very important to determine the routes of transmission and to block them for disease control. The transmission routes can be predicted by population structure analysis of the target pathogen. The purpose of this study is to develop microsatellite markers of *P. helicoides* for population structure analysis. The microsatellites were developed by combining suppression-PCR and thermal asymmetric interlaced PCR. Three primer sets for the microsatellites, (GA)n, (CAC)n(CCA)n and (CTTT)n, respectively, were designed. These microsatellites were verified by conventional PCR with three representative isolates originating from different hosts and locations. There were polymorphisms within each isolate and between the isolates, indicating that these microsatellites would be valuable as markers. In further study, these microsatellite markers will allow analysis of the population structure of the *P. helicoides* isolates from different hosts and locations, and the transmission routes will be predicted by determining the distribution of individual populations. Furthermore, the microsatellite markers will allow fingerprinting of the pathogen and analysis of the origins of the primary inoculum and the dispersal of the secondary inoculum in a greenhouse.

7.33 GENETIC DIVERSITY IN THREE POPULATION SUBDIVISIONS OF *ASCOCHYTA* SPP. ACROSS HIMACHAL PRADESH, A NORTH-WESTERN STATE OF INDIA. **R.P. Kaushal.** *Department of Plant Pathology, HPAU, Palampur 176062, H.P., India. Email: rpkaushal@gmail.com*

Three *Ascochyta* spp. populations comprising of 37 isolates causing *Ascochyta* blight in pea, a premier legume crop of Himachal Pradesh, were analyzed to understand genetic diversity and the probable rate of spread of pathotypes. Genetic diversity was calculated on the basis of allele frequencies of 13 random amplified polymorphic DNA markers using Nei's genetic diversity formulae. Isolates of *Ascochyta* spp. were scored for variation at 13 putative random amplified polymorphic DNA (RAPD) loci. Allele frequency at single locus varied from 0.00 to 1.00. Diversity within each population (H_S) was high with values ranging from 0.36 to 0.40. Highly significant genetic differentiation was detected among all three subpopulations (Nei's coefficient of gene differentiation [G_{ST}] = 0.09, P = 0.004) thus indicating little gene flow over long distances. Haplotype diversity in the three populations were 0.57 (Sangla), 0.53 (Jinghali) and 0.54 (Chamba). Phenograms developed through POPGENE software among populations revealed three distinct clusters of isolates within each subpopulation. Genetic distance between the populations was very low between the Chamba and Sangla populations (0.09), thereby indicating high diversity among the two populations. Based on NTSYS pc 2.0, the 37 isolates fell into two major clusters accommodating 18 and 19 isolates respectively. There was no correlation between geographic origin and phenogram as the isolates were grouped regardless of their site of collection.

7.34 TREFLAN-INDUCED OXIDATIVE STRESS IN BARLEY CULTIVARS VARYING IN TREFLAN TOLERANCE. **Y. Kazhura and E. Semenchik.** *Department of Genetics, Belarusian State University, Minsk 220050, Belarus. Email: kazhura@tut.by*

The effect of oxidative stress was studied in roots of two barley cultivars with contrasting sensitivity to the herbicide Treflan (a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine). Treflan treatment increased benzidine peroxidase activity in roots of barley 'Gonar'. After 72 h of 1 mg/l Treflan treatment, peroxidase activity in roots was more than twice as high as in control roots. In contrast, peroxidase activity in the roots of cv. 'Staly' did not differ from the control. Treflan-induced changes in glutathione content were absent in both cases, but the glutathione pool was much smaller in 'Gonar' plants exposed and unexposed to Treflan. Herbicide-induced increase in the activity of root peroxidases and in glutathione content correlated with damage rate of the microtubule cytoskeleton and root growth inhibition. Higher peroxidase activity, higher inhibition of root growth, reduced glutathione content and severe damage to the microtubule cytoskeleton in the herbicide-sensitive 'Gonar' suggest that enhanced oxidative stress generated by Treflan treatment is significantly greater than in the herbicide resistant 'Staly'.

7.35 THE CAUSAL AGENT OF DWARF BUNT – THE MOST DANGEROUS SOILBORNE FUNGUS IN THE CZECH REPUBLIC. **M. Kochanova, E. Prokinova, M. Vanova and P. Rysanek.** *Czech University of Life Science, Faculty of Agrobiology, Food and Natural Resources, Department of Plant Protection, Kamycka 129, 16521 Prague 6 Suchdol, Czech Republic. Email: kochanova@af.czu.cz*

Tilletia controversa – dwarf bunt causal agent – is the most dangerous fungal soilborne organism in the Czech Republic and one of the most dangerous organisms in many countries all over the world. *T. controversa* spores stay alive in soil up to ten years. Each season over that time, spores constitute a threat for wheat. Sori with three million spores in place of wheat grains inside smutty spikes are broken during harvesting. They contaminate soil, all the crop and the harvester. Spores covering the grain surface are the main reason for refusal of grain in Czech and foreign grain markets. In 2006 dwarf bunt presence was reported from every Czech wheat growing area. Its occurrence depends upon weather during crop growth. In the near future Czech bunt epidemics may be repeated. Methods for bunt diagnostics in its early stages are necessary. Confident detection of dwarf bunt in soil can warn about fields with spore loads – places at risk of of bunt outbreaks in the next seasons. This work was supported by Ministry of Agriculture project QH71105.

7.36 DIFFERENTIATION OF *TAPESIA YALLUNDAE* AND *T. ACUFORMIS* CAUSING CEREAL EYESPOT DISEASE IN POLAND. **M. Korbas, K. Pieczul, J. Horoszkiewicz-Janka and I. Świerczynska.** *Institute of Plant Protection, ul. Miczurina 20, 60-318 Poznan, Poland. Email: m.korbas@ior.poznan.pl*

Eyespot is a widespread and economically important disease of cereals in Poland. Two species, *Tapesia yallundae* (anamorph *Pseudocercosporella herpotrichoides* W-type) and *T. acuformis* (anamorph *P. herpotrichoides* R-type) were previously recognized as agent of this disease. Identification of the pathogens is usually possible by traditional tools like analysis of morphological features and differences in host range as well as fungicide sensitivity.

R-type isolates are equally pathogenic to rye and wheat and produce slow-growing colonies with uneven margins while W-type isolates are highly pathogenic only to wheat and form fast-growing, smooth-edged colonies. Both fungi have a two-allele heterothallic mating system, with mating types MAT-1 and MAT-2. Nowadays molecular tools like PCR are frequently used to distinguish the *Tapesia* species and identify their genetic features such as mating types. The aim of this study was the molecular identification of *Tapesia* species and their mating groups. One hundred isolates collected in 2006 to 2007 from all over Poland were used. We employed species-specific PCR to distinguish the two *Tapesia* species causing eyespot of cereals and to estimate their frequency in Poland. Multiplex PCR was used to determine the distribution of mating types (MAT-1 and MAT-2) for both pathogens. We found that both species and mating types of *T. yallundae* and *T. acuformis* are present in the Polish population, which indicates the possibility of sexual crossing between isolates. On the basis of the analysis performed we estimate their frequency in Poland.

7.37 DETECTION OF DNA AND RNA PLANT VIRUSES FROM PLANT TISSUE PRINTS ON NITROCELLULOSE MEMBRANES BY PCR. S.G. Kumari, B. Rodoni, M. Loh, J. van Leur and A. Freeman. *International Center for Agriculture Research in the Dry Areas (ICARDA), P.O. Box 5466, Aleppo, Syria. Email: s.kumari@cgiar.org*

Nanovirus (*Faba bean necrotic yellows virus* and *Subterranean clover stunt virus*) DNA and Luteovirus (*Bean leafroll virus, Beet western yellows virus* and *Soybean dwarf virus*) RNA were successfully extracted from plant tissue prints on nitrocellulose membranes (NCM). Viral nucleic acids were recovered from both unprocessed and Tissue-blot immunoassay (TBIA)-processed plant tissue prints and found suitable for use in molecular diagnostic techniques (PCR, cloning and sequencing). Usable viral nucleic acid was recovered from both freshly blotted and two-year old plant tissue prints. It was also found that a single plant tissue print from a faba bean stem provided sufficient nucleic acid for use in molecular protocols to detect viral pathogens. The success of these tests showed that plant tissue prints on NCM are stable at room temperature for at least two years, for either Nano- or Luteoviruses. However, RNA was not recovered from some old processed blots of Luteoviruses. This technology potentially eliminates the need to store either fresh or dry plant tissue. Also, problems associated with the transportation of plant samples from remote growing areas to diagnostic laboratories for testing are alleviated, as blotted plant tissue prints on NCM can be sent through the mail. Finally, samples can easily be archived for comparison and used as a source of viral pathogen for future downstream applications, such as PCR, cloning and sequencing. Results showed that the use of NCM is a practical, economical and sensitive method for sampling, storing and retrieval of viral pathogens.

7.38 A MOLECULAR PROCEDURE FOR ANALYSING AND COMPARING COMMUNITIES OF MICROFUNGI IN WHEAT ROOTS. H. Kwaśna, G.L. Bateman and E. Ward. *Department of Forest Pathology, August Cieszkowski Agricultural University, ul. Wojska Polskiego 71c, 60-625 Poznań, Poland. Email: kwasna@au.poznan.pl*

A procedure involving transformation and sequence analysis of ITS1/2 rDNA was evaluated as a means of analysing and comparing the composition of microfungal communities in wheat roots. Roots from a fourth wheat crop with much take-all (*Gaeumannomyces graminis* var. *tritici*) contained communities that were more diverse than those in healthy roots from a first wheat crop. Microbial communities in the healthy root sample were dominated by the protist *Polymyxa graminis* and the oomycetes *Pythium* spp. Fungi that were frequent on the diseased roots, in addition to the take-all fungus, included *Microdochium* spp. and *Ophiosphaerella* spp. New and additional evidence for the ecological roles of these fungi obtained by the procedure is presented.

7.39 DEVELOPMENT OF DIAGNOSTIC MOLECULAR MARKERS FOR RAPID IDENTIFICATION OF *XANTHOMONAS ORYZAE* PV. *ORYZAE* AND *X. ORYZAE* PV. *ORYZICOLA* USING CONVENTIONAL AND MULTIPLEX PCR. J. Lang, J. Hamilton, C.R. Buell, G. Diaz, K. Hollar, M.A. Van Sluys, N. Tisserat and J. Leach. *Campus Delivery 1177 Colorado State University Fort Collins, CO, 80521, USA. Email: Jan.Leach@ColoState.edu*

Xanthomonas oryzae pv. *oryzae* and *X. oryzae* pv. *oryzicola* cause bacterial blight and leaf streak of rice, respectively. Although their symptomology is distinct, the two pathogens are difficult to differentiate from each other based on cultural or biochemical methods. Thus, there is a clear need for molecular-based tools to identify and track these pathogens. Genomic analysis tools available in the Comprehensive Phytopathogen Genome Resource (http://cpgr.plantbiology.msu.edu) were used to identify over 150 primer pairs based on open reading frames with potential to distinguish these pathovars. Each primer pair was screened against well-characterized isolates of each pathovar using conventional and multiplex polymerase chain reaction (PCR). After initial screening, robust primers were tested against a larger number of isolates including genetically and geographically diverse strains of both pathovars, several other *Xanthomonas* species and pathovars, and other bacterial plant pathogens including *Pseudomonas*, *Burkholderia* and *Erwinia* spp. A set of primers were established that distinguish *X. oryzae* species and each pathovar (*X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*). These primers were designed to create accurate and efficient multiplex PCR strategies for identification of these important phytopathogens.

7.40 A COMPREHENSIVE GENOME-BASED DIAGNOSTICS RESOURCE AND PIPELINE FOR IDENTIFICATION OF THREATENING PLANT PATHOGENS. J. Lang, J. Hamilton, J. Leach, N. Tisserat, A. Lévesque, T. Powers and C.R. Buell. *Campus Delivery 1177 Colorado State University Fort Collins, CO, 80523-1177, USA. Email: Jan.Leach@ColoState.edu*

Genomic data is a powerful resource for the development of diagnostic markers for plant pathogens, however, its use is limited by the disparate nature of the data and the lack of the requisite expertise of field/extension plant pathologists and diagnosticians to access, apply, and implement this data readily into existing diagnostic programs. We have constructed a comprehensive resource to house publicly available genome sequence and annotation data for plant pathogens including viroids, viruses, bacteria, fungi, oomycetes, and nematodes and provide search tools to facilitate the use of genomic data by the community. We currently store data and linkages to genome projects for >750 plant pathogens. We have developed a ribosomal DNA database of plant pathogens and close relatives to facilitate design of diagnostic markers. This database and resource is coupled to 1) an experimental component to develop genomic based diagnostic tools

for three proof-of-concept pathogens (*Xanthomonas, Pythium, and Meloidogyne*) and 2) an educational component involving training of extension plant pathologists and diagnosticians in genomics, bioinformatics, and molecular diagnostic methods. We will present progress on the Comprehensive Phytopathogen Genome Resource (http://cpgr.plantbiology.msu.edu).

7.41 THE USE OF RETROTRANSPOSONS TO STUDY THE VARIABILITY OF *PYRENOPHORA TRITICI-REPENTIS* POPULATIONS. **L. Leisova and L. Kucera.** *Department of molecular biology, Crop Research Institute, Drnovska 507, 161 06 Prague 6 – Ruzyne, Czech Republic. Email: leisova@vurv.cz*

Eukaryotic genomes are full of transposable elements of two kinds. The first are RNA-mediated retrotransposons using a copy-paste mechanism. The second are DNA transposons with the cut-and-paste mechanism. Both types of element were detected in *Pyrenophora tritici repentis* – a fungus alternating sexual and asexual stages in its life cycle. Its host plants are wheat and wild grass species. A high genetic dissimilarity exists within its population. In total, 132 isolates of *P. tritici-repentis* were studied, using SSAP and IRAP. These detect DNA sequence variability within the LTR and IR parts of transposable elements respectively. In SSAP analysis, 180 polymorphic bands were detected using four primer combinations. In IRAP analysis two primers were applied (TfoI and MITE) and 15 polymorphic bands were detected. UPGMA analyses clearly distinguished between pathogenic and non-pathogenic isolates of *P. tritici-repentis*. Detailed results will be presented and discussed. The study was supported by the Grant Agency of the Czech Republic, project no. 521/06/1544.

7.42 COMPARSION BETWEEN REAL-TIME PCR AND VISUAL GRADING OF WHEAT PATHOGENS. **C. Lerenius, C. Filipsson and A. Jonsson.** *Department of Soil Sciences, Div. of Precision Agriculture, SLU, P.O. Box 234, SE 53223 Skara, Sweden. Email: anders.jonsson@mv.slu.se*

Quantitative real-time PCR assays were developed for detection of *Septoria tritici*, *Drechslera tritici-repentis* (DTR) and *Stagonospora nodorum* on winter wheat. More than 300 wheat samples were collected from field experiments in southern and central Sweden during 2006 and 2007. The samples were drawn from trials for fungicide and cultivar testing and at different development stages of the wheat. The dominating plant pathogens were recognized by visual inspection at sampling, and level of infection was estimated visually and reported as % infected leaf area. After mixing and homogenizing dried wheat samples, DNA was extracted using one of two commercial kits for plant DNA extraction. Newly designed primers and probes and a previously published primer-probe set were used to specifically detect each pathogen using real-time PCR. The amount of plant pathogen DNA was determined using relative quantification and a wheat-specific gene was used as reference. The determinations were done at two different laboratories. Although visual checks often indicated the presence of only one fungus, real-time PCR results showed that all three pathogens were present in the majority of samples. A surprisingly good correlation (R^2=0.6 for *S. tritici* and R^2=0.56 for DTR) was observed between visual grading and real-time PCR determination for wheat samples taken during 2006, especially when one pathogen dominated on the leaf (R^2=0.75 and 0.67). In conclusion, the PCR methods developed enable an objective measurement of fungal infection levels in wheat.

7.43 A SURVEY OF CENTRAL WEST ASIA AND NORTH AFRICA FOR VIRUSES IN THE *LUTEOVIRIDAE* FAMILY. **M. Loh, S.G. Kumari, J. Van Leur, A. Freeman, R. Ford and B. Rodoni.** *CRC for National Plant Biosecurity, Department of Primary Industries, 621 Burwood Highway, Knoxfield, VIC 3180, Australia. Email: m.loh@crcplantbiosecurity.com.au*

Luteoviridae cause some of the most devastating diseases to legume crops with up to 95% crop losses in some instances. Yield losses have been reported in all cool-season legume crops, some of the most destructive cases occurring in Central West Asia and North Africa (CWANA). Over the past 20 years, serologically based surveys have been conducted in Australia and the CWANA region. 80,000 samples have been tested for 14 viruses within the *Luteoviridae* and *Nanoviridae*. The most damaging diseases associated with yellowing and stunting symptoms have been attributed to viruses belonging to the *Luteoviridae*, some yet to be fully characterised. Diagnostic techniques which fail to detect key viral plant pathogens, or generate ambiguous test results, have important consequences for virus resistance breeding programs. The aim of the current study is to compare molecular and serological methods to detect luteoviruses within historical plant tissue samples. For this, a molecular survey was conducted on freeze dried cool-season food legume samples collected worldwide over the past 20 years, previously tested virus positive by serological methods. These were tested for the viruses *Bean leafroll*, *Soybean dwarf*, *Beet western yellows*, *Beet mosaic*, *Potato leafroll*, *Chickpea chlorotic stunt*, and *Pea enation mosaic* and *Luteoviruses* using specific, diagnostic PCR tests. Preliminary results indicated that a) antisera and serological methods used for past surveys were not sufficiently sensitive or specific to detect luteoviruses in some samples and that b) PCR primers and molecular tests require further development for accurate and sensitive diagnosis and discrimination of legume-infecting *Luteoviridae*.

7.44 A MULTIPLEX PCR ASSAY DETECTING SEVERAL PATHOGENIC ASCOMYCETES. **M. Lummerzheim, L.G. Morello, J. Ferreol and N. Maillac.** *Laboratoire d'Agrophysiologie, Ecole d'Ingénieur de Purpan, 75 Voie du TOEC, 31076 Toulouse Cedex 3, France. Email: marie.lummerzheim@purpan.fr*

We have developed a multiplex PCR method allowing the simultaneous identification of *Phaeoamoniella chlamydospora, Botryosphaeria obtusa, Botryosphaeria dothidea and Eutypa lata*. These fungal pathogens have been identified, amongst others, to be causal agents of grapevine trunk diseases. Petri disease on young grapevines and esca on older vines are caused by *P. chlamydospora* in association with some other fungi. A number of *Botryosphaeria* species, amongst which *B. dothidea* and *B. obtusa*, have been isolated and found to be associated with dieback of grapevines worldwide, and have been identified as causal agents of black dead arm (BDA). *Eutypa lata* causes *Eutypa* dieback which affects vineyards all over the world. These xylem-inhabiting ascomycetes have in common a slow growth and induce extremely complex and variable symptoms on *Vitis vinifera*, making disease identification using traditional isolation methods problematic. We have tested this multiplex PCR assay on pure fungal DNA, crude mycelium, inoculated vine-stocks and naturally infected cordons and wood. It has proven to be quick and reliable for fungal detection and disease diagnosis in suspected vine-stocks in natural and greenhouse conditions.

7.45 SIMULTANEOUS DETECTION OF FIFTEEN POTATO VIRUS AND VIROID PATHOGENS USING A CDNA MACROARRAY. **T. Maoka, Y. Maruta and T. Hataya.** *National Agricultural Research Center for Hokkaido Region, 1 Hitsujigaoka, Toyohira-ku, Sapporo, Hokkaido, Japan. Email: maokat@affrc.go.jp*

Twelve viruses, *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Potato aucuba mosaic virus* (PAMV), *Potato leafroll virus* (PLRV), *Potato mop-top virus* (PMTV), *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus S* (PVS), *Potato virus X* (PVX), *Potato virus Y* (PVY), *Tomato ringspot virus* (ToRSV) and *Tomato spotted wilt virus* (TSWV) have been reported as the causal agents of potato diseases in Japan. For simultaneous detection of these viruses, a detection method using complementary DNA (cDNA) in a macroarray was established. The specific regions of these viral genomes were cloned into suitable plasmid vectors, respectively. From the insert cDNAs, 300-600 bp of capture probes were amplified by polymerase chain reaction (PCR). Each of the capture probes for viruses were immobilized on a rectangular (65mm × 25mm) nylon membrane. Starting with a total plant RNA extract, cDNA syntheses was carried out using a random nonamer primer. Using cDNA as template, subsequent synthesis by PCR was performed by using 12 sets of primers for each virus. Amplified cDNAs were labeled with biotin and used as a target in hybridization analyses. Hybridization signals are easily observable with the naked eye. The hybridizations correctly identified each of the potato viruses without cross-hybridization. In addition to these viruses, *Tobacco rattle virus* (TRV), *Potato virus T* (PVT), and *Potato spindle tuber viroid* (PSTVd) were added to the cDNA macroarray, although these pathogens do not occur on potatoes in Japan. This technique can be practically used for simultaneous detection of pathogens in breeding, quarantine and foundation seed production.

7.46 SCREENING FOR *BOTRYOSPHAERIA* SPP. IN ITALIAN NURSERIES AND VINEYARDS. **G. Marchi, F. Peduto, S. Essakhi, A. Spagnolo, L. Mugnai and G. Surico.** *Dipartimento di Biotecnologie agrarie sez. Patologia vegetale, Piazzale delle Cascine 28, 50144 Firenze, Italy. Email: guido.marchi@unifi.it*

Until 20 years ago, only two species of the fungal genus *Botryosphaeria* Ces. & De Not. were reported as being pathogenic on grapevine: *B. stevensii* causing black dead arm, and *B. rhodina* causing cane dieback and bunch rot. Today, data from all over the world indicate that the situation is much more complex than previously thought, since at least 11 species of *Botryosphaeria* are now reported as potentially pathogenic on grapevine. In order to better understand the epidemiology of these fungi in Italian vineyards, a seminested PCR assay that targets the internal transcribed spacer region (ITS1-5.8S-ITS2) of the nuclear ribosomal DNA (rDNA) of *Botryosphaeria* spp. was developed. This assay was applied to *Botryosphaeria* DNA extracted from the xylem sap and from wood samples collected from nursery propagation material (canes of mother plants, grafted cuttings, rooted cuttings) and from tissues underneath pruning wounds on the trunk or the cordon of standing vines. The results showed that *Botryosphaeria* spp. posses a remarkable ability to colonize grapevine wood tissues since fungal DNA was detected with very high frequency at the wound sites and in cuttings and rooted cuttings. This last finding suggested that propagation material may be a factor in spreading *Botryosphaeria* spp. over long distances. The nucleotide sequence data indicate that *B. dothidea* and *B. obtusa* are by far the commonest species in the samples analyzed. Molecular analyses were complemented by pathogenicity tests.

19.1 PCR METHOD FOR DIAGNOSTICS OF *VERTICILLIUM DAHLIAE* IN OILSEED RAPE. **P. Matusinsky, T. Spitzer and R. Mikolasova.** *Agrotest fyto, Havlickova 2787, Kromeriz 767 01, Czech Republic. Email: matusinsky@vukrom.cz*

Verticillium wilt is a disease responsible for yield losses in oilseed rape in seaside areas of northern Europe. Along with a rising proportion of oilseed rape in crop rotations, this disease is expected to gain more importance also in the Czech Republic. Besides oilseed rape, *V. dahliae* attacks many host species (sunflower, potato, cotton, olive, etc.). Existing methods used in diagnostics of *V. dahliae* in some other crops or mycological cultures were examined. They were tested on isolates of *V. dahliae* and *V. longisporum* cultured on agar media and on oilseed rape tissues. For us, some primers worked well only with pure mycelium, and were not suitable for use in oilseed rape tissues. The method published by Mercado-Blanco *et al*. (2003), originally used to detect defoliating and nondefoliating *V. dahliae* pathotypes in infected olive plants, does not react with oilseed rape DNA and can detect DNA of *V. dahliae*. After minor modification this method can be used to detect *V. dahliae* in oilseed rape. The method does not, however, distinguish between *V. longisporum* and *V. dahliae*.

19.2 DEVELOPMENT OF PLANT HEALTH CLINICS IN INDIA – STATUS, STRATEGIES AND CHALLENGES. **N. Mehta.** *Department of Plant Pathology, CCS Haryana Agricultural University, Hisar 125 004, India. Email: nareshmehta@hau.ernet.in*

The basic objective of plant clinics is to provide comprehensive diagnostic and advisory services encompassing all possible causes of ill health whether biotic or abiotic. The plant clinic will be useful in providing all the valuable qualitative and quantitative local information which will help the farmers to improve their decision making. By providing advice based on sound principles, plant clinics have an important role to play in reducing dependence on pesticides. The emphasis of plant clinics in broad terms should be on human resources and physical infrastructures. Clinics should have laboratories with diagnostic facilities for the whole range of plant diagnostic services integrated under one roof for the benefit of clients desiring simultaneously several diagnostic services. Plant pathologists working in plant clinics should be trained in the latest advances like information technology, or application of computer software for forecasting disease epidemics, and laboratories should be equipped with the audio, video and internet facilities for early dissemination of information to avoid losses. A clinic should be located within or near the campus of a University or Research Institute and easily approachable by the clients. There should be provision of electronic displays with scrolling text so that important messages regarding plant health care can be displayed. A mobile plant clinic with modest diagnostic facilities and trained professionals can do on-the-spot diagnosis. Clinics have to play a greater role by periodically organizing plant health camps, issuing handouts, anticipating disease outbreaks and providing solutions or options tuned to farmers' needs with the utmost clarity.

19.3 GENETIC VARIABILITY BETWEEN THE ISOLATES OF *COLLETOTRICHUM GOSSYPII* OF COTTON. **Y.R. Mehta, and A. Mehta.** *IAPAR, C.P. 481, CEP 86001-970, Londrina, PR, Brazil. Email: yrmehta@iapar.br*

Cotton is attacked by *Colletotrichum gossypii* (CG) and *C. gossypii* var. *cephalosporioides* (CGC). Both pathogens are seed-

transmitted and as they are morphologically similar, distinguishing them is extremely difficult. In the present study attempts were made to detect genetic variability among isolates of CG and CGC using three molecular techniques and 53 isolates collected from different seed and leaf samples and cultivars from the States of Paraná, São Paulo, Mato Grosso, Minas Gerais, and Paraiba, during 1999-2003. Based on pathogenicity tests, 21 isolates were classified as CG and 32 as CGC. The banding patterns obtained by RAPD analysis using eight primers, formed two major groups. The first group contained 94% of the isolates originating from naturally infected seeds and the second group contained 95% of the isolates originating from naturally infected leaf samples. Similar results were obtained by RIC/REP-PCR analysis, where the first group contained 93% of the isolates originating from seeds and the second group contained 78% of the isolates originating from infected leaves. When the amplified product of its region was digested with eight restriction enzymes, similar banding patterns were observed for all the isolates. Results of RAPD and ERIC/REP-PCR demonstrated the existence of genetic variability among the seed isolates and leaf isolates irrespective of their classification as CG or CGC, probably because of the presence of a pathogenicity factor in the leaf isolates but not in the seed isolates.

19.4 VARIABILITY IN *ALTERNARIA HELIANTHI* CAUSING BLIGHT OF SUNFLOWER. R.K. Mesta, V.I. Benagi, S. Kulkarni and Shankergoud. *Agricultural Research Station, Devihosur, 581 110, Haveri, Karnataka, India. Email: rkmesta@yahoo.com*

A. helianthi was isolated from infected sunflower leaves collected from 17 locations in India. Based on morphological characters, the isolates were categorized into eight groups, Ah1–Ah8 and were further studied for cultural and physiological variability. Ah5, Ah6, Ah7 and Ah8 were found superior to other isolates with respect to radial growth and dry mycelial weight. A temperature of 30º C and pH range of 7–8 was found optimum for the growth of *A. helianthi* isolates, but Ah5, Ah7 and Ah8 had wider adaptability. Variation among the isolates was assessed through production of symptoms by toxin inoculation, isozyme studies, and using polyacrylamide gel electophoresis (PAGE). Ah5, Ah8 and Ah7 were found equally virulent as they produced more necrotic lesions/ leaf. Ah3 and Ah6 were moderately virulent and Ah1, Ah2 and Ah4 were less virulent. Peroxidase banding patterns showed three bands in Ah5, Ah6, Ah7 and Ah8. The other isolates were short of one band. Cluster analysis gave 3 distinct groups consisting of Ah1 + Ah4, Ah2 + Ah3, and Ah5 + Ah6 + Ah7 + Ah8 which were closely related within the groups. The catalase banding pattern revealed that Ah5 produced three bands, whereas other isolates gave either 1 band or 2. Cluster analysis revealed that isolates Ah1 and Ah3; and Ah6, Ah7 and Ah8 were highly related.

19.5* QUANTITATIVE MEASUREMENTS OF CYP51 ALTERATIONS IN POPULATIONS OF *MYCOSPHAERELLA GRAMINICOLA* USING PYROSEQUENCING. J. Motteram, H.J. Cools, S. Gilbert and B.A. Fraaije. *Plant Pathology and Microbiology Department, Rothamsted Research, AL5 2JQ, Hertfordshire, UK. Email: juliet.motteram@bbsrc.ac.uk*

Septoria leaf blotch (SLB) caused by *Mycosphaerella graminicola* has been the most important foliar disease of winter cereals in the UK since the mid-1980s. Annual yield losses due to the disease have been estimated at around £30 million, in spite of expenditure of £ 80–90 million on fungicides. Field resistance, conferred by mutations in the target site, to methyl benzimidazole carbamates (MBCs) and quinone outside inhibitors (QoIs) has developed in this pathogen. Field performance data from HGCA-funded experiments over the past ten years have shown a gradual erosion of azole efficacy against SLB. The loss of azole efficacy has been linked with alterations in the target-encoding sterol-14α-demethylase gene (*CYP51*). CYP51 sequence analysis of 136 strains isolated from one field in Kent in 2006 revealed up to 12 different amino acid alterations in 10 different variants. Three substitutions, V136A, A379G and I381V, were found at high frequencies and located in regions predicted to impact on substrate/inhibitor recognition. Most other altered residues are likely to be compensatory, required to maintain enzyme activity when residues important for function are changed. Because Pyrosequencing can detect multiple alleles in close proximity, is sensitive and its design is less affected by sequence constraints in comparison with allele-specific real-time PCR, we chose Pyrosequencing to target the afore-mentioned alterations. DNA from leaf populations sampled after treatments showed differential selection by different azoles. Treatments of prochloraz resulted in increased V136A frequencies whereas tebuconazole clearly selected for I381V. Interestingly, none of the strains tested so far carry both substitutions.

19.6* SOIL DIAGNOSIS BY DETECTION OF *POTATO MOP-TOP VIRUS* USING BAIT PLANT BIOASSAY AND RT-PCR-MICROPLATE HYBRIDIZATION. T. Nakayama, T. Hataya, S. Tsuda, H. Fuwa, M. Shimizu, M. Mori and T. Maoka. *National Agriculture Research Center for Hokkaido Region, Toyohira, Sapporo, 062-8555, Japan. Email: takato@affrc.go.jp*

For the first time in 25 years, in November 2005 an incidence of spraing (brown rings or arcs) caused by *Potato mop-top virus* (PMTV) occurred in potatoes (cv. Sayaka) in a field in Tokachi district of Hokkaido, the northernmost island of Japan. In order to study the epidemic of PMTV, we developed a soil diagnosis method that consisted of a bait-plant bioassay for trapping the vector of PMTV, *Spongospora subterranea*, a causal agent of powdery scab of potatoes. This was followed by RT-PCR-microplate hybridization (MPH) for detecting PMTV from the roots of bait plants. The roots of bait tomato plants (cv. Momotaro) were immersed in a suspension of the soil sample and incubated at 18 ºC for 9 days, with 16 h light and 8 h dark, followed by extraction of total RNA from the roots and processing by RT-PCR-MPH using PMTV-specific primers and a digoxigenin (DIG)-labeled probe. Finally the level of infestation with PMTV was decided according to the colorimetric reaction and measurement of absorbance at 405 nm. With our method, PMTV was reliably detected from soil samples in fields where some of the harvested tubers bore the typical spraing symptom. Sampling of 220 fields located within about 7 km radius of the spraing-contaminated field showed that 133 fields (60.5%) had already been infested by PMTV although none had shown incidence of spraing in harvested tubers.

19.7 MULTIPLEX PCR FOR SIMULTANEOUS DETECTION OF MAJOR FUNGAL PATHOGENS ON MANGO FRUITS IN TAIWAN. H.F. Ni, H.R. Yang and R.S. Chen. *Department of Biochemical Science and Technology, National Chiayi University, Chiayi 600, ROC. Email: rschen@mail.ncyu.edu.tw*

Several fungal pathogens, including *Lasiodiplodia theobromae*, *Phomopsis* spp., and *Collectortrichum gloeosporioides*, can unpredictably affect the quantity and quality of mango fruits in Taiwan.

The detection and identification of these fungi become important for disease control, but are usually time-consuming by conventional isolation methods. In order to rapidly identity these fungi from mango, a multiplex PCR method was developed in this study. The internal transcribed spacer (ITS) regions of the rRNA of these fungi were amplified, cloned, and sequenced. Based on the sequences of ITS region, 3 pairs of specific PCR primers, LtF/LtR, PF/PR, and CgF/CgR, were designed for the identification and detection for *L. theobromae*, *Phomopsis* spp., and *C. gloeosporioides*, respectively. The primers were subsequently shown to amplify predicted-size fragments from the DNA of tested fungi. These fungi could be simultaneously detected by these pathogen-specific primers in a multiplex PCR reaction. The present method provides a rapid, simple, and reliable alternative to conventional methods for identifying fungal pathogens of mango.

19.8* IMPROVED QUANTIFICATION OF PATHOGEN DNA FROM SOIL USING PRESSURE CYCLING TECHNOLOGY. P. Okubara, K. Schroeder, C. Li, R. Schumacher and N. Lawrence. *USDA ARS Root Disease & Biological Control Research Unit, P.O. Box 646430, Pullman, 99164-6430, Washington, USA. Email: pokubara@wsu.edu*

Detection and quantification of *Rhizoctonia*, *Pythium* and other soilborne pathogens are inconsistent at low pathogen populations and in hard-to-extract samples, despite use of sensitive diagnostic assays such as real-time PCR. An efficient and reproducible extraction system in which soil samples are subjected to cycles of hydrostatic pressure (from ambient to as high as 35,000 psi) under controlled conditions has been used to improve the extraction of DNA from *Rhizoctonia* and *Pythium* in soil. This novel technology, called Pressure Cycling Technology (PCT SPS, Pressure BioSciences, Inc., West Bridgewater, Massachusetts, USA) improved the extraction of *Rhizoctonia* and *Pythium* DNA from agricultural soils up to 30-fold and 2-fold, respectively, compared to samples without the pressure cycling treatment. Using PCT SPS, we obtained detectable amounts of *Rhizoctonia* DNA from wheat roots that were previously recalcitrant to homogenization. In 80% of the root samples, pathogen DNA was extracted in amounts high enough to be quantified using real-time PCR. Furthermore, as PCT SPS is a closed system, samples were free from contamination that can occur during mortar-and-pestle extractions. PCT SPS confers a significant advantage in germplasm screening, food security assessment, and characterization of host and microbe nucleic acids.

19.9 SPECIFIC DETECTION OF *XANTHOMONAS ARBORICOLA* PV. *PRUNI* BY PCR USING PRIMERS BASED ON HRP GENES. S.Y. Park, Y.S. Lee, S.H. Lim and J.S. Jung. *Department of Biology, Sunchon National University, 540-745 Suncheon, Republic of Korea. Email: jjung@sunchon.ac.kr*

A PCR-based method has been developed for the rapid detection of *Xanthomonas arboricola* pv. *pruni*, the causal agent of bacterial shot hole on *Prunus* spp. Oligonucleotide primers targeting DNA regions related to the hrp gene cluster of *X. arboricola* pv. *pruni* were designed. Primer pairs Xap5 and Xap3 directed the amplification of 548-bp and 246-bp DNA fragments, respectively, from genomic DNA of all known *X. arboricola* pv. *pruni* strains tested, but not from that of other *X. campestris* pathovars or other bacterial species. To avoid false-negative results arising from the presence of amplification inhibitors in plant extracts, PCR products amplified with 16S rDNA primers and genomic DNA were included in agarose gel electrophoresis. The limit of detection of this primer set was 30 pg of total genomic DNA. PCR using the specific primers designed in this study, was useful for detection of *X. arboricola* pv. *pruni* from naturally infected *Prunus* samples.

19.10 SENSITIVE DETECTION OF *AGROBACTERIUM VITIS* IN GRAPEVINE PROPAGATION MATERIAL. F. Peduto, G. Marchi and G. Surico. *Dipartimento di Biotecnologie agrarie sez. Patologia vegetale, Piazzale delle Cascine 28, 50144 Firenze, Italy. Email: fpeduto@unifi.it*

In the last few years in Italy there has been an increase in the incidence of grapevine crown gall, caused by *Agrobacterium vitis*, in very young vines (1-2 years) planted in soils where the disease was previously absent. In order to determine the spread of *A. vitis* in grapevine propagation material, we implemented the sensitivity and specificity of a pre-existing PCR assay which targets the chromosomal gene *pehA*, a well-characterized pathogenicity determinant of this bacterial species, by designing a new internal primer and developing a two-round hemi-nested PCR protocol. This method was used to detect *A. vitis* DNA in symptomless samples collected from vines at 6 different stages of the propagation process (from vineyards of rootstock mother plants to vineyards of rooted graft cuttings) of a batch of 300,000 vines (cv. Merlot cl. 181 on SO4 rootstock). Although the bacterium was isolated on sucrose nutrient agar from propagation material only after the forcing stage, the DNA of *A. vitis* was detected at all stages, with a 73% average frequency of positive samples per stage. Sequence analysis of the products of the second round of PCR (169 bp), showed an identity that varied from 92 to 99% among the isolates so far collected. To gain more information about the population structure of *A. vitis*, the isolates are being characterized following a Multi-locus Sequence Typing (MLST) approach.

19.11 REAL-TIME PCR FOR DETECTION AND QUANTIFICATION OF *XANTHOMONAS AXONOPODIS* PV. *DIEFFENBACHIAE* ON ANTHURIUM. F. Péréfarres, E. Jouen, I. Robène-Soustrade, L. Gagnevin, A. Laurent and O. Pruvost. *UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical, CIRAD-Université de la Réunion, 97410 Saint-Pierre, Réunion, France. Email: soustrad@cirad.fr*

Effective control of *Xanthomonas axonopodis* pv. *dieffenbachiae*, the causal agent of anthurium bacterial blight, requires efficient screening of quarantine plants against this pathogen. A reproducible real-time PCR method that targets a portion of a gene from the LPS cluster, encoding a putative ABC transporter, was developed to detect *X. axonopodis* pv. *dieffenbachiae* in anthurium samples. The specificity of the assay was confirmed with a panel of strains belonging to *X. axonopodis* pv. *dieffenbachiae* and other pathovars and species. The assay was conducted with serial 10-fold dilutions of purified bacterial DNA and pure cultures (12 strains tested). It was possible to construct standard curves with a high correlation coefficient (r2 = 0.99) in the range of 15 ng to 1.5 pg for purified DNA and 10^7 to 10^2 CFU/ml for bacterial cultures. DNA extraction methods were compared for maximum DNA yield from anthurium plants. Homogenization of tissues with a Homex grinder (Bioreba) followed by purification using the DNeasy blood and tissue kit (Qiagen) was the most efficient method. The assay was successfully used to monitor the in vivo infection of anthurium experimentally inoculated with *X. axonopodis* pv. *dieffenbachiae*. This Real-Time PCR assay provides a rapid method for the early detection of the bacterium in symp-

tomless anthurium tissues. It should therefore be a very useful diagnostic tool for indexing propagation material in nurseries and for surveillance of international movement of *X. axonopodis* pv. *dieffenbachiae* on anthurium.

19.12 RAPID AND SENSITIVE DETECTION METHODS FOR FUNGI CAUSING WOOD DISEASES OF GRAPEVINE. S. Pollastro, A. Pichierri, W. Habib, N. Masiello and F. Faretra. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola, 165/A, Bari, Italy. Email: stefania.pollastro@agr.uniba.it*

Phaeomoniella chlamydospora (W. Gams, Crous, M.J. Wingf. *et* L. Mugnai) Crous *et* Gams, *Fomitiporia mediterranea* M. Fisch and *Phomopsis viticola* (Sacc.) Sacc. are fungal pathogens inducing severe wood diseases of grapevine that are poorly or not at all controlled by fungicides. These fungi can be transmitted by propagation materials and hence become established in new vineyards. Molecular techniques were exploited in order to improve the diagnosis of these fungal pathogens. Nested PCR-based methods were developed starting with RAPD (random amplified polymorphic DNA) markers identified as specific for each pathogen from which SCAR (sequence characterized amplified regions) primers were designed. The specificity of each SCAR primer pair was assessed in PCR experiments using numerous micro-organisms commonly associated with grapevine. The SCAR primers $OPA10_{721}B$ and $OPA10_{721}E$ proved suitable to detect *P. viticola* in shoots, canes and leaves. The primers $OPA13_{844}D$ and $OPA13_{844}F$ allowed detection of *P. chlamydospora* in samples of plant materials and bleeding, soil, and water. The primers $OPA2_{673}A$ and $OPA2_{673}B$ proved suitable for the detection of *F. mediterranea* in grapevine wood. Depending on each primers-pathogen combination the sensitivity of detection ranged from 0.1 to 1 ng of DNA template. All the molecular protocols were validated through comparison with the traditional methods of isolation and growth on agar media. The new protocols, more rapid and sensitive than the classical techniques, are currently being applied in large-scale screening on the health of grapevine propagation materials.

19.13 DEVELOPMENT OF A NEW METHOD TO DETECT LIVING *XANTHOMONAS CAMPESTRIS* IN CRUCIFEROUS SEED LOTS BY BIO-PCR. L. Porcher, R. Mathis, E. Fargier, B. Briand, J. Guillaumès, V. Grimault, L. Guyot, G. Darrieutort, N. Valette and C. Manceau. *GEVES-SNES, Rue Georges Morel B.P. 90024, 49071 Beaucouzé Cedex, France. Email: rene.mathis@geves.fr*

Xanthomonas campestris (Vauterin *et al*., 1995) is a bacterial species that groups seedborne bacteria pathogenic on cruciferous plants. A new BIO-PCR procedure has been proposed to improve the sensitivity and the specificity of *X. campestris* detection in seed lots. A new culture medium was developed, adapted to the BIO-PCR method (the YPACvc medium) and supporting the growth of all *X. campestris* strains. Seven pairs of primers described in the literature were tested for specificity using a set of 46 *X. campestris* strains, a few closely related xanthomonads and a set of saprophytic bacteria isolated from cruciferous plants. The ranges of detection of primer sets within *X. campestris* are different and they can identify pathovars when used in combination. A simplified DNA extraction was set up prior to a duplex PCR reaction using an *X. campestris* specific-primer pair, with universal primers used in the positive control. A subsequent duplex PCR reaction was designed to identify seed lots contaminated with *X. c.* pv. *campestris* only. This BIO-PCR protocol allows the analysis of cruciferous seeds lots in 3 days when current methods need 3 weeks. It has been validated on sixty seed lots (including seed lots previously treated with chemicals or disinfected) and is currently being tested in international validation procedures by panels of laboratories of the International Seed Health Initiative (ISHI) and the International Seed testing Association (ISTA).

19.14 MORPHO-PATHOLOGICAL AND GENETIC ANALYSIS USING RAPD MARKERS OF *SCLEROTINIA SCLEROTIORUM* COLLECTED FROM DIFFERENT HOSTS. L. Prasad and V. Choudhary. *Department of Plant Pathology, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Modipuram, Meerut 250 110, India. Email: lpchaudhary@rediffmail.com*

Diseased samples were collected from eight different plant families in NWPZ of India and experiments were carried out at SVBPUA&T, Meerut. Morphological studies indicated that fungus colony and sclerotium colour and shape of eight isolates from different host were varied on PDA. Pathogenic reactions were evaluated on 17 selected hosts using the fungal disc method of inoculation, and all isolates were found infectious. Cabbage and chickpea plants were found susceptible to all isolates. Interestingly, none of the 8 isolates were able to cause infection in garlic plants. Eight RAPD 10-mer primers were used to analyze the polymorphism among *S. sclerotiorum*. The specific marker bands obtained from random primers OPA-05, OPF-09 (200 Kb), OPF-17, OPF-19 and OPG-19 were found linked and thus, can be used as a marker band for *S. sclerotiorum* isolated from their specific host genotype. Statistical analysis was carried out using NTSYS-PC software; genetic similarity coefficients were found ranging from 0.52 to 0.85. Three clusters were formed and cluster I contained 6 isolates; within-group the maximum (0.85) similarity was shown by the isolates from *Mullugo* and *Solanum*. Clusters II and III contained only one isolate each, which came from *Corchorus* and *Ocimum*, respectively. The results showing the presence of *S. sclerotiorum* disease on weeds indicated an alarming situation to crop plants as the isolates were able to cause disease across hosts belonging to different families. Further research is required for confirmation.

19.15 MOLECULAR VARIABILITY AND DETECTION OF *FUSARIUM* SPECIES BY PCR-BASED RAPD, ISSR AND ITS-RFLP ANALYSIS. R.D. Prasad and T.R. Sharma. *Directorate of Oilseeds Research, Rajendranagar, Hyderabad 500030, India. Email: ravulapalliprasad@gmail.com*

Molecular variability among 45 isolates belonging to 8 sections of the genus *Fusarium* was studied by using PCR-based RAPD, ISSR and ITS-RFLP analysis. RAPD analysis using 12 random primers amplified 1145 loci of sizes ranging from 0.4 kb to 5.5 kb, and cumulative analysis of similarity values placed different *Fusarium* species in 8 clusters. The clear grouping at species level and more precise clustering at *formae speciales* level resulted with DNA markers generated by RAPD primers. The microsatellite primer gave a reproducible banding pattern (0.6 to 3.5kb), and generated 138 ISSR markers, grouping the *Fusarium* isolates in 4 clusters. Analysis based on 334 ITS markers generated by the two primer pairs and restriction enzyme combinations separated the 45 *Fusarium* isolates, into 3 main groups containing various species of *Fusarium*. The ITS-RFLP analysis could not distinguish inter- and intra-specific variation among *Fusarium*

species. DNA banding patterns specific to the species and *formae speciales* of *F. oxysporum* and other *Fusarium* species obtained in the present study will be highly useful for the differentiating *Fusarium* isolates at molecular level.

19.16 GENETIC VARIABILITY AMONG STRAINS OF *XANTHOMONAS CAMPESTRIS* AND DEVELOPMENT OF MULTIPLEX MOLECULAR DIAGNOSTIC METHODS. **N. Punina.** *Center 'Bioengineering' RAS, Moscow, 117312, Russia. Email: hinenkelte@yandex.ru*

The genus *Xanthomonas* includes bacteria responsible for important agricultural diseases. These bacteria are disseminated through seed and planting stock worldwide, and identification of them is a critical problem in many countries. Most of conventional diagnostic methods identify the strains to species level, and application is limited to known phytopathogenic strains. Xanthomonads may belong to either natural populations with high genetic diversity or to epidemic clonal groups with narrow internal genetic variation. It is suggested that markers for different taxonomic levels must be included at PCR assay. Genes *gyrB, cytP450, Xcc0006,* and *Xcc0007* (annotated at genome of *X. campestris* ATCC 33913) were sequenced in a collection of *Xanthomonas* strains representing *X. campestris, X. vesicatoria,* and some other species. Sequence comparisons revealed high intra- and inter-specific variability. Cluster analysis of the nucleotide sequences revealed 3 major groups of *X. campestris* separated by bootstrap values over 95%, and 8 subgroups – at bootstrap values above 65%. The taxonomic position of the strains was compared with their reaction to *X. campestris*-specific diagnostic PCR primers reported elsewhere. Conclusions on their specificity were made and sets of primers for multiplex PCR analysis were suggested.

19.17* NANOBIOTECHNOLOGY FOR *PHYTOPHTHORA* DIAGNOSIS. **M. Riedel, S. Julich, R. Möller, J. Felbel, S. Wagner, A. Breitenstein and S. Werres.** *Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection in Horticulture, Messeweg 11/12, D - 38104 Braunschweig, Germany. Email: m.riedel@BBA.de*

Phytophthora species are important plant pathogens worldwide. Many of them like *P. cinnamomi* or the quarantine organismn *P. ramorum* attack a wide range of host plants. Others are highly specialised on single hosts like *P. alni* which only attacks alder or the quarantine organism *P. fragariae* which prefers strawberries (var. *fragariae*) and raspberries (var. *rubi*). To prevent the spread of these pathogens with latently infected plants, easy to handle diagnostic systems with high sensitivity are of great importance. Furthermore these methods should give results within a short time. That is of special interest for those *Phytophthora* species which are listed as quarantine organisms. In a three year project a chip-formatted PCR in combination with an electrical DNA Chip will be adapted to the detection of *Phytophthora* spp. in plant tissue. The main aim is to develop and optimize a microchip used in a miniaturized thermocycler to perform PCR and amplify selected DNA segments from different *Phytophthora* species. This new nanobiotechnology enables a high sample throughput, significantly shortened cycling times and the reduction of sample material and expensive analytic chemicals.

19.18 DEVELOPMENT OF A *PHYTOPHTHORA* LUCID KEY. **J.B. Ristaino, M.J. Haege and C.H. Hu.** *Box 7616 Department of Plant Pathology, NC State University, Raleigh, NC, 27695, USA. Email: jean_ristaino@ncsu.edu*

Species in the genus *Phytophthora* are responsible for disease and destruction of a wide variety of plants. There are a number of species in the genus and it is difficult to distinguish between them based only on morphological features and the hosts that they infect. The purpose of this project was to develop a Lucid key that can provide the means to identify major *Phytophthora* species based on morphological and molecular characters. The Lucid key was created through the use of many references and sources and for each species includes information such as cultural characteristics, optimal growing temperatures, asexual and sexual reproductive structures, host species, disease symptoms and control methods. Pictures of each species in culture and its asexual and sexual reproductive structures are incorporated in the key. DNA sequence of the internal transcribed spacer regions and a portion of the *cox* 1 gene (Bar Code of Life region) were also used for identification in the matrix-based the key. The identification of *Phytophthora* species is a difficult task, and the Lucid key was created to help provide individuals with easily accessible tools to distinguish species based on a number of important morphological and molecular characteristics. The key will be available in cd format from APS Press.

19.19* *PHYTOPHTHORA INFESTANS* IDENTIFIED IN ARCHIVAL POTATO TUBERS FROM TRIALS AT ROTHAMSTED, 1876-1879. **J.B. Ristaino, C.H. Hu and B.D.L. Fitt.** *Box 7616 Dept. Plant Pathol., NC State Univ., Raleigh, NC 27695 UK. Email: Jean_Ristaino@ncsu.edu*

In previous work we examined the global migration and evolutionary history of *Phytophthora infestans* in modern potato crops, identified an Andean origin for the pathogen and studied the strain that caused the 19th century potato famine from archival materials. Our current objectives are to evaluate temporal changes in the genetic structure of populations of *P. infestans* in blighted tubers sampled from 1876-1879 from soil fertility trials done at Rothamsted. Ten replicated soil fertility treatments were applied to the potatoes, including combinations of manures and synthetic fertilizers. It is "assumed" that the pathogen populations were clonal and that a single genotype and no recombination occurred during this period. A small region of ribosomal DNA was amplified and sequenced from 19 diseased tuber samples and *Phytophthora infestans* was confirmed in all 19 samples. A TaqMan®-based real-time PCR assay was developed and the pathogen was detected in 17 of the 19 samples and an additional 9 samples that were not tested previously. DNA was detected at levels of 100 fg. The type I haplotype has been identified from mitochondrial DNA sequences obtained from all 19 samples. Three samples from 1877 were identified as Ia haplotype. Further sequencing of other mitochondrial regions is being done to identify the haplotype. Quantitative PCR was used to measure amounts of *P. infestans* DNA in the blighted tubers in relation to disease severity. Potatoes grown in the manured plots could be considered samples from one of the longest running "organic" soil amendment trials and were done before development of synthetic fungicides and resistant potato cultivars.

19.20 DETECTION AND IDENTIFICATION OF TREE FRUIT PHYTOPLASMA IN A SINGLE MULTIPLEX REAL-TIME PCR ASSAY. **M. Rott and M. Belton.** *Centre for Plant Health, Canadian Food Inspection Agency, 8801 East Saanich Road,Sidney, British Columbia, Canada V8L 1H3. Email: rottm@inspection.gc.ca*

A set of PCR primers were developed for the amplification of the four common phytoplasma infecting tree fruit; *European stone fruit yellows* (ESFY), *apple proliferation* (AP), *western X disease* (WX) and *pear decline* (PD). In order to identify the phytoplasma in a real-time PCR assay, unique TaqMan probes were designed that were specific to each phytoplasma and labelled with FAM, Hex, Cy5 or Texas Red, respectively. By combining the primer set with either the two stone fruit specific phytoplasma (ESFY and WX) or the two pome fruit specific phytoplasma (AP and PD), all phytoplasma could be readily detection in single or mixed infected samples. Interestingly, all four TaqMan probes could be combined in a single reaction, and using a five channel real-time PCR machine, each phytoplasma could be detected and identified in individual and mixed infections that included all four phytoplasma. While stone fruits are not reported to be infected with AP or PD, and likewise, pome fruits are not reported to be infected with ESFY or WX, the ability to test for all four phytoplasma in one simple assay will make it much easier to test a wide range of tree fruits for phytoplasma infection.

19.21 DETECTION AND QUANTIFICATION BY MOLECULAR METHODS OF *PHOMA TRACHEIPHILA* IN PLANT TISSUE AND SOIL. **M. Russo, F.M. Grasso, G. Licciardello and V. Catara.** *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. Email: vcatara@unict.it*

A real-time PCR assay was evaluated to estimate the presence and quantity of *Phoma tracheiphila* in potentially contaminated sources (plant and soil) and/or to monitor the colonization *in planta*. The assay using TaqMan chemistry (FAM/3' BHQ-1 labeled probe) showed linearity over a 7-log range, with a detection limit of 0·01 pg DNA. *P. tracheiphila* was detected both in woody tissues and in leaves of symptomatic and symptomless plants. High resolution and sensitivity were observed during the early stages of colonization, and the inferred fungal DNA concentration was proportional to the extent of wood colonization. The assay was used to study the relationship between disease severity and pathogen DNA content in artificially inoculated leaves of different citrus species and cultivars. The DNA content in two trials with 4- and 12-month-old seedlings was positively correlated with the disease index. Real-time PCR was also applied to in-vitro produced *P. tracheiphila* philaconidia and sterile and non-sterile soil substrate spiked with known propagule numbers. Under our conditions, as little as 100 *P. tracheiphila* phialoconidia were detected per gram of spiked soil samples, while no amplicon was detected from non-seeded soil samples. The inferred DNA concentration was proportional to the amount of inoculum in spiked samples. The estimated number of phialoconidia was about one log less than those effectively added. DNA extraction methods and the real-time PCR assay seem valuable tools to monitor possible sources of inoculum and to evaluate cultivar susceptibility.

19.22 MOLECULAR IDENTIFICATION AND GENETIC DIVERSITY OF IRANIAN POPULATIONS OF *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI*. **L. Sadeghi, A. Alizadeh and N. Safaei.** *Department of Plant Pathology, University of Tarbiat Modares, Tehran, Iran. Email: azizollahalizadeh@yahoo.com*

We aimed to identify and study the genetic diversity of the agent causing take-all symptoms on wheat, using morphological, biochemical and molecular methods. In recent years, take-all has led to heavy crop losses in Iran. During a survey in 2005-06, 53 pathogen isolates were recovered from diseased samples in different provinces of Iran including, Fars, Golestan, Markazi, Mazandaran and Tehran. Tests indicated that all isolates were pathogenic on wheat; eight isolates were also pathogenic on oats producing numerous perithecia on infected host roots. Using *G. graminis* var. *tritici* (Ggt) specific primers, all our isolates were identified as Ggt. In addition, these primers showed that except for two isolates of type B, all isolates were of type A. On the basis of morphology, biochemistry and geographical distribution, 26 representative isolates were selected for genetic diversity studies. Of the 12 PCR primers tested, five were used for RAPD-PCR analysis on the basis of their reproducibility, polymorphism and resolution. Cluster analysis showed that at 55% similarity, the isolates could be divided into 18 clades including 12 single-member groups, five with two-member groups and one group with three isolates. Additionally, the two type B isolates were distinguished from type A using these five primers. ERIC-PCR analysis separated the isolates into five clades at 55% similarity in which the first and second clades contained 20% and 68% of isolates. The remaining three clades each had a single member. Pooled analysis of RAPD and ERIC-PCR results divided the isolates into 19 groups at 60% similarity. Both RAPD and ERIC-PCR separated the two B type isolates from the A type isolates. The Ggt isolates pathogenic on oat were not distinguished as a group. A single isolate with both simple and lobed hyphopodia was confirmed to be Ggt rather than Ggg as originally believed. The Iranian Ggt population thus shows a high genetic diversity which may indicate an ability to overcome control measures such as chemicals or resistant cultivars. This is the first report on genetic diversity of Ggt in Iran.

19.23 DIAGNOSIS OF *GRAPEVINE LEAFROLL-ASSOCIATED VIRUS 3* BY RECOMBINANT ANTIBODIES. **P. Saldarelli, L. Cogotzi, A. Giampetruzzi, V. Elicio, G. Nölke, M. Orecchia, G. Martelli, R. Fischer, S. Schillberg.** *CNR–Institute of Plant Virology, and Department of Plant Protection and Applied Microbiology, University of Bari, Via Amendola 165/A, 70126 Bari, Italy. Email: p.saldarelli@ba.ivv.cnr.it*

Availability of purified antigens and problems related to the management of animal-rearing facilities are major drawbacks in the production of serological reagents for plant virus diagnosis. In this regard, *Grapevine leafroll-associated virus 3* (GLRaV-3), a major pathogen of grapevine and a certification pathogen for nursery productions, presents additional challenges because it is phloem-limited and has a low titre in infected host tissues. Thus, a number of different home-made and commercial serological reagents have been produced whose standardization was hampered by the above problems. We have developed a fully recombinant DAS-ELISA kit based on the use of an *Escherichia coli*-expressed single chain Fragment variable (scFv) protein, which was selected by phage display and tested for its binding to GLRaV-3 particles. The *E. coli* expression of the scFv reagent was optimized and our kit was compared with a commercial one based on traditional rabbit antibodies. Both kits behaved the same in terms of sensitivity, ability to detect a large number of GLRaV-3 isolates and shelf life. However, the bacterial production of scFv was cost affordable, making its transfer to industrial production feasible. Our scFv disclosed a cross reactivity with GLRaV-1, another

member of the genus *Ampelovirus* but not with the distantly related closterovirus *Grapevine leafroll-associated virus 2*. This system opens new avenues to the standardization of serological diagnosis for GLRaV–3, thus allowing its extensive adoption as a common tool which is perfectly reproducible and does not require the availability of purified antigens.

19.24 CYCLEAVAGE ISOTHERMAL CHIMERIC AMPLIFICATION OF NUCLEIC ACIDS (CYCLEAVAGE-ICAN) FOR THE SENSITIVE, RAPID AND SIMPLE DIAGNOSIS OF VIROIDS. **T. Sano, S. Isono, T. Tsubame, Y. Tsushima, N. Urasaki, S. Kawano, R. Uemori, T. Ooura, O. Takeda and H. Mukai.** *Plant Pathology Laboratory, Faculty of Agriculture and Life Sciences, Hirosaki University, Bunkyo-cho 3, Hirosaki, Aomori 036-8561, Japan. Email: sano@cc.hirosaki-u.ac.jp*

Isothermal and chimeric primer-initiated amplification of nucleic acids (ICAN) (Shimada *et al*., 2002) utilises BcaBEST DNA polymerase, Tli RNaseH, and two DNA-RNA chimeric primers. The several detection systems, a combination of ICAN and luminescence detection by probe hybridization, have been reported for *Mycobacterium tuberculosis, Chlamydia trachomatis, Neisseria gonorrhoeae* (Shimada *et al*., 2003), *Salmonella* species (Isogai *et al*., 2005), *N. gonorrhoeae* (Horii *et al*., 2006) and *Candidatus* Liberibacter asiaticus, the Citrus huanglongbing pathogen (Urasaki *et al*., 2007). We applied the method for detection of *Chrysanthemum stunt viroid*, *Citrus exocortis viroid*, *Apple scar skin viroid* and *Apple fruit crinkle viroid* and obtained positive reactions, a red fluorescence. The reaction was completed in just over 1 hour (reverse transcription for 5 minutes followed by ICAN reaction for 60 min). The sensitivity was almost the same as or superior to the conventional RT-PCR and Northern hybridization methods. Since neither electrophoresis nor PCR apparatus is necessary, the method is easily applicable for practical diagnostic purposes.

19.25 NANOPARTICLE-BASED BIO-BARCODE TECHNOLOGY FOR SENSITIVE (PLANT) PATHOGEN DETECTION. **C.D. Schoen, O. Mendes, M. Dieho, M. Vasic, R. van Doorn, A. van Amerongen, H. Zuilhof and P.J.M. Bonants.** *Plant Research International B.V., Department of Biointeractions and Plant Health, Droevendaalsesteeg 1, 6708 P.B. Wageningen, The Netherlands. Email: cor.schoen@wur.nl*

High-throughput multiplexed DNA detection is important for detection of single nucleotide polymorphisms and pathogens. DNA microarrays utilize fluorescence readout and have become a core technology for the parallel interrogation of a large number of nucleic acid sequences. However, microarray technology coupled with molecular fluorophore probes has limitations; these include the necessity for target amplification, post-amplification fluorophore labeling, slow binding kinetics between target sequence and capture strand, the need for multiple-laser excitation sources and complex, expensive instrumentation. The quest for improved and uniform labelling and detection methodologies has led to development of alternative approaches such as nanoparticle labels. Nanoparticles have been used in biotechnology over the last 4 decades as immunocytochemical probes as well as biological tags. During the last decade, however, there has been an increasing interest in using nanoparticles in DNA detection. Recent advances in functionalizing particles with oligonucleotides and tailoring their surface properties have paved the way for developing a series of new and practical bio-detection systems. Combining new capabilities for controlling particle size and composition with versatile methods of surface modification allows for the design of optically and chemically encoded nanoparticle probes. These probes, in biomolecule detection assays, have numerous advantages over conventional assays. In addition to the optical and recognition properties imparted by surface molecules, metal nanoparticles also conduct electricity and are quite useful as catalysts due to their high surface to volume ratio. For nucleic acid targets, all of these properties have allowed for the design of numerous assays. Examples are reviewed and compared with the most relevant conventional techniques.

19.26 A NASBA METHOD FOR EVALUATING VIABILITY OF *XANTHOMONAS AXONOPODIS* PV. *CITRI* IN FRESH FRUITS. **G. Scuderi, M. Golmohammadi, J. Cubero, M.M. López, G. Cirvilleri and P. Llop.** *Department of Phytosanitary Sciences and Technologies – Section Plant Pathology, University of Catania, 95123 Catania, Italy. Email: gscuderi@unict.it*

Xanthomonas axonopodis pv. *citri* (Xac) causes citrus bacterial canker (CBC), a serious quarantine disease of most citrus species in many areas worldwide. For lack of effective bactericides, citrus canker control mainly relies on eradication of diseased trees. To minimize the risk of introducing CBC in producing areas where the pathogen is not present, it is important to implement earlier diagnosis of CBC and identification of the bacterium, as well as to find new treatments and compounds to eliminate possible inoculum. For these reasons, efficient methods for assessing bacterial viability are necessary. Some genes involved in survival and pathogenicity of Xac have already been described, and different pairs of primers have been evaluated by NASBA using a rapid and simple hybridisation and detection system to assess Xac viability. After heat treatments of Xac strain 306, bacterial RNA was extracted, assayed with the primers designed and the results analysed, looking for genes that were suitable as markers for viability. The genes with best results were analysed by real-time RT-PCR, and both methods compared. The practical application of these methods to determine the treatments and reagents for controlling citrus canker will open new ways to evaluate measures recommended for the treatment of fresh fruit, to avoid the risk of spread of the bacterium.

19.27 CHARACTERISATION OF *FUSARIUM OXYSPORUM* ISOLATES PATHOGENIC ON LAMB'S LETTUCE (*VALERIANELLA OLITORIA*). **V. Sendhilvel, G. Gilardi, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: veltnau@rediffmail.com*

A new wilt of lamb's lettuce (*Valerianella olitoria*) was detected in northern Italy in 2003 and the causal agent of the disease was identified as *Fusarium oxysporum*. Pathogenicity tests and vegetative compatibility analysis were carried out on different isolates of the fungus. The pathogenicity of six isolates was tested on radish (*Raphanus sativus*), wild (*Diplotaxis tenuifolia*) and cultivated (*Eruca sativa*) rocket, cabbage (*Brassica oleracea* var. *sabauda*), cauliflower (*Brassica oleracea* var. *botrytis*), Brussels sprout (*Brassica oleracea* var. *gemmifera*), broccoli (*Brassica oleracea* var. *italica*), and turnip (*Brassica rapa* var. *rapa*). The results indicated that isolates of *F. oxysporum* from lamb's lettuce belong to the forma specialis *conglutinans*. Vegetative compatibility grouping analysis showed the presence of VCG 0101 pathogenic on lamb's lettuce. Among the 12 isolates from lamb's lettuce tested, 10 belongs to VCG 0101 and are classified as *F. oxysporum* f. sp. *conglutinans*, while two are self-incompatible.

19.28 SUBCELLUAR DISTRIBUTION OF COAT PROTEIN AND βC1 PROTEIN ASSOCIATED WITH *TOMATO LEAF CURL JAVA BEGOMOVIRUS*. **P. Sharma and M. Ikegami.** *Department of Life Science, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amemiyamachi, Aoba-ku, Sendai, Miyagi 981-8555, Japan. Email: neprads@gmail.com*

Tomato leaf curl disease is among the most limiting factors that affect tomato production in South East Asia. This disease is caused by a complex consisting of the monopartite begomovirus *Tomato leaf curl Java virus* (ToLCJAV) and a satellite DNAβ component. We have previously shown that the βC1 gene is required for symptom induction, is a determinant of pathogenicity and acts as a suppressor of PTGS. The CP of monopartite geminiviruses is absolutely essential for virus movement. Analysis of the CP amino acid sequence revealed several potential nuclear location signals particularly within the first 60 N-terminal amino acids of the CP. The presence of an NLS in this region was confirmed by nuclear import of GFPCP in yeast one-hybrid system. Deletion of 190 amino terminal residues completely abolished nuclear import. We have further shown that first 30 amino acids include an NLS typical of bipartite geminiviruses, which are localized to nuclei. These results indicate that the ToLCJAV NLS resides in the N terminus of the protein. When the N terminal sequence was removed (GFP CPΔ15-20) there was evidence to suggest that seven deleted amino acids play an important role in the nuclear targeting of ToLCJAV CP. A construct carrying a specific deletion of the 60 amino-terminal residues resulted in exclusively cytoplasmic localization. In contrast, the GFP βC1 fusion protein was localized to the cell cytoplasm.

19.29 IMMUNODETECTION OF HYDROXYPROLINE-RICH GLYCOPROTEINS IN PEARL MILLET COLEOPTILE CELL WALLS IN HOSTS RESISTANT TO *SCLEROSPORA GRAMINICOLA* INFECTION. **H.S. Shetty and S. Deepak.** *Downy Mildew Research Laboratory, DOS in Applied Botany and Biotechnology, University of Mysore, Manasagangotri, Mysore 570006, India. Email: hss_uom@hotmail.com*

Hydroxyproline-rich glycoproteins (HRGPs) which cross-link and are deposited in coleoptile cell walls of pearl millet (*Pennisetum glaucum* (L.) R. Br) can form resistance barriers against the phytopathogenic oomycete *Sclerospora graminicola* (Sacc.) Schroet. HRGPs accumulated in pearl millet coleoptiles in response to *S. graminicola* inoculation were purified and characterized. Immunocytochemical localization by tissue printing and confocal immunofluorescence microscopy indicated that cell walls of parenchymatic cells and the vascular tissue of the coleoptile are the sites of HRGP deposition. *In vitro* studies in the presence of horseradish peroxidase and hydrogen peroxide (H_2O_2) showed cross-linking of pearl millet HRGPs. The results suggest that cell wall strengthening in pearl millet during *S. graminicola* infection is brought about by P/HRGP cross-linking through isodityrosine. $H_2O_2$was found to accumulate in two bursts at 1 and 6 h.p.i., whereas in the susceptible cultivar only an early single peak was detected. Using purified P/HRGPs from pearl millet cell walls, polyclonal antibodies (Pab-P/HRGPs) were raised in rabbits. Pab-P/HRGPs were used in ELIZA to screen various pearl millet genotypes for fast, sensitive and specific detection of P/HRGPs induced upon infection. Maximum hydroxyproline-rich glycoprotein accumulation was observed in seedlings raised from susceptible seeds treated with chitosan and *Pseudomonas fluorescens*. The levels of HRGPs in the cell walls can be enhanced by treatment with various biotic and abiotic elicitors; this opens up possibilities for management of this disease by modification of cell wall HRGPs.

19.30 DEVELOPMENT OF MULTIPLEX PCR TESTS FOR THE DETECTION OF GRAPEVINE FUNGAL PATHOGENS. **J.B. Shiller, M.N. Pearson and A.B. Graham.** *Corbans Viticulture, 8 Bristol Rd, Whenuapai, Auckland, New Zealand. Email: anna@corbansviticulture.co.nz*

The grapevine fungal pathogens *Phaeomoniella chlamydospora, Eutypa lata, Phomopsis viticola* and *Cylindrocarpon* spp. cause young vine decline, dieback, and cane and leaf spot. This paper outlines the development of a multiplex diagnostic PCR for the detection of these pathogens from pure and mixed cultures and from infected grapevine wood. The ITS regions of multiple isolates of each species were amplified and sequenced and primer sets were designed for *E. lata* and *P. chlamydospora*, producing amplicons of 384 bp and 463 bp respectively. A single primer pair was developed for three *Cylindrocarpon* species known to be pathogenic to grapevine, which produces an amplicon of 403 bp. *P. viticola* was detected using previously published primers OPA1791 which produce an amplicon of 194 bp. The specificity of each set of primers was tested against DNA extracts from each of the target organisms and other fungi commonly isolated from grapevine wood. Following this, the primer pairs were tested in concert in triplex PCR reactions and the PCR conditions and reaction mixtures were optimized to eliminate non-specific bands. The sensitivity of each test was established. Our multiplex PCR test should be useful for screening young vines at the nursery stage and understanding the epidemiology of these diseases in established vineyards.

19.31 GENETIC DIVERSITY OF PATHOGENIC AND NON-PATHOGENIC POPULATIONS OF *PHYTOPHTHORA CAPSICI* ISOLATED FROM PEPPER PLANTS AND SOIL. **W.X. Sun, Y.J. Jia and X.G. Zhang.** *Department of Plant Pathology, Shandong Agricultural University, Taian, 271018, P. R. China. Email: zhxg@sdau.edu.cn*

Thirty-six *Phytophthora capsici* strains and one *P. parasitica* strain were evaluated for pathogenicity on pepper (*Capsicum annuum* L.) plants. The strains came from a range of geographic locations and were collected primarily from stems or roots of pepper plants with symptoms of blight, and from soil. Among the *P. capsici* strains, 13 were non-pathogenic (NP) and 23 were pathogenic to pepper. Genetic diversity was assessed by sequence analysis of the rDNA internal transcribed spacers (ITS1 and ITS2) and the 5.8S rDNA gene, and by RAPD analysis. The strains grouped into two ITS clusters. All pathogenic strains clustered in one group, consisting of four subgroups. The NP strains resolved into two groups, with 84.6% in ITS group II, and 15.4% in ITS group I. Pathogenic and nonpathogenic strains also separated into different clusters based on RAPD data, although two of the NP strains grouped with the pathogenic strains. The population of pathogenic strains was less diverse than that of the NP strains. No relationship was found between the genetic pathogenicity profiles and geographic origin.

19.32 POPULATION VARIATION AND SPREAD OF *MYCOSPHAERELLA CRYPTICA* IN AUSTRALIA. **K.M. Taylor.** *CRC for Forestry and School of Biological Science and Biotechnology, Murdoch University, South St, Murdoch, Perth, WA 6150, Australia. Email: katherine.taylor@murdoch.edu.au*

Mycosphaerella cryptica is an important leaf pathogen of *Eucalyptus* species. It is distributed in Australia and New Zealand and

appears to be native to Australia. The two most common plantation eucalypt species in Australia, *E. globulus* and *E. nitens*, can both be severely affected by the disease, which can cause severe blighting and defoliation of juvenile leaves, particularly in the eastern States of Australia. Although most research into *M. cryptica* has focused on these host species, it can also infect over 50 other eucalypt hosts, and has been noted with increasing frequency and severity on native species in Western Australia. Several microsatellite markers were developed for *M. cryptica* to determine the spread of the pathogen throughout Australia. Isolates of the pathogen were collected from numerous areas and hosts in Australia, including Western Australia, Tasmania, South Australia, Victoria and Queensland. The pathogen population appears to be highly variable between locations and host species, with some spread potentially due to human transport of host material.

19.33 REAL-TIME PCR DETECTION OF SORGHUM ERGOT *CLAVICEPS* PATHOGENS *C. AFRICANA*, *C. SORGHI*, AND *C. SORGHICOLA*. **P.W. Tooley, M.M. Carras and A. Sechler.** *USDA-ARS Foreign Disease-Weed Science Research Unit, 1301 Ditto Ave., Ft. Detrick, MD, 21702, USA. Email: paul.tooley@ars.usda.gov*

Sorghum ergot is a serious disease that has caused major losses in sorghum-growing regions worldwide. *Claviceps africana* is now the most widely distributed species causing ergot in many countries including the U.S., whereas both *C. africana* and *Claviceps sorghi* exist in India. A third species (*Claviceps sorghicola*) has been described causing sorghum ergot in Japan. As the three species are morphologically very similar, a DNA-based assay is desirable for rapid identification in cases where ergot-infected sorghum is found entering the U.S. and other countries. We designed primers and probes specific for the above three *Claviceps* species and tested them using purified DNA and ergot samples from the greenhouse and field. Real-time PCR was performed using an ABI Prism 7700 Sequence Detection System in a total volume of 25 microliters. Species-specific primers and probes were developed from the intron 3 region of the β-tubulin gene (*C. africana*, *C. sorghi*) or the EF-1α gene intron 4 (*C. sorghicola*). No amplification was obtained in any of the three ergot species-specific assays when DNA from six other *Claviceps* species was used as template. The lower limit of detection was 1 pg genomic template DNA in all three assays. A future goal is the development of a multiplex assay using the primers and probes described here. The assays we describe will provide useful tools for detecting sorghum ergot in seed and grain shipments and for determining which species are present in the samples, thereby aiding the regulatory decision-making process.

19.34 DETECTION OF *BEAN COMMON MOSAIC VIRUS* STRAIN BLCM IN COWPEA BY SEROLOGICAL AND MOLECULAR TECHNIQUES APPLICABLE FOR ROUTINE SEED HEALTH TESTING. **A.C. Uday Shankar, H.S. Prakash and O.S. Lund.** *DOS in Applied Botany and Biotechnology, University of Mysore, Manasagangotri, Mysore 570006, India. Email: hsp@appbot.uni-mysore.ac.in, prakash.hs@rediffmail.com*

Conventional seed health testing procedures are cumbersome and at times unreliable. Testing of seeds for viral infection is crucial for providing adequate supplies of virus-free seeds. All seeds sown may not germinate or may contribute to disease dynamics in the field. Cowpea seeds were sprouted for three days and used for assay. Cowpea seeds infected with *Bean common mosaic virus* strain blackeye cowpea mosaic (BCMV-BlCM), as detected by DAC-ELISA were pooled with known healthy samples at ratios of 1:1 to 1:50. DAC-ELSA could detect one infected seed in a batch of four healthy, followed by one infected in a batch of five healthy by IC-RT-PCR. In other experiments, seeds were germinated for 7 days. Leaf discs were harvested from primary leaves. Infected leaf discs were pooled with known proportions of discs from healthy leaves. DAC-ELISA could detect one infected in a batch of five healthy, followed by one infected in a batch of seven healthy by IC-RT-PCR. Total RNA extracted from combinations of BCMV-BlCM-infected and healthy leaves were employed as templates for cDNA synthesis for RT-PCR. RT-PCR could detect BCMV-BlCM at a ratio of 1:20 infected to healthy in leaf extracts. DAC-ELISA could detect one infected cotyledon and embryonic axis among 16 healthy. IC-RT-PCR could detect one infected intact cotyledon and embryonic axis among 20 healthy. RT-PCR could detect one infected intact cotyledon and embryonic axis among 25 healthy.

19.35 IDENTIFICATION OF *LEPTOGRAPHIUM* SPECIES USING A CUSTOM-DESIGNED SPECIES-DIAGNOSTIC MICROARRAY. **N. Van Zuydam, B. Wingfield, K. Jacobs, S. Lezar and M. Wingfield.** *Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, RSA. Email: natalie.vanzuydam@fabi.up.ac.za*

Species of the anamorph genus *Leptographium* include several tree pathogens and the majority are vectored by bark beetles that infest pine trees. This relatively small genus contains species that are morphologically very similar and some are closely related. The species of *Leptographium* are phylogenetically well defined and supported by defining morphological characteristics, which makes the genus an ideal model to test a custom-designed species-diagnostic array. The aim of the study was to use available sequence data in the design of a prototype array for *Leptographium* and to investigate its efficacy against three test organisms. Partial sequences for the β-tubulin, elongation factor-1α and internal transcribed spacer region 2 (ITS2) were used to design 37 species-specific probes and primer pairs that identify 26 species of *Leptographium*. These probes were challenged with PCR targets from *L. dryocoetidis, L. elegans* and *L. leptographioides*. The test probes showed the best hybridisation efficiency when hybridised to the correct PCR target and the three test species were identified using the prototype array. A few test probe failures and cross-hybridisations were observed but these did not affect the overall ability of the array to identify the test species.

19.36 DEVELOPMENT OF A SENSITIVE REAL-TIME PCR PROTOCOL FOR DETECTION OF *XANTHOMONAS FRAGARIAE* IN STRAWBERRY PLANT TISSUE. **J. Vandroemme, S. Baeyen, P. De Vos and M. Maes.** *Institute for Agricultural and Fisheries Research, Burg. van Gansberghelaan 96, 9820 Merelbeke, Belgium. Email: joachim.vandroemme@ilvo.vlaanderen.be*

Xanthomonas fragariae (Xf) is the causal agent of angular leafspot on strawberry and is considered a quarantine organism in strawberry propagation material within the EU. Reliable screening of planting material for latent infections is an important challenge in the control of this disease. A real-time PCR assay based on Taqman chemistry was developed for the detection of Xf. Primers and probe sequences were based on a DNA fragment amplified in the Xf-specific multiplex PCR of Pooler *et al*., 1996. Specificity of the assay was tested with an extended range of Xf strains and isolates, with other *Xanthomonas* spp. and with

unidentified bacterial isolates from strawberry plants. The DNeasy Plant Mini Kit (Qiagen) was adapted to allow preparation of PCR-quality DNA from 100 mg of fresh strawberry tissue. For this, extra purification steps were needed; polyvinylpyrrolidone (PVP) was added to the kit lysis buffer and the eluted DNA was filtrated over polyvinylpolypyrrolidone (PVPP). The PCR master mix was supplemented with T4 protein 32 (GP32), which further improved the sensitivity of detection. The final protocol enabled Xf detection in undiluted DNA extracts from strawberry tissue, while with the default sample preparation and real-time PCR mix, the sample had to be diluted 100 times to obtain reliable detection. Xf was detected down to 300 colony-forming units in a 100 mg of strawberry leaf material. The assay offers a new tool for sanitary control of plant material with low or latent *X. fragariae* infections.

19.37 COMPARISON OF DIAGNOSTIC APPROACHES FOR *PHYTOPHTHORA RAMORUM* FROM CALIFORNIA FORESTS. **A.M. Vettraino, S. Sukno, A. Vannini and M. Garbelotto.** *University of Tuscia, S. Camillo de Lellis snc, 01100 Viterbo, Italy. Email: vettrain@unitus.it*

The sensitivity, specificity, and robustness of five methods for detection of *Phytophthora ramorum* including culturing the pathogen on two selective media, an ELISA-based polyclonal assay, a nested PCR-based assay, and a TaqMan real time PCR assay, were compared on symptomatic plant material sampled in fields at different times and from different host species, and processed in two different laboratories in the USA. With all methods *P. ramorum* could be detected in all sites, sampling periods, and in both laboratories. About 80% of samples collected were found positive for *P. ramorum.* Over 41 species were analysed, and 3 species *M. fabaceous*, *R. ursinus* and *Symphoricarpos* sp. were recorded as new hosts of *P. ramorum*. All diagnostic assays were highly correlated with one another, and with disease symptoms, but only the ELISA-based assay was not affected by sampling period, laboratory processing, hosts and substrates. Assays involving pathogen culture were strongly affected by time of sampling, host species, and the processing laboratory. The two PCR-based assays were more robust than culturing, but differed from one another. While the nested PCR assay was more sensitive and least affected by time of sampling, host species, provenance of samples, and testing laboratory, it was also prone to a higher rate of false positives. Conversely, the TaqMan assay was more specific to its target, but it proved to be less sensitive and more significantly affected by time of sampling, provenance of samples and the testing laboratory.

19.38 IMPROVED RISK ASSESSMENT OF *SCLEROTINIA* STEM ROT IN OILSEED RAPE BY QUANTITATIVE PCR-ASSAY. **A-C. Wallenhammar, C. Almqvist, A. Redner and A. Sjöberg.** *HS Konsult AB, P.O. Box 271, SE 701 45 Örebro, Sweden. Email: ac.wallenhammar@hush.se*

The impact of *Sclerotinia* stem rot depends on weather conditions and the timing of ascospore release, and causes severe damage from year to year in spring oilseed rape (SOSR) in central Sweden. Disease forecasting available for farmers today is a regional risk assessment, based on local climate and field data. This work aims to improve risk assessment and to identify fields where a fungicide application is justified, by determining the presence of inoculum (ascospores) on the petals by a quick and accurate method, namely quantitative PCR. This method is validated by the agar petal test. Petals from 80 fields of SOSR were assessed by the agar test in 2006–2007 and a corresponding disease incidence was assessed in the fields. Based on the petal-test results, samples with varying infection levels were chosen for qPCR-assay. A linear relationship between percent infested petals and percent positive PCR-assays (R^2= 0.8809) was found. The method was developed further during 2007. The weather in 2006 prior to and during flowering was dry, hence 7.6% of the petals were infected and the corresponding disease incidence was determined as 2.4%. In 2007 humid conditions prevailed; 30% of the petals were infected, with a corresponding disease incidence as low as 7.3%. These results indicate that the conditions causing flower parts to stick to the leaves and stems are important for disease development and that this part of the infection process needs to be further clarified.

19.39 MOLECULAR DETECTION OF MATING-TYPE OF *SETOSPHAERIA TURCICA* CAUSING NORTHERN MAIZE LEAF BLIGHT. **Y.Y. Wang, J.G. Dong and G.Z. Zhang.** *Department of Plant Pathology, China Agricultural University, P. R. China. Email: zhanggzh@cau.edu.cn*

Northern maize leaf blight caused by *Setosphaeria turcica* is an important fungal disease and severely damages production. A molecular method for detecting the pathogen's mating type has been developed in this study. According to the sequence of HMG (high mobility group) box (Accession No.E15510), the conserved region of MAT1-2 gene, a pair of primers HMG-U/HMG-L was designed to detect the MAT1-2 of the pathogen. A DNA band about 250bp was amplified from MAT1-2 isolates. Comparing the sequence of the Alpha box, the conserved region of MAT1-1 gene, from 16 fungal species of Pleosporales, a pair of degenerate primers was designed to detect the MAT1-1 of the pathogen. A band of 345bp was amplified from MAT1-1 isolates. Based on the sequence of the band, a pair of specific primers Alpha-U/Alpha-L was designed and a DNA band about 200 bp was amplified from MAT1-1 isolates. With the two pairs of specific primers, we detected the mating types of 83 isolates. The results indicated that 46 isolates were MAT1-2 and the others were MAT1-1. In order to confirm the PCR results, the mating types of all isolates were examined in pairing cultures on Sach medium. The results matched with PCR very well. The PCR method developed in this study for detecting the mating type of the *S. turcica* was rapid, exact and more reliable than the traditional method.

19.40 T-RFLP USED TO IDENTIFY VASCULAR PATHOGENS IN GRAPEVINES AFTER HOT WATER TREATMENT. **B.S. Weir and A.B. Graham.** *Corbans Viticulture Ltd, 8 Bristol Road, Whenuapai, 0618, New Zealand. Email: b.s.weir@rhizobia.co.nz*

Terminal restriction fragment length polymorphism (t-RFLP) has previously been used to evaluate diversity in microbial populations in soil and leaf litter. The method was adapted to monitor endophytic and pathogenic fungal populations in the xylem of grapevines during nursery propagation. Tissue samples were taken from young grapevines for fungal isolation onto MEA, and DNA was extracted from sub-cultures of isolates using the REDextract™ kit (Sigma). Isolates were identified to genus by sequencing the ITS region, and for *Phaeoacremonium* and *Botryosphaeria* to species by β-tubulin sequences. The DNA extracts from up to three isolates of each identified genus or species was used to obtain standard t-RFLP profiles. The extracts were PCR-amplified with terminally labelled ITS1F and ITS4 primers. The ITS fragments

were digested separately with *Hae*III and *Cfo*I and run on an ABI 3100 Genetic analyser, with a 1000-bp standard. The electropherogram was analysed with GeneMapper 3.5. Data including peak size, height, and area, was exported from GeneMapper into an R statistical package TRAMPR, where peaks were analysed and included in the database. DNA was extracted from xylem samples of grapevines four months after grafting and again at eight months. This method was able to distinguish between all of the fungal pathogens associated with vine decline and dieback diseases, as well as a range of common grapevine endophytes. The ability of t-RFLP to identify pathogenic and endophytic species in grapevine wood samples is a significant advance.

19.41 fAFLP MARKERS TO STUDY THE GENETIC DIVERSITY OF *GUIGNARDIA* SPP. E. Wickert, A.P. Aukar, E.G.M. Lemos and A. de Goes. *São Paulo State University, Campus of Jaboticabal, Jaboticabal, SP, Brazil. Email: agoes@fcav.unesp.br*

Citrus black spot (CBS) is caused by *Guignardia citricarpa*, and all commercial citrus cultivars are susceptible. fAFLP molecular markers have been used to discriminate pathogenic and endophytic isolates, and make inferences about the genetic structure and phylogenetic relations between isolates. Isolates from different geographic regions, hosts, types of tissue, and fruit with different types of symptoms were used. AFLP molecular markers were found to be effective to study genetic diversity and to evaluate the phylogenetic relations between *Guignardia* spp. isolates and allowed pathogenic isolates (*G. citricarpa*) to be distinguished from isolates considered endophytic (*G. mangiferae*). The endophytic and pathogenic isolates proved to be genetically close, although the low genetic divergence observed was statistically significant. The endophytic isolates had higher intraspecific genetic diversity than the pathogenic isolates which, although present at higher numbers and being from more diverse origins, had lower diversity. The pathogenic group had the same haplotype colonizing different hosts, in distinct geographic regions, tissues, and various types of lesions. The fact that the endophytic and pathogenic isolates belong to distinct populations was corroborated, in our samples, by the lack of haplotypes shared by both groups. In the phylogram, factors such as geographic origin, host, type of tissue and symptoms from which the isolates were derived had little influence on the cluster they formed; the determinative factor in the cluster was either the pathogenic or endophytic character of the isolates.

19.42 DISTRIBUTION OF *PHYTOPHTHORA CINNAMOMI* ACROSS DISEASE SITES IN WESTERN AUSTRALIA. N. Williams, G.E.StJ. Hardy and P.A. O'Brien. *Centre for Phytophthora Science and Management, School of Biological Science and Biotechnology, Murdoch University, Murdoch, WA 6150, Australia. Email: p.obrien@murdoch.edu.au*

Phytophthora cinnamomi is currently causing disease in a wide range of native Australian plant species. We have carried out a systematic survey of the distribution of the pathogen at seven disease sites in Western Australia using both PCR and baiting to detect the pathogen in soil samples. A nested PCR assay based on ITS sequences was developed for detection of the pathogen in soil. This can detect 1 pg of *P. cinnamomi* DNA. When baiting was used for detection, the number of positive samples ranged from 1–10%. Analysis of material from the selective plates by the PCR assay significantly increased the number of positive samples. When DNA extracted directly from soil was analysed by nested PCR, over 90% of the samples were positive. This was not due to cross-contamination between samples, as stringent quality control was in place to detect such cross-contamination.

19.43 MONOCLONAL ANTIBODIES AGAINST THE RECOMBINANT NUCLEOCAPSID PROTEIN OF *TOMATO SPOTTED WILT VIRUS* AND THEIR APPLICATION TO VIRUS DETECTION IN CHINA. J. Wu, C. Yu, F. Deng and X. Zhou. *Institute of Biotechnology, Zhejiang University, Hangzhou, 310029, P. R. China. Email: wujx@zju.edu.cn*

Tomato spotted wilt virus (TSWV) is the type member of the genus *Tospovirus* and causes significant losses in a wide range of economically important ornamental and vegetable crops worldwide; it is transmitted by several species of thrips, mainly the western flower thrips to over 1000 susceptible species of monocot and dicot plants in temperate and tropical regions. The nucleocapsid protein gene (777 nt), located on the ambisense S RNA segment was amplified using RT-PCR with primers FCP-F (5'ATCGGATCCATGTCTAAGGTTAAGCTCAC -3', a *Bam*HI site was underlined) and FCP–R (5'- ATCCTCGAGTTAAGCAAGTTCTGTGAGTTTTGC -3', a *Xho*I site was underlined) and then cloned. The cloned gene was expressed using the *Escherichia coli* pET-32a expression system and the expressed recombinant protein was checked by Western blot with anti-TSWV antibodies. The recombinant protein was purified using Ni-NTA Agarose, a metal chelate affinity chromatography, and used for the production of mouse monoclonal antibodies (MAbs). Three Mabs were produced and selected by indirect enzyme-linked immunosorbent assay (ELISA) and Western blot assays. TAS-ELISA and immunocapture RT-PCR (IC-RT-PCR) methods were then established for reliable and efficient detection of the viruses.

19.44 MOLECULAR ANALYSES REVEAL GENETIC COMPLEXITY IN *CITRUS TRISTEZA VIRUS* DEKOPON ISOLATE AND ITS APHID-TRANSMITTED PROGENY. R.K. Yokomi, M. Saponari, Z. Weng and Z. Xiong. *CNR–Istituto di Virologia Vegetale, Sezione di Bari, Via Amendola, 165/A, 70126 Bari, Italy. Email: m.saponari@ba.ivv.cnr.it*

An isolate of *Citrus tristeza virus* (CTV) called Dekopon, found in a hybrid mandarin variety in Fresno County, CA, was characterized before and after aphid transmission (AT). The isolates were analyzed by SSCP, genotyping with multiple molecular markers in RT-PCR, sequencing of the major CP and p33 ORFs, and bioindexed. These analyses revealed a complex population of phylogenetically distinct CTV genotypes, comprised of a Dk genotype in the VT superclade, a VT-like genotype, and an unidentified genotype related to but genetically distinct from T30. Segregation of this CTV complex was observed in the AT progeny. The Dk and the VT-like genotypes induced seedling yellows and stem pitting which were generally more severe than the parental isolate. AT isolates with the T30-like genotype produced no or mild symptoms alone, suggesting that some cross protection may occur even though the Dk genotype, associated with strong seedling yellows reaction, was the dominant population in the parent. The Dk component was transmitted by aphids 81% of the time while the VT and T30 genotypes were transmitted 45% and 63% of the time, respectively. Phylogenetic analysis of the CP and p33 ORF sequences suggested that the Dk genotype is related to the NUagA isolate. Genomic sequences of the AT progeny isolates containing a predominant single genotype were

obtained from a genome-wide analysis with a CTV resequencing microarray. The resequencing analysis achieved call rates of 95.8% to 99.8% and call accuracies of 98.4% and 99.6%, and is being used to further characterize the AT isolates.

19.45 MOLECULAR PHYLOGENY OF KOREAN AND CHINESE *GYMNOSPORANGIUM* SPECIES. **H.Y. Yun, S.G. Hong and K.Y. Lee.** *Department of Forest Sciences, College of Agriculture and Life Sciences, Seoul National University, 56-1 Shillim-dong, Kwanak-gu, Seoul 151-921, Republic of Korea. Email: botany95@dreamwiz.com*

Phylogenetic relationships of three *Gymnosporangium* species, *G. asiaticum, G. japonicum*, and *G. yamadae* as main species from Korea and China, were investigated based on 28S rDNA sequences. *Gymnosporangium clavariiforme*, *G. cornutum*, *G. clavipes*, *G. juniperi-virginianae*, *G. libocedri* and *G. fuscum* were included for comparison. 28S rDNA sequences were analyzed for 34 telial and 7 aecial isolates from Korea, and 69 telial and 4 aecial isolates from China. Each species formed monophyletic groups with strong bootstrap support. *G. asiaticum* was divided into two subgroups, one of which contained three isolates and the other contained 11 isolates. Correlation by geographical distribution was not supported by molecular phylogenetic analyses. Evolution of morphological characteristics of the aecial stage in *G. asiaticum* and *G. yamadae* was evaluated by mapping the character states on the phylogenetic tree. Among nine morphological aecial characteristics tested, seven, including aecial host, aecial type, peridium open type, aeciospore surface structure type, peridial cell shape, peridial cell surface structure type (side wall of peridial cell and peridial cell surface structure type), and inner wall of peridial cell, were relatively well conserved and had value for distinguishing between *G. asiaticum* and *G. yamadae*.

19.46 BROWN ROT FUNGI ON STONE AND POME FRUITS IN CHINA. **X.Q. Zhu, L.Y. Guo and X.Y. Chen.** *Department of Plant pathology, China Agricultural University, Beijing, P. R. China. Email: mycolozhu@cau.edu.cn*

A total of 316 isolates of *Monilinia* spp. from symptomatic stone and pome fruits in China were identified based on morphology, the PCR method described by Ioos, and the sequence of ITS region amplified with the universal primers ITS1 and ITS4. Among these isolates, 276 were *M. fructicola*, 37 were *M. fructigena*, and three were *M. laxa*. Isolates of *M. fructicola* and *M. fructigena* were found on peach, plum, apricot, apple and pear, while *M. laxa* was isolated only from apricot and cherry. The ITS region of 19 isolates (9 *M. fructicola*, 2 *M. laxa* and 8 *M. fructigena*) from China were sequenced and compared with those of isolates from other countries (18 *M. fructicola*, 18 *M. laxa*, 15 *M. fructigena*, one *M. mali* and one *Monilia polystroma*), retrieved from GenBank. Multiple alignments and a phylogenetic tree were constructed using CLUSTALW 1.8. The 72 isolates were separated into 7 groups. For *M. fructicola*, the isolates from China were grouped together with those from other countries. However, For *M. fructictigena* and *M. laxa*, the isolates from China were separated from isolates from other countries into different groups. Results of this study revealed that *M. fructicola* is the most prevalent on stone fruits while *M. fructigena* is the most prevalent on pome fruits.

MYCOTOXINS

33.1 *FUSARIUM* INFECTION AND MYCOTOXIN CONTENT IN WHEAT AND OAT AFTER SINGLE OR MULTIPLE ARTIFICIAL INFECTIONS. **H.U. Aamot, O. Elen, J. Razzaghian , E. Lysøe and S.S. Klemsdal.** *Bioforsk, Norwegian Institute for Agricultural and Environmental research, Plant Health and Plant Protection Division, Ås, Norway. Email: heidi.udnes.aamot@bioforsk.no*

Fusarium head blight (FHB) is a widespread and destructive disease of cereals caused by a number of *Fusarium* species. FHB can reduce cereal quality by producing a number of toxic metabolites that have an adverse effect on animal and human health. Under field conditions a mixture of *Fusarium* species exists. Little is known about how the presence of a mixture of different *Fusarium* species in the same sample affects mycotoxin production. The aim of this study was to examine the correlation of the infection level of different *Fusarium* species and the corresponding mycotoxin content in single and multiple infections in grain. Wheat and oats, grown under greenhouse conditions, were spray-inoculated with five *Fusarium* species frequently found in Norwegian grain (*F. graminearum, F. culmorum*, *F. langsethiae*, *F. avenaceum,* and *F. poae)* in single and multiple combinations. Infections in oat proved difficult to establish, and chemical toxin analysis of harvested grain showed that the mycotoxin content in oat was generally lower than in corresponding wheat samples. Inoculations with *F. langsethiae* were not successful, and neither T-2 nor HT-2 was detected in wheat or oat. Neither was it possible to detect *F. langsethiae* in the kernels when analysed by qPCR. In 2007 the inoculation experiment was repeated, this time with adjustments to ensure infection establishment. This time all *Fusarium* species studied became well established in the kernels. The interactions between the *Fusarium* species regarding establishment of the fungus on the developing kernels and the production of the mycotoxins will be discussed.

33.2 THE OCCURRENCE OF AFLATOXINS AND FUMONISIN IN MAIZE INFECTED WITH COMMON SMUT. **H.K. Abbas, R.M. Zablotowicz, C.A. Abel and H.A. Bruns.** *USDA-ARS, Crop Genetics and Production Research Unit, Stoneville, Mississippi, 38776, USA. Email: hamed.abbas@ars.usda.gov*

Mycotoxins produced by *Aspergillus and Fusarium* spp. when present in maize, can cause serious toxicological problems in animals and humans. Maize can also be infected with the fungus *Ustilago maydis* the causal agent of common smut, producing structures that are edible. Little is known about the relationship between the occurrences of mycotoxin contamination in maize infected with common smut. In 2006 a high degree of common smut was observed in fields planted with two maize isolines 34B23 and 34B24Bt, thus at harvest the disease incidence was evaluated and maize was sampled from plants that were infected or free from smut symptoms. There was no significant effect of the expression of the Bt gene with ~17 % infected ears in both isolines. Grain harvested with symptoms of smut contained 2437 $\mu g\ kg^{-1}$ total aflatoxin compared to ~50 $\mu g\ kg^{-1}$ in maize not infected with smut. Grain from smut infected grain contained ~1000-fold greater colony forming units (cfu) of *A. flavus* compared to uninfected grain. Fumonisin concentration averaged 201 $mg\ kg^{-1}$ in smut-infected grain, while uninfected grain contained only 3.4 $mg\ kg^{-1}$. Although smut-infected maize may be a gourmet foodstuff, these results raise toxicological concerns associated with human intake of this product. The levels of aflatoxin, fumonisin and corresponding *A. flavus* cfu observed in our initial

study are many orders of magnitude greater than safety limits established by both U.S. and European guidelines.

33.3 BIOCONTROL OF AFLATOXIN IN MAIZE USING NON-TOXIGENIC *ASPERGILLUS FLAVUS* STRAIN K49. **H.K. Abbas, R.M. Zablotowicz, C. Accinelli, M.E. Lyn, J.R. Wilkinson, H.A. Bruns and C.A. Abel.** *USDA-ARS, Crop Genetics and Production Research Unit Stoneville, Mississippi, 38776, USA. Email: Hamed.Abbas@ars.usda.gov*

Aflatoxin (AF) produced by *Aspergillus flavus* can be a major problem in Mississippi Delta maize (*Zea mays* L.) resulting in great economic losses if AF levels are greater than 20 μg kg^{-1}. Although research has been directed at reducing maize AF contamination, no consistent control methods are currently available. This paper summarizes the development of a non-toxigenic strain of *A. flavus* (K49) that, when applied to maize as soil or foliar inoculants, suppresses AF. Five years of field testing showed these non-toxigenic isolates reduced AF contamination by 60-94% in Mississippi field trials. The non-toxigenic K49 displaces toxigenic *A. flavus* populations in soil and those colonizing maize kernels. K49 is a suitable biocontrol agent because it does not produce any aflatoxins or cyclopiazonic acid. Although K49 possesses several genes in the aflatoxin biosynthesis pathway of one of the regulatory genes, aflR, has not been detected in this strain. Ecological monitoring studies indicate that K49 has superior soil colonization, and pin-par inoculation technique indicates K49's potential for rapid maize colonization. Thus the reduction of AF contamination by competitive exclusion of toxigenic *A. flavus* isolates makes K49 an excellent candidate for use in biocontrol. Various strategies are being employed to improve delivery systems and formulations, and molecular techniques are being employed to assess the efficacy of this unique biocontrol agent.

33.4 ROLE OF *OSTRINIA NUBILALIS* IN VECTORING *ASPERGILLUS FLAVUS* IN A MAIZE FIELD IN NORTHERN ITALY. **C. Accinelli, H.K. Abbas, R.M. Zablotowicz, S. Maini and A. Vicari.** *Department of Agro-Environmental Science and Technology, University of Bologna, 40127 Bologna, Italy. Email: cesare.accinelli@unibo.it*

The European corn borer (ECB), *Ostrinia nubilalis* Hübner, is a major pest of maize in Europe and in several agricultural areas of the United States. In addition to causing direct yield losses, the ECB is thought to act as a vector carrying spores (propagules) of the aflatoxin-producing fungus *Aspergillus flavus*. The objective of this study was to investigate the role of ECB larvae and moths in dispersal of *A. flavus* propagules in a maize field in northern Italy. In an initial sample of more than 200 first-flight ECBs, only 4.5% carried detectable *A. flavus* propagules, as determined by cultural methods. PCR detection of genes of the aflatoxin biosynthesis pathway including afllD, aflG, aflP, aflR and aflS, showed a higher percentage (11.0%) of positive moths. *A. flavus* propagules and aflatoxin genes were only observed in larvae at the end of the first generation. Higher incidence was observed in moths and larvae of the second generation. Cultural methods showed that 48.0% and 95% of the moths and larvae analyzed respectively carried *A. flavus*. As observed with individuals of the first generation, the PCR test was more sensitive. Laboratory bioassay conducted with 2nd instar ECB larvae showed that spores of aflatoxin-producing *A. flavus* strain F3W4 and the non-aflatoxin producing strain K49 did not affect larval growth or mortality. In contrast, presence of aflatoxin B1 (≥ 5.0 μg ml^{-1}) caused significant decrease in larval growth and increased mortality ($P < 0.01$). These findings indicated that ECB adults and larvae can play an important role in carrying *A. flavus* propagules especially late in the season.

33.5 PERSISTENCE OF AFLATOXIN AND FUMONISIN IN BT AND NON-BT CORN RESIDUES. **C. Accinelli.** *Department of Agro-Environmental Science and Technology, University of Bologna, 40127 Bologna, Italy. Email: cesare.accinelli@unibo.it*

Aflatoxin and fumonisin produced in maize infected with *Aspergillus* and *Fusarium* species can cause toxicological problems. The objective of this study was to determine the overwintering mycotoxin levels in various crop residues (leaves and stalks, cobs, and cobs containing kernels) on the soil surface three to six months after harvest. Two maize hybrids, one expressing the Bt endotoxin, Cry1Ac and a non-Bt hybrid isoline were grown in Elizabeth, MS in a randomized complete block. Samples were oven-dried at 55 °C, ground and analyzed for aflatoxin, fumonisin and *A. flavus* colony-forming units (CFU). Grain from the Bt hybrid contained less aflatoxin ($p=0.044$) than the non-Bt hybrid (109 versus 200 μg kg^{-1}, respectively). Average total aflatoxin levels in leaves and stalks were ~2 μg kg^{-1}, cobs ~20 μg kg^{-1} and cobs containing grain (~509 μg kg^{-1}) with higher total aflatoxin levels ($p<0.01$) occurred in Bt than non-Bt residues. Total fumonisin levels averaged 0.7 μg g^{-1} in leaf and stalk residues, versus 7 μg g^{-1} in cobs, and 55 μg g^{-1} in cobs with grain with no difference between isolines. Aflatoxin levels remaining in residues were ~30% of that in December while fumonisin levels were < 2%. Maize cobs containing grain maintained ~ log (10) 5.6 CFU g^{-1} of residue compared to < 4.4 CFU in other maize residues or 3.5 CFU in soil. The presence of high mycotoxin levels in maize residues may negatively impact wildlife, and livestock. The persistence of *A. flavus* CFUs indicates that maize residues are a significant source for contamination.

33.6 VARIABILITY IN *ASPERGILLUS FLAVUS* L. EX. FRIES INFECTING GROUNDNUT (*ARACHIS HYPOGAEA* L). **S.S. Adiver and M.G. Kiran Kumar.** *Oil Seeds Scheme, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad-580 005, Karnataka State, India. Email: shivaputra_adiver@rediffmail.com*

Aflatoxins are a problem in groundnuts due to infection of *Aspergillus flavus*. Surveys conducted in 14 districts of Karnataka (India) to assess the severity of *A. flavus* revealed a high incidence in Belligatti when assessed on agar plates. It was also found high in two other districts, Davanagere and Tumkur, using a blotter test. In market-collected samples, highest incidence was in Dharwad and Hubli. *A. flavus* produced greenish to brownish colonies, with pyriform to globbose conidia, 8 × 7 μm to 5 × 4 μm in size. The fungus produced larger colonies on potato dextrose agar and smaller ones on host-extract agar. All selected isolates of the fungus (22 isolates) recorded significant variation in growth on solid and in liquid media. The study also indicated substantial cultural variability among these isolates. The isolates showed significant variation for production of aflatoxins in groundnut kernels. Higher production of toxin by Shimoga isolate and lower production by Mudhol isolate was obtained following seed inoculation. In culture, there were similar trends in toxin production. The isolates showed variability in peroxidase and polyphenoloxidase zygogram patterns. Based on this the isolates could be categorized into distinct groups. In inoculated seeds, infection led to reduction in sugars (reducing, non-reducing and total sugars),

proteins, oil content and seed germination. Germination percentage varied with different groundnut genotypes tested. In in vitro seed colonization, cultivar Dh-86 showed a moderate level of resistant to *A. flavus.*

33.7 A BIOCHEMICAL APPROACH TO ELUCIDATE THE PATHWAY OF PATULIN DEGRADATION BY A BIOCONTROL YEAST. I. Aloisio, S.A.I. Wright, D.V. De Felice, P. Tremonte and R. Castoria. *Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università del Molise, Campobasso, Italy. Email: castoria@unimol.it*

Patulin contaminates apple fruits as a consequence of blue mould, a postharvest disease caused by *Penicillium expansum*. The basidiomycetous yeast *Rhodotorula glutinis* strain LS11 is a biocontrol agent of postharvest pathogens. *R. glutinis* LS11 intracellularly degrades patulin to desoxypatulinic acid, a product that is non-toxic to microorganisms that are inhibited by patulin. On stored apples, the yeast lowers the accumulation of patulin even in fruits infected by *P. expansum.* Exposure to a high concentration (500 ppm) of patulin dramatically inhibits/delays the growth of *R. glutinis* LS11, which needs to gradually get accustomed to the mycotoxin. To test if patulin degradation by strain LS11 is induced, this yeast was grown in the presence of 10 ppm of patulin. After incubation, half of the cells were further incubated with 125 ppm of mycotoxin, the other half being used for extraction of proteins. Both kinds of sample were assayed for patulin degradation. HPLC analyses showed that patulin degradation was significantly faster in LS11 cells preconditioned with 10 ppm of patulin (desoxypatulinic acid was also detected as the major degradation product) as well as with intracellular protein extracts from cells grown in the presence of the same toxin concentration, as compared to respective controls. SDS-PAGE showed a differential band of approximately 45 kDa in intracellular proteins extracted from LS11 cells incubated with 10 ppm of patulin. Further studies are in progress to purify and characterize this protein. This will help to elucidate the patulin degradation pathway and to identify the enzymes involved.

33.8* PREVENTIVE AND CORRECTIVE ACTION TO MINIMISE OCHRATOXIN A IN GRAPES AND WINE. P. Battilani, J. Cabañes, P. Giorni, Z. Kozakiewicz, A. Lebrihi, A. Lichter, S. Minguez, G. Mule, N. Magan, A. Pietri, V. Sanchis, A. Silva, E. Tjamos, A. Venâncio and G. Zinzani. *Institute of Entomology and Plant Pathology, Faculty of Agriculture, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. Email: paola.battilani@unicatt.it*

Ochratoxin A (OTA) is a mycotoxin produced in grapes in the vineyard by black aspergilli (mainly *A. carbonarius*). Ochratoxin A is a highly harmful metabolite classified as a possible human carcinogen. EC regulation No. 123/2005 has fixed 2µg/kg as the limit for OTA in grape juice, must and wine, confirmed in the regulation in force (1881/2006). Black aspergilli are present on bunches from setting and their incidence is highest at ripening. OTA is detected close to ripening, rarely at veraison. It can be present in symptomless bunches but high levels are associated with mouldy berries; those damaged by *Lobesia botrana* or by powdery mildew are more contaminated with OTA. Meteorological conditions influence black aspergilli and OTA content in grapes, but cropping systems also play a role. OTA present in grapes is released into must during crushing and maceration, while a decrease in OTA is observed during alcoholic and malolactic fermentations. Common adjuvants used in must and wine clarification contribute to decreasing OTA. Good pest & disease control, reasonable irrigation and fertilizer supply, timely harvest at ripening, with no delay especially when damaged and mouldy berries are present, minimizing the time interval between harvest and crushing, discarding bunches with visible mould growth or extensive damage, represent the main preventive actions. The more useful corrective actions are as follows: use of chemical adjuvants at the recommended levels for this type of wine, to adsorb OTA; allowing must to settle before alcoholic fermentation; and/or the use of a selected lactic acid bacterial strain to promote malo-lactic fermentation in the wine.

33.9 ROLE AND IMPORTANCE OF DIFFERENT INFECTION PATHWAYS ON THE FINAL *FUSARIUM* INFECTION AND FUMONISIN CONTAMINATION OF MAIZE. M. Blandino, G. Tamietti, F. Vanara and I. Visentin. *Department of Agronomy, Forestry and Land Management, University of Turin, Via L. da Vinci 44, Grugliasco (TO), Italy. Email: massimo.blandino@unito.it*

The contamination of almost all Italian maize grain lots by fumonisins B_1 and B_2 is clearly above the limits for food safety (Reg. 1881/2006). Nowadays, field prevention, through good agricultural practices, and the selection of tolerant hybrids are the only possible solutions to significantly reduce the final contamination. In order to improve agricultural practices, it is crucial to obtain more information on inoculum sources and infection pathways of *Fusarium verticillioides*. In the 2006-07 period in northern Italy, maize was grown in two fields in ploughed and sod soil, with or without crop residues on the surface; different levels of seed infection, fungicide application and artificial inoculation at flowering, protection against European corn borer (*Ostrinia nubilalis* Hb) were also applied. *F. verticillioides* and other *Fusarium* species were isolated from the maize seeds, the plants at different phenological stages, and the grains at milk, waxy and physiological maturity. Fumonisins B_1-B_2 kernel contamination was quantified by HPLC. The infection rate of primary roots and epicotyl was unaffected by the soil cultivation techniques, but was increased by *F. verticilioides* seed inoculation. Stem colonization was quite low, ranging from 0 to 10%. The kernels were *Fusarium*-free during growth; infections started at the milk stage and increased during ripening. Infection rate and fumonisin contamination were increased by crop residues and artificial inoculation at flowering; fungicide ear treatments had only a moderate effect. *O. nubilalis* clearly promoted ear infection and fumonisin contamination.

33.10 STRATEGY FOR REDUCTION OF MYCOTOXIN IN MAIZE KERNELS DURING CULTIVATION. M. Blandino, A. Reyneri and F. Vanara. *Department of Agronomy, Forestry and Land Management. University of Turin, Via L. da Vinci 44, Grugliasco (TO), Italy. Email: massimo.blandino@unito.it*

Mycotoxins in cereals are an economic and health problem. Mycotoxin production begins mainly in the field and is influenced by environmental conditions during ripening and by cultural techniques. The aim of this research was to evaluate the effect of combined agronomic techniques on mycotoxin contamination in maize kernels. Three experimental fields were prepared in northern Italy in 2005, 2006 and 2007. The following treatments were compared at each site: 2 hybrids with different precocity and 4 combinations of agronomic technique (seeding time, seeding density, N fertilization and European Corn Borer (ECB) control, combined with the

following practices: TAR–high risk technique, involving late planting, high seed density and N fertilization; TAM–medium risk technique, characterized by normal planting, high seed density and N fertilization; TAC–correct technique, using early planting, low seed density and balanced N fertilization; TAA–technique the same as TAC, plus a chemical treatment against ECB. The TAM and TAC treatments on average reduced *Fusarium* ear rot severity by 60% compared to TAR. TAA gave a reduction of ear rot severity by 87% compared to TAR. The results showed a mean reduction of fumonisin contamination of 2, 3 and 9 times for TAM, TAC and TAA respectively compared to TAR. A significantly increased contamination of deoxynivalenol was found for both hybrids but only for late planting, while aflatoxin contamination was low at all sites.

33.11 OCCURRENCE OF BLACK ASPERGILLI ON GRAPES IN ITALY AND RELATIONSHIP WITH PRESENCE OF OCHRATOXIN A IN MUST. **M. Borgo, G.L. Lucchetta, D. Bellotto, GL. Dal Cortivo, I. Bazzo, L. Stringher and E. Angelini.** *CRA Centro di Ricerca per la Viticoltura, Conegliano (TV), Italy. Email: michele.borgo@entecra.it*

Ochratoxin A (OTA) in wine is linked to contamination with *Aspergillus* spp. During 2001-2006, we surveyed grape samples for the presence of mycotoxin-producer species, collected in several vineyards in northern, central and southern Italy, in three different periods during ripening. After homogenisation, each sample was divided into two parts, one for mycological analyses, the other for OTA determination by ELISA. *A. niger* was the prevalent species in the samples, followed by *A. carbonarius*, mostly found in samples from southern Italy. The occurrence of black *Aspergillus* species was positively correlated with high temperatures in summer and negatively with rainfall and a decrease in temperature before harvest. The years and the environment had a determining influence on the development of black *Aspergillus* populations. Semi-quantitative determination of OTA in must samples showed it to be present in about 40% of the samples. In particular, samples from southern Italy showed the highest occurrence (75%) and also the highest concentration of OTA, sometimes higher than 2 mg/ml. The values decreased progressively towards the north of Italy.

33.12* STUDIES OF DOTHISTROMIN, AN AFLATOXIN-LIKE MYCOTOXIN PRODUCED BY A PINE NEEDLE PATHOGEN. **R.E. Bradshaw, A. Schwelm and S. Zhang.** *Bio-Protection Centre, Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand. Email: R.E.Bradshaw@massey.ac.nz*

Dothistroma needle blight is a fungal disease of pine trees that has been prevalent in southern hemisphere plantation forests for many decades. The disease has recently reached unprecedented epidemic levels in parts of Europe and Canada, and climate change has been implicated in these outbreaks. Dothistromin toxin is produced by the fungal pathogen *Dothistroma septosporum* and accumulates in red bands on needles that are characteristic of the disease. Since the toxin was considered a potential target for disease control we aimed to understand its role in the disease process and the genetic basis of its biosynthesis. These studies revealed several unexpected results. (1) Despite earlier assumptions, dothistromin is not a pathogenicity factor. Dothistromin-deficient mutants made by targeted gene replacement are pathogenic to pine needles and there is no evidence of reduced virulence. (2) Dothistromin is unusual among fungal secondary metabolites in being produced at a very early stage of growth, rather than in late exponential or stationary phase. The early onset of dothistromin production, along with its broad-spectrum toxicity, led to our current hypothesis that dothistromin has a role in protection against faster-growing competitor organisms on the needle surface. (3) The genes for dothistromin biosynthesis are present in a 'fragmented cluster' on a mini-chromosome. This is in contrast to biosynthetic and regulatory genes for dothistromin's close chemical cousin, aflatoxin, which are tightly clustered on a main chromosome in *Aspergillus* spp. Hence studies of dothistromin raise fundamental questions about the evolution and regulation of metabolic gene clusters in fungal plant pathogens.

33.13* STRATEGIES TO REDUCE *FUSARIUM* AND MYCOTOXIN CONTAMINATIONIN NORWEGIAN CEREALS. **G. Brodal, O. Elen, I.S. Hofgaard, H.U. Aamot, E. Lysøe and S.S. Klemsdal.** *Bioforsk . Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskolevn 7, N-1432 Ås, Norway. Email: guro.brodal@bioforsk.no*

Increasing levels of *Fusarium* toxins, particularly deoxynivalenol (DON), T-2 /HT-2 and moniliformin, have been recorded in Norwegian cereals during the last few years. Previously *F. avenaceum*, *F. culmorum*, *F. poae* and *F. tricinctum* were the most common *Fusarium* species found on cereals in Norway. However, more recently *F. graminearum* has occurred more frequently, and the T-2/HT-2 toxin producing species *F. langsethia* has also been detected, especially in oats. Investigations were made to clarify if there has been a change in the composition of *Fusarium* species. We are aiming to establish a three-step screening system in order to identify grain lots with high levels of *Fusarium* toxins: 1- Identify 'high-risk' fields/lots, based on information on cultivation practice and climatic conditions, through the use of a FHB-prediction model. 2- Analyze the 'high-risk' lots using a rapid test selected for its capacity to detect *Fusarium* toxins in a large number of grain samples at low cost. 3- Forward selected samples (based on analyses in step 2) for chemical mycotoxin analyses. Since 2004, we have run chemical mycotoxin analyses on grain samples from oat and spring wheat grown in Norway under different climatic conditions. Prediction models will be developed to estimate the risk of *Fusarium* infection and mycotoxin development in cereal fields, based on data for weather and cultivation practices. Preliminary results from using the prediction models will be presented.

33.14 MAPPING *RHIZOCTONIA SOLANI* PHYTOTOXIN-SENSITIVITY GENES IN RICE. **S.A. Brooks.** *USDA ARS, Dale Bumpers National Rice Research Center, 2890 Highway 130 East, Stuttgart, Arkansas, 72160, USA. Email: ricegenes@mac.com*

Rhizoctonia solani is a necrotrophic pathogen that causes disease on all major crops grown worldwide. Isolates from anastomosis group one (AG1) sub-group IA cause sheath blight disease of rice, which costs southern United States rice producers an estimated $50M USD annually in yield losses. Rice is a model plant for genetic analyses and host-parasite interactions; however, tolerance to this disease remains a mystery. Previous attempts to identify sheath blight tolerance genes have failed due to difficulties obtaining precise phenotypic data, which limits the resolution of genetic (QTL) maps. Recently, however, the phytotoxin produced by *R. solani* was isolated and used to demonstrate a correlation between disease susceptibility and toxin sensitivity ($r = 0.66$), establishing the toxin phenotype as a potential component

of disease tolerance. Phenotypic evaluation of toxin sensitivity in 154 F2 progeny from a cross between Cypress (*tox-S*) and Jasmine 85 (*tox-I*) revealed a 9:7 segregation ratio for *tox-S* : *tox-I*, indicating an epistatic interaction between the two genes. This discovery of Mendelian inheritance of a precise phenotypic trait enabled a map-based gene cloning approach to identify both phytotoxin sensitivity genes.

33.15 A GENETIC APPROACH TO ELUCIDATE THE PATHWAY OF PATULIN DEGRADATION BY A BIOCONTROL YEAST. R. Castoria, D.V. De Felice, S. D'Alonges, G. Ianiri, J. Heitman, A. Idnurm and S.A.I. Wright. *Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università del Molise, Campobasso, Italy. Email: sandra.wright@unimol.it*

The basidiomycetous yeast *Rhodotorula glutinis* (teleomorph *Rhodosporidium* spp.) can be isolated from diverse environments. *R. glutinis* strain LS11 was isolated from apple, and was subsequently found to protect apples from postharvest rots caused by *Penicillium expansum* and *Botrytis cinerea*. It is also able to degrade and detoxify the mycotoxin patulin, which is formed in apples infected with *P. expansum*. Patulin is a very potent toxin and only minute concentrations are permitted in commercial apple juice. The extreme sensitivity of *Escherichia coli* to patulin and its insensitivity to the degradation products was utilized to develop an assay for screening of mutants defective in patulin degradation. In order to find the genes responsible for enzymatic degradation, we generated *Agrobacterium*-mediated insertion mutants and are in the process of constructing a genomic library. The genes of mutants that do not degrade patulin are being identified. The genome library is being constructed in *Cryptococcus neoformans* strain JEC43, a basidiomycetous yeast strain that is sensitive to patulin and is unable to degrade it. To have additional genetic tools for *R. glutinis* LS11 uracil auxotrophs were selected on minimal medium supplemented with 5-FoA (5-fluoro-orotic acid). This approach yielded mutants in genes for enzymes involved in uracil biosynthesis, *URA3* (orotidine-5'-phosphate decarboxylase) or *URA5* (orotate phosphoribosyltransferase). In order to identify the gene mutated, each auxotroph was complemented with the *URA3* and *URA5* genes of *C. neoformans*. Of eight auxotrophs analysed, two were auxotrophs for URA3 and six for URA5. The transformants were confirmed phenotypically and genotypically.

33.16 MOLECULAR AND IMMUNOLOGICAL DETECTION OF *FUSARIUM* INFECTION AND FUMONISIN CONTAMINATION IN MAJOR FOOD CROPS IN SOUTHERN INDIA. S. Chandra Nayaka, A.C.Uday Shankar, S.R Niranjana and H.S. Prakash. *Department of studies in Applied Botany and Biotechnology, University of Mysore, Manasagangotri, Mysore, India. Email: moonnayak@gmail.com*

Fumonisins, are group of mycotoxins mainly produced by *Fusarium verticillioides* and *F. semitectum*, have adverse health effects on humans and livestock that ingest fumonisin-contaminated food products and feeds. Maize, sorghum and rice are excellent substrates for the growth of *Fusarium* sp., and fumonisin (FB) production. In order to understand the magnitude of *F. verticillioides* and *F. semitectum* infection and FB contamination, surveys were conducted in different agro-ecological zones of southern India, and 112 samples of maize, rice and sorghum were collected from different sources. These samples were analyzed for FB_1 and FB_2 contamination by HPLC and Competitive Direct ELISA. Analysis indicated that post-harvest was the favorable stage for infection by *F. verticillioides* and *F. semitectum* and FB production. More than 60 % of the maize kernels, 30% sorghum seed samples and 23% of rice seed samples tested were contaminated with FB, ranging from 3 to 927 µg/kg. A PCR for specific detection of toxigenic and non-toxigenic *F. verticillioides* was standardized. Specific primers VERT1, VERT2 and for toxigenic strains, VERTF1 and VERTF2 were designed based on IGS sequence. The primers specific to the toxigenic *F. verticillioides* were highly effective and amplified the expected 400 bp product. This study demonstrated the potential utility of PCR using specific primers for detection of *F. verticillioides* in major food crops.

33.17 SEQUENCE-BASED IDENTIFICATION AND FUMONISIN PRODUCTION OF A POPULATION OF *FUSARIUM* SPECIES CAUSING BAKANAE DISEASE OF RICE IN THE PHILIPPINES. C.J.R. Cumagun, C.L. Padilla, F. Van Hove, M.T. Gonzàlez-Jaén and P. Marin. *Crop Protection Cluster, University of the Philippines Los Baños, College, Laguna, 4031, Philippines. Email: christian_cumagun@yahoo.com*

Forty *Fusarium* strains were isolated from rice stems, shoots and grains in the Philippine provinces of Nueva Ecija and Laguna. All isolates were identified as *Fusarium fujikuroi* based on the elongation factor -1α sequence except three isolates which were identified as *F. proliferatum*, *F. sacchari* and *F. oxysporum*. Only five isolates produced fumonisins in liquid culture; concentrations, estimated by ELISA, ranged from 0.025 ppm to 0.238 ppm. High performance liquid chromatography (HPLC) analysis of 20 isolates revealed seven isolates as fumonisin producers with production ranging from 0.86 µg/g -210 µg/g. Amplification of a partial sequence of the *fum1* gene (a key gene in fumonisin biosynthesis) is ongoing. The production of fumonisins of *F. fujikuroi* in rice implies the need to explore a larger population of this pathogen to promote food safety.

33.18 BIOCHEMICAL AND MOLECULAR STUDY OF TRIAZOLE RESISTANCE AND ITS EFFECT ON OCHRATOXIN PRODUCTION BY *ASPERGILLUS OCHRACEUS*. E.G. Doukas, A.N. Markoglou and B.N. Ziogas. *Laboratory of Pesticide Science, Agricultural University of Athens, Greece. Email: ziv@aua.gr*

Mutants of the ochratoxigenic *Aspergillus ochraceus* Wihl. NRRL-5175, moderately resistant to the triazole fungicide epoxiconazole, were readily isolated after UV mutagenesis. Cross-resistance studies with other fungicides showed that the mutation(s) for resistance reduced the sensitivity of mutant isolates to the triazole fungicides flusilazole and difenoconazole and the imidazole imazalil. Furthermore, in the majority of strains studied, a reduction of the sensitivity to more than one of the specific inhibitors fenpropimorph (morpholine), fludioxonil (phenylpyrrole), iprodione (dicarboximide), cyprodinil (anilinopyrimidine), fluazinam (phenylpyridinamide), boscalid (anilide) and pyraclostrobin (Qo inhibitor) was observed. For all the mutant strains the mutation(s) did not affect sensitivity to the benzimidazole carbendazim and there was a negative cross-resistance relationship with the non-specific inhibitor chlorothalonil. Study of fitness parameters of the epoxiconazole-resistant isolates, showed that these mutations did not affect or negatively affect characteristics like mycelial growth and conidial production. Chromatographic analysis of ochratoxin A and B production by the wild-type and resistant strains, on both artificial medium and on wheat grains, showed that the mutation(s) negatively affected or caused the loss of ochratoxin production.

Moreover, a correlation between conidial and ochratoxin production was not observed. Molecular analysis using degenerate oligonucleotides based on conserved areas of 14α-demethylase, the target site of triazoles, from other fungal species, revealed the presence of two different homologue genes, cyp51A and cyp51B, in *A. ochraceus*. Sequence analysis showed a number of different single point mutations in cyp51A, but their role in fungicide-resistance needs further investigation.

33.19 DETECTION OF CERCOSPORIN PRODUCED BY *CERCOSPORA* IN TISSUES OF *AGAVE TEQUILANA* VAR. 'AZUL'. **L. Fucikovsky, R. Arrequin and M.I. Chávez.** *Instituto de Fitosanidad, Colegio de Postgraduados, Carretera Mexico-Texcoco km 36.5, Montecillo, Edo. de México, C.P. 56230, Mexico. Email: fucikovs@colpos.mx*

Agave tequilana is a Mexican plant from which the alcoholic drink tequila is made. The plant suffers from *Cercospora* fungus and in 2001-2002 this killed up to 60% of the plants in the states of Jalisco, Nayarit, Guanajuato and Tamaulipas, producing large 10 cm, oval , sunken, greyish, necrotic areas with brownish superficial fructifications on leaves. The plant turns yellow, usually when it is 4 year old. The purified fungus was introduced into basal leaf tissues and after 10 to 20 days, lesions were noted. After 3 to 4 months the plants turned yellow. The fungus was reisolated from the diseased tissues. When the affected stem (*piña*) was cut in half, the internal tissue was white and in 20 minutes the affected parts turned red, indicating the possibility of a toxin, because no fungus could be isolated from it. This was confirmed using the red diseased tissue, by analysing the H-NMR spectrum which showed signals corresponding to phenolic hydroxyl protons at δ 14.81, aromatic protons at δ 7.13, a dioximethylene at δ 5.93, and methoxyl groups at δ 4.05. Signals from the 2-propanol fragment were located as a methyl doublet at δ 0.28, and signals at δ 3.49, 2.72 and 3.23, indicating cercosporin. This was confirmed by comparison with an authentic sample of commercially available cercosporin from *C. hayii* and suggests that the toxin is cercosporin is probably causing the plant's death.

33.20 CONTROL OF *ASPERGILLUS FLAVUS* IN MAIZE USING BIOLOGICAL AND NATURAL MATERIALS. **Y.A.Y. Gibriel, F.I. Mursy, S.M. Mohsen, A.S. Hamza and G. Moghazy.** *P.O. Box 259, Maadi, Cairo 11728, Egypt. Email: yousef1000@yahoo.com*

It was found that *Saccharomyces cerevisiae* was not efficient in preventing the growth of *Aspergillus flavus* and aflatoxin production in stored maize. *Lactobacillus acidophilus* was able to decrease the growth of the fungus and its production of aflatoxin in storage. *Bacillus subtilis* was the strongest treatment among others which could inhibit the fungal growth and aflatoxin production when storage period reached 21 days. With addition of 0.05 ml marjoram per 100 g of maize, reduction of the *A. flavus* count reached 62.5% at 21 days of storage. Raising the level of marjoram to 0.1 ml inhibited the growth *A. flavus* completely at 7 days and up to the end of storage, also preventing aflatoxin production. This was also noted with higher marjoram levels. The minimum inhibitory level of mint to be used against the development of *A. flavus* was 0.3 ml per 100 g maize. This level was also able to prevent aflatoxin production. The minimum inhibitory concentration of salt to prevent *A. flavus* growth in maize was 1 g salt/100 g maize. This concentration can be successfully used in the storage of maize up to 21 days, and is also suitable to prevent aflatoxin production.

33.21 THE EMERGING PROBLEM OF *ASPERGILLLUS FLAVUS* IN SOUTHERN EUROPE. **P. Giorni, N. Magan, A. Pietri and P. Battilani.** *Via Emilia Parmense 84, 29100 Piacenza, Italy. Email: paola.battilani@unicatt.it*

In 2003, the summer was hot and dry in Europe and *Aspergillus* section *Flavi* caused severe problems on maize. High levels of aflatoxins (AFs) in grain produced in northern Italy resulted in the production of severely contaminated milk, with AFM_1 above the legal limit. As a consequence, batches of milk had to be discarded with severe losses for farmers. A quick response to this outbreak enabled short-term help to be given to farmers, with appropriate pre- and post-harvest guidelines, and research support for rational management of the maize chain. Several aspects were considered, starting from the characterisation of *A.* section *Flavi* isolates, regarding their ecology and nutrition, niche overlap with *Fusarium verticillioides*, and the effect of weather and cropping system on AF contamination and the influence of post-harvest storage conditions on fungi and mycotoxin contamination dynamics. The Italian population of *A.* section *Flavi* included almost all *A. flavus*, with 70% being AF producers. These strains seemed less thermophilic than those reported in other areas, with variable adaptation to ecological conditions. *A. flavus* was more competitive and dominated *F. verticillioides* when a_w was <0.90 or 0.90 and 30°C. Grain contamination at harvest was significantly influenced by meteorological conditions, especially dryness. Temperature lower than 10°C, 0.80 a_w or CO_2 at 50% limited *A. flavus* activity. All these data will be used to develop a predictive model to be included in a Decision Support System aimed to minimise consumer exposure to AFs.

33.22 THE EFFECT OF FUNGICIDE APPLICATIONS ON CONTENT OF MYCOTOXINS PRODUCED BY *FUSARIUM CULMORUM* AND *F. AVENACEUM*. **J. Horoszkiewicz-Janka, M. Korbas, E. Jajor and K. Pieczul.** *Institute of Plant Protection, Department of Mycology, ul. Miczurina 20, 60-318 Poznan, Poland. Email: J.Horoszkiewicz@ior.poznan.pl*

Fungi of the *Fusarium* genus infecting wheat ears cause quantitative and qualitative losses. The consequence of fusariosis can be reduction of grain weight, creasing of grains, weakened germination, further infection of grains with *Fusarium* and, mycotoxin contamination. Decrease of mycotoxin content in grains is possible by reducing ear infection with *Fusarium* with fungicides. However, not all active substances control fungi responsible for fusariosis effectively. In 2006 and 2007 plot experiments in 4 replications using 3 winter wheat cultivars were performed in our Field Experimental Station. At flowering, ears were inoculated by spraying with a spore suspension of *F. culmorum* and *F. avenaceum*. Two days after inoculation, fungicides (either triadimenol + tebukonazol or famoksat + flusilasol) were sprayed. Ear infection by fungi was evaluated at grain maturity. The average percentage of infected ear surface as well as quantitative and qualitative parameters of the crop were determined. Protein content was analysed and gluten content and sedimentation index were determined. Mycotoxin content was determined using HPLC, and content of deoxynivalenol (DON), nivalenol (NIV) and zearelone (F-2) was determined. Detection limit for NIV and DON was 0.01 mg/kg and 0.3 μg/kg for F-2. Fungicide treatment reduced ear infection by *Fusarium* spp. and significantly reduced mycotoxin content in grain.

33.23 SIRODESMIN: ROLE IN VIRULENCE OF *LEPTOSPHAERIA MACULANS* AND ORIGIN OF BIOSYNTHETIC GENE CLUSTERS. **B. Howlett.** *School of Botany, the University of Melbourne, VIC, Australia. Email: bhowlett@unimelb.edu.au*

Sirodesmin PL is a secondary metabolite toxin produced by *Leptosphaeria maculans*, which causes blackleg disease of *Brassica napus*. Its biosynthesis involves a cluster of 18 co-regulated genes and disruption of the two module non-ribosomal peptide synthetase gene prevents the production of sirodesmin PL. The non-ribosomal peptide synthetase mutant has less antibacterial and antifungal activity than the wild type and is half as effective in colonising stems, as shown by quantitative PCR analyses, indicating sirodesmin's role as a virulence factor in stems. The expression pattern of promoter GFP-fusions of the non-ribosomal peptide synthetase and an ABC transporter *in planta* is consistent with the distribution of sirodesmin PL as revealed by mass spectrometry experiments. Sirodesmin is a member of the epipolythiodioxopiperazine (ETP) class of toxins, which are involved in animal and plant diseases. Surveys of genome sequence data show putative ETP gene clusters in 14 taxa; these clusters are discontinuously distributed amongst ascomycete lineages. Such clusters appear to have a single origin and have been inherited relatively intact rather than assembling independently in the different lineages. Movement of entire clusters by horizontal gene transfer is the most parsimonious hypothesis to explain the discontinuous distribution of clusters. The ability of fungi to transfer gene clusters that encode toxins or virulence factors presents important implications, particularly for fungi that are plant and animal pathogens.

33.24 PREDICTION OF DEOXYNIVALENOL CONTENT IN WHEAT USING SPECTRAL REFLECTANCE. **O. Jirsa, K. Klem, I. Polisenska and J. Babusnik.** *Agrotest Fyto, Ltd., Havlickova 2787/121, Kromeriz, Czech Republic. Email: jirsao@vscht.cz*

The aim of the present investigation is to develop a method for screening and identification of samples contaminated by *Fusarium* mycotoxins using a sensor system based on measuring spectral reflectance. Relationships between spectral reflectance, deoxynivalenol (DON) content and amount of *Fusarium*-damaged kernels (FDK) in winter wheat samples were examined. Spectral reflectance was measured with an Avantes AVS S2000 spectrophotometer, a system transmitting the signal through a single optic fibre. The reflectance spectra of whole grains were assessed in the range from 400 to 1100 nm. Two types of samples were measured and the results compared: i) samples collected from one field and an identical cultivar, differing only in graded DON content obtained by various fungicidal treatments, and ii) a set of field samples from different locations and three harvest years. The samples were measured as a bulk and also some single kernel measurements were made. The curve of spectral reflectance for each sample was averaged from four separate measurements. The results were analyzed at 10-nm steps and the reflectance at these wavelengths was used for statistical analysis. For the samples of type i), correlation analysis demonstrated a close relationship between reflectance and DON, and also reflectance and FDK for wavelengths from 500 to 700 nm and around 1000 nm. For samples of type ii), significant year-to-year variation was observed. The results were obtained within research projects supported by the National Agency for Agricultural Research, Ministry of Agriculture, No. QG4007.

33.25 OCCURRENCE OF *FUSARIUM* SPECIES AND MYCOTOXINS IN ASPARAGUS SPEARS. **Z. Karolewski, A. Waskiewicz, M. Kostecki, L. Irzykowska, J. Bocianowski, P. Golinski, M. Knaflewski and Z. Weber.** *August Cieszkowski Agricultural University, Dabrowskiego 159, 60-594 Poznan, Poland. Email: karolew@au.poznan.pl*

The occurrence of *Fusarium* spp. and associated mycotoxins in asparagus spears in Poland was evaluated. In 2005 spears of two cultivars were collected from two locations, Swidwowiec (white spears of cv. Eposs and green spears of cv. Gynlim) and Poznan (green spears of cvs. Eposs and Gynlim), on sandy and sandy loam soil, respectively. In 2006 white and green spears of 'Eposs' were collected from Swidwowiec. Two years of observation showed that *Fusarium oxysporum* was detected on average in 24.2% of white and 3.4% of green spears, with *F. proliferatum in* 13.3% of white and 0.3% of green spears. *F. culmorum*, *F. heterosporum*, *F. scirpi*, *F. solani* and *F. verticillioides* occurred occasionally. *F. oxysporum* and *F. proliferatum* were found more frequently in the base than in the top of spears, and in epidermal more than in vascular parts. Chemical analyses revealed that fumonisin B_1 (FB_1) and moniliformin (MON) were present in the spears at levels expressed in µg of toxin per kg of spear fresh weight. In 2005, asparagus samples contained the mycotoxins as follows (µg kg^{-1}): FB_1 31.2-4095.3 and MON ND (not detected)-870.0 while in 2006 the levels were, respectively, ND-7.4 and ND-144.0. FB_1 and MON occurred both in spears in which *Fusarium* fungi were detected and in which they were not detected. It is necessary to find all sources of the mycotoxins, and more extensive work is needed to explain this fact.

33.26 DETECTION OF FUMONISIN-PRODUCING STRAINS OF *FUSARIUM VERTICILLIOIDES* AND GENES RELATED TO TOXIN PRODUCTION. **V. Karthikeyan, P. Titone, D. Spadaro, M.L. Gullino and A. Garibaldi.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: karthickeyanv@yahoo.com*

Fusarium verticillioides (Sacc.) Nirenberg (teleomorph *Gibberella moniliformis*) occurs worldwide on rice, and is known to produce various mycotoxins, including fumonisins which constitute a potential health hazard in rice and rice products. In order to have an early detection PCR-based technique, two primers, VERTF1 and VERTF2 (based on the IGS region of the the multicopy rDNA unit) were used on 42 strains of *F. verticillioides* isolated from rice collected from different locations of rice growing areas in north-western Italy. DNA was extracted using a modified CTAB method and quantified by spectrophotometer. Among the 42 strains, 17 were confirmed as fumonisin producers, amplifying a PCR product of 385 bp. The same 42 strains were subjected to another set of PCR primers specific for the gene *fum5*, and 15 of them gave a band at 845 bp, confirming the presence of a polyketide synthase. The primer specificity was confirmed with the strains ITEM-231 and ITEM-1746 of *F. verticillioides* coming from the ITEM collection of ISPA (Bari, Italy). The early detection of biosynthetic and regulatory genes will improve the control of fungal growth and toxin production and would help to minimize the application of chemicals.

33.27 MODELLING OF INTERACTING ENVIRONMENTAL FACTORS ON GROWTH OF MYCOTOXIGENIC SPOILAGE MOULDS. **N. Magan, D. Aldred and R. Parra.** *Applied Mycology Group, Cranfield Health, Cranfield University, Bedford, MK43 0AL, UK. Email: n.magan@cranfield.ac.uk*

Mathematical modelling has proved to be a valuable tool in the food industry to predict microbial growth as a function of environmental factors such a pH, temperature and water availability. However, less attention has been paid to filamentous fungi because of their mycelial growth habitat. Many studies have also only considered single factors in modelling fungal growth whether using growth/no growth thresholds, or empirical mechanistic approaches. The use of secondary polynomial model development using surface response contour plots has received particular attention for mycotoxigenic moulds. Using *Aspergillus* section *Nigri* group, *A. flavus* group and *Fusarium* species as examples we have examined the development of models which take into account interacting environmental factors of a_w and temperature using polynomial functions and validated these against different models for microbial growth available in the literature (e.g. Miles, Davey and Rosso). These types of approaches could have significant impacts on the development of prevention approaches as part of a HACCP system for minimising entry of mycotoxins into the food and feed chain.

33.28 THE OCCURRENCE OF TOXIGENIC *FUSARIUM* FUNGI IN GRAIN OF WINTER RYE AND TRITICALE AS AFFECTED BY FUNGICIDE USE. **A. Mankevičienė, I. Gaurilčikienė and S. Supronienė.** *Lithuanian Institute of Agriculture, Instituto av. 1, LT-58344 Akademija, Kėdainiai distr., Lithuania. Email: audre@lzi.lt*

Experiments conducted at the Lithuanian Institute of Agriculture in 2004 were designed to investigate the contamination of grain of winter rye 'Duoniai' and triticale 'Tornado' with *Fusarium* fungi and mycotoxins produced by them, as affected by spraying the crops with the fungicides propiconazole, tebuconazole, and azoxistrobine at the beginning of anthesis (BBCH 63). We analysed winter rye and triticale grain sampes for *Fusarium* species composition, and in rye grain we detected *F. avenaceum* (Fr.) Sacc, *F. sporotrichioides* Sherb., *F. poae* (Peck) Wollenw, *F. culmorum* (W. G. Sm.) Sacc., *F. graminearum* Schwabe, *F. solani* (Mart.) Sacc., *F. incarnatum* (Desm.) Sacc. And *F. sambucinum* Fuckel. In triticale grain we detected *F. culmorum*, *F. poae* and *F. heterosporum* Nees. The grain samples from winter rye plots sprayed with azoxistrobine were the most heavily affected by *Fusarium* (42.5%) and the highest contents of DON (691 μg kg^{-1}) and T-2 toxin (153.6 μg kg^{-1}) were identified in them. In the control treatment only 18.3% of grains were affected by *Fusarium* and lower contents of DON (69 μg kg^{-1}) and T-2 toxin (22.8 μg kg^{-1}) were determined. Tebuconazole reduced the amount of *Fusarium*-affected grain by 14.3%, but had no effect on mycotoxin production. Propiconazole and azoxistrobine had no effect on the spread of *Fusarium* in triticale grain; however, tebuconazole reduced the amount of the *Fusarium*-contaminated grain from 5.8 to 1.5%. The grain of triticale not sprayed with fungicides was more heavily contaminated with DON (427 μg kg^{-1}).

33.29 EFFECT OF PHENYLPYRROLE RESISTANCE MUTATIONS ON MYCOTOXIN PRODUCTION BY *ASPERGILLUS CARBONARIUS* AND *PENICILLIUM EXPANSUM*. **A.N. Markoglou, K. Vattis, K. Dimitriadis, E.G. Doukas and B.N. Ziogas.** *Pesticide Science Laboratory, Agricultural University of Athens, Greece. Email: markan@aua.gr*

Mutants of *Aspergillus carbonarius* and *Penicillium expansum* highly resistant to phenylpyrroles were readily isolated after mutagenesis and selection on media containing fludioxonil. Cross resistance studies with other fungicides showed that the mutation(s) for resistance to phenylpyrroles also reduced the sensitivity of mutant strains, but only to the aromatic hydrocarbon and dicarboximide fungicides. However, an increased sensitivity to strobilurin-type fungicides was observed in *A. carbonarius* mutant strains. Study of ecological fitness parameters showed that the resistance mutation(s) may or may not affect mycelial growth rate, sensitivity to high osmolarity, sporulation, conidial germination and pathogenicity. *In vitro* studies on the effect of mutation(s) on ochratoxin A (OTA) production showed that some mutant strains of *A. carbonarius* produced OTA at similar or even higher concentrations than the wild-type parent strain on culture medium. Contrary to the above, a total loss in ochratoxigenic ability was observed in the remaining mutant strains. However, studies with artificially inoculated grapes, showed that some non-ochratoxigenic mutant strains appeared to be ochratoxigenic. In the case of *P. expansum* all mutant strains produced patulin at concentrations lower than the wild-type parent strain, but a much greater amount of citrinin was produced in these isolates. In addition, in most of these mutant strains mycotoxin production was further increased when the mutants were grown on fludioxonil-amended medium. Our data indicate, for the first time to our knowledge, the potential risk of increased mycotoxin contamination of grapes and apples after intensive use of phenylpyrrole and dicarboximide fungicides.

33.30 SOME FACTORS INFLUENCING PATULIN PRODUCTION BY *PENICILLIUM EXPANSUM* IN APPLES AND PEARS. **A.M. Menniti, F. Neri, R. Gregori and M. Maccaferri.** *CRIOF-DIPROVAL, Alma Mater Studiorum, Università di Bologna, Viale Fanin 46, 40127 Bologna, Italy. Email: annamaria.menniti@unibo.it*

The objective of our study was to examine the effects of five *Penicillium expansum* isolates on patulin production in cultivars of apples and pears, in relation to incubation time, and incidence and severity of decay. These factors were evaluated in apples and pears inoculated with *P. expansum* and kept at 20 °C for short periods of time. The ability of five *P. expansum* isolates to grow and produce patulin in inoculated 'Golden Delicious' apples varied among the strains from below the limit of quantification to 662 μg kg^{-1}. The strains showed different pathogenicity and different capacities to produce the toxin. Variety and species of pome fruit influenced patulin production. In the same conditions of infection, *P. expansum* isolate PE97.IT produced higher patulin content in apples than in pears. The highest patulin production was 386 μg kg^{-1} in 'Golden Delicious'. No blue mould symptom appeared in pears inoculated with *P. expansum* and no patulin was detected in fruit after 3 days at 20°C. However, patulin increased with incubation time after 6 and 8 days. No patulin was detected in healthy pear tissue but it was high in the decayed area. Patulin control is therefore possible by using only healthy fruit, sorting damaged and rotten fruit before processing.

33.31 BEAUVERICIN PRODUCTION MARKEDLY DIFFERS BETWEEN GROUP 1 AND 2 ISOLATES OF *FUSARIUM SUBGLUTINANS* FROM MAIZE. **A. Moretti, G. Mulé, M. Láday, A. Ritieni, L. Hornok and A. Logrieco.** *CNR–Institute of Sciences of Food Production, Via Amendola 122/O, 70126 Bari, Italy. Email: antonio.logrieco@ispa.cnr.it*

Fusarium subglutinans (teleomorph *Gibberella subglutinans*), a globally distributed pathogen of maize that causes stalk and ear

rot, mostly in temperate regions, produces a range of mycotoxins including beauvericin (BEA). *F. subglutinans* has recently been split into two major groups, based on DNA sequence data for six nuclear regions. Since BEA production in this species varies widely in terms of both incidence and amount, with many strains reported as BEA non-producers, a Pan-European collection of the fungus originating mostly from maize was grouped by RFLP and tested for BEA production. Of the 62 isolates in Group 1, 48 (77%) produced from 10 to 532 μg/g of BEA, whereas none of the 39 Group 2 isolates synthesized detectable amounts of the toxin. The high correlation between RFLP group and BEA production is consistent with the existence of two reproductively isolated populations within *F. subglutinans* and shows that the toxicological risk from *F. subglutinans* isolates depends on the group to which they belong.

33.32 TOXIN PRODUCTION AND GENETIC DIVERSITY OF *FUSARIUM FUJIKUROI*, AGENT OF RICE BAKANAE DISEASE IN ITALY. **A. Moretti, S. Somma, AM Picco, M. Rodolfi and A. Ritieni.** *CNR–Institute of Sciences of Food Production, Via Amendola 122/O, 70126 Bari, Italy. Email: antonio.moretti@ispa.cnr.it*

In the last few years bakanae disease of rice, caused by *Fusarium fujikuroi*, has emerged as a major disease in northern Italy. Although only *F. fujikuroi* can cause the abnormal growth of stems that is the main typical symptom of bakanae, additional species of *Fusarium* are frequently associated with the disease and they are very similar morphologically to *F. fujikuroi*. This pathogen can also produce many toxins, such as fumonisins, beauvericin, and enniatins, which have different biological activities. We here report a biological, molecular and toxin-production characterization of *F. fujikuroi* strains isolated from plants of different rice varieties affected by abnormal stem growth. The plants came from different sites in northern Italy in 2004 and 2005. One hundred and thirty-six strains of *F. fujikuroi* were isolated from stems and roots of rice plants. Morphological identification, sexual fertility tests and sequencing of β-tubulin and calmodulin genes were used to identify *F. fujikuroi*. Genetic diversity of strains was investigated using AFLP analysis. *In vitro* toxin production of cultures was analyzed showing that beuavericin, enniatins and fumonisins were produced by many strains. These data show that *F. fujikuroi* is a pathogen frequently occurring on rice in Italy and the disease could become endemic in northern Italy. The ability of *F. fujikuroi* strains to produce fumonisins, beauvericin and enniatins and be sexually fertile is worrisome for the possibility that new pathotypes with higher toxin levels may be produced through sexual recombination and selected.

33.33 MOLECULAR IDENTIFICATION OF OCHRATOXIN-PRODUCING *ASPERGILLUS CARBONARIUS* ON GRAPES. **K. Muthusamy, D. Spadaro, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: davide.spadaro@unito.it*

Ochratoxin A (OTA) is a dangerous nephrotoxic and carcinogenic mycotoxin, produced by various *Aspergillus* and *Penicillium* species in different food products. OTA has also been detected in grape-derived products, such as wine. Ochratoxin contamination of grapes takes place in the field and is mainly caused by black aspergilli, among which *Aspergillus carbonarius* is the most important. To prevent OTA in grapes or other foodstuffs, there should be a rapid and specific method for early detection of toxigenic fungi in the vineyard. For grapes, this is particularly critical around harvest time, when contamination risk and OTA production become high. Morphological or microscopic methods are time-consuming and, often, mycological expertise is necessary. Many studies have described PCR-based methods that can be automated, giving more specific, sensitive and rapid detection of the target organism. In the present study, we designed two sets of specific PCR primers, AcPKS-F1/AcPKS-R1 and AcPKS-F4/AcPKS-R4 from a DNA sequence of a polyketide synthase (PKS) gene from *A. carbonarius* AC06, which amplify a 187 bp product. The primers were tested on different species of *Aspergillus*. Among the toxigenic fungi, i.e. *A. niger*, *A. tubingensis*, *A. flavus*, *A. japonicus*, *A. aculeatus* and *A. ochraceus*, only *A. carbonarius* gave a positive result, confirming the specificity of both primer sets for this key ochratoxigenic species on grapes.

33.34 *FvVE1* REGULATES FUMONISIN PRODUCTION AND PATHOGENICITY IN *FUSARIUM VERTICILLIOIDES*. **K. Myung, S. Li, N. Zitomer, H.K. Abbas, A.H. Glenn and A.M. Calvo.** *Department of Biological Sciences, Northern Illinois University, 1425 W. Lincoln Hwy., DeKalb, Illinois 60115, USA. Email: amcalvo@niu.edu*

The *veA* homologous genes are essential for biosynthesis of sterigmatocystin in *Aspergillus nidulans* and aflatoxin production in *A. parasiticus* and *A. flavus*. Whether *veA* homologs have a role in regulating secondary metabolism in other fungal genera is unknown. In this study, we examined the role of the *veA* homologous gene *FvVE1* on production of fumonisin toxins in the important plant pathogen *F. verticillioides*. Our studies indicate that deletion of *FvVE1* suppresses fumonisin production on corn (maize) and rice medium. Furthermore, corn plants grown from seeds inoculated with *FvVE1* deletion mutants did not show disease symptoms while plants grown from seeds inoculated with the *F. verticillioides* wild type and complementation strains clearly showed disease symptoms under the same experimental conditions. In this latter case, the presence of lesions coincided with accumulation of fumonsins in the plant tissues, and only these plant tissues had elevated levels of sphingoid bases, indicating disruption of sphingolipid metabolism. The effects of *FvVE1* deletion on toxin production and plant disease were found to be the same in two separate mating types. Our results strongly suggest that *FvVE1* is necessary for fumonisin biosynthesis and pathogenicity by *F. verticillioides*. The conservation of *veA* homologs among ascomycetes suggests that *veA* could play a pivotal role in regulating secondary metabolism and pathogenicity in other fungi. We propose *veA* as a potential target for implementation of a control strategy to prevent the devastating health and economic effects of plant pathogenic fungi.

33.35 MOLECULAR DETECTION AND BIOCONTROL OF TOXIGENIC *FUSARIUM VERTICILLIOIDES* IN MAIZE (*ZEA MAYS*, L.) GROWN IN INDIA. **S.R. Niranjana, S. Chandra Nayaka, N.M. Carmen and H.S. Prakash.** *DOS in Applied Botany and Biotechnology, University of Mysore, Manasagangotri, Mysore-570006, India. Email: srniranjana@hotmail.com*

Fusarium verticillioides is an important fungal pathogen in maize. It causes both pre- and post-harvest losses and also produces Fumonisins, secondary metabolites which are toxic and highly carcinogenic. We have attempted to develop molecular probes for the detection of toxigenic and non-toxigenic *F. verticillioides* in maize seed samples. We also screened *Trichoderma*

harzianum isolates from native rhizospheres for their potential to control toxigenic *F. verticillioides*. In PCR a single fragment about 800 bp indicated the presence of *F. verticillioides*, and a further 400 bp fragment was seen toxigenic isolates of *F. verticillioides*. Eight isolates of *T. harzianum* were obtained, and isolate Th-8 showed better antifungal activity than carbendizim. Th-8 was formulated in different solid substrates such as wheat bran, paddy husk, talcum powder and cornstarch. Maize seeds of kanchan, pioneer and sweet corn were selected for laboratory and field studies and these seeds were treated with conidial suspensions of *T. harzianum* at the rate of 1×10^8 spore/ml with formulation at the rate of 10 g/kg. It was found that the pure culture of Th-8 was more effective in reducing ear rot disease and fumonisin incidence, and it also increased seed germination, vigour index, yield, and thousand-seed weight. Next in efficacy was talc formulation, compared to the carbendizim-treated and untreated control. Treatment with Th-8 stimulated an early and higher expression of various defence enzymes like PAL, PO and PPO, which resulted in considerable reduction of ear rot disease.

33.36 *FUSARIUM* INFECTION AND MYCOTOXIN CONTENTS OF OATS UNDER DIFFERENT TILLAGE TREATMENTS. **P. Parikka, V. Hietaniemi, S. Rämö and H. Jalli.** *MTT Agrifood Research Finland, FI-31600 Jokioinen, Finland. Email: Paivi.parikka@mtt.fi*

Fusarium infection and mycotoxin contents of oat 'Belinda', 'Freja', 'Roope' and 'Veli' were studied in Finland in a field trial in 2004–2006 under autumn ploughing and direct drilling. The infection of developing kernels was investigated from panicle emergence until harvest. Trichotechene mycotoxins were analysed in grain 2-3 weeks before harvest and at harvest. The weather in 2004 was very rainy and cool, in 2005 warm and less rainy, while in 2006 it was warm and dry. *Fusarium* infection was detected immediately after panicle emergence on all cultivars. The first species identified was a T-2/HT-2- producing species *F. langsethiae*, but also *F. poae* was found in the early stages of kernel development. The deoxynivalenol-producing species *F. culmorum* and *F. graminearum* colonized the kernels later. In 2005 and 2006, *F. poae* was most abundant in autumn-ploughed plots. Direct drilling increased the infection of *F. avenaceum* and reduced *F. culmorum*. Tillage did not have much effect on *F. graminearum* and *F. sporotrichioides* infections. However, it increased *F. langsethiae* infection. Direct drilling decreased DON and NIV contents in grain, but seemed to increase T-2/HT-2 contens of oats. The amounts of DON detected were not high, the highest contents being 790 μg/kg on 'Roope' in 2006. T-2/HT-2 toxins were detected on all the cultivars, the amounts being highest in 'Belinda', 950 μg/kg in 2006. The nivalenol contents were high in 2005 due to heavy *F. poae* infection.

33.37 *FUSARIUM* PATHOGENS ON CEREALS AND OCCURRENCE OF THEIR TOXINS IN THE CZECH REPUBLIC. **I. Polisenska, J. Salava, L. Tvaruzek, G. Wolf, J. Weinert and P. Matusinsky.** *Agrotest Fyto, Ltd., Havlickova 2787/121, Kromeriz, Czech Republic. Email: ivana.polisenska@vukrom.cz*

The PCR method was used to observe the presence of *Fusarium avenaceum*, *F. culmorum*, *F. graminearum* and *F. poae* on samples of winter wheat and spring barley grown in the harvest years 2006 and 2007 at various locations in the Czech Republic. The winter wheat samples were also examined for relationships between the amount of *F. graminearum* and *F. culmorum* mycelial biomass and the content of deoxynivalenol (DON), zearalenone (ZEA) and T-2 toxin. The results were correlated with evaluation of the percentage of visually scabby kernels and percentage of kernels infected by *Fusarium* spp. pathogens, which was determined by incubation in a humid environment. Quantitative assessment of the presence of *F. graminearum* and *F. culmorum* as well as analyses of mycotoxin content were carried out using ELISA. Detection and quantification of the pathogens producing mycotoxins in cereal grain can provide much information significant for understanding disease epidemiology, developing control measures or improving resistance by breeding or biotechnology. It is also important for the determination of associations and study of relationships among qualitative parameters influenced by presence of the fungus. The contribution of these assessments to the estimation of mycotoxin contamination level is based on knowledge of the closeness of this relationship.

33.38 STUDIES ON OTA CONTAMINATION OF MUSTS AND WINES IN SOUTHERN ITALY. **S. Pollastro, C. Dongiovanni, C. Giampaolo, R.M. De Miccolis Angelini, F. Mingolla, A. Pichierri, M. Di Carolo, P. Pollastro and P. Natale.** *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. Email: stefania.pollastro@agr.uniba.it*

Ocratoxin A (OTA) is a common contaminant of food and drink all over the world. In 1996, it was first detected in wine, becoming a prominent safety problem for all grape derivatives. Nine years of studies, carried on in about 170 vineyards in the southern Italy, on over 25 cultivars, showed that *Aspergillus carbonarius* (Bainier) Thom, one of the fungi involved in secondary bunch rots, is the main source of OTA contamination in wine. Over 95% of *A. carbonarius* isolates proved able to produce OTA although secretion was broadly variable in time and amount. In vineyards, the fungus increased from the end of July to the end of August-September, being particularly abundant at vintage time on black-berry cultivars. For quantitative detection of *A. carbonarius* on grapes and in musts, a semi-selective medium and a real-time Scorpion PCR protocol were developed and validated. Detection of 10^5-10^6 conidia g^{-1} of must resulted in 80% probability level to have an OTA concentration higher than 1–1.5 ng kg^{-1} of must (EU maximum tolerable limit in wine: 2 ng kg^{-1}). Integrated management of cultural and crop protection practices in vineyards was found essential for preventing or reducing OTA contamination. Anilynopirimidines, fungicides commonly used on grapevine against grey mould, were the most effective in reducing *A. carbonarius* and OTA contamination. Several factors (grapevine cultivar and clone, growing area, training system, control of fungal diseases and pests, and agronomic techniques) exerted a heavy influence on the risk of OTA contamination.

33.39 MYCOTOXIN MENACE AND PEOPLES' ACCESS TO HEALTHY AND SAFE FOOD IN INDIA. **B.N. Reddy and C.R. Raghavender.** *Mycology and Plant Pathology Laboratory, Department of Botany, Osmania University, Hyderabad 500007, India. Email: reddybn1@yahoo.com*

Mycotoxins are gaining importance due to their deleterious effects on human and animal health. They occur frequently in the tropics because of high temperature, moisture, unseasonal rains and flash floods which further enhance fungal proliferation and mycotoxin production. The prevalence and level of human exposure to mycotoxins in India is alarming. The chronic health risks

are particularly prevalent in India where the diets are highly prone to mycotoxigenic fungi and mycotoxins due to poor harvesting practices, improper storage and transport. For the past three decades, several disease outbreaks due to mycotoxins were reported in the country wherein poultry, cattle and humans had lost their lives. The involvement of mycotoxins, especially incidence of aflatoxins and ergot alkaloids are much higher in these outbreaks than the fumonisins and trichothecenes (T-2, DON). Disease outbreaks due to mycotoxins continue to be problems of significant public health importance in the country. India has a staggering human population of more than 1 billion, of whom nearly 40% live below the poverty line. It is very difficult to imagine their access to completely safe and toxin-free food since people are forced to consume less expensive, poor quality food grains because of their poor purchasing ability. Mycotoxin contamination may develop as a result of fungal action before and after harvest and also during storage. The task ahead is challenging, particularly in highly populated parts of the world. Therefore, the strict control of food quality is necessary to avoid such disease outbreaks.

33.40 TOXIGENICITY OF *ALTERNARIA ALTERNATA*, THE CAUSAL AGENT OF ALTERNARIOSIS OF GROUNDNUT (*ARACHIS HYPOGAEA* L.). **M.N. Reddy.** *Department of Microbiology, S P Mahila University, Tirupati-517502, India. Email: mopuri_nr@yahoo.com*

Alternaria alternata (Fr.) Keissler, the causal agent of alternariosis/veinal necrosis of groundnut (*Arachis hypogaea* L.) was toxigenic when cultured on various laboratory media. The symptoms suggest that the fungus may be producing a powerful toxic metabolite(s) during pathogenesis. The cell free extract/culture filtrate induced necrosis and chlorosis on groundnut leaves, inhibition of root and shoot growth of germinating seeds and wilting/dehydration of cut shoots of seedlings. Five non-specific phytotoxins isolated from the culture filtrate, partially purified by TLC and identified by UV and IR spectral analyses using authentic samples, included alternaric acid, alternariol, alternariol monomethyl ether, altenuic acid and tenuazonic acid. Toxigenicity was monitored with standard bioassay techniques. Tenuazonic acid was also detected in the infected tissues, indicating that this toxin is produced by the pathogen during pathogenesis. The results demonstrate the pathophysiological significance of tenuazonic acid in the disease syndrome, indicating that it is probably a toxin *in vivo*. The toxin killed cell suspensions obtained by enzymatic digestion of groundnut leaflets only. The results are discussed in the light of the existing literature.

33.41 SOME OXYLIPINS CAN REGULATE TOXIN SYNTHESIS AND CONIDIOGENESIS IN *ASPERGILLUS PARASITICUS* AND *ASPERGILLUS OCHRACEUS*. **M. Reverberi, F. Punelli, A. Ricelli, S. Zjalic, M. Scarpari, M. Punelli, A. Dobson, A.A. Fabbri and C. Fanelli.** *Università La Sapienza, Dipartimento di Biologia Vegetale, Largo Cristina di Svezia 24, 00165 Roma, Italy. Email: massimo.reverberi@uniroma1.it*

In *Aspergillus parasiticus* and *Aspergillus ochraceus*, producers of aflatoxin and ochratoxin A (OTA) respectively, modulation of oxidative stress drives toxin biosynthesis and affects fungal growth and differentiation. Contamination by these fungi induces the formation of 9- and 13-hydroperoxyoctadecadienoic acid (HODE) in maize and wheat seeds; 9-HODE, from maize seeds, triggers conidiogenesis and toxin synthesis in *A. parasiticus* whereas 13-HODE inhibits both events. During seed colonisation, oxidative stress occurs in *A. parasiticus* mycelium and is sensed by the oxidative stress transcription factor Apyap1 which promotes the activation of antioxidant defences. The disrupted mutant D*Apyap1* promotes the release of peroxides on the seed surface, with respect to WT, and the are seeds also induced early to form lipoperoxides on their coat. This event induces earlier formation of aflatoxin in D*Apyap1* in comparison with WT. Also in wheat seeds infected by *A. ochraceus*, OTA synthesis and conidiogenesis seem to be modulated by the relative percentage of 9- and 13-HODE. In *A. ochraceus* several lipoxygenases *(lox)* form oxylipins which are secreted partially outside the mycelium. The knock-out mutant D*lox* presents a quantitative alteration of some oxylipins and D*lox*-contaminated wheat seeds produce more 13- than 9-HODE. Further OTA synthesis and conidiogenesis are both inhibited. In conclusion some oxylipins produced by mycotoxigenic fungi can affect lipoperoxidation pathways in infected seeds, which, in turn, produce lipoperoxides affecting toxin synthesis and conidiogenesis of *A. parasiticus* and *A. ochraceus*.

33.42 *CANDIDA SAKE* AND *PANTOEA AGGLOMERANS* AS BIOCONTROL AGENTS AGAINST PATULIN PRODUCTION IN STORED APPLES. **V. Sanchis, H. Morales, J. Usall, A.J. Ramos and S. Marin.** *Food Technology Department, CeRTA-UTPV, Lleida University, Spain. Email: Vsanchis@tecal.udl.cat*

Patulin, a mycotoxin produced primarily by *Penicillium expansum*, is currently of great concern because of its undesirable effects on human health. It has been proved that patulin can damage organs and tissues in animals and some studies revealed carcinogenic and teratogenic effects. Patulin is found mainly in low quality apples diverted to production of apple by-products. The use of chemicals is the most common procedure to prevent postharvest rots but legislation is becoming more and more restrictive. The use of biocontrol agents (BCAs) as an alternative is currently being proposed. The aim of this study was to evaluate the effect of two BCAs (*Candida sake* CPA-2 and *Pantoea agglomerans* CPA-1) on *P. expansum* growth and patulin accumulation in cold storage and further deck storage. Wounded apples were inoculated with a cell suspension of either *C. sake* or *P. agglomerans* and with a *P. expansum* conidial suspension. Apples were cold-stored at 1ºC until lesion diameter reached 2 or 4 cm. Half the apples of each treatment were further stored at 20ºC for three days before patulin analyses. The BCAs were found to control blue rot and patulin accumulation during cold storage. *C. sake*, however, seemed to be more efficient than *P. agglomerans*. Subsequent storage at room temperature should be avoided as the controlling effect of BCAs under cold storage weakens due to the accelerated development of rot during deck storage. The authors are grateful to the Spanish government (MEC, project AGL2004-07549-C05-01) for funding.

33.43 MANAGEMENT OF *FUSARIUM VERTICILLIOIDES* IN MAIZE. **A. Scandolara, A. Marocco, A.Pietri, V. Rossi, E. Mazzoni and P. Battilani.** *Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. Email: paola.battilani@unicatt.it*

Fusarium verticillioides is a maize pathogen causing ear and stalk rots worldwide; in grain it produces a family of mycotoxins called fumonisins. Fumonisin B_1 (FB_1), the most important, can damage health if eaten by animals or humans. The European Commission has fixed maximum admissible levels for fumonisins

(B_1+B_2) in maize and derived products, that are frequently exceeded in southern Europe. Since 2002, several research projects have been developed in Italy, to understand the maize–*F. verticillioides* pathosystem aiming at developing a Decision Support System. The main topics were: 1. ecophysiology of *F. verticillioides*; 2. trigging conditions for grain infection; 3. the role of cropping system on fumonisin production; 4. selection of maize lines resistant to infection; 5. direct control actions against *F. verticillioides*. The fungus is active from 5 °C to 40 °C, optimum 25–30°C, and above 0.90 a_w, optimum 0.99. Inoculum is always present in the field and, whether air- or splash-borne, easily reaches the ear where it can start infection along with growth stages, till ripening. Fumonisin is detected in grain from the early dough stage, and a cumulative effect is normally observed, that increases also when a_w of kernels is lower than 0.90. Some steps in crop management, like geographic region, hybrid variety, seeding period, manuring, irrigation and harvest time can influence fumonisin production, but meteorological conditions play the main role. Promising results have been obtained studying genetic resistance, but the use of new hybrids, and the control of the European corn borer and the fungus can contribute to reducing fumonisin contamination.

33.44* HIGH INCIDENCE OF *FUSARIUM AVENACEUM* (NECTRIACEAE, *GIBBERELLA*) AND MONILIFORMIN IN APPLES WITH WET CORE ROT SYMPTOMS. **H.-J. Schroers, J.L. Sørensen, U. Thrane, K.F. Nielsen, M. Žerjav, A. Munda and J. Frank.** *Agricultural Institute of Slovenia, Hacquetova 17, 1001 Ljubljana, Slovenia. Email: hans.schroers@kis.si*

In 2004-2006, fungi causing wet apple core rot (WACR) in various cultivars were inventoried in Slovene orchards. In 2730 mature Gloster apples, the full yield of 21 trees in 2004, ca 10% of apples developed white, rose or reddish mycelium in the core and a wet, light-brown rot extending destructively into the surrounding apple flesh. Almost consistently, *Fusarium avenaceum* (Fa) was isolated. A less exhaustive screening of apples in various Gloster, Golden Delicious, Fuji and Jona Gold orchards confirmed Fa as the predominant agent of WACR. However, infection rate of Golden Delicious apples was less than 2%. Fa strains grown on yeast extract sucrose agar formed, among others, the metabolites antibiotic Y, two chlamydosporols, aurofusarin, rubrofusarin and several enniatines. These were identified qualitatively by HPLC equipped with diode array detection, collecting UV spectra (HPLC-DAD/UV) of metabolites. In 9 of 15 apples naturally infected with Fa and showing WACR, 2000-9000 ppb moniliformin was detected by HPLC-DAD/UV analysis; the other samples had 150–900 ppb moniliformin. Artificial inoculations of rot-free apples with representative strains of Fa indicated that moniliformin is formed after 3–7 days in small amounts (measured by LC/MS) and can reach levels of 2000-4500 ppb (measured by HPLC-DAD/UV) within 3 weeks. Considering that apples containing WACR can be removed from the production line only with great difficulty, these results indicate that Fa can be a mycotoxin-related threat to human health. Mycotoxin analyses were done at the BioCentrum–DTU, Technical University of Denmark.

33.45 ARTIFICIAL NOSE DETECTION OF MYCOTOXINS IN MAIZE FLOUR. **S. Schürch, K. Gindro, T. Zesiger, O. Viret and F. Mascher.** *Agroscope Changins-Wädenswil Research Station, C.P. 1012, 1260 Nyon, Switzerland. Email: stephanie.schuerch @acw.admin.ch*

Several *Fusarium* species are known to colonise maize ears and contaminate the grains with toxins belonging to different chemical groups. These mycotoxins pose severe health problems to animals consuming contaminated feed. Therefore, a quick and relatively cheap method is necessary to detect material which contains mycotoxins. In preliminary tests, the use of an artificial nose to detect mycotoxin contamination in wheat samples yielded promising results. In the present study, we aimed at calibrating this artificial nose to detect contaminated maize flour. The ears of 14 maize varieties were artificially inoculated with *Fusarium graminearum*. The mycotoxin content of the ground grains was determined by HPLC analysis. Four mycotoxins were examined: deoxynivalenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol and zearalenone. The headspace (volatile fraction) of the samples was analysed with an artificial nose comprising a quadrupole mass spectrometer. By principal component analysis we identified the masses best correlated with mycotoxin content. First results showed that the artificial nose can distinguish contaminated from uncontaminated samples. Further development to obtain a quantitative response is under way.

33.46 *FUSARIUM* SPECIES AND THEIR MYCOTOXINS ON TRANSGENIC AND NONTRANSGENIC MAIZE HYBRIDS. **L. Slezakova.** *Crop Research Institute, Drnovska 507, 161 06 Praha 6 – Ruzyne, Czech Republic. Email: Slezakova@seznam.cz*

Potentially toxigenic species of *Fusarium* are widespread and common, infecting maize and causing a wide range of diseases. They reduce grain yield and quality and their secondary metabolites – mycotoxins, which can occur in infected grain, have adverse effects on humans and livestock. The most frequent and best known mycotoxins are deoxynivalenol, fumonisins, zearalenon, HT-2 and T-2 toxin. Toxigenic fungi enter maize through different routes, very often through damage created by insects, especially the European corn borer (ECB), the major pest of maize in the Czech Republic. This damage to plants and their ears is often the initial infection site for toxigenic species. Bt-transformed maize can reduce the occurrence of toxigenic species and minimize the risk of mycotoxin contamination in maize. Bt-maize contains the gene from the soil bacterium *Bacillus thuringiensis* expressing the Cry 1 Ab protein which is toxic to, and protects the maize from the European corn borer. During the years 2002-2007 different samples of corn (preharvest, harvest and postharvest) from different localities of the Czech Republic were collected. The main aim of this study was to analyse the spectrum of toxigenic species occuring in different samples of maize (preharvest ears, harvest and postharvest grains). The most frequent species were *Fusarium subglutinans, F. verticillioides, F. proliferatum, F. sporotrichioides* and *F. graminearum.* The efficacy of Bt-maize was showed in lowered occurrence of potentially toxigenic species. This study was supported by project No. 1B 53043 of Ministry of Agriculture of the Czech Republic.

33.47 WINES PRODUCED IN PIEDMONT, NORTHERN ITALY: PRESENCE OF *ASPERGILLUS* SPP. IN VINEYARDS AND OF OCHRATOXIN A IN WINES. **D. Spadaro, A. Lorè, A. Ciavorella, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: davide.spadaro@unito.it*

Ochratoxin A (OTA), produced on grapes by *Aspergillus* section *Nigri* (black aspergillus), in particular by *A. carbonarius*, constitutes a threat to health, particularly because it is nephrotoxic. The presence of black aspergillus is a key factor in the period be-

tween veraison and harvest, producing OTA in the grape bunches. During 2006, between change of berry colour and harvest, in three Piedmontese vineyards, the population of *Aspergillus* spp., and in particular *A. carbonarius* was monitored. Each vineyard was divided in plots and nine different chemical or biological treatments effective against *Botrytis cinerea* were applied. A low level of *A. carbonarius* was found, promoted by the adverse weather conditions that year. The study was repeated during 2007. The occurrence of OTA in wines was measured using an immunoaffinity column for clean-up, and liquid chromatography with detection by fluorescence. We analysed 594 wines, both red (mainly Barbaresco, Barbera, Barolo, Dolcetto, Nebbiolo and Roero) and white (Arneis, Chardonnay, Favorita, Moscato). The incidence of contamination was 39.6% with a low mean concentration level, never exceeding the limit of 2.0 ppb, established by European legislation. The white wines were, as expected, less contaminated than the red. Wines produced after the 2006 vintage contained a higher incidence of OTA (81.6% of samples contaminated), but never at worrying levels (a mean concentration of 0.089 ppb).

33.48 CONTAMINATION OF ITALIAN APPLE, PEAR, PEACH AND APRICOT JUICES WITH PATULIN PRODUCED BY *PENICILLIUM EXPANSUM*. **D. Spadaro, S. Frati, A. Ciavorella, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: davide.spadaro@unito.it*

Patulin is a secondary metabolite produced by *Penicillium expansum*, the causal agent of blue mould on apples. The pathogen is generally associated with damaged fruit or fruit already infected by other microorganisms in orchard as well as in postharvest conditions. A survey on the occurrence of patulin in commercial pure and mixed apple juices marketed in Italy was conducted during 2005. Patulin was detected in 34.8% of the samples. Mean levels of patulin were significantly lower in mixed than in pure apple juices, and levels of contamination were comparable in clear and cloudy juices. A similar incidence of positive samples was found in conventional and organic apple-based juices. Most of the studies on the incidence and level of patulin were carried out on apple-derived products. During 2006, a second survey was made on the presence of patulin in apricot, pear and peach juices commercialized in Italy. Patulin was found in 54.0% of the samples. Mean levels were significantly lower in apricot and pear than in peach juices. Except for one sample, the level of patulin was lower than 50 $\mu g\ kg^{-1}$. The high incidence of patulin contamination in apricot, pear and peach juices shows that its presence in other than apple products is not negligible.

33.49 EFFECT OF BIOCONTROL AGENTS ON ACCUMULATION OF PATULIN BY *PENICILLIUM EXPANSUM* IN DIFFERENT APPLE CULTIVARS. **D. Spadaro, S. Frati, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: davide.spadaro@unito.it*

Patulin is a secondary metabolite produced by different fungal species attacking food products, such as fruit, vegetables and cereals. The presence of patulin is usually associated with blue mould rot caused by *Penicillium expansum*, a post-harvest pathogen of apples and pears. Chronic health effects of patulin in rodents include genotoxicity, immunotoxicity, and neurotoxicity, while its effects on humans are not yet clear. In many areas, populations of *P. expansum* have developed that are resistant to the few fungicides admitted in the postharvest environment, so that alternative practices, such as biocontrol using antagonistic microorganisms could become important. Different biocontrol agents were evaluated for their capacity to reduce *P. expansum* attacks on apples, and their effect on patulin concentration in the final juice. The parts of the fruit attacked by the pathogen were analysed for patulin content, through extraction with ethyl acetate, purification with SPE columns and HPLC-DAD detection. Experiments were done with different apple cultivars, and cv Golden delicious appeared to be the most susceptible. Trials were carried out in controlled conditions, storing fruits at 20±1°C for 7 days or at 4±1°C for 28 days and later at 20±1°C for 7 days. Some strains of the yeast *Metschnikowia pulcherrima* significantly reduced *P. expansum* development. We will continue to research the mechanisms involved in biocontrol of patulin content.

33.50 MYCOTOXIN PRODUCTION BY *FUSARIUM PROLIFERATUM* AND *FUSARIUM VERTICILLIOIDES* ISOLATED FROM HOPS IN SERBIA. **S. Stankovic, J. Levic, T. Petrovic and V. Krnjaja.** *Maize Research Institute "Zemun Polje", Belgrade-Zemun, 11080, Republic of Serbia. Email: sstojkov@mrizp.co.yu*

Hop (*Humulus lupulus* L.) is commercially important as an essential flavoring in beer and is cultivated on no more than 600 ha in Serbia, although industry requirements are much greater. Fungi from the genus *Fusarium* can cause wilting, chlorosis and canker of hops in most hop-growing countries. Among them, *F. sambucinum* is the most frequent species. *F. proliferatum* has rarely been described as a parasite of hops, and for *F. verticillioides* no data is available. The objectives of this study were to investigate the toxicological profile of the *F. proliferatum* and *F. verticillioides* strains isolated from diseased hop plants, to study their possible fertility and assign them to a specific mating population. For fertility test, all strains were crossed twice to standard tester strains of the MPs A through G (Klittich and Lelslie, 1988). Fumonisin B_1, beauvericin and fusaproliferin extractions and analyses were performed by HPTLC according to the procedures described by Munkvold *et al*. (1998), Logrieco *et al.* (1993 and 1996) and Logrieco *et al*. (1996), respectively. Mycotoxin production of strains, tested for pathogenicity, was evaluated showing that all isolates of *F. proliferatum* and *F. verticillioides* produced 250 to 3000 $\mu g\ g^{-1}$ fumonisin B_1; five out of six isolates of *F. proliferatum* produced 400 to 500 $\mu g\ g^{-1}$ beauvericin; three strains of *F. proliferatum* produced 400 to 450 $\mu g\ g^{-1}$ fusaproliferin and all isolates of *F. verticillioides* produced up to 400 $\mu g\ g^{-1}$ fusaproliferin. All tested strains of *F. proliferatum* were MATD-2 and *F. verticillioides* MATA-1.

33.51 DETECTION OF *FUSARIUM VERTICILLIOIDES* IN MAIZE KERNELS BY QUANTITATIVE REAL-TIME PCR. **A. Susca, G. Mulè and A. Logrieco.** *CNR–Institute of Sciences of Food Production, Via Amendola 122/O, 70126 Bari, Italy. Email: antonio.logrieco@ispa.cnr.it*

Fusarium verticillioides is considered the main agent of fumonisin accumulation in maize plants. Molecular detection and quantification of *F. verticillioides* may generate new information and novel systems to control and reduce maize fungal colonization and to prevent fumonisins entering the food chain. TaqMan technology was developed to quantify *F. verticillioides* genomic DNA in naturally contaminated maize kernels. Specific primers and TaqMan probes were designed on the calmodulin gene se-

quence and quantification was based on a calibration curve constructed with serial dilutions of total DNA of *F. verticillioides* type-strain. The assay, combined with rapid DNA isolation based on magnetic purification, was well adapted for maize analyses, allowing throughput of a large number of reproducible assays (96-well format) in less than 4 h. This real-time PCR, which was found highly specific and sensitive, can be used to better understand the epidemiology of the fungus, and host/pathogen or environment/fungus interactions in order to improve management of maize ear rot disease.

33.52 *FUSARIUM* IN MAIZE: BIOLOGY, EPIDEMIOLOGY AND INTEGRATED CROP MANAGEMENT. **A. Suty-Heinze, I. Haeuser-Hahn and S. Dutzmann.** *Bayer CropScience AG, Alfred Nobel Str. 50, 40789 Monheim, Germany. Email: Isolde.Haeuser-Hahn@bayercropscience.com*

Different *Fusarium* species including *F. graminearum* and *F. verticillioides* cause damage to all parts of maize over the whole vegetation period. Depending on the climatic conditions and especially temperature, the dominating species will vary. Besides yield reduction, *Fusarium* spp. produce mycotoxins that have considerable negative impact on food and feed quality especially silage due to their toxicity but are also their suspected negative influence on ethanol production. Different fungicides used as a seed treatment or as foliar application are able to control different seed-, soil- and air-borne *Fusarium* species. Additionally, insecticides able to reduce the impact of vectors such as the European corn borer, which support infection with *Fusarium* spp., have a positive effect in decreasing the pest population. This allows reduction of disease caused by *Fusarium* spp. in the different plant organs, reduction of mycotoxin contamination of maize cobs.

33.53 *FUSARIUM* SPECIES AND MYCOTOXINS OF OAT IN ONTARIO, CANADA. **L. Tamburic-Ilincic.** *University of Guelph, Ridgetown Campus, 120 Main St. E, Ridgetown, Ontario, N0P 2C0, Canada. Email: tamburi@ridgetownc.uoguelph.ca*

Fusarium graminearum (Schwabe) [teleomorph: *Gibberella zeae* Schw. (Petch)] is a predominant species of *Fusarium* that is pathogenic to cereals in Ontario, Canada, and produces the mycotoxin deoxynivalenol (DON) in grain. The first objective of this study was to determine the *Fusarium* spp. and concentrations of mycotoxins from commercial oat fields grown in the same area as wheat and barley in Ontario. The second objective was to estimate DON level across cultivars grown in the Ontario Performance Trial after spray-inoculation with *F. graminearum*. Grain samples were retrieved from oat fields randomly selected across Ontario from 2005 to 2007. The top three *Fusarium* species were *F. graminearum, F. sporotrichioides* and *F. poae.* The highest concentrations of measured DON, HT-2, and T-2 were 0.3 ppm, 0.5 ppm and 0.2 ppm, respectively. Under natural infections, significant differences in DON content were observed among farm field locations, but not among cultivars. However, potential differences in DON accumulation amongst the cultivars grown in the Ontario Performance Trial were identified after spray-inoculation with *F. graminearum*. In this test, DON concentrations ranged from 0.1 ppm to 1.1 ppm depending on the cultivar in each of 2006 and 2007. This study supports the importance of monitoring *Fusarium* spp. and concentrations of mycotoxins in oat, especially during weather conditions that favor *Fusarium* infection and mycotoxin accumulation of susceptible oat cultivars.

33.54 MYCOTOXIC METABOLITES PRODUCED BY *FUSARIUM* SPECIES ASSOCIATED WITH *FUSARIUM* HEAD BLIGHT AND FEED REFUSAL DISORDERS IN WESTERN AUSTRALIA. **D. Tan, E. Ghisalberti, G. Flematti, M.J. Barbetti and K. Sivasithamparam.** *School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, WA 6009, Australia. Email: tand12@student.uwa.edu.au*

Fusarium head blight (FHB) in wheat is one of the world's most destructive plant diseases, decreasing both grain yield and quality. In 2004, FHB was detected on quality assurance samples of wheat grain and on summer cereal crop residues in Western Australia. There have been earlier reports on FHB outbreaks from eastern Australia, Canada, China, southern and eastern Europe, Japan, South America, and the USA. Accounts of the occurrence of *Fusarium* as plant pathogens are widespread and worldwide, and various *Fusarium* spp. have been found to attack a wide range of plants, including pasture species, cereals, maize, cotton, banana, and tomato. Sheep in six different locations in Western Australia were recently reported to partially or completely refuse to ingest annual *Medicago* pods, which were later found to be contaminated with a number of different *Fusarium* species known to produce deoxynivalenol (which causes vomiting and feed refusal in animals) and/or diacetoxyscirpenol (which causes reduced feed intake in animals) as part of their array of toxigenic secondary metabolites. Similar incidents have also been reported in South Africa and the central United States. This study aims to isolate and characterize the secondary metabolites produced by Western Australian *Fusarium* species, associated with cereal and pasture species, which have caused *Fusarium* head blight and/or feed refusal disorders in sheep, respectively.

33.55 SOURCES OF ERGOT INFESTATION IN WHEAT. **J.E. Thomas and V. Fanstone.** *National Institute of Agricultural Botany, Huntingdon Road, Cambridge, CB3 OLE, UK. Email: jane.thomas@niab.com*

Ergot (*Claviceps purpurea*) has increased in prevalence in the UK over the last five to ten years. Grass margins around fields that are sown as part of arable environmental schemes may be one possible source of ergot spores. Increasing levels of weedy grasses as a result of changing management practice may also harbour ergot inoculum. Ergot sclerotia were collected from grass species in field margins and the arable environment during a three year monitoring period. Inoculum from single sclerotial cultures was used to infect wheat plants in the field or glasshouse. Infectivity was assessed by counting ergot numbers produced. There was wide variation in infectivity of ergots from within a single host grass species and between species. Cocksfoot (*Dactylis glomerata*) is commonly used in sown grass margins, and is widespread in the arable environment. Over sixty samples of ergot were received from this species, and while some produced low numbers of ergot on wheat, others were highly infective. This grass species should be regarded as presenting a potential source of inoculum for wheat crops, and its use in field margins could increase the level of infective inoculum. Ergots from *Holcus lanatus* (Yorkshire Fog) and *Festuca pratensis*, (meadow fescue) which are both sown in field margins, had consistently low infectivity on wheat and could be used to reduce the risk of grass margins as sources of ergot inoculum.

33.56 EARLY EVALUATION OF MYCOTOXIN CONTAMINATION RISK IN MAIZE.E. Torelli, E. Gobbi, G. Bianchi, M. Baldini, R. Gubiani, F. Saccardo, S. Cividino, R. Locci and G. Firrao. *Dipartimento di Biologia Applicata alla Difesa delle Piante, Università di Udine, Via Scienze 208, Udine, Italy. Email: firrao@uniud.it*

In north-east Italy the occurrence of mycotoxins, and particularly fumonisin, on maize infected before harvest is of great concern for food and feed safety. To prevent the introduction of contaminated grain lots into the food chain, there is an urgent need for rapid methods for early assessment of contamination, since extraction and analysis of samples is time-consuming, and not suitable for routine analysis of grain at the time of delivery to drying and storage services. Here, we report the development and the evaluation of methods for early assessment of contamination risk. They include: (i) aereobiological analysis of fungal spores with a cyclone-type air sampler during maize harvest; (ii) proximal imaging analysis with near infrared illumination; and (iii) electronic nose detection of volatile metabolites associated with *Fusarium* infection. The prediction data were correlated with the content of different toxins (fumonisin, aflatoxin, ochratoxin) in field-collected maize samples as determined by ELISA and HPLC, and with quantitative data obtained by Real Time PCR of *FUM1* (a gene involved in fumonisin biosynthesis) and by ELISA of *Fusarium*-specific exopolysaccharides. Agronomic and environmental data (hybrid, seed date, harvest date, water content at harvest, irrigation, and pest management) were also integrated with the aim of developing risk assessment models and protocols for the Friuli Venezia Giulia region.

33.57 EFFECTS OF FOLIAR FERTILIZERS AND FUNGICIDES ON MYCELIAL GROWTH AND DEOXYNIVALENOL PRODUCTION OF *FUSARIUM GRAMINEARUM IN VITRO* AND *IN VIVO*. J.A. Verreet, M. Beyer, H. Klink, M. Klix, J. Guo and N. Scheider. *University of Kiel, Institute of Phytopathology, Germany. Email: javerreet@phytomed.uni-kiel.de*

Fungi of the genus *Fusarium* colonize various host plants, including crops such as wheat, essential for human nutrition. In wheat, *Fusarium* species cause *Fusarium* head blight (FHB), a disease that is accompanied by yield losses and contamination of the grain by various mycotoxins with adverse effects on animal and human health. Beside the influence of the cropping system (e.g. susceptibility of variety, crop rotation, soil management) on the epidemiology of *Fusarium* species, the control of head blight largely depends on a single group of active ingredients (DMIs) having the same mode of action, so the development of fungicide resistance seems to be only a question of time. Hence, compounds with different modes of action would be useful for integrated control strategies that focus on decreasing the risk of fungal resistance towards fungicides. Our objective was to evaluate the effects of foliar fertilizers on disease severity and DON production of *F. graminearum*. The *in vitro* and *in vivo* results illustrate the efficacy of fertilizers on the suppression of mycotoxin synthesis. The effects on different mycotoxins will be discussed in the context of spray timings, mycotoxin content, yield and mode of action.

33.58 MOLECULAR CHARACTERISTICS OF FUMONISIN-PRODUCING *FUSARIUM* SPP. FROM ASPARAGUS. S. von Bargen, O. Martinez, M. Gossmann and C. Büttner. *Section Phytomedicine, Lentzeallee 55/57, Humboldt-Universität zu Berlin, 14195 Berlin, Germany. Email: phytomedizin@agrar.hu-berlin.de*

Fusarium proliferatum causes, together with other *Fusarium* spp., crown rot of asparagus (*Asparagus officinalis*). This species as well as *F. oxysporum*, *F. redolens*, *F. subglutinans* and *F. verticillioides* are described as producers of the mycotoxin fumonisin. *F. proliferatum* isolates obtained from perennial asparagus plantings from Austria and Germany were included in a study of genetic variability and detectability of two essential genes of the fumonisin-gene cluster and compared with *Fusarium* species infecting crops. Genetic fingerprinting of 45 isolates revealed genetic heterogeneity of *F. proliferatum* by establishment of fourteen different fingerprint groups. Most isolates differentiated into three main fingerprint clusters, but no association was found between fingerprint group and origin of the isolates. By gene-specific PCR it was shown that, in all isolates tested, both initial genes of the fumonisin biosynthetic pathway -*FUM1*, encoding a polyketide synthase as well as *FUM8*, an aminoacyltransferase gene – were detectable. In *F. oxysporum*, *F. redolens*, *F. subglutinans* and *F. verticillioides* isolates these genes were sporadically detected. Verification of *FUM* genes suggests that these fungal strains are able to produce fumonisins and therefore can cause contamination of asparagus spears with this mycotoxin. Amplified *FUM* gene fragments exhibited nucleotide polymorphisms as was shown by sequencing, but were not discriminated by PCR-RFLP. Sequence variability of *Fum1* and *Fum8* gene fragments of the *F. proliferatum* isolates was below 1%. Interspecific divergence was considerably higher. Gene fragments from *F. verticillioides* shared 84% (*Fum1*) and 77% (*Fum8*) sequence identity with *F. proliferatum* sequences.

33.59 THE INFLUENCE OF CARBENDAZIM-RESISTANCE ON TRICHOTHECENE PRODUCTION BY *GIBBERELLA ZEAE*. Y.J. Zhang, X. Zhang, M.G. Zhou, J.X. Wang and P.S. Fan. *College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, P. R. China. Email: mgzhou@njau.edu.cn*

Gibberella zeae (anamorph *Fusarium graminearum*) causes head blight of cereals and contaminates grains with mycotoxins such as deoxynivalenol (DON). Previous work in our laboratory indicated carbendazim-resistance had no influence on mycelial growth, sporulation and production of perithecia. To investigate the influence of carbendazim-resistance on trichothecene production of *G. zeae*, 29 carbendazim-resistant and 29 wild-type sensitive strains of *G. zeae* generated from single conidia were randomly selected and incubated in GYEP medium under shake-culture in the dark. After 7 days, cultures were analyzed for trichothecene toxins by gas chromatography equipped with electron capture detector (GC-ECD). We found that the mycotoxin chemotype IA (deoxynivalenol, 3-acetyl deoxynivalenol) predominated in all Chinese strains both of carbendazim-resistance and wild-type. However, toxin production varied greatly in different strains and slightly in the conidium progenies. By comparing mycotoxin levels between tested sub-populations of carbendazim-resistant and wild-type sensitive strains, we found that total DON (sum of deoxynivalenol and 3-acetyldeoxynivalenol) produced in carbendazim-resistance strains was significantly ($p = 0.0065$) more than that in wild-type sensitive ones. DON toxins produced by carbendazim-resistance strains were 88.7 μg/g dry weight of mycelium in average, while it was only 23.4 μg/g in sensitive strains. Under field conditions, the influence of carbendazim-resistance on trichothecene production of *G. zeae* is still not clear. In order to elucidate the relationship between trichothecene production and carbendazim-resistance, *Tri* genes from carbendazim-resistant and wild-type strains will be used for gene expression analysis.

NATURAL COMPOUNDS AND DISEASE CONTROL

27.1 EFFECTS OF VOLATILE COMPOUNDS OF THE LIPOXYGENASE PATHWAY ON *COLLETOTRICHUM ACUTATUM*. **F.T. Arroyo, J. Moreno, P. Daza, L. Boyanova, B. De los Santos and F. Romero.** *IFAPA-Centro Las Torres-Tomejil, Apdo Oficial, Spain. Email: franciscot.arroyo@juntadeandalucia.es*

Volatile compounds characterising strawberry fruit aroma were tested for their antifungal activity against *Colletotrichum acutatum*, one of the causal agents of strawberry anthracnose. In this study, the effects of aldehydes, alcohols and esters, which are generated from the oxidative degradation of linoleic and linolenic acids through the lipoxygenase and hydroperoxide lyase pathway, were evaluated on development of *C. acutatum* conidia. (E)-2 Hexenal was the most effective of all tested volatile compounds to inhibit spore germination. Secondary conidia production or microcyclic conidiation and appressoria formation were also inhibited by strawberry fruit volatile compounds at similar quantities used for spore germination tests. Nevertheless, the maturation of appressoria was more sensitive, especially the pigmentation of the structure and formation of the penetration peg. Both processes were inhibited at 1.35 $\mu g l^{-1}$ for (E)-2 hexenal. Because this compound was the most effective to inhibit development of *C. acutatum* conidia, a study of its effect on conidial cells was carried out by transmission electron microscopy (TEM). The compound altered the structure of cell walls and plasma membranes causing disorganization and lysis of organelles, and eventually cell death.

27.2 NEW LEAF SPOT DISEASES OF CASHEW NUT AND THEIR MANAGEMENT BY AQUEOUS PLANT EXTRACTS. **A. Arya.** *Botany Department, Faculty of Science, The M.S. University of Baroda, Vadodara 390002, India. Email: aryaarunarya@rediffmail.com*

Search for environmentally sound pesticides received impetus following the publication of Silent Spring in 1962. Synthetic pesticides are toxic, pollutive and non-biodegradable; at the same time pathogens develop resistance against these compounds. Bioactive products of plant origin that are less persistent in the environment and are safe to mammals and other non-target organisms are the focus of attention today. Use of neem (*Azadirachta indica*) and basil (*Ocimum sanctum*) is recommended against a variety of pests. Pyrethrum, rotenone, ryania, *Acorus*, and nicotine (present in tobacco) have long been used as pesticides. Cashew nut is an important plantation crop of the country, valued for its nutritious seeds, wood and cashew nut oil. In Gujarat, cashew plantation has recently been initiated. Surveys of seedlings and young plants in nurseries revealed the presence of *Monascus*, *Gloeosporium* and *Cladosporium* rot of cashew nut leaves. Disease symptoms were recorded and isolations were made to obtain the pathogens in pure culture. It was found that these are new host records. We tested crude leaf extracts of six common plants *in vitro* and *in vivo* in an effort to find a control for these diseases. Aqueous leaf extracts of *Annona squamosa*, *Callistemon lanceolatus*, *Datura metel* and *Biota orientalis* where found effective against three leaf spot pathogens at 25% concentration.

27.3 ACTIVITIES OF PLANT EXTRACTS IN BIOCONTROL OF PHYTOBACTERIA. **G.M. Balestra, A. Quattrucci and A. Rossetti.** *Department of Plant Protection, University of Tuscia, Via S. Camillo de Lellis snc, 01100 Viterbo, Italy. Email: balestra@unitus.it*

In organic agriculture, bacterial pathogens may be controlled using cupric salts plus appropriate agronomical practices such as seed certification and balanced use of fertilizer. Here we report the results of using, *in vitro* and *in vivo*, extracts of plants in the Liliaceae and Moraceae at 10 g l^{-1} and 300 g l^{-1} concentrations, respectively to inhibit the growth and survival and to reduce the damage caused by bacterial pathogens on tomato plants (*Clavibacter michiganensis* subsp. *michiganensis*, *Pseudomonas syringae* pv. *tomato*, and *Xanthomonas vesicatoria*) and on kiwifruit (*P. syringae* pv. *actinidiae*, *P. syringae* pv. *syringae*, and *P. viridiflava*). In *in vitro* tests, both extracts showed antibacterial activity against all bacterial strains selected and at different concentrations (10^6–10^8 cfu ml^{-1}). In *in vivo* experiments, both natural extracts proved able to control the bacterial pathogens of tomato and kiwifruit in the plant phyllosphere for at least 10 days, even in the case of a high bacterial concentration (10^8 cfu ml^{-1}). For tomato bacterial pathogens, disease incidence and disease severity were reduced, at least for a time.

27.4 AN ORGANIC STRATEGY FOR OLIVE KNOT CONTROL. **G.M. Balestra, F. De Cesare, A. Vincenzi, A. Quattrucci and M. Muganu.** *Department of Plant Protection, University of Tuscia, Via S. Camillo de Lellis snc, 01100 Viterbo, Italy. Email: balestra@unitus.it*

We investigated the influence of compost activity to reduce survival of the olive knot pathogen (*Pseudomonas savastanoi pv. savastanoi* (Psav) and the damage (knots) it causes. Three-year-old olive plants cv. 'Frantoio', grown in pots with peat with or without 20% or 40% compost concentrations were experimentally contaminated with and without artificial injuries, to monitor disease severity and epiphytic survival respectively. We also studied whether hydrolytic enzyme activities in the rhizosphere of olive plants grown in compost could affect the epiphytic survival of Psav. A dose-dependency was observed. The greatest Psav reduction occurred at 20% compost amendment, in terms of both epiphytic survival (below 1×10^1 CFU/cm^2 after 14 days) and disease severity (33.3% after 90 days) while olive plants grown in absence or with 40% compost concentration, showed 3×10^2 CFU/cm^2 values of bacteria survival and 60% of disease severity after 14 and 90 days, respectively. At 20% compost amendment, beside the greatest phytobacteria suppression, rhizosphere acid phosphatase and β-glucosidase activities were greater than in peat and were negatively correlated with pathogen dynamics, thus suggesting that these enzyme activities might be involved in Psav biocontrol. The effect of 20% compost concentration was probably due to an indirect effect on olive plant growth.

27.5 *IN VITRO* TESTS AND FIELD TRIALS TO ASSESS THE EFFICACY OF A NATURAL FUNGICIDE TO CONTROL AIRBORNE STRAWBERRY DISEASES IN HUELVA (SOUTH-WESTERN SPAIN). **C. Blanco, B. de los Santos and F. Romero.** *Department of Plant Protection, Centro IFAPA Las Torres-Tomejil, 41200 Alcalá del Río, Sevilla, Spain. Email: cesar.blanco.ext@juntadeandalucia.es*

Strawberry (*Fragaria* × *ananassa* Duch.) is a worldwide crop. The Huelva province (south-western Spain) is one of the most important production area in Europe, with an acreage of more than 7,000 ha and a total production over 237,773 t in 2005. Powdery mildew (causal agent *Sphaerotheca macularis*) and grey mould (causal agent *Botrytis cinerea*) are two of the most important airborne strawberry diseases. Powdery mildew has been described in all areas were strawberries are grown and was con-

firmed in south-western Spain in 2002, reaching yield losses of 40%. Symptoms affect aerial parts of the plant. Disease control relies on application of chemical fungicides and sulphur. Grey mould is also widely present in strawberry crops, causing great yield losses and affecting fruit in post-harvest storage. Disease control strategies rely on application of chemical fungicides. DEFEND® is a natural product with fungicide activity. *In vitro* tests showed 100% growth inhibition for *B. cinerea.* During the 2006-2007 strawberry growing seasons, DEFEND® was used to control powdery mildew and grey mould at an experimental farm in Huelva. Its efficacy was compared to strawberry Integrated Production management (IP) fungicide schedules. Marketable fruit yield and incidence of both diseases were not statistically different on plants treated with DEFEND® than on plants under the IP management. This is valuable preliminary work to assess the product as an alternative to chemical treatments in order to reduce chemical inputs on IP strawberry crops.

27.6 ANTIMICROBIAL ACTIVITY OF CULTURE FILTRATES OF EDIBLE MUSHROOMS. **J.T. Chen and J.W. Huang.** *Department of Plant Pathology, National Chung Hsing University, Taichung, ROC. Email: jtchen@wufeng.tari.gov.tw*

Culture filtrates of 21 edible mushrooms were screened for antimicrobial activity against the following plant pathogens: *Colletorichum higginsianum, Fusarium oxysporum* f.sp. *lactucae, Pythium aphanidermatum, Rhizoctonia solani, Acidovorax avenae* subsp. *citrulli, Erwinia carotovora* subsp. *carotovora, Erwinia chrysanthemi, Pseudomonas syringae, Xanthomonas axonopodis* pv. *vesicatoria, Xanthomonas campestris* pv. *campestris and Xanthomonas campestris* pv. *oryzae.* None of the culture filtrates were inhibitory to mycelial growth of *C. higginsianum*, *R. solani* and *P. aphanidermatum*. Only *F. oxysporum f. sp. lactucae* was weakly inhibited by culture filtrates of *Agaricus blazei*, *Agrocybe cylindracea*, *Grifola frondosa*, *Ganoderma lucidum*, *Lentinus edodes*, and *Lepista nuda*, but they were not inhibited by culture filtrates of other mushrooms. A paper-disc agar-diffusion method was used to test the effect of mushroom culture filtrates on the growth of plant-pathogenic bacteria. Culture filtrates of *L. edodes* and *L. nuda* showed the strongest inhibitory effect against *X. campestris* pv. *campestris*. The culture filtrate of *A. cylindracea* showed different degrees of suppression against the bacteria tested, and the strongest suppression against *E. chrysanthemi*. Spraying green pepper plants with culture filtrate of *L. nuda* significantly reduced the severity of bacterial spot in the greenhouse.

27.7 SECONDARY METABOLITES FROM ANTAGONISTIC PGPR STRAINS OF *BACILLUS AMYLOLIQUEFACIENS* AND *BACILLUS SUBTILIS* FROM ARGENTINA. **G.H. Chiessa, V.A. Barrera, G. Sarti, L. Gasoni, S.S. Miyazaki and K. Kobayashi.** *Instituto de Microbiología y Zoología Agrícola, INTA, C.C. 25, 1712 Castelar, Argentina. Email: gchiessa@cnia.inta.gov.ar*

Different *Bacillus* strains are antagonistic bacteria widely applied in agriculture because of their ability to control plant pathogens and to improve plant growth. Better understanding of the interactions between antagonistic agents and plant pathogens is needed to optimize methods of application. Several bacterial strains isolated from natural and cultivated soils were tested for biocontrol activity against *Fusarium oxysporum* and *Rhizoctonia solani*. Liquid cultures of the most effective isolates were applied in greenhouse and field experiments on lettuce, potato and table beet. We finally selected two isolates to determine the secondary metabolites involved in interaction with the plant pathogens. In previous studies these isolates were identified using physiological and molecular analysis as *B. amyloliquefaciens* and *B. subtilis*. Secondary compounds were extracted with ethyl acetate from liquid cultures amended with tryptophan at pH 7 and 30°C. After filtration, extracts were dried under vacuum. Solubility fractionation was done using organic solvents and water, with further characterization by scanning spectrophotometry. The metabolites were produced during the bacterial growth phase and proved to be soluble in water. Absorbance peaks at 360 and 420 nm were observed for both strains. In addition, absorbance peaks from *B. amyloliquefaciens* were four times higher than from *B. subtilis*. Further studies are in progress to fully characterize and identify these metabolites.

27.8* CONTROL OF *PHYTOPHTHORA INFESTANS* AND *OIDIUM NEOLYCOPERSICI* IN TOMATO AND *BREMIA LACTUCAE* IN LETTUCE WITH ETHANOL EXTRACTS OF *INULA VISCOSA*. **Y. Cohen and S. Karavani.** *The Mina & Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat gan, Israel. Email: ycohen@mail.biu.ac.il*

Inula viscosa is a perennial wild composite native to the Mediterranean Basin. Our previous data showed that extracts made from air-dried leaves with a mixture of acetone and n-hexane (i) effectively control foliar diseases of potato, tomato, grape and wheat (Wang *et al*. 2004, Phytopathology 94, 1042-1047) and (ii) the active antifungal ingredients were mainly the sesquiterpenoid lactones tomentosin, inuviscolide and costic acid (Cohen *et al.* 2006, Phytopathology 96, 417-424). We now show that paste extracts made from *I. viscosa* leaves with ethanol and used in acetone as a carrier are highly effective (MIC=0.25%) against foliar diseases of tomato (late blight and powdery mildew) and lettuce (downy mildew). Formulated extracts were found efficient in controlling epidemics of *P. infestans* or *O. neolycopersici* in the field (good as the commercial standard or better), suggesting that they might be suitable for organic farming and/or low-input farming. Tomentosin and inuviscolid were shown to induce G2/M arrest and apoptosis in human melanoma cells (Rozenblat *et al*. 2007, Biochemical Pharmacology 74, *doi:10.1016/j.bcp.2007.08.024*). The biological mode of activity of these compounds against fungal plant pathogens is currently being studied in a similar manner. We acknowledge European Community financial support under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the Integrated Project QUALITYLOWINPUTFOOD, FP6-FOOD-CT-2003-506358.

27.9 THE USE OF ESSENTIAL OILS FROM LEMON GRASS AND CLOVE TO CONTROL POSTHARVEST ANTHRACNOSE OF MANGO FRUIT. **R. Duamkhanmanee.** *Faculty of Agricultural Technology and Agro-Industry, Rajamangala University of Technology, Suvarnnabhumi, Thailand. Email: d_raweewon@yahoo.com*

The efficacy of essential oil from lemon grass (*Cymbopogon citratus* Stapf.) and clove (*Eugenia caryophyllus*) was evaluated for control of postharvest anthracnose of mango fruit 'Nam Dokmai', compared with a fungicide before and after inoculation with spore suspension of *Colletotrichum gloeosporioides.* The study consisted of four experiments which were conducted at Rajamangala University of Technology, Suvarnnabhumi, Ayudhya

Huntra, using a completely randomized design with four replications. The result showed that mango dipped in lemon grass oil at 4,000 ppm concentration in hot water before and after inoculation gave the minimal disease score of 2.40 and 1.85 respectively. In a clove oil pre-inoculation study, we found that mango dipped in clove oil at 4,000 ppm in hot water gave the minimal disease score of 1.85. Hot water treatment after inoculation gave the lowest disease score, which was 1.45. The second and the third ranks were 1.85 and 1.95, which resulted from the application of carbendazim and clove oil in hot water respectively.

27.10 NATURAL ESSENTIAL OIL FROM LEMON GRASS AND CLOVE TO CONTROL POSTHARVEST ANTHRACNOSE IN A MANGO ORCHARD. **R. Duamkhanmanee.** *Rajamangala University of Technology, Suvarnnabhumi, 60 Moo 3 Huntra District Amphur Pranakorn Sri Ayudhya, Ayudhya Province, 13000, Thailand. Email: d_raweewon@yahoo.com*

This research aimed to study the efficiency of essential oil from lemon grass (*Cymbopogon citratus* Stapf.) and clove (*Eugenia caryophyllus*) on controlling postharvest anthracnose of mango fruit, 'Nam Dok Mai', caused by *Colletotrichum gloeosporiodes*. The experiment was conducted at Rajamangala University of Technology, Suvarnnabhumi, Ayudhya Huntra using completely randomized design with three replicates. There were eight treatments: lemon grass oil and clove oil at 4,000, 5,000 and 6,000 ppm, compared with carbendazim at 1,000 ppm and non-chemical control spraying. The best control was achieved by spraying with lemon grass oil at 5,000 ppm. The result showed an alternative way to control post-harvest anthracnose of mango fruit as a substitute for chemical treatments.

27.11 NATURAL COMPOUNDS INDUCING RESISTANCE IN WHEAT AND OILSEED RAPE AGAINST THEIR FUNGAL PATHOGENS. **V. Grigova, V. Sasek, L. Vechet and L. Burketova.** *Laboratory of Pathological Plant Physiology, Institute of Experimental Botany ASCR, Na Karlovce 1a, 160 00 Praha 6, Czech Republic. Email: grigova@ueb.cas.cz*

One of the alternative plant protection strategies is to induce resistence or elicit plant defence mechanisms that prepare plants for pathogen attack before appearance of disease in the field. Plants are treated with synthetic or biological inducers, which activate defence mechanisms without participation of the pathogen. Our experiments are based on our previous research, when an outstanding decline of infection with *Blumeria graminis* sp. *tritici* was found on the susceptible wheat cultivar Kanzler following treatment of plants by both synthetic inducers and plant extracts. We focus here on stimulation of plant defence responses by inducers (ethephone, benzothiadiazole, salicylic acid, glycine betaine, aminobutyric acid, chitosan, jasmonic acid), collagen hydrolysates, and extracts prepared from knotweeds (*Reynoutria sachalinensis*, *Reynoutria bohemica*, *Reynoutria japonica*), ginger (*Zingiber officinale*) and oak bark (*Quercus robur*) in the pathosystems: wheat–*B. graminis* sp. *tritici* (powdery mildew) and oilseed rape– *Leptosphaeria maculans* (blackleg). Efficiency of the inducers was evaluated in inoculation tests, with disease symptoms measureded using image analysis and scale, and monitoring expression of genes encoding PR-proteins and other genes involved in the defence response. The work is supported by grant No. QH72117 from the Ministry of Agriculture of the Czech Republic.

27.12 EFFECTS OF ESSENTIAL OILS AND SURFACTANTS ON CONIDIAL GERMINATION OF *BOTRYTIS CINEREA*. **T.F. Hsieh and C.H. Chen.** *Floriculture Research Center, Agricultural Research Institute, COA, KuKeng, YunLin, ROC. Email: tfhsieh@wufeng.tari.gov.tw*

Essential oils from citronella grass (*Cymbopogon nardus* Rnedl.), peppermint (*Mentha piperita* L.) and lemongrass (*C. citratus* Stapf.) and the surfactants AOS97P (sodium α-olefin sulfonate), AS95N and AS97P (sodium lauryl sulfate) were tested for their activity against germination of *Botrytis cinerea* conidia. Each essential oil or surfactant was diluted to 125, 250, 500 and 1,000 ppm and tested for antifungal activity by the glass slide method and water agar plate method. Results showed that the latter method was better for testing the antifungal activity of essential oils. Among three essential oils tested, lemongrass oil was the best in suppression of conidial germination. The volatile substance from lemongrass oil at 250 ppm was also inhibitory to conidial germination. Our results also showed that AS95N and AS97P at 250 ppm, and AOS97P at 1000 ppm were inhibitory to conidial germination. When AS95N was tested as a surfactant, the rate of conidial adhesion to cover glasses was significantly decreased at 250 ppm. Lemongrass oil and surfactant AS95N were chosen as the active ingredients of a plant protectant "Mold Stop". Gray mold of *Phalaenopsis* caused by *B. cinerea* was significantly decreased when *Phalaenopsis* petals were sprayed with lemongrass oil or AS95N at 250 ppm, or Mold Stop at 500 or 1000 ppm. They were as effective as the chemical Topsin M at 400 ppm.

27.13 INDUCIBLE DEFENCES ELICITED BY A NEW CHITOSAN FORMULATION PREVENT POWDERY MILDEW INFECTION IN GRAPEVINE AND MELON. **M. Iriti, G. Di Tommaso, S. D'Amico, V. Picchi and F. Faoro.** *Istituto di Patologia Vegetale, Università di Milano, Italy. Email: franco.faoro@unimi.it*

Elicitors, such as microbe-associated molecular patterns (MAMP) or products of avirulence (avr) genes, can trigger plant defence mechanisms when recognized by host receptors (Iriti and Faoro 2007, Mycopathologia, 164, 7-64). It has been shown that chitosan, a deacetylated chitin derivative, acts as a MAMP, by binding to a specific receptor (Cheng and Xu 2005. J. Integrat. Plant Biol. 47, 452-456), thus eliciting systemic immunity in some pathosystems. In this study, a new chitosan formulation (Valagro, 5-1570) has been tested as an inducer of resistance against powdery mildew in grapevine and melon. Two grapevine cultivars (Trebbiano and Montepulciano) were sprayed weekly with solutions of 5-1570 at different concentrations, or with commercial formulations of Penconazole and Methildinocarp as negative controls, from 21 May to 13 August. All the 5-1570 concentrations tested were effective in controlling powdery mildew, though the best concentration was 0.05%. In this case, disease severity was 2.39% in the treated grapes, compared with 87.5% in the untreated ones and 0.92% in fungicide-treated plants. Similar results were obtained in melon, confirming the efficacy of the formulation. The polyphenol profiles of treated grape berries at veraison and at harvest were investigated, showing that with Trebbiano no significant differences from the controls were found in most of the examined compounds. Instead, in Montepulciano berries some polyphenol classes, particulary flavonols and stilbenes (including trans-resveratrol), were significantly higher in 5-1570-treated plants.

27.14 PHLOEM MOBILITY OF SALICYLIC ACID AND ITS HALOGEN DERIVATIVES. **C. Jousse, J.-F. Chollet , F. Rocher and J.-L. Bonnemain.** *UMR CNRS 6161 & 6514, Université de Poitiers, 40 Avenue du Recteur Pineau, F-86022 Poitiers Cedex, France. Email: cyril_j@yahoo.com*

Salicylic acid (SA) is an essential secondary signal for both local resistance and systemic acquired resistance (SAR). In response to pathogen attack, SA level rises in phloem sap. This is well known but SA membrane transport in plant cells is poorly documented. Systemicity of SA and some halogen derivatives was predicted with Bromilow and Kleier models. The ability of exogenous SA and halogen derivatives to accumulate in phloem of castor bean (*Ricinus communis* L.) was evaluated by HPLC analyses of phloem sap. SA accumulated in the phloem to high levels (~10-fold at pH 4.6). The analog 3,5-dichlorosalicylic acid (3,5-ClSA), predicted to be immobile according to the models, also moved in the phloem at a concentration near to that of the incubation medium. The analog 5-chlorosalicylic acid (5Cl-SA), predicted to be poorly mobile, also accumulated in phloem sap (~6-fold at pH 4.6). The discrepancies between the predictions and our data suggest that the ion-trap mechanism may not be the sole process involved in uptake of SA and its halogen derivatives. The halogen derivatives could be considered in practice as stimulators of the plant defence system as they are known to be more efficient than SA itself.

27.15 ANTIFUNGAL COMPOUNDS IN ROCKMELON FRUIT AND THEIR POSSIBLE INVOLVEMENT IN DISEASE RESISTANCE. **V. Kumar and R. McConchie.** *Faculty of Agriculture, Food and Natural Resources, The University of Sydney, NSW 2006, Australia. Email: v.kumar@usyd.edu.au*

Resistance of rockmelon fruit to disease decreases with maturity. Although latent infections occur in field conditions, disease development is rare until the fruit are mature. The resistance of rockmelon fruit of different ages grown under glass was determined by inoculating the fruit with *Fusarium oxysporum* f. sp. *meloni*. The disease severity data showed that fruit of 7, 14 and 21 days after anthesis (DAA) showed greater resistance against the pathogen as compared to 28, 35 and 42 DAA. This decrease in resistance of fruit could be attributed to the lower level of antifungal compounds in mature fruit compared to young fruit. Extraction of antifungal compounds from fruit of all ages indicated that there are at least two phenolic antifungal compounds with R_f 0.35 and 0.18 on TLC plates. Presence of both antifungal compounds in the acid-hydrolysed fraction indicates that both are glycosylated. The concentration of both compounds decreased with maturity. Agar plate bioassay showed that both compounds had antifungal activity against *F. oxysporum* f. sp. *meloni* and *Cladosporium cladosporioides*.

27.16 CHITOSAN AND *PYTHIUM OLIGANDRUM* IN CONTROL OF *PHYTOPHTHORA INFESTANS*. **H. Kurzawinska and S. Mazur.** *Department of Plant Protection, University of Agriculture, Kraków, Poland. Email: smazur@chello.pl*

In Poland potato late blight caused by fungus *Phytophthora infestans* (Mont.) de Bary is still an economically important and very dangerous disease affecting potatoes. Our experiments were carried out both *in vitro* and *in vivo*. We evaluated *in vitro* the bio-preparations Biochikol 020 PC (B.A.S. chitosan) and Polyversum (B.A.S. *Pythium oligandrum*) on the linear growth of *P. infestans* mycelium. The preparations were applied at 3 concentrations. The studies were carried out using the Kowalik & Krechniak method (1961). The field experiment was conduced in 2005-2007 at the Mydlniki Experimental Station of the Academy of Agriculture near Kraków. The aim was to test the effect of dressing potato sets and spraying plants with Biochikol 020 PC and Polyversum on the top leaves and tubers infected by *P. infestans*. As a standard fungicide, Vitavax 2000 FS (B.A.S. karboxin and thiuram) was used. In vitro, *P. infestans* mycelium linear growth was significantly inhibited (in comparison to the control) only at 2% concentration of Polyversum and Biochikol 020 PC. However, the field evperiment showed that all preparations tested reduced *P. infestans* infection in top leaves and tubers. The studies were financed by The Ministry of Science and Information (grant No. PO6R 001 29).

27.17 ISOLATION AND CHARACTERIZATION OF A 63 KDA PROTEIN WITH ANTIMICROBIAL ACTIVITY FROM LEAVES AND STEM OF *ASPARAGUS COCHINCHINENSIS*. **Y.-C. Lai and L.-C. Chen.** *Department of Plant Pathology, National Chung Hsing University, Taichung 402, ROC. Email: lcchen@dragon.nchu.edu.tw*

Gel filtration on Sepharose 4 B, ion-exchange on DEAE cellulose and C_{18} reverse-phase HPLC were used to isolate an antifungal protein, ACAP *(Asparagus cochinchinensis* antimicrobial protein), from the leaves and stem of *Asparagus cochinchinensis* (Lour.) Merr. The ACAP with a molecular mass of 63 kDa was identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The SDS-PAGE profile of purified ACAP under nonreducing conditions had a major band of 63 kDa. Under reducing conditions, the 63 kDa band was absent and the intensities of 33 and 30 kDa bands were enhanced. This protein precipitated only between pH 4 and 9 and was stable in phosphate buffer between 4 and 75°C. It could inhibit the mycelial growth of some phytopathogenic fungi such as *Rhizoctonia solani* and *Botrytis cinerea* in vitro, and exhibited antibacterial activity toward *Ralstonia solanacearum*. Specific agglutination activity, SOD activity, rRNA N-glycosidase activity, glycosylase activity, deoxyribonuclease activity and phosphatase activity were also characterized. However, chitinase activity, ribonuclease activity and β-1,3-glucanase activity were absent. ACAP was labeled with a fluorescent dye using NHS–fluorescein to elucidate its antifungal activity at the cellular level. The fluorescent signal indicated that ACAP interacted with the fungal mycelium of *R. solani*. Cell-free protein synthesis inhibition assay showed that ACAP inhibited protein synthesis in rabbit reticulocyte lysate. The IC_{50} (the concentration of ACAP giving 50% translation inhibition) of ACAP was approximately 388 ng/ml. All these characters demonstrate that this protein has potential antimicrobial activity to defend the plant from disease.

27.18 OPTIMIZING CONDITIONS FOR THE FERMENTATION OF NATAMYCIN BY *STREPTOMYCES LYDICUS* A02. **W.C. Liu, J.Y. Qiu, T. Liu, C.G. Lu, D.W. Liu and L.X. Chen.** *Institute of Plant & Environment Protection, Beijing Academy of Agriculture & Forestry Sciences, Beijing 100097, P. R. China. Email: liuwccn@yahoo.com.cn*

Streptomyces lydicus A02, an antagonistic strain isolated from forest soil in a Beijing suburb, was able to produce efficient antifungal substances which strongly inhibited many plant pathogenic fungi. A main active component was identified as Natamycin, a

macrolide polyene antibiotic widely used as a natural biological preservative in the food industry. It is a new use for *S. lydicus* to produce Natamycin for the control of plant diseases. Here we report the result of optimization for Natamycin production in shake-flask fermentation conditions by strain A02. The components of the fermentation medium were selected by changing one variable at a time. The concentration of each component was optimized with uniform design. The optimized formulation was: 1.5% soybean meal, 0.1% peptone, 1.2% soluble starch, 1.2% sucrose, 0.5% NaCl, 0.025% $MgSO_4 \cdot 7H_2O$, 0.02% KH_2PO_4, 0.1% $CaCO_3$, initial pH7-8. For determining an appropriate combination of other culture factors, an orthogonal experiment was planned. The result showed that the optimized fermentation process was as follows: 2.5 ml of liquid seed cultured at 28°C for 24–28 h was inoculated into a 500 ml flask with 50 ml of optimized fermentation medium and incubated at 31°C, shaken at 240 rpm for 120 h. The diameter of the inhibiting zone to *Botrytis cinerea* presented by the fermented broth was up to 52 mm, over 90% higher than that produced by the broth with basic medium and initial fermentation process.

27.19 EFFECT OF CRUDE PLANT EXTRACTS OF ZHIMU AND RENDONGTENG ON THE IN VITRO GROWTH OF PLANT PATHOGENIC FUNGI. **C.-T. Lo, S.-Y. Lue and L.-S. Shi.** *Department of Biotechnology, National Formosa University, 64 Wenhua Rd. Huwei, 63208 Yulin, ROC. Email: ctlo@sunws.nfu.edu.tw*

Use of natural plant products including Chinese herbs and their products in agroecosytems is now emerging as one of the prime means to protect crop produce and the environment from pesticidal pollution, which is a global problem. In this study, we used the inexpensive Chinese herb plants zhimu (*Anemarrhena asphodeloides* Bge.) and rendongteng (*Lonicera japonica* Thumb.) to determine their effect on phytopathogenic fungi. *In vitro* tests for the efficacy of plant crude extract of *R. anemarrhenae* and *L. japonica* against *Rhizoctonia solani*, *Sclerotium rolfsii*, *Colletotrichum higginsianum*, and *Alternaria brassicicola* were done. Crude extracts of the two plants, unless otherwise stated, were prepared as follows. One kg of the herbs was cut into small pieces, extracted twice with MeOH (2-3 l × 2) under reflux, and then concentrated under reduced pressure to give 135 to 140 g of dark brown syrup. The test used 8 replications on mycelial growth and conidial production of the tested pathogens on PDA with the extract at concentrations of 0, 0.1, 0.5, 1.0, and 5.0 %. The crude extract of *R. anemarrhenae* at >200ppm significantly reduced mycelial growth over 70% and inhibited 90% sporulation of the fungi tested. However, the crude extract of *L. japonica* at 1000 ppm could inhibit only 40–70% of mycelial growth of the fungi tested.

27.20 ANTIFUNGAL ACTIVITY OF *PENICILLIUM STRIATISPORUM* PST10 AND ITS BIOCONTROL EFFECT ON *PHYTOPHTHORA* ROOT ROT OF CHILLI PEPPER. **Y. Ma, Z.Z. Chang, J.T. Zhao and M.G. Zhou.** *50 Zhongling street, Nanjing, Jiangsu Province, P. R. China. Email: myzjtzyn@hotmail.com*

Penicillium striatisporum Pst10 was isolated from the rhizosphere of chilli peppers. In dual culture agar plate assays, this isolate showed very high antagonistic effects on mycelium growth of *Phytophthora* spp., *Cladosporium cucumerium, and Sclerotinia sclerotiorum.* In *in vitro* assays, the toxicity of sterilized liquid culture filtrates (SLCF) of Pst10 grown in potato dextrose broth (PDB) was tested against *Phytophthora capsici* mycelium growth and sporangia/spore formation or germination. SLCF completely inhibited mycelium growth and even at a 100-fold dilution led to abnormal mycelium. A 20-fold dilution of SLCF inhibited formation and germination of sporangia and spores. Three antifungal substances were separated by thin-layer chromatography (TLC) from organic solvent extracts of Pst10 liquid culture filtrate. We obtained four different fractions by HPLC, and each one had antifungal activity. One fraction of the four was identified as Calbistrin A, a member of a rare class of fungal metabolites. Composted pig manure slightly increased the colonization of the chilli rhizosphere by Pst10. In pot tests, the incidence of *Phytophthora* blight of chilli was significantly reduced when artificially infested soil was treated with conidia and SLCF of Pst10. This appears to be the first study reporting the potential of *Penicillium striatisporum* as a biological control agent of soil-borne plant pathogens.

27.21 APPLICATION OF BIOACTIVE PLANT SUBSTANCES FROM OLIVE TISSUES AND GRAPES IN NON-CHEMICAL DISEASE CONTROL. **T. Mavrakis, L. Skaltsounis, N. Magan and F. Ververidis.** *Department of Plant Sciences, P.O. Box 1939, Technological Educational Institute of Crete, Heraklion 710 04, Greece. Email: ververidis@teicrete.gr*

Grape or wine polyphenols have beneficial effects for human health, andhave been linked to several functions in plants, such as defence against invading pathogens. In a similar manner, phenolic compounds in *Olea europaea* tissues have pharmacological properties and are considered natural antioxidants, thus inhibiting the Gram-positive microorganisms involved in olive fruit fermentation. Oleuropein, the main phenolic compound present in *O. europaea* fruits and leaves and its aglycon, obtained from oleuropein by hydrolysis, are well-known pharmacologically active molecules with potential applications as antimicrobial agents in some fairly common olive tree diseases. Moreover, oleuropein (derived from olive tissues) and its derivatives (derived from olive mills waste waters) have a variety of biochemical roles, including anti-inflammatory and antithrombotic activities. Our aim is to exploit these natural antioxidants by using them as phytoprotective agents against various known economically important pathogens in Greece. Our current findings show high antimicrobial activity of oleuropein against phytopathogenic bacteria. We have determined the minimum inhibitory concentration (MIC) of those compounds against various economically important pathogens such as Gram-positive or Gram-negative bacteria. They have also been shown to inhibit or delay the rate of radial growth of some plant-pathogenic fungi. The antimicrobial activity of these substances has been tested *in vivo* against various commercial varieties of vegetables and fruits and compared with other known organic chemicals (chitosan) or often used agrochemicals. This work is funded by a research grant PEP-Crete 2006 (GSRT) awarded to F.V.

27.22 BIOPESTICIDES AND OTHER NATURAL PRODUCTS EVALUATED FOR MANAGING POWDERY MILDEW IN CUCURBITS CROPS. **M.T. McGrath.** *Department of Plant Pathology, Cornell University, Long Island Horticultural Research and Extension Center, 3059 Sound Avenue, Riverhead, NY, 11901-1098, USA. Email: mtm3@cornell.edu*

Several types of US EPA-defined biopesticides (botanical oils, hydrogen dioxide, potassium bicarbonate, microbial pesticides) and other products approved or being developed for disease con-

trol in organically-produced crops were evaluated for powdery mildew (caused by *Podosphaera xanthii*) in pumpkin through three field experiments conducted in 2004 to 2006. All 14 products tested are protectants (no mobile activity). All provided some control on upper leaf surfaces when applied weekly beginning after mildew detection at the action threshold (1 of 50 older leaves affected) using a tractor-sprayer. The best level of control obtained on upper leaf surfaces based on AUDPC values in the three experiments was 52 to 76% for Eco E-RASE (jojoba oil), 46 to 87% for Organocide (sesame oil), and 57 to 92% for Microthiol Disperss (sulfur). None of these were significantly different from the 72 to 93% control obtained with Bravo Ultrex (chlorothalonil), a conventional protectant fungicide commonly used by growers to control powdery mildew. Three other products provided control that was not significantly different from that obtained with Bravo in one of the two experiments in which they were tested. Control was 86% with Prev-Am (sodium tetraborohydrate decahydrate), 77% with JMS Stylet-oil (mineral oil), and 73% with AgriLife (citric acid). Three parallel experiments were conducted in 2007 to evaluate MilStop (potassium bicarbonate) and Organocide on resistant and susceptible cultivars of muskmelon, butternut squash and pumpkin.

27.23 *IN VITRO* AND *IN VIVO* SYNERGIC EFFECT OF ANTIFUNGAL PLANT METABOLITES AGAINST *SCLEROTIUM ROLFSII*. **R. Montes-Belmont.** *Centro de Desarrollo de Productos Bioeticos, Instituto Politécnico Nacional, Apdo postal 24, 62731, Yautepec, Morelos, Mexico. Email: rbelmont@ipn.mx*

Finding alternatives to methyl bromide is an unfinished task in control of soilborne diseases. In previous work several plant metabolites were found to inhibit *in vitro* the development of *Sclerotium rolfsii* the causal agent of southern blight disease; minimum lethal doses of these compounds ranged from 0.01% to 5%, so only those substances that could be applied at very low concentrations would be likely to be used as a potential control measure. In this work combinations of the metabolites carvacrol, geraniol, eugenol, thymol and citronellol at half of their lethal doses were tested. Combinations of two or three metabolites were added to potato dextrose agar media, then sown with disks of *S. rolfsii* mycelium; after 10 days data on fungal growth inhibition were recorded. The best 8 combinations of metabolites were applied to soil infested with *S. rolfsii*, and tomato plants were transplanted in this in a complete randomized greenhouse experiment. Results showed that only citronellol lost its fungicidal property in combination with the other metabolites, and the other treatments had a synergic effect. The best treatments were geraniol-eugenol, and eugenol-carvacrol with less than 10% of dead plants, followed by geraniol-eugenol-thymol with 12% of dead plants. The control treatment had 70% of dead plants. Eugenol-carvacrol treatment also increased the height and weight of plants.

27.24 FUNGICIDAL ACTIVITY OF CHITOSAN AGAINST *COLLETOTRICHUM* SPP. **A. Moret, Z. Muñoz and S. Garcés.** *Departament de Biologia Vegetal, Facultat de Biologia, Avgda. Diagonal 645, 08028 Barcelona, Spain. Email: mmoret@ub.edu*

The objectives of this study were to evaluate the antifungal properties of chitosan and to assess its role in the protection of tomato fruit against a *Colletotrichum* sp. isolated from infected tissues of *Dracaena sanderiana*. The isolate was tested in vitro using PDA amended with five concentrations of chitosan (0, 1, 1.5, 2, and 2.5%). Chitosan significantly ($p<0.05$) inhibited the radial growth of this fungus, with a marked effect at the three highest concentrations after 7 days of incubation. The estimated concentration that reduced radial growth to 50 % (EC_{50}) was 2.28%. Tomato fruits coated with aqueous solutions of 1.0 and 2.5% (w/v) chitosan, were artificially inoculated with *Colletotrichum* and incubated at 4 ºC and 25 ºC for 10 days. Lesion diameter and percentage of diseased fruits were recorded. Fruits coated with chitosan at the higher concentration (2.5%) and stored at 4 ºC gave the best results for delay of softening and disease incidence. There were significant differences ($p<0.05$) between the effects of incubation temperature at each chitosan concentration. Lesion size at the highest chitosan concentration varied from 0.5 to 1.1 cm in fruits stored at 4ºC for 10 days, with disease incidence of 16.67%, compared with lesions ranging from 0.8 to 1.3 cm in fruits incubated at 25ºC and an incidence of 58.3%.

27.25 GROWING CROPS OF *BRASSICA JUNCEA*, THEN INCORPORATING THEIR RESIDUES GIVES COMPLEMENTARY CONTROL OF *RHIZOCTONIA* ROOT ROT OF SUGAR BEET. **N. Motisi, F. Montfort, T. Doré and P. Lucas.** *Biology of Organisms and Populations applied to Plant Protection, INRA Agrocampus Rennes, BP 35327 F-35653 Le Rheu, France. Email: natacha.motisi@rennes.inra.fr*

Brassica species are nowadays increasingly used as catch crops with the aim of suppressing soil-borne pathogens, though effectiveness of this practice is not always demonstrated. A way to understand the irregular efficacy of catch crops in the field is to separate the mechanisms by which they act upon soil-borne pathogens. In this study, we assumed that catch crops, during their growth phase, can have a negative effect on soil-borne pathogens and that this effect is enhanced after crushing the crop at flowering and incorporating residues into the soil. To test this, a 2-year field study was done with different managements of a *Brassica juncea* catch crop, in a sugar beet-winter wheat rotation, to analyze its action on sugar beet root rot caused by *Rhizoctonia solani*. Three treatments, mustard pulled out at flowering (MP), mustard crushed at flowering and incorporated into soil (MC) and bare soil (BS) as control were set up and assessed for their effect on root rot incidence and severity. In both years, disease incidence was higher in BS plots than in either MP or MC plots and was significantly higher in MP plots than in MC plots only in the second year. MC treatment seemed to reduce disease severity compared to MP and BS treatments. These results suggest that both root and aerial parts of mustard can reduce rhizoctonia root rot and that growing mustard has a suppressive effect on disease incidence while incorporation of mustard as green manure could give further control by reducing disease severity.

27.26 USE OF VOLATILE PLANT COMPOUNDS AS POSTHARVEST BIOFUMIGANTS TO CONTROL FRUIT DECAY. **F. Neri, M. Mari and P. Bertolini.** *CRIOF-DIPROVAL, University of Bologna, Via Gandolfi 19, 40057 Cadriano (BO), Italy. Email: fiorella.neri@unibo.it*

Some volatile compounds naturally occurring in plant products commonly used in the human diet were evaluated as fruit postharvest biofumigants against *Penicillium expansum*, *Monilinia laxa*, *Phlyctema vagabunda* and *Botrytis cinerea*. The most consistent fungicidal activity was found with some isothiocyanates (allyl-isothiocyanate, 4-methyltiobutyl-isothiocyanate and butenyl-isothiocyanate), followed by *trans*-2-hexenal, carvacrol, citral and *trans*-cinnamaldehyde; other compounds such

as hexanal, (-)-carvone, *p*-anisaldehyde, eugenol and 2-nonanone gave progressively lower inhibition. The *in vitro* activity of the volatiles was not always confirmed *in vivo.* Among the isothiocyanates tested, allyl-isothiocyanate provided the best control of brown rot in peaches and nectarines (80-100% efficacy, with 0.04 mg l^{-1}) without negative effects on fruits. Allyl-isothiocyanate (0.7 mg l^{-1}) also produced a significant reduction of blue mould infection on pears (over the 50%), although some phytotoxicity symptoms appeared on fruit skin after cold storage. *Trans*-2-hexenal significantly reduced *P. expansum*, *M. laxa* and *B. cinerea* infections in pome fruits, stone fruits and soft fruits (grapes and strawberries), respectively, whereas it failed to control *P. vagabunda* rot on apples. The results with *trans*-2-hexenal (12.5 μl^{-1}) on 'Golden Delicious' apples were particularly interesting, where the compound greatly reduced blue mould (98% efficacy) and fruit patulin content, without any detrimental effects on fruit. In contrast, *trans*-2-hexenal caused phytotoxic symptoms in apricots, nectarines, peaches, strawberries and 'Abate Fetel' pears, and off-flavours in plums, 'Conference' and 'Bartlett' pears, 'Royal Gala' apples and 'Italia' grapes. Carvacrol, citral or *trans*-cinnamaldehyde had little positive effect or failed to control the decays.

27.27 PROTECTION OF PRUNING WOUNDS ON KIWIFRUIT BY *TRICHODERMA HARZIANUM* AND EFFECTS ON THE HOST PLANT. L. Neri, R. Baraldi, F. Osti and S. Di Marco. *CNR-Istituto di Biometeorologia,Via Gobetti 101, 40129 Bologna, Italy. Email: s.dimarco@ibimet.cnr.it*

Decay of kiwifruit (*Actinidia deliciosa* var. *deliciosa*) is a recently discovered chronic wood disease, widespread in Italy, which causes reduced productivity and longevity of vineyards. The disease is highly dangerous, is usually underestimated and is difficult to eradicate once present. Different pathogenic fungi, above all *Phaeoacremonium aleophilum* and *Fomitiporia mediterranea*, are known to cause the disease. The main route for infection in plants appears to be through pruning wounds. In order to develop low-impact strategies for sustainable agriculture, we investigated the use of *Trichoderma harzianum* as a potential defence agent on summer and winter pruning wounds, to prevent disease development. During the summer, 1-year-old shoots from potted plants were cut and immediately treated with a *T. harzianum* suspension; the formation of healing callus on wounds was followed, and a better developed callus, together with a shorter callusing period, were observed in treated plants compared to the control. The physiological mechanisms involved in wound healing were investigated by measuring the concentration of the growth promoter hormone indole-3-acetic acid (IAA) in the pruned shoots, using gas chromatography-mass spectrometry (GC-MS). Changes in the level of endogenous free IAA were observed during the first 8 days after pruning, suggesting a possible implication in callus formation. Furthermore, the response of winter pruning wounds, treated with *Trichoderma*, to artificial infection with *Phaeoacremonium aleophilum* was investigated, through re-isolation of the pathogen from inoculated shoots. The results are discussed.

27.28 STRATEGIES FOR THE CONTROL OF *COLLETOTRICHUM CAPSICI*, CAUSE OF ANTHRACNOSE OF PEPPER. A. Nwankiti, E.J. Ekefan and C. Nduagu. *Crop and Environmental Protection, P.O. Box 2373, University of Agriculture, Makurdi, Nigeria. Email: alphookey213@yahoo.com*

Partial or complete control of anthracnose of pepper can be achieved, depending on the plant extract and dosage used. Leaf extracts of plants at 1% w/v stimulated radial growth of *Colletotrichum capsici* from 12.6% (control) to 26.7% and significantly reduced sporulation from 85.9% (control) to 48.4%. The stem and bark extract of *Cochlospermum planchonii* completely inhibited *C. capsici* spore production. Radial growth in treatments containing a stem-bark extract of *Ocimum gratissium* was significantly lower at all concentrations compared to other extracts from other plants. Percentage reduction in radial growth was highest (50%) when the stem-bark extract of *O. gratissium* was used at 1% w/v than when 3% w/v (44.3%) and 5% w/v (41.9%) were used. The root-bark extract and the concentrations at which they were tested were more toxic to spore production (80.0–64.3%) than on radial growth (66.6–58.4%). Although the stem-bark extract of *V. amygdalina* stimulated sporulation 41% at 5% w/v. The root-bark extract significantly reduced sporulation (64.3%) at 5% w/v. The incidence of seed-borne *C. capsici* in naturally infected pepper seeds was significantly ($P<0.05$) reduced by root-bark extracts of *V. amygdalina* and *Azadirachta indica* (87.5%) compared with *A. senegalensis* (62.5%) and the control. Chemical screening showed the presence of glycoside, saponin, tannin, alkaloid and flavonoid in the stem and root bark of plant extracts which showed antifungal activities.

27.29 GAMMA-IRRADIATION AS A METHOD OF CONTROLLING ROOT-KNOT NEMATODES. G. Palazova and D. Miteva. *Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, "Acad. G. Bonchev" Str. Building 25, Sofia, 1113, Bulgaria. Email: gpalazova@yahoo.co.uk*

Meloidogyne spp. root-knot nematodes are widespread, highly pathogenic plant parasites. Countermeasures are costly and may not be effective. The goal of our work was to investigate the influence of gamma-irradiation occurred in the plant endoparasitic nematodes with the appropriate dose radiation treatment. The susceptible host-plant banana (*Musa* spp.) carrying the nematodes was subjected to gamma-irradiation with ^{137}Cs. The roots were treated to release the eggs from the egg masses according to Hussey and Barker (1973) and the hatched larvae were introduced into sterile soil containing the non-invasive host-plants (*Impatiens valeriana* L.). Gamma-irradiation of the host-plants with low doses (10 kGy) was stimulating for the physiology including blossoming, fasciculation, and leaf mass, and at the same time this dose of 10 kGy totally destroyed the mature female nematodes in the root galls. The dose of 10 kGy led to a high level of mortality of the eggs in egg mass formation and a small percentage of hatch from eggs into vermiform second stage juvenile larvae (J2), which were sterile and incorporated in host-plants without reaching mature females. The eggs protected within a gelatinous matrix retained barely 5% vitality. In this study we showed that the 10 kGy dose of gamma-irradiation is lethal for root-knot nematodes and at the same time it is stimulating for the host-plants.

27.30 INVESTIGATION OF DIFFERENT *PLEUROTUS* SPECIES AS BIOCONTROL AGENTS AGAINST *HETERODERA SCHACHTII* IN THE GREENHOUSE. P. Palizi, E. Mohammadi Goltapeh, E. Pourjam and N. Safaei. *Dept. of Plant Pathology, College of Agriculture, Tarbiat Modares University, P.O. Box 14115-336, Tehran, Iran. Email: pariisa_p@yahoo.com*

The aim of the study was to determine the extent and ability of oyster mushrooms to attack and kill the beta cyst nematode

(*Heterodera schachtii*). The ability of *Pleurotus* species *P. ostreatus*, *P. sajor-caju*, *P. florida*, *P. flabellatus*, *P. ostreatus* (sporeless) and *P. eryngii* to prey on the cyst nematode was confirmed. Nematodes were inoculated on water agar plates on which a single sparse fungal colony of one of the fungi grew. Nematodes were immobilized quickly after being inoculated on plates of each *Pleurotus* species, with killing of about 95.96%, 87.56%, 87.75%, 74.5%,75% and 54.32% nematodes, respectively. The efficacies of old mushroom compost from cultures of *P. ostreatus* and *P. sajor-caju* in controlling cysts on *Beta vulgaris* (ICI) were studied under greenhouse conditions. The results showed that 100 and 200 grams of old mushroom compost per 3 kilograms pot could significantly control cysts, reducing by more than 85% cysts in soil, cysts on the roots and larvae inside roots compared with the contol treatment.

27.31 ANTAGONISTIC EFFECTS OF EXTRACTS OF OYSTER MUSHROOMS (*PLEUROTUS* SPP.) ON THE NEMATODE *PRATYLENCHUS VULNUS*. **P. Palizi, E. Mohammadi Goltapeh, E. Pourjam and N. Safaei.** *Dept. of Plant Pathology, College of Agriculture, Tarbiat Modares University, P.O. Box 14115-336, Tehran, Iran. Email: pariisa_p@yahoo.com*

Nematicidal activities of culture filtrates of six *Pleurotus* species, *P. ostreatus*, *P. sajor-caju*, *P. florida*, *P. flabelatus*, *P. ostreatus* (sporeless) and *P. eryngii* against *Pratylenchus vulnus* were studied *in vitro*. The fungi were grown on malt extract agar for 21 days at room temperature (25°C). The cultures were filtered through Whatman paper and sterilized by milipore filters (0.45 µm). *Pratylenchus vulnus* nematodes were added to 2 ml of each culture filtrate, and distilled water was used as control. Extracts of *P. ostreatus* could paralyse 88% of nematodes whereas those of *P. ostreatus* (sporeless) and *P. eryngii* were not significantly different from the control. The fatty acid compositions of different oyster mushrooms were studied by using filtrates. The fatty acids in the filtrates were obtained by Folch system extraction with chloroform/methanol (2:1) and derivation of their methyl esters. The fatty acids were identified and quantified by gas chromatogeraphy. Fatty acid compositions varied among species and the dominant ones in all oyster mushroom species were palmitic, oleic, stearic and linoleic acids. Linolenic acid levels were low in all species, and *P. eryngii* extracts, least effective on the nematodes, did not contain measurable levels of linolenic acid. The most important fatty acid (most effective agent for killing nematodes) in oyster mushroom species was linoleic acid, since concentrations of this acid were high in all six fungal species.

27.32 ANTIFUNGAL ACTIVITY OF SALACEYIN A AGAINST *COLLETOTRICHUM ORBICULARE* AND *PHYTOPHTHORA CAPSICI*. **C.N. Park, D. Lee and B.S. Kim.** *Division of Biotechnology, College of Life Sciences & Biotechnology, Korea University, Seoul 136-713, Republic of Korea. Email: biskim@korea.ac.kr*

Through a screening program to identify endophytes that produce biologically active secondary metabolites, we identified two novel salicylic acid derivatives (salaceyin A and salaceyin B) from the culture broth of *Streptomyces laceyi* MS53 that was isolated from a stem of *Ricinus communis* L. The antifungal activity of novel salicylic acid derivatives, salaceyin A, 6-(9-methyldecyl) salicylic acid, and salaceyin B, 6-(9-methylundecyl) salicylic acid were evaluated against plant pathogenic fungi. Salaceyin A showed antifungal activity against *Cladosporium cucumerinum, Colletotrichum orbiculare* and *Phytophthora capsici* at 64 µg ml^{-1} while salaceyin B was less effective. Salaceyin A showed potent *in vivo* control against *Phytophthora* blight in pepper plants. The disease was effectively suppressed at 500 µg ml^{-1}, which was comparable to the commercial fungicide, metalaxyl. Salaceyin A suppressed anthracnose development on cucumber leaves in a concentration-dependent manner. The control efficacy of salaceyin A against *C. orbiculare* infection was similar to chlorothalonil when applied prior to pathogen inoculation.

27.33* BIOFUMIGANT EFFECT OF *BRASSICA CARINATA* ON *PHYTOPHTHORA* SPP. IN STRAWBERRY FIELDS. **M. Porras, E. Romero, C. Zurera, C. Barrau and F. Romero.** *IFAPA-Centro Las Torres-Tomejil, CICE, Junta de Andalucia, Apartado 41200, Alcalá del Río, Sevilla, Spain. Email: maria.porras.ext@juntadeandalucia.es*

Biofumigation is based on the action of volatile compounds, essentially isothiocyanates, produced by the hydrolysis of members of the *Cruciferae.* Biofumigation with *Brassica carinata* and soil solarization were tested for effectiveness in reducing *Phytophthora* spp. soil populations and enhancing strawberry production. Experiments were conducted in a strawberry farm in Moguer (Huelva, SW Spain), for two consecutive growing seasons from October to May (2005-06 and 2006-07). Plots, never treated with methyl bromide, were naturally infested by *Phytophthora* spp. Treatments were soil solarization (S), biofumigation+solarization (B+S), and the untreated control (C). Biofumigation with *B. carinata* (10 $kg.m^{-2}$ at 10-cm depth aprox.) was done in July and plots were solarized and drip-irrigated from July to September, using clear 50-µm low-density polyethylene mulch. Of all treatments, B+S increased plant growth (foliar surface), fruit weight, and strawberry yield the most, each year. Plant growth differences were observed after solarization alone and the untreated control, with foliar surface (cm^2) of B+S/S/C of 502/414/351, and 435/346/228 in January 2006 and 2007, respectively. Furthermore, solarization alone increased plant growth and strawberry yield relative to the control. Fruit weight (g/fruit) of B+S/S/C was 25/22/17 and 26/24/23 in 2006 and 2007, respectively. In addition, both treatments reduced *Phytophthora* soil populations relative to the control. The current work, supported by Project ANDALGHORT Common Initiative Interreg España-Portugal, contributes to the development and optimization of biofumigation with *Brassica* and soil solarization as alternatives to the traditional use of chemicals in strawberry production.

27.34 COMPARATIVE STUDY OF DIFFERENT BRASSICA SPECIES TO CONTROL *PHYTOPHTHORA CACTORUM* AND *VERTICILLIUM DAHLIAE*. **E. Romero, C. Zurera, M. Porras, C. Barrau and F. Romero.** *IFAPA-CICE Centro Las Torres-Tomejil. Junta de Andalucìa, Apdo. Oficial 41200, Alcalá del Río (Sevilla), Spain. Email: eva.romero.ext@juntadeandalucia.es*

Biofumigation is a term used to describe the supression of soil-borne pathogens and pests by the incorporation of plant residues such as those of Brassicas into the soil. The suppressive effect of Brassicas has been attributed to glucosinolates, which have little biological activity but whose hydrolysis products are highly biocidal. Interest in biofumigation has increased recently due to prohibition of several synthetic pesticides and soil fumigants. The objectives of this work were to evaluate the biocidal potential of several *Cruciferae* species to control the strawberry pathogens *Phytophthora cactorum* and *Verticillium dahliae*, as well as to investigate biofumigant potentials in different cruciferous

crop tissues during their growth cycle. Five *Brassica* species, *B. carinata*, *B. juncea*, *B. nigra*, *B. napus* and *B. oleracea*, were selected on the basis of their glucosinolate content. Studies were carried out for two consecutive growing seasons 2005-06 and 2006-07. Three sowing dates were considered from October to August. Samples were taken at three stages of plant development: leaves (five leaves), flowers (all the flowers open) and seed (seed in lower full size pods). Different concentrations of fresh macerated shoot tissue were assayed in glass jars. Three *P. cactorum* and two *V. dahliae* isolates were tested and their colony radial growth measured daily. The results revealed significant differences in biofumigant effect depending on the species tested. *B. carinata*, *B. juncea* and *B. nigra* had the greatest suppressive effect. Seed was the most effective growth stage.

27.35 LOQUAT SCAB CONTROL BY NATURAL EXTRACTS. FURTHER TRIALS ON TREATMENT EFFICACY. G. Scarito, A. Salamone, D. Giacalone, F. Barone and F. Calabrese. *Dip. SENFIMIZO - Sez Patologia vegetale e Microbiologia agraria, Università degli Studi di Palermo, V.le delle Scienze 2, 90128 Palermo, Italy. Email: salamone@unipa.it*

Loquat scab, caused by *Spilocaea eriobotryae* (Cav.) Hugh., is the most important disease of loquat (*Eriobotrya japonica* Lindl.) in Italy. It can be controlled by chemical products that are not permitted in organic agriculture. Our work has evaluated natural products to control the disease. Concentrations at 1 and 0.5 ml/l of oregano and clove essential oils were tested on 10 year-old loquat plants grown in an organic field near Palermo (Sicily). The essential oils were emulsified in a soy oil-based commercial product. Both extracts were compared with copper sulphite (1.5 ml/l) treatment and a control. Each treatment was repeated 4 times using a split plot system (4 trees/block). All sprays were applied every 15 days (from November to May 15th). Incidence and severity of disease were checked on 100 leaves of different maturity collected every 6 months and on 100 fruits at harvest. The results showed that both essential oils had inhibitory activity at the highest concentrations, more evident on leaves than on fruits, compared with untreated trees. No statistical differences were found in comparison with copper sulphite treatment. No phytotoxicity was found at either concentration.

27.36 CONTROL OF WHEAT ROOT ROTS UNDER FIELD CONDITIONS WITH COMPOUNDS PRODUCED BY *FUSARIUM SAMBUCINUM* STRAIN FS-94. L. Shcherbakova, L. Dorofeeva, G. Devyatkina, G. Sokolova and D. Fravel. *Russian Research Institute of Phytopathology, Golitsyno, Moscow reg., 143050, Russia. Email: larisa@vniif.rosmail.com*

Appreciable yield losses from root rot epidemics on wheat caused by *Fusarium* fungi are frequently observed in Russia. Chemical fungicides are not always effective in controlling fusarial root rots on wheat, and biocontrol agents may provide an alternative or additional method for managing them. Laboratory experiments with *F. sambucinum* strain FS-94 isolated from wheat rhizosphere showed that the strain was non-pathogenic on wheat, did not produce the trichothecene mycotoxin diacetoxyscirpenol in submerged culture, and could not grow at human body temperature. FS-94 synthesized high-molecular weight, non-fungitoxic compounds (HMC) which were extracted with 1M KCl buffered with 50mM phosphate, pH 6.0, purified on Sephadex G-50 and dissolved in water. These compounds prevented *F. culmorum* growth and sporulation on artificially-infected wheat seedlings, reduced pathogen infectivity, and induced plant resistance. To confirm the protective effect of the HMC under field conditions, small-plot trials were conducted in 2006 and 2007. Prior to sowing, wheat seed naturally infected with soilborne pathogens (predominately *Fusarium*) were soaked in water or various concentrations of HMC for 3 hours or overnight. The treatments with HMC significantly reduced incidence and severity of root rots throughout the growing season. Maximal efficacy against *Fusarium* spp. was observed when seeds were soaked overnight in 200 µg/ml of HMC, resulting in a 56% reduction in disease incidence and a 63% reduction in disease severity at the tillering stage. Seed treatment also protected plants from *Helminthosporium* and *Rhizoctonia* root rots and basal stem rot. Yield in plants treated with HMC was 16.7% greater than in untreated plants.

27.37 BIO-POLYMERS AND METALAXYL SEED PRIMING ENHANCE GROWTH AND DISEASE RESISTANCE IN PEARL MILLET TO *SCLEROSPORA GRAMINICOLA*, AN OOMYCETE CAUSING DOWNY MILDEW. H.S. Shetty and J. Sudisha. *DOS in Applied Botany and Biotechnology, University of Mysore, Manasagangotri, Mysore 570006, India. Email: hss_uom@hotmail.com*

Priming the plant and seed induces a physiological state in which plants are able to activate defense responses faster or better. Recently much evidence suggests that several pesticides act by priming plants and this shows promise for developing practical applications in crop disease management. Plant-based gum exudates are biopolymers which contain plant growth regulating hormones with enormous therapeutic potential without any side effects. In this study, four gum exudate-producing plants (*Acacia arabica*, *Moringa oleifera*, *Carica papaya*, and *Azadirachta indica*) were evaluated for synergistic effects of the biopolymer and metalaxyl, in seed priming to improve pearl millet [*Pennisetum glaucum* (L.) R. Br] seed quality, growth parameters, and resistance to downy mildew disease caused by *Sclerospora graminicola* (Sacc.) Schroet. Susceptible seeds were primed with biopolymers and metalaxyl (Apron 35 SD) and tested under *in vitro* and greenhouse conditions. Biopolymers showing synergism with metalaxyl gave enhanced seed germination and vigour. *Acacia arabica* and *Azadirachta indica* gum alone and or with 3 gm/kg of Apron showed higher seed germination (91%). Seed priming with 6 gm/kg of Apron gave 89% seed germination and was par with the control. A similar trend in vigour was noted among treatments. Seed priming with biopolymers alone gave various disease protection levels when compared to controls. *A. arabica* and *A. indica* gums along with 3 gm/kg of Apron gave best results, offering a significant disease protection of 86% and also promoting growth. The advantages in disease protection of seed priming with biopolymers plus metalaxyl are discussed.

27.38 EFFECT OF ORGANIC AMENDMENTS FROM SOLANACEAE AND BRASSICACEAE PLANTS AND *TRICHODERMA HARZIANUM* ON *VERTICILLIUM* WILT OF PEPPER. U. Smolinska and B. Kowalska. *Department of Plant Protection, Research Institute of Vegetable Crops, ul. Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland. Email: usmolin@inwarz.skierniewice.pl*

Verticillium dahliae is a soilborne plant pathogen causing vascular wilt disease of pepper (*Capsicum annuum* L.). The objective of this study was to find new methods to protect pepper against this pathogen. One of the alternative management strategies is

application of beneficial microorganisms or plant material containing biologically active compounds. In the present work both methods were used together. We wished to determine 1) the effect of plant residues from *Bassicaceae* and *Solanaceae* and *Trichoderma harzianum* on microsclerotia survival in the soil; 2) the effect of organic amendments and *T. harzianum* on development of disease symptoms on pepper; 3) the anatomical and cytological changes occurring in the diseased plants; 4) the effect of organic amendments on suppressive properties of soil and peat substrate; 5) any negative influence on following crops. Dry residues from plants, rapeseed meal and water extracts from these materials, decreased amounts of microsclerotia formed on *V. dahliae* colonies and in the soil. The results obtained in *in vitro* tests were verified in greenhouse experiments. Addition of the plant material decreased the population of the pathogen in soil and increased yield of pepper. The organic amendments enhanced the suppressive potential of the soil by increasing the population of actinomycetes, fluorescent *Pseudomonas*, and spore-forming bacteria. The phytotoxic effect of the compounds released during decomposition of the plant residues, was studied in a growth chamber using the Phytotoxkit test.

27.39* PLANTS CONTAINING PYRROLIZIDINE ALKALOIDS: A POTENTIAL SOURCE FOR NEMATODE CONTROL? T. Thoden, M. Boppré, T. Burzlaff and J. Hallmann. *Forstzoologisches Institut, Albert-Ludwigs-Universität, Am Fohrenbühl 25, D-79252 Stegen, Germany. Email: tim.thoden@fzi.uni-freiburg.de*

Crotalaria, a plant genus widely used as a green manure and fibre crop in tropical and subtropical regions, is well known for its potential to control plant-parasitic nematodes. Although the mode of action still awaits clarification, *Crotalaria* spp. do contain pyrrolizidine alkaloids (PAs), a class of secondary plant metabolites with a variety of reported chemo-ecological properties. So far, PAs have been detected in hundreds of plant species worldwide and we think that other PA-containing plant species besides *Crotalaria* might be interesting alternatives for nematode management strategies, especially in regions where *Crotalaria* spp. can not be grown for climatic, edaphic or other reasons., We therefore tested the nematicidal potential of pure PAs and plants containing PAs. Our *in vitro* studies showed nematicidal effects of different types of pure PA on *Meloidogyne incognita* at concentrations between 70–10,000 ppm. Furthermore, a mixture of purified PAs from *Chromolaena odorata* suppressed the host-finding of *M. incognita* on lettuce even at concentrations of 70 ppm. Our *in vivo* studies showed that cropping of the PA-containing plants *Senecio bicolor* and *Ageratum conyzoides* reduced the population density of *M. incognita* juveniles. In addition, the incorporation of plant material from *A. conyzoides*, *S. bicolor* and *C. odorata* into soil significantly reduced *M. incognita* infestation of tomato and lettuce. Therefore, PA-based methods like cropping and/or the incorporation of PA-containing plant material might be an environmentally sound alternative for nematode control.

27.40 BIOASSAY OF SPORE-GERMINATION INHIBITION IN *FUSARIUM GRAMINEARUM*. T. Yi and T. Ikeda. *College of Biosafety Science & Technology, Hunan Agricultural University, Changsha 410128, P. R. China. Email: yituyong@hotmail.com*

We extracted several substances from eight varieties wheat seed flour and bran using three organic solvents, 0.1M Tris-Cl (pH 7.5) and water, and bioassayed these extracts and seventeen chemicals for inhibition of spore germination in *Fusarium graminearum in vitro*. The results showed: (1) there are some water-soluble, temperature-sensitive substances in wheat seed flour that can inhibit *F. graminearum* spore germination; (2) except for 25 ng/ml Corystein and 250 ng/ml Naringenine, the other tested chemicals did not inhibit spore germination.

27.41 ERADICATION OF PATHOGENIC BACTERIA BY INCORPORATING FRESH CROP DEBRIS: EVALUATION UNDER CONTROLLED CONDITIONS. M.J. Zanón and C. Jordá. *Instituto Agoforestal Mediterráneo (IAM), Universidad Politécnica de Valencia, Camino de Vera, s/n, 46022 Valencia, Spain. Email: mazanal@etsia.upv.es*

In Spain, tonnes of plant debris are generated after harvest and are left on the soil surface to become important sources for pathogens and diseases. Different techniques include the incorporation of these remains in the soil. Knowledge of the effect of this incorporation is necessary, especially when plant remains are infected with potential pathogens. The results obtained in this research should be useful in different areas like biofumigation, or combined biofumigation and solarization. Biofumigation is an importan technique that can substitute the application of methyl bromide as a non-chemical soil disinfection method operating through volatile substances released into the soil atmosphere by decomposing amendments. The use of crop debris as biofumigant material has been shown to have the same effects on the control of plant diseases as conventional pesticides. Furthermore, incorporation of these plant remains into soil could be a solution for their negative environmental impact. A method for the eradication of *Clavibacter michiganensis* subs. *michiganensis* and *Ralstonia solanacearum* from fresh tomato debris, which was artificially infected, was tested for its efficacy in several experiments carried out under laboratory and greenhouse conditions. The results confirm that these bacteria can be eradicated under controlled conditions after thermal treatments at 45ºC, but the pathogens were never eradicated after treatments at 25ºC. It is important to take these results into account with regard to the effect of different soil disinfection techniques or ecological alternatives.

Nematology and plant diseases

29.1* EVALUATION OF *POCHONIA* AND *TRICHODERMA* FROM BENIN AS BIOCONTROL AGENTS AGAINST ROOT-KNOT NEMATODES. A. Affokpon, G.K. Mutua, J. Coosemans and D.L. Coyne. *Laboratory of Phytopathology and Plant Protection, K.U. Leuven, W. de Croylaan 42, 3001 Heverlee, Belgium. Email: affokpon_antoine@yahoo.fr*

Two isolates of the fungi *Pochonia chlamydosporia* and *Trichoderma asperellum,* isolated from Benin, were evaluated for their biocontrol potential against *Meloidogyne* spp. (root-knot nematodes) in pots on tomato. Three application rates of *P. chlamydosporia* (5×10^3, 7.5×10^3 and 10×10^3 chlamydospores per g soil) and of *T. asperellum* (1.5×10^5, 2.25×10^5 and 3.75×10^5 spores g^{-1} soil) at two application timings (2-week pre-planting and "at planting") were evaluated at nine weeks after planting using soil naturally infested with nematodes (initial *Meloidogyne* populations of 300 juveniles per 100 g soil). For a given fungal application rate, the time of fungal application did not significantly influence densities of *Meloidogyne* second stage juveniles (J2) in soil or in roots. Both *P. chlamydosporia* and *T. asperellum* were able to reduce J2 soil populations by 75% and 35% respectively, with no

differences between application rates. Root J2 densities were reduced by up to 57% and 42% respectively, compared to non-fungal control pots. Only the pre-plant fungal application was able to reduce significantly nematode root galling. Egg mass parasitism by *P. chlamydosporia* was not influenced by fungal application timing, with greatest colonization (62%) obtained at the application rate of 10×10^3 chlamydospores g^{-1} soil. Although fewer egg masses (< 3%) were parasitized by *T. asperellum*, the proportion of colonized egg masses increased at the pre-plant application rate of 3.75×10^5 spores g^{-1} soil. Densities of *P. chlamydosporia* in the rhizosphere had increased by up to 6.2-fold of the initial application at nine weeks after planting, while *T. asperellum* densities had declined.

29.2 ASSESSMENT OF FUNGAL PARASITISM OF *HETERODERA SCHACHTII* BY *POCHONIA CHLAMYDOSPORIA* VAR. *CHLAMYDOSPORIA*. **E. Ayatollahy, S. Fatemy and H.R. Etebaryan.** *Plant Protection Department, P.O. Box 11365/4117, Abooreihan Campus, Tehran University, Tehran, Iran. Email: ebrahim_ayatollahi@yahoo.com*

Heterodera schachtii is the most destructive pest of sugar beet in Iran. Among nematophagous fungi, *Pochonia chlamydosporia* var. *chlamydosporia* has provided considerable control of nematodes and is one of the most promising natural enemies with commercial potential for root knot and cyst nematodes so far tested. Our aim was to investigate the pathogenicity on *H. schachtii* of an isolate of *P. c.* var. *chlamydosporia* isolated from sugar beet fields in Iran. Discs of 5 mm diameter from the margin of actively growing mycelia on PDA were placed in the center of each of three 9-cm Petri dishes containing 0.8% water agar and 100 ppm antibiotiqs. Three fungus-free females and/or cysts were placed next to fungal blocks or uninfected PDA blocks as control in three replicates. After three weeks at 20°C, females and cysts were crushed between a slide and cover slip and different developmental stages of eggs and percentage of diseased eggs were determined with the aid of a stereomicroscope. The fungus showed high pathogenicity, and infected more than 70% of eggs within females and 90% of cysts. However, the fungus was capable of colonizing mature eggs in cysts; most of diseased eggs were immature.

29.3 ROOT PATHOGENS IN *CITRUS* NURSERIES OF FARS PROVINCE (IRAN). **K. Ayazpour, A. Ghanaatian and M. Pakniyyat.** *Plant protection department, Islamic Azad University of Jahrom, Iran. Email: Kayazpour@yahoo.com*

In different seasons during 2002-2003 most of the *Citrus* nurseries of Fars province were visited and samples showing symptoms of wilt, dieback, and decline were removed to the laboratory with care. After washing the roots, 5 mm lengths of root were treated in 10% chlorax for 1-3 minutes and put on the culture media WA and PDA. Fungi isolated and purified by hyphal tip or single spore culture were inoculated on lemon seedlings and pathogenic isolates were studied taxonomically. 52 pathogenic isolates were found, belonging to the five following fungi: *Rhizoctonia solani*, *Fusarium solani*, *Pythium aphanidermatum*, *Phytophthora citrophthora*, and *Phytophthora parasitica. F. solani* is described for the first time as a cause of seedling damping-off from Iran. 105 Samples of soil and roots from different part of commercial citrus nurseries were collected, screened and centrifuged to separate out the nematodes, and preparations were made. The following species were identified: *Tylenchulus semipenetrans*, *Helicotylenchus pseudorobustus, Paratylenchus hamatus, Xiphinema pachtaicum.* Which *Helicotylenchus pseudorobustus* and *Xiphinema pachtaicum* are reported for the first time in Fars province.

29.4 BIOLOGICAL MANAGEMENT OF *MELOIDOGYNE JAVANICA* BY *TRICHODERMA HARZIANUM*. **B. Khattak and Saifullah.** *Department of Plant Pathology, Agricultural University Peshawar, Pakistan. Email: baharkk75@yahoo.com*

Trichoderma harzianum was isolated from areas of NWFP, Pakistan infested with root knot nematodes, *Meloidogyne javanica* (Treub) Chitwood. Samples of *T. Harzianum* were collected from various localities of Malakand and Swat, and tested against *M. javanica* in *in vitro* conditions. Isolates Th_1 and Th_9, from Jabban and Shamozai were found more aggressive against *M. javanica*. The effect of *T. harzianum* culture filtrates (CF) at different concentration levels (standard, 1:1, 1:10, and 1:100), were studied. Hatching of *M. javanica* eggs was significantly inhibited by the culture filtrates, and this inhibition was positively correlated with increase in the filtrate concentration. Maximum inhibition of egg hatching (80.36%) was observed at standard concentration, followed by 68.08% at 1:1 and minimum inhibition (10.66%) was recorded at the lowest concentration. Isolates Th_1 and Th_9 significantly inhibited egg hatching. Parasitism of *M. javanica* eggs and juveniles was also observed. The highest egg infection (90.00%) was recorded with Th_9.

29.5 BIONEM WP: BIOLOGICAL CONTROL OF NEMATODES IN ANNUAL AND PERENNIAL CROPS. **D. Blachinsky, M. Lazare, J. Antonov, A. Bercovitz, K. Feldman, N. Markov and M. Keren Zur.** *Agrogreen Minrav Group - Kiryat Minrav Hi-Tech Park, P.O. Box 153, Ashdod 7710, Israel. Email: dafna@agrogreen.co.il*

"BioNem WP" is a bionematicide based on the naturally occurring bacterium *Bacillus firmus*. The product can be applied pre- or post-planting through the irrigation system or by spraying. "BioNem WP" is registered and used commercially in Israel for a variety of annual and perennial crops. Pre-plant application of "BioNem WP" in eggplants reduced nematode populations and related root damage. The protection level was comparable to that of fumigants/volatile nematicides used on the same farm. Mid-season application on already infested peppers reduced the nematode population and increased yield late in the season. Many perennial crops are severely damaged by nematodes.Volatile nematicides are not suitable for application during the growing season, and non-volatile nematicides require numerous applications; this kind of control regime results in environmental damage, resistance build up and biodegradation of chemicals leading to decreased efficacy. Drip irrigation of "BioNem WP" in the early winter in a peach orchard reduced the root knot nematode population, which remained at a low level until the following autumn, unlike the chemical standard (cadusaphos), and resulted in yield increase. The effect of a spring application was slightly less. We suggest that early application (before root flash) of "BioNem WP" is the key for optimal nematode control.

29.6 THE REACTION OF THREE TOMATO ROOTSTOCKS TO *MELOIDOGYNE INCOGNITA* IN CHINA. **Z. Cao, D. Dong, X. Wang, L. Han and L.M. Gullino.** *Department of*

Ecology and Ecological Engineering, College of Resources and Environmental Science, China Agricultural University, Beijing 100094, P. R. China. Email: zhipingc@cau.edu.cn

The root-knot nematode (*Meloidogyne incognita*) and soilborne pathogens are economically important in vegetable production. Due to the increasingly restricted use of fumigants, development of resistant rootstock cultivars in vegetable production becomes urgent. The objective of this study was to evaluate three resistant tomato rootstocks (Beaufort F1, Energy F1 and He-Man F1) and tissue culture seedlings of Beaufort F1 for control of root-knot nematode. Pot trials showed that all three rootstocks and tissue culture seedlings of Beaufort F1 have high resistance to root-knot nematode, their root-knot index, egg-mass densities and root-knot size were decreased compared to the susceptible control (Hezuo908). In the field experiment, the root-knot indexes of the three rootstocks were only about 10%, but the susceptible control (FA189) was 100% at harvest time, and yields were enhanced 16-20%. Tissue culture seedlings of Beaufort F1 had lower root-knot index values than FA189, but higher than Beaufort F1. Yields were similar for tissue culture seedlings and Beaufort F1.

29.7 PLANT-PARASITIC NEMATODES INFECTING GRAPEVINE IN SOUTHERN SPAIN AND THEIR HOST-PARASITE RELATIONSHIPS. P. Castillo, B.B. Landa, H. Rapoport, N. Vovlas, C. Gutiérrez-Gutierrez, J. Navas-Castillo, J.A. Navas-Cortés, F. Pérez-Camacho and R.M. Jiménez-Díaz. *Instituto de Agricultura Sostenible, CSIC, Apdo. 4084, 14080 Córdoba, Spain. Email: pcastillo@ias.csic.es*

The primary goal of this study was to determine the extent of soil infestation and rootstock infection by plant-parasitic nematodes in 75 commercial vineyards distributed over the main grapevine-growing areas of Andalusia, southern Spain. Plant-parasitic nematodes potentially damaging grapevine rootstocks in the surveyed area included root-knot (*Meloidogyne arenaria*, *M. incognita*, *M. javanica*), ring (*Mesocriconema xenoplax*), and root-lesion (*Pratylenchus vulnus*) nematodes, as well as the virus-vector, dagger nematodes (*Xiphinema index*, and *Xiphinema italiae*). High level of field infestations by *X. index*, and *X. italiae* (more than 50 nematodes/100 cm^3 soil) were found in vineyards affected with severe decline and reduced vigour in two locations. Soil infestations by *X. index* and *X. italiae* have the potential to impair root function. Moreover, infection of grapevines by *Grapevine fanleaf virus* (GFLV) transmitted by those nematodes can further result in a reduction in the lifespan of vineyards by about 12-20 years. Therefore, research is in progress for the detection of GFLV in the nematode-infected plants and in nematodes from soil that might be related to the severe plant decline. Histopathological observations of *Meloidogyne*-infected rootstocks revealed important alterations in roots which would negatively affect capacity for nutrient and water uptake and transport. Furthermore, our histological observations and data on nematode reproduction in infected roots indicate that some of the most commonly used rootstocks in Andalusia, including Paulsen 1103, Richter 110, Rupestris du Lot, and SO4, which previously were considered moderately resistant to *Meloidogyne* spp., should now be considered susceptible to the populations of these nematodes prevailing in vineyard soils in Andalusia.

29.8 BIONOMICS AND IDENTIFICATION OF THE ROOT-LESION NEMATODES *PRATYLENCHUS SPP.* (NEMATODA: PRATYLENCHIDAE). P. Castillo, N. Vovlas and A. Troccoli. *Instituto de Agricultura Sostenible, CSIC, Apdo. 4084, 14080 Córdoba, Spain. Email: pcastillo@ias.csic.es*

Root-lesion nematodes of the genus *Pratylenchus* are recognised worldwide as a major constraint of crops of primary economic importance, including horticultural, woody and ornamental plants. *Pratylenchus* species rank second only to root-knot nematodes in economic impact on crops worldwide, because of their wide host range and distribution. They are migratory endoparasites that cause severe root damage whilst feeding primarily in the cortical parenchyma. The genus *Pratylenchus* comprises 68 nominal species of worldwide distribution. Nevertheless, most economic damage to herbaceous, vegetables and fruit crops throughout the world is attributable to a dozen of the commonest species, including *P. brachyurus* in maize, cotton, peanut, pineapple, potato and tobacco; *P. coffeae* in coffee, citrus, sugarcane and tea; *P. goodeyi* in banana; *P. neglectus* in cereals and legumes; *P. penetrans* in grasses, forages, fruit trees and strawberries; *P. pratensis* in cereals, grasses, ornamentals and strawberries; *P. scribneri* in potato, soybean and strawberries; *P. thornei* in cereals and legumes; *P. vulnus* in pome and stone fruit trees, ornamentals and roses; and *P. zeae* which parasitizes maize, rice, sugarcane and wheat. This contribution summarises specialised information on general morphology and diagnostic traits of *Pratylenchus* spp. and their usefulness in taxonomy, including new biochemical and molecular diagnostic technologies. Numerous aspects of the biology, life cycle, epidemiology and ecology, pathogenicity and host-parasite relationships of *Pratylenchus* spp., as well as their interactions with beneficial and pathogenic fungi will be discussed.

29.9 *IN VITRO* EFFICACY OF *BACILLUS THURINGIENSIS* ANTAGONISM ON ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* (NEMATODA: TYLENCHIDAE). R. Hernández, M.E. Márquez, M. Escobar, A. Lemes, E. Laguardia and E. Fernández. *Plant Health Research Institute Calle 110 No. 514 e/ 5ta. B y 5ta. F, 11600 Playa, La Habana, Cuba. Email: rhernandez@inisav.cu*

Antagonist effects *in vitro* of *Bacillus thuringiensis* (Berliner) LBT-25 (BT-25) liquid culture fractions on the root-knot nematode *Meloidogyne incognita* were tested. The nematocide and nematostatic actions of BT-25 were demonstrated by light microscopy through the appearance of egg necrosis, vacuolization in the bodies of first-stage juveniles (J_1), lesser mobility of the second stage juveniles (J_2) and a significant reduction of hatching ($P \leq 0.05$). All formulated BT-25 fractions, including those exposed to 120 °C for 15 minutes, caused irreversible toxicity to 100% of eggs and J_1 juveniles. Biomass from non-formulated product reduced hatching of *M. incognita* more than 80%.

29.10 SURVEY OF NEMATODES ASSOCIATED WITH LOCAL LAND RACES OF UPLAND RICE IN THE PROVINCES OF DAVAO DEL NORTE AND DAVAO DEL SUR, THE PHILIPPINES. M.N. Infante, M.F. Doblas and R.G. Reloba. *Regional Crop Protection Center, Southern Mindanao Integrated Agriculture Research Center, Department of Agriculture, Regional Field Unit XI, Davao City, Philippines. Email: marilou.infante@lycos.com*

The staple food among Filipinos is rice, most of which is made up of lowland varieties, while upland rice is the staple food

for a number of our indigenous people in the uplands, aside from some root crops. For some, rice is also a source of livelihood. Niche markets exist for these local land races of upland rice in Davao Region. These are aromatic and soft when cooked, hence are very palatable. Twenty-two local land races of upland rice from 4 barangays were sampled from Davao del Norte and 5 plant-parasitic nematode species were collected, namely *Criconemoides* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., *Rotylenchus* sp., *and Tylenchus* sp. Two municipalities with 13 local land races of upland rice from 6 barangays were sampled from Davao del Sur. Eight plant-parasitic nematode species were collected: *Criconemoides* sp., *Rotylenchus* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., *Pratylenchus* sp., *Rotylenchus* sp., *Tylenchus* sp., and *Meloidogyne graminicola*. *M. graminicola* was collected from the local land race Tenda from the municipality of Malita. *Meloidogyne* sp. can produce 350–500 eggs/female and has a 28–30 day life cycle. Upland rice is harvested after 5-6 months. Hence, profiling of the upland rice areas in the Davao Region is important since *M. graminicola* could be a serious threat to the upland rice production of our indigenous people.

29.11 INDUCTION IN GROUNDNUT (*ARACHIS HYPOGAEA*) OF THE OXIDATIVE ENZYME PEROXIDASE BY APPLICATION OF *PSEUDOMONAS FLUORESCENS*, AS A DEFENCE AGAINST THE ROOT KNOT NEMATODE, *MELOIDOGYNE ARENARIA*. **P. Kalaiarasan.** *Department of Nematology, Tamil Nadu Agricultural University, Coimbatore 641 003, India. Email: kalainem@rediffmail.com*

Pseudomonas fluorescens isolates Pf1, PfCBE, PfPOL and PfBSR were found to protect groundnut from the root knot nematode, *Meloidogyne arenaria*. Induction of peroxidase by application of *P. fluorescens* isolates against challenge inoculation with *M. arenaria* in groundnut was studied. Plant growth was significantly higher in all treatments with the bacterium. Isolate Pf1 performed significantly better than the other three isolates. The bacterial treatments also reduced the level of nematode infestation. Higher levels of peroxidase activity in bacterized groundnut inoculated with nematodes were observed. Isoform analysis revealed the induction of a single PO1 isoform and higher levels of PO2 isoform in bacterized groundnut inoculated with nematodes. The nematode-suppression ability of the bacterial isolates was related to their root colonizing ability. The result suggested that induction of defense enzymes in the phenylpropanoid pathway may contribute to restriction of nematode infection in groundnut.

29.12 MANAGEMENT OF *MELOIDOGYNE INCOGNITA* IN COTTON USING ROTATION. **S.R. Koenning.** *Plant Pathology Department, North Carolina State University, Raleigh, NC, 27695-7616, USA. Email: stephen_koenning@ncsu.edu*

An experiment of six years duration was conducted to determine the influence of corn (*Zea mays*) and soybean (*Glycine max*) grown in rotation with cotton (*Gossypium hirsutum*) on population levels of *Meloidogyne incognita*, the southern root-knot nematode and on cotton yield. Corn was a good host for *M. incognita*, whereas a resistant or susceptible soybean cultivar allowed only limited reproduction of this pathogen. Inclusion of soybean in the rotation resulted in increased cotton yield compared to continuous cotton (P= 0.05). Rotation with corn was inferior to rotation with soybean most years. The root-knot tolerant cotton cultivar Stoneville 5599BR generally yielded more than susceptible Fibermax 989BR.

29.13 CEREAL CYST NEMATODE (*HETERODERA AVENAE*) IS CAUSING DAMAGE ON WHEAT IN HENAN, THE BREAD BASKET OF CHINA. **L. Hong-lian, J.M. Nicol, Y. Hongxia, W. Xujin and Y. Weixing.** *College of Plant Protection, Henan Agricultural University, Zhengzhou, Henan 450002, P. R. China. Email: honglianli@sina.com*

China is the world's largest wheat producer with over 120 Mt and average yields around 4 tonnes per hectare. Cereal cyst nematode (CCN) *Heterodera avenae* was first identified in China in Hubei province and is now found in more than 10 provinces in China representing about 2/3^{rds} of the total wheat production area. Henan is one of the most important wheat producing provinces with a large area (5M ha, 4.8 t/ha) and accounts for more then 26% of China's annual wheat production (2006). In Henan province more than 60% surveyed samples have CCN with high population densities (12-107 eggs/g soil) compared with other published reports. In 2006 preliminary yield loss studies were conducted on two widely grown cultivars under natural field conditions in farmers' fields. The experiment consisted of 7 replicates with and without the application of Temik15G® (22.5 kg/ha). Temik effectively reduced the soil population of *H. avenae* by 52.5% and significantly increased the yields of both cultivars tested, Wenmai 4 (by 28.8%) and Wenmai 6 (by 40.3%). Furthermore significant correlations were found with the number of cysts per root system and yield with both cultivars. These preliminary experiments clearly demonstrate, even without complete control of CCN, that this nematode is causing economic yield loss on wheat production in Henan province. Work has begun in Henan in collaboration with CIMMYT to confirm this preliminary yield loss data, identify the pathotype, study the biology and ecology of this population and screen both Chinese and foreign wheat germplasm to identify resistance.

29.14* ISOLATION OF AN EXPANSIN GENE FROM A SPECIFIC cDNA LIBRARY OF THE ANTERIOR END OF *BURSAPHELENCHUS XYLOPHILUS*. **S. Lin, L. Guo, H. Jian and G. Zhang.** *Department of Plant Pathology, China Agricultural University, Beijing 100094, P. R. China. Email: hengjian@cau.edu.cn*

Pine wilt disease caused by pine wood nematode (*Bursaphelenchus xylophilus)* has been a disaster in Chinese forests. For isolation and identification of parasitic genes of this nematode, a specific cDNA library of the anterior end of body will be constructed in this research. About 250 anterior ends (about one fourth of body length) and 250 posterior ends (about half the body length) of the nematode were cut by an ophthalmic bistoury, and the mRNAs were isolated using Dynabeads mRNA® DIRECT™ Micro kit, respectively. The cDNAs were synthesized by SMART™ cDNA library construction kit (Clonetech). A specific cDNA library of the anterior end was constructed by modified solid-phase subtractive hybridization. About 6,000 positive colonies were obtained and the fragment length of colonies ranged from 200 to 1200 bp. One clone designed as BXOB14 was 42%, 46% and 39% identical with EXPB1, EXPB2 from *G. rostochiensis* and putative avirulence protein (*CAC27774.1*) from *M. incognita*, respectively. A full-length cDNA of putative expansin of the pine wood nematode, which contained an open reading frame encoding 149 amino acids, was obtained by the RACE technique. The detailed role of pine wood nematode expansin is not clear.

29.15* BIOCHEMICAL AND MOLECULAR BASIS OF INTERACTION BETWEEN *FUSARIUM UDUM* AND *HETERODERA CAJANI* ON PIGEONPEA WITH REFERENCE TO HOLDING/BREAKING WILT RESISTANCE. **S. Lingaraju and G.R. Nagabhushana.** *Department of Plant Pathology, U.A.S., Dharwad 580005, Karnataka, India. Email: lingaraju_s@rediffmail.com*

Vascular wilt (caused by *Fusarium udum*) and pearly root (caused by *Heterodera cajani*) are constraints for pigeonpea (*Cajanus cajan*) productivity in India. It is now known that the wilt resistance character of some pigeonpea cultivars may/may not be broken due to the interaction of *F. udum* and *H. cajani.* The mechanism(s) underlying such responses in pigeonpea cultivars/genotypes were investigated. Changes in several biochemical constituents in a genotype whose wilt resistance holds up in an interaction was compared with that of a genotype whose wilt resistance breaks. Further, genetic variation among several such pigeonpea genotypes losing or holding wilt resistance was studied through a genetic marker, RAPD (random amplified polymorphic DNA). Protein extracts obtained from root and stem pigeonpea samples collected at flowering stage from different treatments were used for estimation of peroxidase (PO), polyphenol oxidase (PPO), and phenyl ammonia lyase (PAL). Also, total phenols and total sugars were estimated. A higher accumulation of defense enzymes (PO and PPO) and total sugars in a genotype which maintained resistance suggested that the build-up of these biochemicals to higher levels was instrumental. Changes in PAL and total phenols, though minimal as seen in our study, may also be important. The molecular marker employed did not differentiate the genotypes in a dendrogram analysis. Our investigations showed that a higher activity of defense enzymes and biochemicals is necessary to hold the resistance character of wilt-resistant pigeonpea cultivars in a disease-complex situation.

29.16 AGRI-TERRA: A NEW MATERIAL FOR THE MANAGEMENT OF PHYTONEMATODES. **E.C. McGawley and M.J. Pontif.** *Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA, 70803, USA. Email: emcgawley@agctr.lsu.edu*

Nematologists, plant pathologists and entomologists in California, Florida, Idaho, Louisiana, Minnesota, North Carolina and New York (USA) and in Spain, China, the United Kingdom, Mexico, Saudi Arabia, Italy, Belgium, Australia, India, Indonesia, Korea, Pakistan, Vietnam and Morocco have evaluated the efficacy of Agri-Terra and the Agri 50 family of pesticides against insects, phytoparasitic nematodes, fungi and insects. Agri-Terra has demonstrated efficacy against a wide spectrum of nematodes including reniform, root-knot, soybean cyst, lesion, lance, stunt, ring, stubby-root, dagger and spiral on burmuda grass, lantana, pepper, tomato, cotton, soybean, rice, sugarcane, cabbage, endive, lettuce, mustard, tobacco, cucumber, and potato. 2007 was the seventh consecutive year of trials evaluating the efficacy of Agri-Terra against plant-pathogenic nematode species associated with major crops in Louisiana. Trials have been conducted in microplots (a controlled environment in which the efficacy of an experimental nematicide can be evaluated without interference from unknown and unmanaged biological and non-biological conditions) and in small and medium-scale fields on LSU Agricultural Center Research Farms. A 10 gallon per acre (1% concentration applied to soil as an at-planting, in-furrow, fine mist spray treatment) of Agri-Terra is the most optimal treatment for soybean, resulting in harvest plant weights, pod numbers and pod weights that were significantly greater than those of non-inoculated controls. This concentration/rate combination of Agri-Terra is also the optimal treatment for cotton, producing statistically significant increases in plant growth and boll production while providing excellent nematode control.

29.17 NEMATICIDAL EFFECT OF FURFURAL AND SOME BIOCONTROL AGENTS ON CITRUS NEMATODES INFESTING CITRUS TREES. **M.M.M. Mohamed.** *National Research Centre, Plant pathology, Depart, Dokki, Giza, Egypt. Email: moawad_bondok@yahoo.co.uk*

Studies of the effect of furfural and nematode-trapping fungi on yields of citrus infested with citrus nematode (*T. semipentrans*) were carried out under field conditions. Both furfural and nematode-trapping fungi increased the yield of Valencia orange (*Citrus sinensis* L.) when they were applied at the rate of 3000 ppm for furfural, 1l and 1kg per 10l water in the drip irrigation system. The furfural treatment was superior to the nematode-trapping fungi treatments. Citrus nematode populations in soil and citrus roots were decreased by furfural and nematode-trapping fungi treatments compared with untreated trees (controls). Citrus nematode populations were decreased more by furfural than by the nematode-trapping fungi during the first three months after application.

29.18 BIOLOGICAL CONTROL OF *MELOIDOGYNE JAVANICA* BY *PSEUDOMONAS FLUORESCENS* CHA0, AND *TRICHODERMA HARZIANUM* BI. **S. Mokhtari, N. Sahebani and H. Reza Etebarian.** *Plant Pathology Department, University College of Abureihan, University of Tehran, Iran. Email: etebar@chamran.ut.ac.ir*

A plant growth-promoting rhizobacterium, *Pseudomonas fluorescens* strain CHA0, and a fungal antagonist, *Trichoderma harzianum* strain BI, were evaluated for biological control of the nematode *Meloidogyne javanica*. In a dual-culture plate assay, where colonies of *T. harzianum* and *P. fluorescens* met each other, no further growth of either organism occurred. Culture filtrates from *P. fluorescens* CHA0 and *T. harzianum* BI inhibited nematode egg hatching and caused second-stage juvenile (j_2) mortality. A 60-day glasshouse experiment was conducted to assess the influence of these antagonists alone and in combination on the multiplication of *M. javanica* and growth of tomato. *T. harzianum* alone improved tomato growth and reduced galling and numbers of egg masses more than did *P. fluorescens*. Significantly, the multiplication of *M. javanica* and growth of tomato recorded from combination treatments were not different from *T. harzianum* alone. In this study peroxidase and polyphenoloxidase activities in tomato plants were determinated during 7 days at one-day intervals. The results showed that activity of these enzymes increased at day 1 and reached a maximum at day 5 after nematode inoculation. There was no significant difference between plants treated with *P. fluorescens* CHA0 alone and *T. harzianum* BI+*P. fluorescens* CHA0.

29.19 ROOT KNOT NEMATODE CONTROL IN GLASSHOUSE TOMATOES, PERMITTED IN ORGANIC PRODUCTION. **D.G. Natsiopoulos, I.K. Vagelas, C.I. Podimatas and F.T. Gravanis.** *Technological Education Institute of Larissa, Department of Plant Production, 41110 Larissa, Greece. Email: dimitris_natsiopoulos@yahoo.com*

Root knot nematodes are major pests reducing tomato green-

house production. Non-chemical control methods, permitted in organic production, were tested in a tomato glasshouse experiment. Two fungal species acting as biological agents were used (*Trichoderma viride* and *Gliogladium virens*). Oregano crop residues as well as vetch (*Vicia sativa*), incorporated into the soil, were also employed. Finally fumigation with metham sodium 40 w/v (Laisol 40 SL) was used for comparison and a plot was left untreated, as control. Four plots were designed per treatment with eight plants per plot. The two fungi were inoculated in the tomato root area (10^6 spores per ml, 10 ml spore suspension per plant) and the crop residues were incorporated into the plot soil two weeks before planting. The soil was infested with natural populations of root knot nematodes (*Meloidogyne* spp). Plants were left for three months and root gall index, stem fresh weight and yield were then determined. Results showed that yield and stem fresh weight of the fumigated plots followed by the *G. virens* treatments were significantly higher than for all other treatments. Fumigation as well as treatments with *T. viride* and *G. virens* had the lowest gall index. Treatments with vetch or oregano produced no significant difference from the control treatment in gall index.

29.20 UTILIZATION OF HOST PLANT RESISTANCE IN THE MANAGEMENT OF *PRATYLENCHUS ZEAE* ON MAIZE. **E.O. Oyekanmi, D.L. Coyne and B. Fawole.** *Crop Protection and Environmental Biology Department, University of Ibadan, Ibadan, Nigeria. Email: tunjioyeks@yahoo.co.uk*

Pratylenchus zeae causes more than 50% grain yield reduction and severe root necrosis and also predisposes the roots to secondary infections by soil-borne pathogens. This study was embarked on in order to find an environmentally-friendly and cheap alternative to nematicides. Fifteen selected maize genotypes based on resistance to downy mildew, *Maize streak virus* and *Striga*, were compared in a screenhouse for reaction to *P. zeae*, and six were selected for field work based on resistance to *P. zeae*. Five thousand juvenile and adult *P. zeae* were used to inoculate the maize selections both in screenhouse and in the field. Parameters on *P. zeae* density, reproductive factor (RF), grain yield and maize biomass were assessed at harvest. The RF ranged from 0.05 to 1.48 and *P. zeae* density was between 263.4 and 7433.0. Genotypes Western Yellow, 9450, Oba Super 2 and Oba Super 1 were rated resistant; others were tolerant while Gandajika 8022 and Ikenne88TZSR-Y-1 were susceptible to *P. zeae* infection. A positive regression coefficient ($r^2 = 0.36$) was obtained when $\log_{10}$ (x +1) transformed density of *P. zeae* was regressed on RF. This study established that the resistant genotypes prevented a significant grain yield loss of 40.0%. Most of the genotypes resistant to the nematode were also resistant to downy mildew, *Maize streak virus* and striga. Thus perhaps maize resistance to *P. zeae* could be an indicator for resistance to downy mildew, *Maize streak virus* and *Striga*.

29.21 THE INFLUENCE OF THREE WEED SPECIES ON REPRODUCTION OF *ROTYLENCHULUS RENIFORMIS* ON COTTON AND SOYBEAN. **M.J. Pontif and E.C. McGawley.** *302 Life Sciences Bldg., Baton Rouge, Louisiana, 70808, USA. Email: mpontif@agctr.lsu.edu*

Reniform nematodes that damage cotton and soybean crops can also reproduce on weeds, thereby maintaining populations during the off-season. Microplot studies were conducted to determine the effects of cotton (LA. 887), soybean (Pioneer 96B21), and three endemic weed species, morning glory (*Ipomoea lacunosa*), hemp sesbania (*Sesbania exaltata*), and Johnsongrass (*Sorghum halepense*), on reproduction of the reniform nematode *Rotylenchulus reniformis*. Over three microplot trials, the co-culture of cotton with any of the three weed species significantly suppressed numbers of reniform nematode juveniles in soil. When cultured alone, reniform nematode reproductive values after 60 days for cotton averaged 59.5, while those for MG, HS, and JG when alone averaged 53.3, 25.3, and 20.0, respectively at harvest. Reproductive values for cotton-MG co-culture averaged 44.1. Those for the cotton-HS combination averaged 30.0 and those for the cotton-JG combination averaged 25.0. Reniform reproduction data for soybean in two trials followed a trend similar to that observed for cotton. Suppression of reniform nematode reproduction probably resulted from crowding due to the increased amount of biomass present in microplots containing two plant species and/or from the secretion of allelopathic compounds by weed roots.

29.22 DNA MARKER FOR THE NEMATODE *DELADENUS SIRICIDICOLA*, A PARASITE OF THE WOOD WASP *SIREX NOCTILIO*, A PEST OF *PINUS RADIATA*. **M. Quader, L. Nambiar and D. Smith.** *1Primary Industry Research Victoria, Department of Primary Industries, Knoxfield Centre, 621 Burwood Highway, Knoxfield, VIC 3180, Australia. Email: motiul.quader@dpi.vic.gov.au*

The nematode *Deladenus siricidicola* is a very effective biological agent to control wood wasp (*Sirex noctilio*) an important pest of pines (*Pinus radiata*) in Australia. A number of primers were screened against DNA of *D. siricidicola* to develop a diagnostic DNA marker. One primer set was able to amplify a common PCR product of about 720 bp from isolates of *D. siricidicola*. The potentially diagnostic PCR product was from nematode isolates collected in different parts of Australia (one from South Australia, One from Victoria and seven from New South Wales). DNA sequence analysis showed that the amplified band contained partial 18S rDNA and ITS-2, complete ITS-1 and 5.8S sequences. The partial 18S rDNA sequences were matched with the homologous partial 18S rDNA sequences of Deladenus spp. in GenBank from Belgium. The most similar (21-23%) nematode was *Nacobbus aberrans*. DNA sequences between isolates showed slight variation (1-3 bases) within the amplified region. Based on amplified sequences, nine isolates from three states of Australia were grouped into 7 groups in a phylogenetic tree. This information could be used to study diversity in relation to infectivity of the nematodes to wood wasps, and taxonomy of the nematode.

29.23 ABUNDANCE AND FREQUENCY OF PARASITIC NEMATODES ON COWPEA IN BURKINA FASO AND SENEGAL. **P.A. Roberts, A. Sawadogo, M. Birame Toure, N. Cisse, I. Drabo and J.D. Ehlers.** *Dept. of Nematology, University of California, Riverside, CA, 92521, USA. Email: philip.roberts@ucr.edu*

The impact of parasitic nematodes on cowpea productivity in West Africa has not been determined. Nematodes probably contribute to the poor fertility soil regimes in which cowpea production is low, since nematode infection reduces root function. Surveys were conducted to estimate nematode abundance and frequency from 75 cowpea fields in both Senegal and Burkina Faso. Fields were selected to represent variations in cropping practices and histories in the main production regions. Enumeration of population densities in mid- to late-season root and soil samples provided information on the identity, frequency and levels of

nematode infestations. In Senegal, six nematode genera were found with potential for damage to cowpea (*Meloidogyne, Pratylenchus, Helicotylenchus, Scutellonema, Tylenchorhynchus*, and *Hoplolaimus*). Of these, *S. cavenessi* occurred in 100% of the sites, followed by *Helicotylenchus* (65%), *Meloidogyne* (62%), *Hoplolaimus* (57%), *Tylenchorhynchus* (38%) and *Pratylenchus* (35%). In Burkina Faso samples from the Sahelian zone, the North-Central Sudan Savannah zone, and the meridional Sudan Savannah zone, twelve nematode genera were found, of which six (*Scutellonema*, *Helicotylenchus*, *Meloidogyne*, *Tylenchorhynchus*, *Telotylenchus*, *Pratylenchus*) were abundant in one or more regions. Thus, a pattern of common genera in Burkina Faso and Senegal was found, although species differences occurred, for example, *S. cavenessi* in Senegal and *S. clathricaudatum* in Burkina Faso. Experiments using nematicide treated and nontreated split-plots in infested cowpea fields revealed growth and yield reductions of at least 50% due to nematode infection. These findings provide guidance for genetic improvement of cowpea via incorporating resistance to the most damaging nematode parasites.

29.24 FUNCTIONAL ANALYSIS OF NEMATODE PARASITISM GENES. **M.N. Rosso, M. Magliano, G. Dubreuil, E. Deleury and P. Abad.** *INRA–UNSA–CNRS, Interactions Plantes-Microorganismes et Santé Végétale, 400 Route des Chappes, B.P. 167, 06 903 Sophia Antipolis Cedex, France. Email: rosso@sophia.inra.fr*

Root-knot nematodes are a major pest in agriculture, due to their broad host-spectrum and wide distribution in all temperate and tropical areas. They induce the formation of hypertrophied and polynucleated feeding cells known as giant cells essential to their development. Secretions injected in the plant tissues through the stylet of the nematode are key effectors during the different phases of parasitism. Proteomic and transcriptomic approaches have made it possible to identify proteins potentially involved in the induction of the giant cells and the establishment of the nematode in the plant tissue. Plant parasitic nematodes have been so far refractory to transformation or mutagenesis and the development of reverse genetics is required to exploit the large mass of accumulating data on candidate parasitism effectors. Nematode genes can be silenced by chemically stimulating the uptake of double stranded RNA (dsRNA) by the free-living and non-feeding juveniles but the silencing is generally too brief to cover the nematode life cycle. Alternatively, dsRNA can be ingested by the parasitic stages feeding on transgenic plants expressing hairpin RNA homologous to the nematode gene targeted. An additional reverse genetics tool has been developed using *Tobacco rattle virus* to deliver dsRNA in the nematode feeding cells. Virus-inoculated plants efficiently deliver dsRNA/siRNA to the feeding parasite, and virus-mediated gene silencing in the progeny allows the functional analysis of genes involved in different steps of the nematode life cycle.

29.25 ECO-FRIENDLY MANAGEMENT OF ROOT-KNOT NEMATODES INFESTING TOMATO. **V.K. Singh.** *Plant Pathology Division, S.K. University of Agricultural Sciences and Technology, Bari-Brahmana, Dhiansar 181133, Jammu, India. Email: virendra_singh16@yahoo.com*

To combat root-knot nematode, *Meloidogyne incognita*, on tomato we investigated the effect of soil amendment with leaf powder of some medicinal plants such as neem (*Azadirachta indica*), madar (*Calotropis procera*) and behaya (*Ipomoea fistulosa*) at the rate of 1% (w/w). We also tested the predacious fungi *Arthrobotrys oligospora* and *Dactylaria brochopaga*. All the amendments reduced the number of galls and the nematode population, and resulted in improved growth of tomato plants. However, compared with the other treatments and the control, treatment with neem leaf powder + *A. oligospora* significantly reduced the number of galls and population density of the nematode and increases the shoot and root length, fresh and dry mass of shoots and roots.

29.26* EFFICACY OF ENCAPSULATED *HIRSUTELLA RHOSSILIENSIS* TO CONTROL *HETERODERA SCHACHTII*. **B.E. Slaats, A. Patel, W. Beitzen-Heineke, K.-D. Vorlop, R.A. Sikora and J. Hallmann.** *Federal Biological Research Centre for Agriculture and Forestry, Institute for Nematology and Vertebrate Research, Toppheideweg 88, 48161 Muenster, Germany. Email: j.hallmann@bba.de*

Hirsutella rhossiliensis is a nematophagous fungus that parasitizes numerous plant-parasitic nematodes worldwide. For commercial use of *H. rhossiliensis* as a biological control agent, proper formulation is required. Renewables were investigated as encapsulation material for *H. rhossiliensis*. Following preliminary screening of different capsule materials, alginate plus pectine derivative (ALG+PA5) was selected for further testing. The potential of capsules containing 1% *H. rhossiliensis* to control sugarbeet cyst nematode *Heterodera schachtii* was evaluated in 18-litre pots of heat-treated field soil. Six sugar beet seeds were sown per pot. Eighteen days after germination all seedlings except one were removed and root weight and number of nematodes per root system were recorded. The remaining sugar beets were harvested after four months. Plant top fresh weight (g), root fresh weight (g) and final population density (P_f) of *H. schachtii* were measured. Capsules containing 1% *H. rhossiliensis* reduced nematode infestation up to 57% compared to the untreated control. Sugar beet roots showed distinct differences in size and morphology between treatments. The highest root weight and lowest root branching due to nematode infestation were achieved with fungal capsules. In contrast, empty capsules increased damage caused by *H. schachtii*. Although fungal capsules improved sugar beet yield, the final population density of *H. schachtii* was not reduced.

29.27 DETAILED EVALUATION OF *IN VITRO*-PROPAGATED *MUSA* SPP. FOR RESISTANCE AGAINST THE BURROWING NEMATODE, *RADOPHOLUS SIMILIS*. **D. Suganthagunthalam, A. Elsen and D. De Waele.** *Laboratory of Tropical Crop Improvement, Department of Biosystems, K.U. Leuven, Kasteelpark Arenberg 13, 3001-Heverlee, Belgium. Email: suganthagunthalam.dhakshinamoorthy@biw.kuleuven.be*

The burrowing nematode, *Radopholus similis*, is a most damaging pest of banana. Use of resistant varieties is a promising tool for sustainable nematode management. In this regard, many *Musa* accessions were screened all over the world against *R. similis*, to identify natural sources of resistance. Nine new *Musa* accessions were reported as resistant to *R. similis* by a previous screening study conducted in Uganda using sucker-derived *Musa* plants. Although i*n vitro*-propagated *Musa* plants are often more susceptible and sensitive to pathogenic attacks, they are frequently used for commercial and research purposes. Hence in this study, the *Musa* accessions reported as resistant in the previous study were tested

again against *R. similis* using tissue culture-derived planting material. The varieties were screened in comparison with the standards, i.e. Pisang Jari Buaya and Yangambi Km5 as resistant reference and Grande Naine as susceptible reference cultivars. Of the seven accessions tested, two, i.e., Long Tavoy and Saba were confirmed as resistant. Two other accessions, i.e. Marau and Pisang Mas were partially resistant. Two accessions, i.e. Poro Pora and Kokopo showed some degree of resistance and one accession, Gia Hui was susceptible. Further, nematode penetration and developmental studies were conducted to trace the target for resistant response. Comparative rate and number of penetrating and developing nematodes were studied in susceptible and resistant accessions. Plants were harvested at five different times after nematode inoculation and the penetrated nematodes were counted microscopically by staining them inside the root system with acid fuchsin.

29.28 IMAGE ANALYSIS OF *MELOIDOGYNE* SP. ENCUMBERED WITH *PASTEURIA PENETRANS* SPORES. I.K. Vagelas, F.T. Gravanis, B. Pembroke, S.R. Gowen and A. Kimber. *Department of Plant Production, Technological Education Institution of Larissa, 41110 Larissa, Greece.* Email: *vagelas@teilar.gr*

Pasteuria penetrans is a bacterial parasite of root-knot nematodes that shows great potential as a biocontrol agent. Second-stage juveniles (J2s) of a *Meloidogyne* sp. population were added to a *P. penetrans* spore suspension, derived from a commercial product of the bacterium. When 5-8 spores per J2 were observed to be attached, movements of J2s encumbered (or not) with spores were recorded with a digital camera fitted to an inverted microscope, and the images analysed. The velocity of movement without attached spores was significantly higher than that of nematodes with attached spores. The J2 body movement could be fitted in a rectangle. In our model the shorter side of the rectangle was an effective descriptor of the effect of *P. penetrans* attachment on nematode motion. The longer side of the rectangle describing J2s encumbered with *P. penetrans* spores was significantly shorter than that of the J2s without spores.

29.29 THREE ALTERNATIVES TO METHYL BROMIDE AGAINST ROOT-KNOT NEMATODE IN CHINA. X. Wang, Y. Chen, Z. Cao, D. Dong and L.M. Gullino. *Department of Ecology and Ecological Engineering, College of Resources and Environmental Science, China Agricultural University, Beijing 100094, P.R. China. Email: zhipingc@cau.edu.cn*

Methyl bromide (MB) is still widely used against root-knot nematodes (*Meloidogyne incognita*) in China. However, as one of the substances that deplete the stratospheric ozone layer, it must, in China, be totally phased out by 2015. In this study, three methods alternative to methyl bromide, grafting tomato on resistant rootstock (He-man, *L. lycopersicum* × *L. hirsutum*), calcium cyanamide and neem oil have been tested on greenhouse tomato against root-knot nematode in Shouguang city (Shandong Province, China) in 2006-2007. A root-knot nematode susceptible cultivar, FA-189,served as the control. The gall indexes for the treatments of MB, grafting, calcium cyanamide, neem oil and control was 0, 3.8%, 64.4%, 96.0%, 100.0%, respectively. The tomato yield for MB, grafting and calcium cyanamide treatment increased 58.5%, 61.5% and 53.6% compared to the control. The yield of neem oil treatment increased 28.6% compared to the control but was significant lower than those of the other three treatments. A preliminary economic analysis indicated that the pure profit of the five treatments ranked as grafting (24,926\$ ha^{-1}) > MB (24,370\$ ha^{-1})> calcium cyanamide (22,858\$ ha^{-1})>neem oil (17,070\$ ha^{-1})>control (8,446\$ ha^{-1}). In conclusion, grafting with resistant rootstock is a promising alternative to methyl bromide, calcium cyanamide is also an effective alternative, and neem oil is not an effective alternative.

29.30 NUTRITIONAL REQUIREMENTS FOR MYCELIAL GROWTH OF *STREPTOMYCES RUBROGRISEUS*, A BIOCONTROL AGENT OF ROOT KNOT NEMATODE. H. Xue and H. Jian. *Department of Plant Pathology, China Agricultural University, Beijing 100094, P. R. China. Email: hengjian@cau.edu.cn*

Streptomyces rubrogriseus parasitizes egg masses of the root knot nematode. For exploring the potential of this *Streptomyces* for biological control of nematodes, we investigated the nutritional requirements for mycelial growth of *S. rubrogriseus* in liquid media. Five ratios of carbon to nitrogen, four carbon concentrations, eight kinds of carbohydrate, four nitrogen compounds, five macro-element deficiencies, five trace-elements and five vitamins were studied for their effects on the mycelial growth. The optimized combination for growth was: carbon to nitrogen ration at 40:1; 0.5 mol/l carbon concentration; D(+)-cellobiose; potassium nitrate; manganese and boron. We also found starch, bean flour, potassium hydrogen phosphate, calcium carbonate, sodium chloride and ferric sulphate improve the medium for large-scale fermentation. Meanwhile, two year's pot experiments showed that a dosage of 10^{12} spores of *S. rubrogriseus* per pot can reduce galling index by 51.4%.

29.31 EFFICACY OF CERTAIN BIOTIC AND ORGANIC MATERIALS FOR CONTROLLING ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* ON EGGPLANT. M.M.A. Youssef and H.H. Ameen. *Plant Pathology Department, National Laboratory, National Research Centre, Cairo, Egypt. Email: myoussef_2003@yahoo.com*

Eggplant (*Solanum melongena*) is an important vegetable crop and is subject to attack by the root knot nematode *Meloidogyne* ingognita, causing severe damage. Two control methods (a) soaking eggplant seeds and (b) dipping egg plant roots were adopted in this study. The materials used in both methods were as follows: aqueous solution of acetyl salicylic acid as resistance inducer, aqueous extract of pigeon droppings as organic manure, aqueous extract of prickly pear stem as green manure, aqueous solution of ammonium sulphate 20.5% N) and aqueous solution of a biofertilizer or biocide, Nemaless which contains strains of *Serratia marescens).* All these materials were assessed for their ability to control *M. incognita* infecting eggplant cv. Baladi under net greenhouse conditions. Carbofuran G, nematicide, (conc. 10%) was used for comparison. In the first method the highest percentage of female reduction, was achieved by acetyl salicylic acid (81.8%) followed by carbofuran (70.8%), ammonium sulphate (67.9%) and stem of prickly pear (43.8%). Nemaless was the least useful. In the second method the highest percentage female reduction was achieved by pigeon droppings (68.4%) followed by acetyl salicylic acid (39.5%) and ammonium sulphate (21.1%). Prickly pear was the least successful in reducing nematode females (15.8%). No treatment showed significant effects on plant vigour. Key words: *Meloidogyne incognita*, eggplant, seed soaking, bare-root dip, biotic and organic materials.

PLANT BREEDING AND RESISTANCE STRATEGIES

40.1 ORIGIN OF BARLEY ACCESSIONS WITH MULTIPLE DISEASE RESISTANCE DETERMINED BY SSR ANALYSIS. **J.M. Bonman, Y. Gu, D. Coleman-Derr, E. Jackson, S. Chao and H. Bockelman.** *USDA-ARS, Small Grains and Potato Germplasm Research Unit, Aberdeen, ID, USA. Email: mike.bonman@ars.usda.gov*

Although only 1% of accessions of cultivated barley (*Hordeum vulgare* subsp. *vulgare* L.) in the USDA National Small Grains Collection (NSGC) are of unknown origin, these accessions represent 20% of the accessions with multiple disease resistance (MR). These accessions were originally obtained in 1930 from an N.I. Vavilov nursery in Kharkov, Ukraine. Since many MR accessions in the NSGC originated from Ethiopia, the purpose of this study was to test the hypothesis that the unknown MR accessions were also from Ethiopia. The experiment used 40 simple sequence repeat (SSR) markers to screen 12 MR and 12 susceptible (S) accessions of unknown origin, 12 MR and 12 S accessions from Ethiopia, and 152 accessions randomly chosen from the NSGC barley core subset. SSRs that failed to amplify products in >10% of the genotypes and individual alleles occurring in <5% of the accessions were omitted from the analysis. Results from 24 SSRs amplifying 148 alleles were used to group accessions based on using Ward's clustering procedure. Ten of the MR accessions of unknown origin were clearly grouped with accessions from Ethiopia. The grouping included 10 MR, 5 S, and 3 core subset accessions from Ethiopia and one S accession of unknown origin. Our results support the idea that the MR accessions collected from the Vavilov nursery were originally from Ethiopia. This initial experiment is being repeated using 48 SSRs, 192 accessions, and a more automated genotyping procedure.

40.2 MAPPING OF RESISTANCE TO STEM ROT (*SCLEROTINIA SCLEROTIORUM*) IN *BRASSICA NAPUS*. **L. Buchwaldt, S.R. Rimmer and D.J. Lydiate.** *Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK, S7N 0X2 Canada. Email: Buchwaldtl@agr.gc.ca*

We utilized *Brassica napus* Zhong You 821 as source of quantitative resistance to stem rot (*Sclerotinia sclerotiorum*). A reliable disease screening method was developed involving inoculation of stems with a fixed amount of mycelium grown on glucose rich media to control the level of plant cell-wall degrading enzymes which allowed differentiation between levels of stem rot resistance. The phenotypic data included % collapsed lesions and stem lesion length 7, 14 and 21 days after inoculation which also was used to calculated AUDPC. Microsatellite markers (SSR) were mapped in three populations of doubled haploid (DH) lines, 63 DH3 lines derived from Westar × ZY821, plus 230 DH4 and 185 DH5 lines derived from back a cross of the two most two resistant DH3 lines to Westar. A total of 209 SSR were mapped in DH3, 118 SSR in DH4 and 83 SSR in DH5. The DH populations were phenotyped for *Sclerotinia* resistance by inoculating 5 stems of each DH lines with isolate no. 321 in 2–3 replicated tests. Data analysis with Mapmaker QTL of the 28 most resistant and 28 most susceptible DH4 lines resulted in significant QTLs on linkage group N1, N3 and N19. The variances explained by sclerotinia resistance at each QTL ranged from 15% to 38%. F_1 progeny from crosses between the most resistant lines and Westar were susceptible indicating that resistance is recessive. Most F_1 progeny from crosses among different resistant lines were also resistant, suggesting that important QTL were shared between them and homozygous; while less resistant F_1 progeny probably were heterogeneous at certain QTLs.

40.3 GENES RELATED TO STABLE RESISTANCE TO RICE BLAST IN KOREA. **Y.C. Cho, H.J. Park, Y.H. Jeon, J.U. Jeung, J.P. Suh, J.H. Roh, J.H. Lee, K. Jena, S.J. Yang and Y.G. Kim.** *National Institute of Crop Science, RDA, Republic of Korea. Email: yccho@rda.go.kr*

Rice blast continues to be a potentially devastating disease of rice, affecting yield and decreasing quality. It is necessary to look for novel resistance gene(s) for blast that can express a broad-spectrum of resistance in diverse environmental conditions. Korea experiences the breakdown of blast resistance in about 10-year cycles by the emergence of new isolates with enhanced virulence because of the rapid increase of areas cropped with varieties of similar genetic background since 1978. The four *R* genes *Pia*, *Pii*, *Pik*, and *Pik-m* originating from *japonica* rices and the five *R* genes *Pib*, *Pita*, *Pita-2*, *Piz*, and *Pik-p* originating from *indica* rices were identified in Korean *japonica* varieties. The early-maturing varieties had an average of 3.2 *R* genes in each, medium-maturing rices had 1.8, and mid-late maturing lines contained 1.7, respectively. The varieties resistant to blast were 46.2% in early-maturing, 38.1% in medium-maturing, and 10% in mid-late-maturing varieties, respectively. In haplotype analysis, the regions of *Piz* on chromosome 6 and *Pita* on chromosome 12 were effective across years and regions. The monogenic line IRBL5-M of *Pi5* gene originating from *indica* on chromosome 9 showed stable resistance to blast isolates and different field conditions. The strategy to enhance the durability of blast resistance in Korea would be to pyramid *Piz* resistance genes including *Piz-5* and *Pi9*, *Pita* (*Pita-2*), and *Pi5*. This work was supported by a grant (code 20070301034034) from BioGreen21 Program, RDA, Republic of Korea.

40.4 REACTIONS TO DOWNY MILDEW (*PERONOSPORA DESTRUCTOR*) IN THE LOCAL URUGUAYAN ONION GERMPLASM. **P. Colnago, P. H. Gonzalez, J. M. Cortizas, M. Noguez, S. Peluffo, H. Gonzalez Idiarte and G.A. Galvan.** *Facultad de Agronomía, Universidad de la República, Uruguay. Email: horticrs@fagro.edu.uy*

The differential reaction to *Peronospora destructor* in the Uruguayan local onion (*Allium cepa*) germplasm was studied. A set of 27 onion landraces were assessed in a field trial, as well as in a chamber under controlled conditions of temperature and light. Local cultivars were included as susceptible controls. Disease of natural origin was promoted by sprinkler irrigation and intercalating rows of a susceptible cultivar at high density. The experimental design was alfa-latice with four replications. Disease incidence was evaluated weekly and the data integrated to calculate AUiPC, whereas severity was evaluated only once, after a disease outbreak. Local germplasm showed diversity in the incidence (3 to 95%), the incidence progress (AUiPC), and severity (from 5 to 38% of the leaf area affected). This result is interesting for selection for resistance. However, resistance was positively related to late maturity, in such a way that short- and intermediate-day onion types were the most susceptible landraces, and long-day (late) types were the most resistant. The behavior of this last onion group (Valencia type) should be the result of specific resistance factors, or physiological differences due to developmental stage. Evaluation under controlled conditions with a single experimental inoculation, allowed to quantify differences in (1) frequency of successful infections, (2) the rate of growth from infection to spots (cm), (3) incubation peri-

od, and (4) latency period. The diversity in the reaction, even within each onion type, confirmed the potential of local germplasm to be used as sources of field resistance in onion breeding.

40.5 *PHYTOPHTHORA* DISEASES IN THE ROSACEAE: MAPPING OF GENES INVOLVED IN RESISTANCE. J. Davik, D. Røen, S. Sletner Klemsdal, H. Eikemo and M. Bente Brurberg. *Bioforsk, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway. Email: sonja.klemsdal@bioforsk.no*

We address two *Phytophthora* diseases, strawberry crown rot, *P. cactorum*, and raspberry root rot, *P. fragariae* var. *rubi* (Pfr). Raspberry and strawberry cultivars vary in resistance but most commercial cultivars are susceptible to these two diseases. A universal fluorescent labelling method was used for marker analyses to identify genetic markers linked to resistance. For raspberry, the mapping populations were the progenies from a cross between resistant Asker and susceptible Glen Moy. The resistance of 200 progeny plants will be tested both in the field and in a controlled hydroponic culture environment. The entire segregating raspberry population and both parents have been planted at two field locations: one field where Pfr is known to be present, and on another without disease. Disease development will be recorded for two successive years. Thirty days after inoculation with mycelia of Pfr, disease symptoms were recorded. Commercially grown strawberry is a very heterozygous octoploid. We use the diploid wild strawberry as a model plant. Bioforsk keeps a collection of diploid strawberry genotypes from various locations in Norway. A total of 68 genotypes in this collection were tested for resistance to *P. cactorum*. Varying degrees of resistance were observed. Susceptible as well as highly resistant accessions were identified. Based on these results, genotypes will be selected to develop a segregating population from a resistant × susceptible cross in order to investigate inheritance of the resistance.

40.6 GENE ACTION AND GENE NUMBER CONTROLLING PUSTULE SIZE OF STRIPE RUST (*PUCCINIA STRIIFORMIS*) IN WHEAT. H. Dehghani, M. Moghaddam, M.R. Ghannadha, M. Valizadeh and M. Torabi. *Department of Plant Breeding, Faculty of Agriculture, Tarbiat Modrers University, Tehran, Iran. Email: dehghanr@modares.ac.ir*

Winter wheat cultivars Pool, Kotare, Norseman, and Tancred were studied for slow rusting resistance with three pathotypes of *Puccinia striiformis* by analysis of mean generation. Parental, F_1, F_2, and back cross populations from two crosses between the cultivars were evaluated for pustule size in the greenhouse in a randomized complete block design with three replications for cross-pathotype combination. Genetic components were estimated based on the joint scaling test. All cross-pathotype combinations fitted four- parameter or five-parameter models, indicating at least digenic epistatic interaction. The degree of dominance ranged from complete dominance to overdominance. This means that gene action changed in many cultivars, depending on the race used in the test, and various epistatic interactions were also observed. The broad and narrow heritability ranged from 38% to 63% and 12% to 33%, respectively. Most estimates obtained from twelve formulae indicated that stripe rust pustule size was controlled by a small number of genes. In breeding programs it should be possible to exploit for small pustule size by selection based on the genes acting for components of slow rusting resistance (pustule size).

40.7 BREEDING FOR RESISTANCE TO RICE BLAST CAUSED BY *PYRICULARIA ORYZAE* IN EGYPT. A.A. El-Hissewy. *RRTC, Sakha, Kafr El-Sheikh, Egypt. Email: ahmedelhissewy@yahoo.com*

Rice is one of the most important food crops grown in Egypt. Annually, about 0.6 million ha of rice are grown, producing about 6.0 million tons of grain with a national average about 10.0 tons/ha which is considered the highest per unit area average in the world. Rice blast caused by *Pyricularia oryzae* Cav. Is the main disease of rice in Egypt. Most Egyptian rice varieties are resistant but the most popular varieties, Sakha 101 and Sakha 104 that cover about 40% of the total rice growing area are susceptible and suffer yield losses of about 5.0%. Developing blast resistance is a major objective of the rice breeding program in Egypt through the pedigree breeding method. Every year, more than 100 newly bred lines of rice are tested under both artificial and natural infestation. Basic research on the genetic behaviour of blast resistance is also done. Some promising lines showing high levels of leaf blast resistance are under the yeild testing program, to replace both Sakha 101 and Sakha 104.

40.8* GENETIC VARIATION AMONG *FUSARIUM* ISOLATES FROM ONION AND RESISTANCE TO BASAL ROT IN RELATED *ALLIUM* SPECIES. G.A. Galvan, C.F.S. Koning-Boucoiran, W.J.M. Koopman, K. Burger-Meijer, P.H. Gonzalez, C. Waalwijk, C. Kik and O.E. Scholten. *Facultad de Agronomía, Universidad de la República, Uruguay. Email: horticrs@fagro.edu.uy*

The aim of this research was to study levels of resistance to *Fusarium* basal rot in onion cultivars and related *Allium* species, by using genetically different *Fusarium* isolates. In order to select genetically different isolates for disease testing, a collection of 61 *Fusarium* isolates, 43 of them from onion (*Allium cepa*), was analysed using amplified fragment length polymorphism (AFLP) markers. Onion isolates were collected in the Netherlands (15 isolates) and Uruguay (9 isolates), and received from other countries and fungal collections (19 isolates). From these isolates, 29 were identified as *F. oxysporum*, 10 as *F. proliferatum,* whereas the remaining 4 isolates belonged to *F. avenaceum* and *F. culmorum*. The taxonomic status of each species was confirmed by morphological examination, by DNA sequencing of the elongation factor 1-α gene, and by the use of species-specific primers for *F. oxysporum, F. proliferatum*, and *F. culmorum*. Within *F. oxysporum*, isolates clustered in two clades suggesting different origins of *F. oxysporum* forms pathogenic to onion. These clades were both present in each region sampled. Onion and six related *Allium* species were screened for resistance to Fusarium basal rot using one *F. oxysporum* isolate from each clade, and one *F. proliferatum* isolate. High levels of resistance to each isolate were found in *Allium fistulosum* and *A. schoenoprasum* accessions, whereas *A. pskemense*, *A. roylei* and *A. galanthum* showed intermediate levels of resistance. Among five *A. cepa* cultivars, 'Rossa Savonese' also showed intermediate resistance. Regarding the current feasibility for introgression, *A. fistulosum*, *A. roylei* and *A. galanthum* were identified as potential sources for the transfer of resistance to *Fusarium* into onion.

40.9 EFFECTIVENESS OF INDIVIDUAL *PC58* CROWN RUST RESISTANCE GENES AND SUBSEQUENT SNP MARKER DEVELOPMENT. E. Jackson, J.M. Bonman, G. Hu, E. Obert, S. Harrison, J. Chong, R. Oliver and M. Acevedo. *USDA ARS 1691 S. 2700 W. Aberdeen, ID 83210, USA. Email: Eric.Jackson@ars.usda.gov*

Crown rust, caused by *Puccinia coronata* f. sp. *avenae*, is the most damaging disease of oat (*Avena sativa* L.). Historically, crown rust has been controlled by identifying and employing various major genes. The resistance gene *Pc58*, originally identified in 'TAM O-301', has been widely effective. Recent studies have established that *Pc58* is a complex of genes mapping to 3 loci: two tightly linked genes, *Pc58a* and *Pc58c*, and a third loosely linked gene (*Pc58b*). The objectives of the current study were to determine the effectiveness of each gene in the *Pc58* complex and develop single nucleotide polymorphism (SNP) markers linked to the effective genes. Parents and Ogle/TAM O-301 (OT) mapping population (MP) lines were grown under intense natural disease pressure in Louisiana and Texas over two years, and in plots in Canada inoculated with a composite of Canadian races. Diseased leaf area (DLA) and infection types were measured for parents and individual lines, and for groups of lines containing *Pc58a* and *Pc58b* singly or other combinations of the three genes. OT MP lines containing *Pc58a* had significantly lower (2.5X) DLA than all other lines. Additionally, DLA of lines containing *Pc58b* alone or when not in combination with *Pc58a* did not differ from the susceptible parent. Since *Pc58a* significantly reduced crown rust in all 5 field experiments, candidate SNP markers were developed from tightly linked restriction fragment length polymorphism (RFLP) probes flanking *Pc58a*. Work is underway to validate the SNP markers for use in marker-assisted introgression of *Pc58a* into new breeding materials.

40.10 ISOLATION OF HOST FACTORS INTERACTING WITH *POTATO SPINDLE TUBER VIROID* RNA: A POSSIBLE WAY TO EXPLORE ENGINEERED RESISTANCE TO THESE SUBVIRAL PATHOGENS. K. Kalantidis, M.A. Denti, S. Tzortzakaki, E. Marinou, M. Tabler and M. Tsagris. *Department of Biology, University of Crete, P.O. Box 2208, 71409 Heraklion, Greece. Email: tsagris@imbb.forth.gr*

Viroids are RNA pathogens of higher plants replicating either in nuclei or in chloroplasts. Their genome, a circular single stranded non-coding RNA of ca 260-400 nucleotides (depending on the viroid), replicates in the cell via complementary RNA strands. their pathogenicity is based on specific interactions of the genomic RNA and/or replicative forms with proteins, nucleic acids or possibly other components of the host (Tabler and Tsagris 2004, Trends in Plant Science 9, 339). Isolation of host factors specifically interacting with viroid RNA (RNA binding proteins) might help to elucidate their replication, and also they might offer a gene pool for potential resistance genes. Therefore, we developed a method for isolating RNA-binding proteins from cDNA expression libraries and have isolated a tomato protein, Virp1, which specifically binds to PSTVd in an *in vitro* assay (Martinez de Alba 2003, J Virol. 77, 9685). Virp1 forms a complex with PSTVd in infected cells. We isolated and characterized the *Virp1* genes (at cDNA level) in *Nicotiana tabacum* and *N. benthamiana* plants, and we generated transgenic *N. tabacum* and *N. benthamiana* plants, which suppress the endogenous genes *NtVirp1* and *NbVirp1*, respectively, via RNA-mediated gene silencing. We show that plants in which the endogenous *Virp1* genes have been suppressed to a very low level by RNAi, do not support PSTVd and *Citrus exocortis viroid* replication.

40.11 RESISTANCE TO *BOTRYTIS CINEREA* OF KIWIFRUIT TRANSGENIC FOR THE *OSMOTIN* GENE. P. Magro, D. Martignoni, P. Gutiérrez Pesce, S. Cifeca and E. Rugini. *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis snc, 01100 Viterbo, Italy. Email: magro@unitus.it*

The pathogenic fungus *Botrytis cinerea* parasitizes over 200 host plants; among them, *Actinidia deliciosa* is relatively susceptible. The factors that promote the spread and the growth of *B. cinerea* in plant tissues include climate conditions and the growth stage of the plants; in addition, low temperature, sunburn, wind and injuries are also predisposing factors. Although biotic resistance has been investigated on many food crops, in kiwifruit this work is still at an early stage. However, transgenic kiwi plants were already obtained several years ago including those carrying the *osmotin* gene which codes for a protein toxic for phytopatogenic fungi. In this work fruit characteristics, such as weight, Brix degree, penetrometric resistance and resistance to *B. cinerea* were evaluated. Fruits were collected from eight-year-old plants of the cv Hayward, transgenic for the *osmotin* gene with a constitutive 35*S* promoter (supplied by Prof. R. Bressan, Purdue University), and grown under authorized field conditions. Both whole and injured fruit were inoculated with pathogen spores. After incubation, under controlled conditions, the diameter of the external fungal lesions and the level of soft rot of the pulp, were significantly smaller among the osmotin somaclones than in fruits of untransformed plants, demonstrating the effectiveness of this genetic transformation.

40.12 EVALUATION OF RESISTANCE OF SOME WHEAT ADVANCED LINE TO WHEAT POWDERY MILDEW. M. Monazzah, M. Torabi and S. Rezaee. *Department of Plant Pathology, Science and Research Branch, Islamic Azad University, Tehran, Iran. Email: moaryam_mnazzah@yahoo.com*

Wheat powdery mildew disease is caused by *Blumeria graminis* f.sp. *tritici*. Infected samples of wheat were collected from different provinces of Iran in 2005 and transferred to the greenhouse. Eighteen isolates were evaluated for their virulence genes. Pure spores of each isolate were inoculated on the first leaves of 17 differential lines. Infection types were assessed based on a 0 to 9 scale 16 days after inoculation. From the total of 18 isolates, 14 pathotypes were identified. This indicated the high genetic variability in the population of the pathogen and presence of different pathotypes. Two pathotypes from Varamin with 87.8 % pathogenicity, were the most virulent pathotypes. High frequencies of virulence for genes *Pm*3b, *Pm*5 and *Pm*3d were found. Frequencies of virulence for *Pm*1,2,9 and *Pm* 2,4b,8 were low. Comparing the results of virulence surveys in different provinces during 2000 to 2006 showed an increase in frequencies of virulence for some resistance genes while virulence for some of genes had been decreased. Virulence frequencies are highly influenced by the resistance genes carried by cultivars grown in a particular area. Resistance of 60 advanced lines of wheat to 3 pathotypes from Gorgan, Moghan and Varamin was evaluated in greenhouse conditions. All lines were susceptible in seedling stage. In field experiment, 33 lines were resistant and 27 line were moderately susceptible. Susceptible reactions of those lines in seedling stage and resistance reactions in adult plant stage indicated that these lines may carry adult plant resistance. Since due to the high potential of pathogen for producing the new pathotypes to overcome the resistance of the grown cultivars, it is suggested that virulence studies and work for finding new sources of resistance should be regularly continued.

40.13 THE SEARCH FOR FACTORS THAT REGULATE THE CAP-INDEPENDENT TRANSLATION OF VIRUS GENE EX-

PRESSION. R. Ogura, N. Matsuo and K. Hiratsuka. *Graduate School of Environmental and Information Sciences, Yokohama National University, Kanagawa, Japan. Email: d06ta003@ynu.ac.jp*

Internal ribosome entry sites (IRES) were first identified as sequences in viral genomes that allow cap-independent initiation of translation. Recent studies have revealed IRES-mediated translation mechanisms among plant viruses. It has been shown that a crucifer-infecting tobamovirus, crTMV, contains a 148-nt IRES upstream of the coat protein (CP) gene and the sequence is able to mediate cap-independent translation in plant cells. To identify factors involved in cap-independent translation mediated by the CP-IRES, we conducted genetic screening using transgenic *Arabidopsis* harbouring a bicistronic reporter construct consisting of CP-IRES and two luminescent reporter genes. To isolate mutants with altered IRES-mediated translation efficiency, transgenic seeds were treated with EMS and the resulting M2 seedlings were selected for altered luciferase activity using a cooled CCD camera. After screening of about 30,000 M2 plants, we identified several independent M2 lines with reduced luciferase activity. The IRES activities of these plants were up to 10% of control plant as revealed by Dual-Luciferase assays. Further characterization of the mutants and identification of the mutated genes are currently underway.

40.14* EMS-GENERATED RESISTANCE TO *RHIZOCTONIA* IN AN ADAPTED WHEAT. P. Okubara, C. Steber, T. Paulitz and K. Kidwell. *USDA ARS Root Disease & Biological Control Research Unit, P.O. Box 646430, Pullman, Washington, 99164-6430, USA. Email: pokubara@wsu.edu*

We report the first genetic resistance in wheat to *Rhizoctonia solani* AG-8 and *R. oryzae*, the causal agents of *Rhizoctonia* root rot and pre-emergence damping-off. *Rhizoctonia* resistance was generated in the spring wheat cultivar Scarlet using EMS mutagenesis. Resistant plants, named Scarlet-Rz1, displayed substantial root and shoot growth in the presence 100 to 400 ppg of *R. solani* AG-8 and *R. oryzae* in greenhouse assays. Scarlet-Rz1 was otherwise indistinguishable from wild-type Scarlet in appearance, growth habit and seed production in the greenhouse and field. Seedling resistance was monitored in the BC_1F_2, BC_1F_3, BC_2F_2, BC_2F_3 and BC_2F_4 generations of Scarlet-Rz1, and appeared to be inherited as a single co-dominant gene. Genetic resistance to necrotrophic soilborne pathogens is essential for improving the economic viability of conservation tillage systems, in which damage caused by root pathogens can severely limit yield potential. In the absence of naturally-occurring resistance genes and of effective control practices for *Rhizoctonia* root rot and damping-off, Scarlet-Rz1 is a novel genetic resource for developing other *Rhizoctonia*-resistant wheat cultivars. Our findings also demonstrate the utility of chemical mutagenesis for generating resistance to necrotrophic pathogens in allohexaploid wheat.

40.15 EFFICIENT ACCLIMATIZATION OF MICROPROPAGATED TOMATO PLANTLETS USING ARBUSCULAR MYCORRHIZAL FUNGI. S. Patharajan and N. Raaman. *Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, India. Email: spatharajan@ yahoo.co.in*

Lycopersicon esculentum Miller (Solanaceae) ranks as the leading fresh and processed vegetable crop, which is improved by micropropagation and mycorrhization. In *in vitro* culture, different explants such as leaf, shoot tip, axillary bud and internode were cultured on MS medium supplemented with different combinations and concentrations of plant growth regulators. Good callus was observed in MS medium supplemented with 3.0 mg/l of 2,4-D. Direct shoots and multiple shoots were formed from explants (proximal cut end) on the combinations of BAP (3.0 mg/l) and IAA (3.0 mg/l). Roots were formated in MS medium amended with 2.0 mg/l of IBA at 14 days. After 21 days, well-developed *in vitro* plants were hardened in plastic cups filled with sterile soil and vermiculite (2:1). During acclimatization, surface-sterilized arbuscular mycorrhizal (AM) fungal spores were inoculated to the plants for enhanced survivability of *in vitro* plants in field conditions. Levels of biochemical changes and increased survivability were observed in plants inoculated with spores of *Glomus mosseae*, *Gigaspora margarita* and *Scutellospora erythropa*, compared to the control micropropagated plants.

40.16 EVALUATION OF INTERSPECIFIC PEACH HYBRIDS FOR RESISTANCE TO *PLUM POX VIRUS*. J. Polák, I. Oukropec and P. Zeman. *Crop Research Institute, Division of Plant Health, Drnovska 507, 161 06 Prague, Czech Republic. Email: polak@vurv.cz*

The interspecific hybrids of *Prunus persica*, Barier, Cadaman, Fire, GF-677, MRS, NBS 540-73, and Pumiselect were evaluated for five years for resistance to *Plum pox virus* (PPV). The hybrids were grafted on peach trees artificially infected with PPV. The relative concentration of PPV protein was determined in leaves of hybrids by ELISA, every June. The presence of PPV was checked by RT-PCR. The presence and intensity of PPV symptoms in leaves was evaluated monthly from May to August every year. Hybrid GF-677 was very resistant to PPV, and Cadaman was resistant. These hybrids were selected as candidate sources of resistance for crossing with peach cultivars susceptible to PPV. Supported by the Ministry of Agriculture of the Czech Republic, Project No. MZe 0002700603.

40.17 SEQUENCE-BASED BREEDING: HOW MARKERS TURN INTO SEQUENCE ALLELES. M. Prins, M. de Both and A.P. Sørensen. *Keygene N.V., Agro Business Park 90, P.O. Box 216, 6700 AE Wageningen, The Netherlands. Email: marcel.prins@keygene.com*

Current high-throughput sequencing technologies are revolutionizing the DNA research arena. What will the implications be for molecular plant breeding projects for researchers as well as for plant breeders? Keygene is designing and developing a range of molecular breeding applications using advanced sequencing technologies. Some will replace current molecular marker technologies and some will open up novel possibilities for genetic research. We will present a number of current and future possibilities and emphasize the power of efficient use of a combination of HT sequencing tools for trait discovery and exploitation in plant breeding. To keep up with the speed of data generation, the aggregation of data into information that can be absorbed by the researcher and plant breeder becomes an increasingly demanding task. Also in this field Keygene has developed tools that are applied for rapid identification of plant genes involved in response to insects as well as fungal and bacterial pathogens.

40.18 A PROTEIN ACTIVATOR INDUCES PLANT RESISTANCE AGAINST VIRUS DISEASE. D. Qiu, H. Zeng and X. Yang. *Institute of Plant Protection, Chinese Academy of Agricul-*

tural Sciences, No.12 Zhongguancun South Street, Haidian District, Beijing 100081, P. R. China. Email: dewenqiu@hotmail.com

A protein activator 40-65 kd in size was isolated and purified from fungi including *Botrytis*, *Alternaria*, *Magnaporthe*, *Aspergillus*, *Penicillium*, *Rhizoctonia*, *Trichoderma*, and *Fusarium*. Amino acid and derived nucleotide sequence analysis indicated that it represents a new group of proteins. This activator protein can accelerate plant metabolism, promote resistance and enhance plant growth via ligand-receptor interaction and a series of signaling cascades. This contributes to activate the plant disease-resistance system. Large-scale production of the protein showed that it is stable, of low toxicity and is easily degraded in soil. Application of the protein to many plants in lab or field significantly induced plant disease resistance against *Tobacco mosaic virus* (TMV) and *Cucumber mosaic virus*. The protein was found to suppress TMV accumulation and TMV coat protein concentration in tobacco leaves. This protein also showed 40-80% control for other diseases and 10–20% yield promotion.

40.19 INFLUENCE OF MALE-STERILE CYTOPLASMS ON GRAIN MOULD RESISTANCE IN SORGHUM. S. Ramesh, R.P. Thakur, P. Sanjana Reddy, V.P. Rao and B.V.S. Reddy. *International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India. Email: r.thakur@cgiar.org*

Sorghum grain mould caused by a complex of airborne fungi is highly destructive, particularly in short-duration hybrids and varieties that are grown during the rainy season in humid tropical regions of the world. In this study, a total of 72 hybrids, developed on two sets of isonuclear, alloplasmic A-lines, each in six different nuclear genetic backgrounds with A_1 and A_2 cytoplasmic male-sterility systems using 3 dual restorer (R-) lines, and their 15 parental lines were evaluated for grain mould resistance in a field experiment during the 2004 and 2005 rainy seasons. Panicle grain mould was rated at physiological maturity using a 1-9 scale, where 1= no mould, and 9 = >75% grains moulded on a panicle (PGMR). The A_1 and A_2 cytoplasm-based A-lines were compared for their general combining ability effects while A_1 and A_2 cytoplasm-based hybrids were compared for mean PGMR scores, specific combining ability effects, and for the mid-parent heterosis for PGMR scores. Significant but marginal differences between A_1 and A_2 cytoplasm-based hybrids were found for PGMR scores and mid-parent heterosis only in a few nuclear genetic backgrounds. In set 2, 20 of the 36 hybrids, based on 3A-lines (both on A1 and A2 cytoplasm) were resistant, while all others were susceptible. The results show that nuclear genotype rather than cytoplasm *per se* has influence on PGMR of the hybrids. Thus, there is a good possibility of breeding grain mould resistant hybrids based on A_2 cytoplasm involving diverse grain mold resistant hybrid parents.

40.20* MOLECULAR AND VIRULENCE VARIABILITY OF RUSSIAN ISOLATES OF WHEAT AND RYE STEM RUST (*PUCCINIA GRAMINIS* f.sp. *TRITICI* AND f.sp. *SECALIS*) IN 2001-2005. E.S. Skolotneva, S.N. Lekomtseva, Y.V. Maleeva and I.D. Insarova. *Moscow State University, Department Mycology & Algology, Biology Faculty, Moscow, Russia. Email: lekom37@mail.ru*

Stem rust is a serious cereal disease in Russia with its extensive regions growing wheat and rye. We collected *Puccinia graminis* isolates from barberry, wheat, rye and wild cereals in various regions of Russia in 2001–2005. The race composition of *P. graminis* f. sp. *tritici* (Pgt) was very diverse by Shannon's index, with 43 pathogen races, 2–3 of which dominated annually, identified during this period. Our results suggest that in Central Russia the sexual process contributes to the diversity of Pgt clones from barberry as well as to the variability of race composition on wild cereals. There is a great genetic variability within wheat and rye special forms of *P. graminis*. It has been described using different molecular markers (McCallum *et. al*. 1999; Kim *et. al*. 1992) but little is known about the current population structure in nature. The structure of *P. graminis* f. sp. *secalis* isolates from various grasses was analyzed by several molecular methods. There was some evidence for host specialization of the isolates tested, which were stably correlated with the source of inoculum. Using isozymes and randomly amplified polymorphic DNA markers we performed a complex analysis of Pgt isolates. Isozyme analysis revealed geographic variation among the isolates while RAPD-profiles were independent of geographic origin but more determined by host plant. These results suggest that there are several Pgt variability trends at the molecular level.

40.21 IDENTIFICATION OF A MARKER LINKED TO A RESISTANCE GENE OF *EUCALYPTUS* TO RUST USING NBS PROFILING. J.E.C Teixeira, C.M. Silva, C.A.V. Bonine, D. Gratapaglia and L.E.A. Camargo. *Depto. Fitopatologia, Universidade de São Paulo, Av. Padua Dias 11, 13418-900, Piracicaba, SP, Brazil. Email: leacamar@esalq.usp.br*

The conserved motifs of plant R genes such as the NBS domain have been widely used to PCR-amplify resistance gene analogs (RGAs) from various species. We used NBS-profiling and bulk segregation analysis to identify markers linked to a gene for resistance in eucalyptus to the rust *Puccinia psidii.* Our material was an F1 population derived from a controlled cross between two *E. grandis* × *E. urophylla* hybrid clones that present distinct and extreme reactions to the disease. Plants were grown in three localities (approximately 1,000 plants/locality) and exposed to natural rust infection. Clones of parental plants were included in each trial for comparison. Three pairs of DNA bulks, one for each location, were composed of 20 plants from the extreme resistant and susceptible phenotypes, digested with *Alu*I, and PCR-amplified with degenerate primers that recognize the NBS domain. The bulks were also analyzed with AFLP markers. Six AFLP and one RGA marker revealed polymorphisms between the R and S bulks in all three cases and thus were considered candidates for a full linkage analysis. This was done by genotyping 200 plants randomly selected from all locations with the candidate markers and considering disease reaction as a qualitative variable (resistant = leaves with no or very few pustules; susceptible = leaves with some or numerous pustules). All markers were linked in trans to the resistance allele, the closest one (3.2 cM) being the RGA marker. Currently, we are determining the map location of this gene using a set of reference microsatellite markers.

40.22 EVALUATION OF PINEAPPLE GENOTYPES FOR RESISTANCE TO FUSARIOSIS. J.A. Ventura and H. Costa. *INCAPER, Rua Afonso Sarlo 160, Bento Ferreira, 29052-010, Vitória-ES, Brazil. Email: ventura@incaper.es.gov.br*

Pineapple fusariosis, caused by *Fusarium guttiforme* (syn. *F. subglutinans* f. sp. *ananas*), is a serious disease of pineapple (*Ananas comosus* var. *comosus*) in Brazil, and significantly impacts pineapple fruit production with average losses of 30 to 40%.

Control of fusariosis through management practices and fungicide application is possible; however, genetic control using resistant cultivars is the most effective and economical means of controlling the disease. This study was conducted to determine the disease reaction of 74 pineapple genotypes and breeding lines. Seedling evaluation was done under controlled environmental conditions with a virulent isolate of *F. guttiforme* (E-203). Based on disease reactions, 83.8% of the hybrids were found to be resistant to the *Fusarium* isolate tested. Additionally, six genetically diverse lines were identified to be resistant to fusariosis. However, these sources need be well adapted to the pineapple crop region. Genetic analyses of pineapple genotypes by RAPD with 22 decamer primers confirmed the variability, and showed that the resistant accessions tended to be closer. These results suggest that pineapple germplasm contains a broad genetic base for resistance to *F. guttiforme* in Espirito Santo, Brazil, and the resistant sources identified in this study may be utilized in pineapple breeding programs to develop new resistant commercial cultivars. Support: FAPES, FINEP and CNPq.

40.23 QUANTIFYING DNA OF *LEPTOSPHAERIA MACULANS* AND *PYRENOPEZIZA BRASSICAE* IN OILSEED RAPE TISSUES USING QPCR. J.S. West, S.L. Rogers, A.O. Latunde-Dada, E.J. Pirie, J.F. Stonard, Y.J. Huang, S.D. Atkins and B.D.L. Fitt. *Rothamsted Research, Harpenden, AL5 2JQ, UK. Email: jon.west@bbsrc.ac.uk*

Quantitative PCR has potential as a method to measure quantitative (polygenic, partial or horizontal) resistance. This approach has been tested against *Leptosphaeria maculans* (*Phoma* stem canker, blackleg) *and Pyrenopeziza brassicae* (light leaf spot), two major pathogens of winter oilseed rape in the UK. Pathogen DNA was quantified in tissues from 20 different cvs. *L. maculans* DNA was quantified in leaf petioles and crowns, while *P. brassicae* DNA was measured in main shoot tips or lamellae of newly emerged leaves. There was a good correlation between the amounts of *L. maculans* DNA and final stem canker severity from flowering to harvest. Yield loss was associated with severe canker (>50% stem cross-sectional area affected). Amounts of *L. maculans* DNA were small in crowns before mid-winter and varied greatly in petioles with leaf position and sample date (maximum in late autumn). There were often good correlations between amount of *P. brassicae* DNA and severity of light leaf spot observed after laboratory incubation of samples from crops or *in situ* on plants in crops after periods of dry weather. More *P. brassicae* DNA was found in lamellae of symptomless, recently unfolded leaves, than in shoot tips sampled in winter and the amount of DNA in leaves was more closely correlated with subsequent severity of light leaf spot on plants. These quantitative PCR data are providing new insights to optimise fungicide timing and new information about pathogen growth *in planta*.

40.24* ENHANCED RESISTANCE TO COMMON SCAB DISEASE OF POTATO USING CELL SELECTION TECHNIQUES. C.R. Wilson, R.S. Tegg, A.J. Wilson, G.A. Luckman, A. Eyles, Z.Q. Yuan and A.J. Conner. *Tasmanian Institute of Agricultural Research, University of Tasmania, 13 St Johns Ave, New Town, TAS 7008, Australia. Email: Calum.Wilson@dpiw.tas.gov.au*

Common scab disease is one of the most economically important diseases affecting potato production. Cultural and chemical control options for this disease are limited and often ineffective. Thaxtomin A, a phytotoxin produced by the causal pathogen(s), has recently been confirmed as critical to the disease induction process. Development of tolerance to this toxin should therefore lead to long-term, durable disease resistance, which is an important goal to improve sustainability of potato production. We have used cell selection techniques to obtain thaxtomin A-tolerant potato clones of current commercial varieties. This has the advantage of detecting enhanced resistance phenotypes whilst retaining important commercial characteristics of the parent varieties. We used thaxtomin A as a selection agent against potato callus. Rare variants with resistance to the toxin were recovered, regenerated and tested for disease resistance and agronomic performance. Glasshouse pathology screening has identified a large number of clones (approx. 30% of total) with enhanced disease resistance. The best of these clones show extreme resistance and have yet to succumb to common scab disease even under very high disease pressure. Agronomic field trials have shown that many of the resistant clones yield as well as their parent cultivar and most have cooking characteristics at least as good. These data suggest this approach for generation of disease resistance within commercial clones is feasible and promises to provide a significant disease management tool that will be easily incorporated into current production systems.

Plant pathogenic bacteria

26.1 BIOLOGICAL CONTROL OF BACTERIAL SPOT OF TOMATO CAUSED BY *XANTHOMONAS AXONOPODIS* PV. *VESICATORIA*. K. Abo-Elyousr. *Plant Pathology Dept. Faculty of Agriculture, Assiut University, 71526, Assiut, Egypt. Email: kaboelyousr@yahoo.com*

Xanthomonas axonopodis pv. *vesicatoria* was isolated from infected tomato seedlings grown in an open field in Egypt. All the isolates tested were able to infect tomato plants with different degrees of disease severity. In an attempt to manage this disease, tomato seeds were treated with a formulation of an antagonistic local isolate of *Pseudomonas fluorescens*, under greenhouse and field conditions. All treatments significantly reduced disease severity of bacterial spot in tomato relative to the infected control. Foliar application of *P. fluorescens* showed the greatest disease reduction compared with seedlings treated with the pathogen.

26.2 RECLASSIFICATION OF XANTHOMONADS ASSOCIATED WITH ANACARDIACEAE INTO TWO SPECIES AND THREE PATHOVARS. N. Ah You, L. Gagnevin, P. Grimont, S. Brisse, X. Nesme, F. Chiroleu, L. Bui Thi Ngoc, E. Jouen, P. Lefeuvre, C. Vernière and O. Pruvost. *UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical (PVBMT), CIRAD-Université de la Réunion, 97410 Saint-Pierre, Réunion. Email: gagnevin@cirad.fr*

Mango, cashew, Brazilian pepper and ambarella are host species of *Xanthomonas* strains which have a broad genetic and phenotypic diversity. Until now, it was not determined whether they should be classified as strains of a single pathovar with a broad host range or as several pathovars. A polyphasic approach was used to determine their respective taxonomic positions, their mutual relationships and their relationship to other *Xanthomonas* species. Pathogenicity tests and amplified fragment length polymorphism (AFLP) suggested the distinction of three pathovars presenting a host specialization: pv. *mangiferaeindicae* on mango and Brazilian pepper, pv. *anacardii* on cashew and pv. *spondiae* on

ambarella. AFLP and multilocus sequence analysis (MLSA) were congruent and showed that the three pathovars are related to *X. axonopodis* genetic groups 9.5, 9.6 and 9.4, respectively. DNA-DNA hybridization, AFLP and MLSA indicated that pvs. *mangiferaeindicae*, *anacardii* and *citri* (the causal agent of citrus canker) belong to a single species and that they should not be classified as *X. axonopodis*. Our results agree with the recent elevation of pv. *citri* to species level and propose to include pvs. *mangiferaeindicae* and *anacardii* in this species. Pathovar *spondiae* is genetically related to group 9.4, for which no reclassification has yet been proposed.

26.3 SEROLOGICAL STUDY ON *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* THE PATHOGEN OF LEAF SPOT DISEASE ON CANE-APPLE (*ARBUTUS PAVARII PAMPANINI*) IN JABAL AL-AKHDAR AREA, LIBYA. **A.M.Y. Alawami, H. Younis, A. Naser M. Bobaker and O.M. Elsanosy.** *Plant Protection Dept., Faculty of Agriculture, Omar Al-Mukhtar University, El-Beida, Libya. Email: Azzawami2002@yahoo.com*

Polyclonal antibodies were produced against *Pseudomonas syringae* pv. *syringae* the causal organism of bacterial spot on cane-apple (*Arbutus pavarii* Pampanini) in Jabal Al-Akhdar area, Libya. Results of slide agglutination and Ouchterlony gel double diffusion tests showed positive reactions between the bacterial isolate and its homologous antiserum. By indirect ELISA it was shown that the first antiserum collection (after 2 days) gave the highest quantity of antiserum compared with the second and third collecting time (7 and 14 days respectively). The titre of the antiserum was $1{:}10.24\times10^4$ as determined by indirect ELISA. Indirect ELISA was shown to be efficient, using this antiserum, for comparing different bacterial isolates.

26.4 INFLUENCE OF HOST RESISTANCE AND GRAFTING ON THE INCIDENCE OF BACTERIAL WILT AND ROOT KNOT DISEASE OF EGGPLANT. **R.T. Alberto, N.L. Opina, S.E. Santiago, R.M.Gapasin and S.A. Miller.** *Department of Crop Protection, College of Agriculture, Central Luzon State University, Munoz, Nueva Ecija 3120, Philippines. Email: ralbrtco@mozcom.com*

Six eggplant cultivars were screened in the greenhouse for resistance to strains of *Ralstonia solanacearum*. Three entries showed resistance to the strains of *R. solanacearum* used and two showed moderate resistance. The wild variety of eggplant *Solanum sisymbrifolium* was rated susceptible to bacterial wilt. The results of screening different rootstock and scion combinations for resistance to bacterial wilt showed that grafting using bacterial-resistant rootstocks effectively reduced bacterial wilt infection. A survey conducted on the incidence of bacterial wilt of eggplant in Pangasinan, Nueva Ecija and Batangas showed that wilt incidence ranged from 0–80% in Pangasinan, 5–50% in Nueva Ecija and 0-25% in Batangas during the dry season. Initial results of a wet season survey on the incidence of the disease in Quezon and Batangas showed 10-30% and 36%, respectively. Partial results of biovar classification of 197 Pangasinan isolates evaluated showed that 6.1% belonged to biovar 3 and 93.9% were of biovar 4. For the Batangas isolates, 38.7% were biovar 3 and 61.3% were biovar 4 while 80% were biovar 3 and 20% were of biovar 4 in the Nueva Ecija isolates. For root knot nematode resistance, no eggplant cultivar was rated resistant to *M. incognita* and *M. graminicola.* All fifteen cultivars evaluated showed susceptible to moderately susceptible reactions.

26.5 EFFICACY OF CHEMICAL AND BIOLOGICAL TREATMENTS ON POPULATION SIZE OF *ERWINIA AMYLOVORA*. **Y. Alipour, A. Babaei Ahari and H. Rahimian.** *Plant Protection Organization, Tehran, Iran. Email: shahablpr@yahoo.com*

Effectiveness of biological agents and chemicals to *Erwinia amylovora* on pears was tested under natural conditions during 2000-2001. Pear trees in Sardrood, Eastern Azerbaijan, were sprayed either three or four times with Bordeaux mixture, Bordeaux mixture-oil, and aqueous suspension of *Pantoea agglomerans* during dormant, green tip, 50% bloom and full bloom stages. Significant differences among treatments were observed. Best activity was obtained with Bordeaux mixture plus oil. Oil alone caused the highest population size of *E. amylovora.* Aqueous suspensions of *P. agglomerans* showed lower activity than chemicals.

26.6 EVALUATION OF CHEMICAL AND BIOLOGICAL CONTROLS OF FIRE BLIGHT. **Y. Alipour, A. Babaei Ahari and H. Rahimian.** *Plant Protection Organization, Tehran, Iran. Email: shahablpr@yahoo.com*

The bacterial disease of fire blight caused by *Erwinia amylovora*, one of the most important and serious diseases of pome fruits, has caused economic losses to such trees in some regions of Iran in recent years and is still spreading. In an experiment with chemical and biological control methods, disease control was evaluated under field conditions. For chemical control, Bordeaux mixture and for biological control, the bacterium *Erwinia herbicola* (*Pantoea agglomerans*) were used. For fire blight severity on pear trees, there were significant differences among treatments. Treatment at four stages with oil sprays gave the most disease severity (75.375%) and similar treatment with sprays of Bordeaux mixture plus oil gave the least disease severity (1.875%). Fire blight bacterial population size was significantly different among treatments. Four-stage oil sprays led to the largest population size, and four-stage Bordeaux mixture plus oil produced the smallest populations.

26.7 THE COMPLETE GENOME SEQUENCE OF *CANDIDATUS PHYTOPLASMA* AUSTRALIENSE. **M.T. Andersen, L.W. Liefting, I. Havukkala and R.E. Beever.** *HortResearch, Private Bag 92169, Auckland, New Zealand. Email: mandersen@hortresearch.co.nz*

Phytoplasmas are associated with more than 600 plant diseases world wide. Formerly known as mycoplasma-like organisms (MLOs) these bacteria have yet to be cultured *in vitro* and little is known about their pathogenicity, other than the symptoms they cause reflect a series of perturbations of normal plant development. The application of nucleic acid molecular techniques such as PCR and DNA sequencing have provided considerable insight into the nature of these pathogens. To date the complete genomes of two phytoplasmas belonging to '*Candidatus* Phytoplasma asteris', onion yellows mild strain (OY-M) and aster yellows witches' broom (AY-WB) have been sequenced. We report here the sequencing of a phytoplasma belonging to a separate Candidatus species, '*Candidatus* Phytoplasma australiense'. This phytoplasma is associated with four diseases in New Zealand (*Phormium* yellow leaf, *Cordyline* sudden decline, *Coprosma* lethal decline, and strawberry lethal yellows), as well as several in Australia. We have determined the complete genome sequence of a New Zealand isolate of '*Ca*. P. australiense' from a plant symptomatic for strawberry lethal yellows. The genome of '*Ca*. P. australiense' consists of a

959,779-bp circular chromosome and is the largest phytoplasma genome that has been sequenced to date. Genomic dotplot analysis show that the level of synteny between the genomes of '*Ca*. P. australiense' and OY-M or AYWB is considerably less than that observed between OY-M and AY-WB. Analysis of the putative coding sequences identified a number that are not present in the other genomes. Results of comparative analyses between the three sequenced phytoplasma genomes will be presented.

26.8* GENOME SIZE AND GENOMIC DIVERSITY OF *CLAVIBACTER MICHIGANENSIS* SUBSP. MICHIGANENSIS STRAINS IN TURKEY. H. Basım and E. Basım. *Akdeniz University, Faculty of Agriculture, Department of Plant Protection, 07070, Antalya, Turkey. Email: hbasim@åkdeniz.edu.tr*

Strains of *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), causal agent of tomato bacterial canker, were isolated from greenhouse and field tomato, both important crops in Antalya province and surrounding counties in the western Mediterranean region of Turkey. Total genomes of the Cmm strains were analysed by using rare-cutting restriction enzymes, *Spe*I, *Xba*I, *Swa*I, *Ase*I, *Eco*RI and *Hind*III, and pulsed-field gel electrophoresis (PFGE) (CHEF DR II). Twelve different pulsotypes of Cmm were determined. Total genomes of the strains were compared to those of other Cmm strains from different countries. The PFGE analyses was not enough to differentiate virulent Cmm strains from avirulent Cmm ones. Genome sizes of the Cmm strains were compared with those in the literature.

26.9 CHARACTERIZATION OF *STREPTOMYCES SCABIES* ISOLATED FROM COMMON SCAB LESIONS ON POTATO TUBERS BY MORPHOLOGICAL, BIOCHEMICAL AND PATHOGENICITY TESTS IN THE CHLEF REGION OF WESTERN ALGERIA. M. Bencheikh and S. Benali. *B.P. 920, Chlef 02000, Algeria. Email: bencheikdz@yahoo.fr*

In summer 2002, nine isolates causing common scab symptoms were collected from potato tubers presenting superficial and raised corky lesions in a number of locations from the Chlef region in Algeria. The common scab-inducing organisms were characterized by creamy colonies on yeast malt extract (YMA) and by aerial mycelium which turned brown with age. The organisms were Gram-positive, non-motile, and utilized L-arabinose, D-fructose, D-glucose and rhamnose. They degraded xylose and starch with production of melanin on peptone yeast extract agar-iron(PYI). Additionally, most strains obtained from different locations were identical in morphology and biochemical characteristics. Furthermore, these isolates shared most characters with the reference isolate ATCC49173. Results of pathogenicity tests showed that all isolates were pathogenic on both potato cultivars Desiree and Claustar, with aggressiveness varying from mild to moderately severe. Koch's postulates for the isolates were also fulfilled.

26.10* GENETIC DIVERSITY OF *XANTHOMONAS CITRI* pv. CITRI CAUSING CITRUS CANKER IN ASIA. L. Bui Thi Ngoc, C. Vernière and O. Pruvost. *UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical (PVBMT), CIRAD-Université de la Réunion, 97410 Saint-Pierre, Réunion. Email: verniere@cirad.fr*

Three PCR-based methods were used to assess the genetic diversity of 239 strains of *Xanthomonas citri* pv. *citri* (Xcc) causing citrus canker in Asia, including some strains from international collections. Data from amplified fragment length polymorphism (AFLP) analysis after SacI/MspI restriction, insertion sequence-ligation mediated-PCR (IS-LM-PCR) targeting 3 IS (ISXac-1, -2 and -3) and variable number of tandem repeats (VNTRs) using 14 minisatellite loci grouped into a multiple-locus VNTR analysis (MLVA) were collected by automated capillary electrophoresis. All three methods discriminated between Xcc-A strains exhibiting a broad host range on different citrus species, and Xcc-A* strains with a more restricted host range, mainly on limes. Pathogenicity profiles were confirmed by inoculation on a range of hosts that differentiated these strains using a detached leaf assay. Xcc-A* was detected in South-East Asia, with a larger geographical distribution than previously known. The different genetic clusters of Xcc-A* that could be identified mostly correlated with geographical origin. Xcc-Aw strains from Florida were closely related to Xcc-A* both genetically and pathologically and they could be synonyms. The 239 Xcc strains were best discriminated by VNTRs, yielding 211 different haplotypes, followed by IS-LM-PCR (149 haplotypes) and AFLP (97 haplotypes). IS-LM-PCR and MLVA appeared to be most appropriate for genotyping Xcc strains on a small geographical scale. The three methods were very informative and data analysis could be highly automated, making them useful for population structure analysis and convenient for addressing issues about the epidemiology of this quarantine pathogen.

26.11 BACTERIAL RICE PANICLE STERILITY THREATENS ITALIAN RICE CROPS. P. Cortesi, F. Bartoli and C. Pizzatti. *Institute of Plant Pathology, State University of Milan, Via Celoria 2, 20133 Milan, Italy. Email: paolo.cortesi@unimi.it*

Bacterial panicle sterility is widely distributed throughout rice-growing areas in Italy, threatening rice productivity. The disease has variable incidence, from barely detectable to more than 50% of panicles almost devoid of grains, and strongly reducing production. We have shown that panicle sterility is caused by *Acidovorax avenae* subsp. *avenae* (*Aaa*), a seed-borne bacterium found for the first time on rice in Italy. To estimate the potential of the pathogen to threaten rice production, we studied: i) contamination of rice-seed lots, ii) variation in virulence of bacterial strains and susceptibility of rice varieties, and iii) climatic factors affecting disease severity. One hundred and fifty samples of rice-seed lots, were tested for the presence of *Aaa* with an improved BIO-PCR assay. Surprisingly, almost all samples tested positive. Virulence of *Aaa* strains was tested on seedlings in the greenhouse, where they caused soft rot, and on plants in the field, where they caused significant increase in the incidence of panicle sterility and grain discolouration. *Aaa* strains sampled in Italy showed significant intraspecific variation in virulence, and the Italian rice varieties showed significant variation in susceptibility. The most severe epidemic of panicle sterility recorded was associated with the high temperatures experienced in 2003. Panicle sterility may be an example of an introduced pathogen able to cause severe epidemics and significant loss to rice crops as a consequence of global warming.

26.12 EMERGENCE OF MIDRIB ROT CAUSED BY *PSEUDOMONAS CICHORII* IN GREENHOUSE LETTUCE. B. Cottyn, K. Vanhouteghem, E. Pauwelyn, K. Heylen, P. Bleyaert, P. De Vos, M. Höfte and M. Maes. *Institute for Agricultural and Fisheries Research, Burg. Van Gansberghelaan 96, 9820 Merelbeke, Belgium. Email: bart.cottyn@ilvo.vlaanderen.be*

Greenhouse butterhead lettuce (*Lactuca sativa* L. var. *capitata)* is the most important leafy vegetable produced in Flanders for consumption and export. During the past 10 years, bacterial midrib rot has emerged as a serious threat for lettuce producers. Symptoms consist of a dark brown to greenish black rot along the midrib of one or more inner head leaves. The disease has consistently been associated with plants approaching harvest, which impedes early detection. Symptomatic lettuces were collected from commercial greenhouses throughout Flanders. Dilution plating of extracts of diseased plants onto pseudomonas agar F consistently yielded colonies that fluoresced under UV366 light. Predominant bacteria were identified as *P. cichorii*, using LOPAT tests, whole-cell fatty acid methyl ester analyses, 16S rDNA sequence analyses, and DNA-DNA hybridizations. BOX-PCR analysis revealed three genotypes in this group of *P. cichorii* isolates. They corresponded to distinct morphotypes distinguished by colony appearance. The three genotypes have not yet been found together in the same greenhouse. Isolates of the three genotypes reproduced the symptoms upon spray-inoculating lettuces, even at low concentrations of 102 CFU/ml. Symptom development on lettuce depended on high humidity and head formation. The use of drip irrigation instead of overhead sprinklers was shown to prevent spread of the pathogen by water splash. In comparison to varnish spot in the field, midrib rot is another manifestation of infection of lettuce by *P. cichorii* in the greenhouse.

26.13 *PANTOEA* SPECIES ASSOCIATED WITH BLIGHT AND DIE-BACK OF *EUCALYPTUS*. **T.A. Coutinho, L. Swart, C. Brady, I. Greyling, G. Nakabonge, S.N. Venter, C.A. Rodas and M.J. Wingfield.** *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa. Email: teresa.coutinho@fabi.up.ac.za*

Bacterial blight and die-back of *Eucalyptus* is caused by *Pantoea ananatis* in South Africa. A similar disease is also reported to be caused by *Xanthomonas campestris* pv. *eucalypti.* In surveys undertaken in Argentina, Colombia, Indonesia, Thailand, Uganda and Uruguay a disease resembling that occurring in South Africa was observed. Infected leaves were collected from plants in these six countries and from recent outbreaks of the disease in South Africa. Bacteria were isolated from infected tissue and phenotypic analyses completed. Partial 16S rRNA and *gryB* sequencing was then performed on all strains isolated. F-AFLPs were also used and the resulting profiles compared to those of the type strains of all species in the genus. *Pantoea* species were consistently isolated from infected leaves collected from all countries. *P. ananatis* was isolated from leaves collected in South Africa, Thailand and Uruguay while the recently described species, *P. vagens* prov. nom. was found on leaves collected in Uganda, Colombia and South Africa. *P. dispersa* was isolated from infected leaves collected in Argentina, South Africa and Thailand, while *Pantoea eucalypti* prov. nom., another recently described species, was found on infected leaves collected from Colombia, Uruguay and South Africa. Representative strains of the species were inoculated into a known susceptible *E. grandis* × *E. nitens* clone. *P. ananatis* was found to be the most pathogenic species while the remaining three species were moderately pathogenic. Bacterial blight on *Eucalyptus,* considered in this study, appears to be caused by a complex of *Pantoea* species. These are opportunistic, and symptoms are expressed when host plants are susceptible and environmental conditions are favourable for infection.

26.14 FUNCTIONAL CHARACTERIZATION OF HOPN1, AN EFFECTOR OF *PSEUDOMONAS SYRINGAE* pv. *TOMATO* DC3000 INVOLVED IN THE MODULATION OF CELL DEATH IN PLANTS. **R. Cuartas Lanza, S. Sopeña Torres, P. Rodríguez-Palenzuela and E. López-Solanilla.** *CBGP/ETSIA, UPM. Av. Complutense s/n, 28040 Madrid, Spain. Email: emilia.lopez@upm.es*

Pseudomonas syringae pv. *tomato* DC3000 is the causal agent of bacterial speck in tomato and *Arabidopsis*. The so called Type Three Secretion System (TTSS) and the effector proteins which are translocated inside the plant cell, play an essential role in the virulence of this bacterium. One such effector, named HopN1 (previously named HopPtoN) has been described as a cystein protease, able to suppress cell death associated with the hypersensitive reaction (HR) in resistant plants, as well as disease caused by the bacterium in susceptible plants. This fact is congruent with the hypothesis that both types of cell death must share common mechanisms to be triggered inside the plant. The suppressor activity of cell death associated with HR has been reported in other effectors, suggesting that this phenomenon constitutes a strategy which benefits the growth of the pathogen within the host. The main objective of this work is the characterization of the mechanism of action of HopN1 with respect to its ability to modulate programmed cell death in plants. We are developing the following experimental approaches: 1) Identification of the target of HopN1 in the plant; 2) Analysis of the evolution of bacterial populations (wild-type and mutant strains) inside the plant, in order to unravel the specific contribution of this effector to bacterial virulence; 3) Study of the effect of over-expression of HopN1 on the virulence of other plant pathogenic bacteria with pathogenic behaviour different from that of *P. syringae.*

26.15 SUSCEPTIBILITY OF SOME LESS SENSITIVE APPLE CULTIVARS TO FIRE BLIGHT, TESTED BY ARTIFICIAL FLOWER INFECTION. **K. Dugalic, E. Đermic, B. Cvjetkovic, T. Milicevic.** *The Agricultural Institute Osijek, Juzno predgradje 17, Croatia. Email: bcvjetkovic@agr.hr*

Fire blight, one of the most destructive diseases of rosaceous plants, occurred in Croatia in 1995. An experimental orchard of 27 apple cultivars was established at the Agricultural Institute in Osijek. Some of these cultivars were noted by their breeders as less sensitive to fire blight. In order to evaluate their susceptibility, 5-year-old apple trees were inoculated during flowering (stage F-F2 according Baggiolini) on April 13th 2007. The inoculum originated from the same orchard and contained two isolates of *Erwinia amylovora* (5 OS and 6 OS). For inoculation, bacterial suspensions with 3.9×10^5 cfu/ml were prepared by diluting 100 ml of overnight culture of each isolate. On each tree one branch was sprayed with inoculum. The experiment was performed in 4 repetitions and 8 trees of each cultivar were inoculated. Development of symptoms was monitored and final results were collected on June 6th 2007. Golden Delicious was included as susceptible standard, and was found sensitive. The cultivars that showed high levels of resistance were: Rewena, Realka, Enterprise, Liberty, Arkansas Black, Spartan, Topred Del., Cooper 7A and 7SB2 and Nova Easygro. Apart from sensitivity to *E. amylovora* infection, other pomological parameters (yield, fruit quality) were evaluated. On the basis of all apple cultivar characteristics tested for eastern Croatia, we conclude that best pomological characteristics of all apple cultivars resistant to fire blight were possessed by Cooper 7SB2, Enterprise and Rewena.

26.16 ROLL OF CALCIUM IN SIGNAL TRANSDUCTION DURING THE DEFENCE RESPONSE OF OILSEED BRASSICAS TO WHITE RUST CAUSED BY *ALBUGO CANDIDA*. **S.A. Elahinia.** *Department of Plant Protection, P.O. Box.41635-1314, University of Guilan, Rasht, Iran. Email: saelahinia@gui.ac.ir*

We investigated oilseed brassica (*Brassica juncea* (L.) Czern. and Coss.), genotypes Tourch and Commercial Brown, susceptible and resistant to white rust (*Albugo candida)* respectively. Suspensions of fresh zoospores (25 nm drop) of *A. candida*, Race 7v were inoculated on cotyledons of 8-day-old seedlings. Segments of infected leaves sampled 8 days later were observed by scanning electron microscopy (SEM). Pustules on the resistance genotype were much fewer in number, small and poorly sporulating than on the susceptible host. Also crystal in various shapes were frequently present on the infected areas and the surface of pustules in the resistance genotype. The crystals were characterized by energy dispersive X-ray microanalysis in conjunction with SEM. More calcium was mobilized in infected areas of the resistant host than in the susceptible one. The level of calcium in the uninfected areas of both resistant and susceptible hosts and on the infected areas of the susceptible host was nearly the same. The results indicated that calcium mobilization in the resistant genotype upon infection with *A. candida* correlated with elicitation of a defence response conditioned by resistance genes. This is the first report on the mobilization of calcium in the white rust pathosystem as part of a resistance response.

26.17 INVOLVEMENT OF VARIOUS PSEUDOMONADS IN BACTERIAL SYMPTOMATOLOGY ON FIELD MELONS. **A. Fabi, M. Renzi and L. Varvaro.** *Department of Plant Protection, University of Tuscia, Viterbo, Italy. Email: fabi@unitus.it*

In 2007 a severe outbreak of a suspected bacterial disease occurred over a wide area of melon cultivation in coastal Maremma (central Italy). The symptoms consisted of leaf spots appearing a few days after transplantation and/or soft rots developing on fruits. The symptoms did not clearly correspond with the typical bacterial angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans*. Various bacterial strains were isolated from infected leaves and fruits, then characterized by biochemical, metabolic and nutritional methods and confirmed by the Biolog® System. The bacterial species isolated were strains of *P. syringae* pv. *syringae*, *P. s.* pv. *lachrymans*, *P. viridiflava* and a few other *Pseudomonas* spp. Pathogenicity was tested on the same varieties of melon plants and other host plants in the greenhouse, and Koch's postulate was always satisfied. Identification of the pathogenic strains using PCR is still in progress in order to understand the role of different bacterial species in causing the disease. The relations between disease severity and the weather conditions that occurred in the investigated areas are discussed.

26.18 DETECTION OF BLACKLEG AND POTATO SOFT ROT WITH ITS-PCR AND ITS-RFLP IN IRAN. **R. Firouz, M. Bahar and B. Sharifnabi.** *Department of Plant Protection, College of Agriculture, Isfahan University of Technology, Isfahan, Iran. Email: razieh_firooz@yahoo.com*

Certain species and subspecies of *Pectobacterium* cause blackleg and soft rot diseases in potato. Due to their differences in adaptation to diverse climates, over-wintering and survival in soil or seed tubers, it is important to precisely identify the causal agent of potato blackleg in each region. In surveys of potato blackleg in Iran during 2003-2004, 54 bacterial strains isolated from diseased plants sampled, were recognized as *Pectobacterium* sp. on the basis of morphological and biochemical characteristics and also pectolytic activities. To confirm the results of biochemical tests, after DNA extraction with the method of Cullen *et al.,* primer set G_1/L_1 was used to detect *Pa*, *Pcc* and *Pch* in one PCR reaction. By utilizing this primer set, the ITS regions of the bacteria were amplified. On the basis of band patterns obtained, the isolates were placed in three groups. The first group comprised only the standard isolate *Eca* SCRI1043 and such a pattern was observed in none of the isolates we collected. Isolates identified as *Pcc* by biochemical tests, fell into the second group, and the third group included isolates identified as *Pch* from biochemical and physiological tests. The results obtained by the ITS-PCR method were confirmed by using ITS- RFLP analyses. Thus, species and subspecies of *Pectobacterium* causing potato blackleg and soft rot could be identified more rapidly and accurately using this set primers than by using morphological and biochemical tests.

26.19 COMPARISON OF SEMI-SELECTIVE MEDIA TO DETECT *CURTOBACTERIUM FLACCUMFACIENS* pv. *FLACCUMFACIENS* IN SEEDS OF BEAN (*PHASEOLUS VULGARIS*). **V.C. Frare, M.H.D. Moraes, J.R. Ottoni, D.M. Balani, V. Mondo and J.O.M. Menten.** *USP/ESALQ, Av. Pádua Dias, 11, C.P. 09, Piracicaba, SP, 13418-900, Brazil. Email: vanessa@esalq.usp.br*

We compared the efficiency of the semi-selective media MSCFF and modified CNS in detecting *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff) through the methods of streak and spread (dilution in series 10^{-5},10^{-6},10^{-7}). Four subsamples of 500g each, obtained from 2 samples of crop seeds of cv 'Rubi', were immersed into 500 ml of sterile distilled water for 24 h at 5 ºC. After the assembling, the plates were incubated at 28 ºC, and evaluated after three and six days. A known strain of Cff was used to confirm the evaluation of the media and the PCR. The CNS medium revealed the presence of typical Cff colonies and the MSCFF medium also showed halo formation. Typical colonies were isolated and submitted to PCR (primers CF4 and CF5) and to pathogenicity tests in tobacco leaves and bean pods. Within the three days, in the streak method, there was characteristic growth in MSCFF medium, for the two samples, and in the CNS medium only for the second sample. In the spread method, growth was observed only in MSCFF medium, at the 3 dilutions, for both samples. In the last evaluation only CNS medium was evaluated. By the streak method, growth was observed also in sample 1; in the spread method, growth was seen in the 3 dilutions for sample 1 but only in the 10^{-5} dilution for sample 2. The results of PCR and pathogenicity tests were positive.

26.20 APPROACHES TO STUDYING SURVIVAL OF *XANTHOMONAS AXONOPODIS* pv. CITRI IN LEAVES AND FRUITS. **M. Golmohammadi, A. Redondo, P. Llop, I. Gell, M.M, López, J.H. Graham and J. Cubero.** *Dpto. Protección Vegetal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Ctra de La Coruña km 7,5. Madrid, Spain. Email: cubero@inia.es*

Xanthomonas axonopodis pv. *citri* (Xac) causes citrus bacterial canker (CBC), a serious disease of most citrus species and responsible for economic losses in wet, subtropical citrus industries. In the Xac life cycle, bacteria multiply in lesions, and when free water is available on leaf surfaces, cells egress from stomata

and lesions, to be dispersed by windblown rain droplets. However the lifespan and fate of Xac on leaf and fruit surfaces is uncertain and needs clarification to determine the potential of infected fruit for bacterial spread into export markets free of CBC. To study bacterial survival and viability, two approaches have been tested. First, a reporter based on gfp expression in the bacterium was evaluated for survival studies. For this purpose, Xac strains were transformed with plasmids containing the native *gfp* gene and a *gfp* modified so that the gfp protein is unstable inside the bacterial cell. Xac strains that express Gfp in this labile form only emit fluorescence when they are alive as opposed to the native-gfp containing strains that remain fluorescent even after death. Using native and labile gfp variants, bacteria were visualized in leaf and fruit tissues on surfaces and internally. The second approach was based on using RNAs as targets for PCR detection. Our goal was to develop a protocol based on retrostranscription real-time PCR (RRT-PCR) to not only detect the bacterium but to determine the viability of the bacterial population in planta and in fruit. RRT-PCR is based in the instability of mRNA as compared to DNA (retrotranscription-PCR vs. DNA-PCR). Following this approach the transcription of a variety of Xac genes was evaluated for use as viability markers.

26.21 TWO SEED-BORNE BACTERIAL SPECIES, *PANTOEA ANANATIS* AND *P. ALLII* SP. NOV., ARE ASSOCIATED WITH CENTRE ROT OF ONION. T. Goszczynska, C. Brady, S.N. Venter, I. Cleenwerck, M. Vancanneyt, J. Swings and T.A. Coutinho. *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa. Email: teresa.coutinho@fabi.up.ac.za*

Pantoea species are becoming increasingly important as plant pathogens worldwide. In South Africa, they have been reported to cause diseases of eucalyptus, maize and onion. Centre rot has never been observed locally in onion fields but two *Pantoea* species were isolated from onion seed. The objective of this study was to determine the taxonomic position of these two species. Thirty strains isolated from onion seed in South Africa and strains obtained from diseased onion plants in the USA were examined. All strains were subjected to a polyphasic analysis that including phenotypic characterisation, analysis of the F-AFLP fingerprint profiles, 16S rRNA and *gyrB* gene sequences, and DNA-DNA hybridisations. The type strains of all recognised *Pantoea* species were included. Pathogenicity trials were undertaken with selected South African and American strains. The majority of strains were identified as *P. ananatis*. Nine strains, however, formed an F-AFLP cluster that did not resemble profiles of described *Pantoea* spp. These strains also formed a separate branch in 16S rDNA and *gyrB* phylogenetic trees. DNA homology values among three representative strains of this cluster were between 90 and 100%. These results showed that the strains belong to a single species. The most closely related type strain was *P. ananatis* with 44 to 57% DNA homology. The name *Pantoea allii* sp. nov. is proposed. The results of pathogenicity trials indicated that the disease known as centre rot is caused by *P. ananatis* as well as *P. allii*, as these species induced identical symptoms in the inoculated onion plants.

26.22 EVALUATION OF RESISTANCE OF CULTIVATED WALNUT VARIETIES AND SELECTIONS TO *XANTHOMONAS ARBORICOLA* PV. *JUGLANDIS* IN GREECE. F.T. Gravanis, I.K. Vagelas, C. Rumbos, A. Chatzaki, D. Rouskas and J. Tsiantos. *Technological Education Institute of Larissa, Department of Plant Production, 41110 Larissa, Greece. Email: gravanis@teilar.gr*

Thirty three walnut varieties (16 lateral and 17 terminal fruit fullness varieties) and 12 and 13 walnut selections and crosses, respectively, were evaluated for resistance to *Xanthomonas arboricola* pv. *juglandis* (Xaj) using artificial inoculation *in vitro*. A mixture of four Xaj strains isolated from natural infected walnut nuts in Greece was used. The inoculum had a concentration of $1–2\times10^7$ cfu/ml and was injected into nuts by syringe. Nuts injected with SDW were used as control. The inoculated nuts were incubated in plastic boxes ($120\times80\times70$ cm) kept in growth chambers at 25 °C for two weeks with appropriate moisture maintained by spraying water every day. The experiment was conducted in the second week of June 2007. The inoculated nuts had a diameter of 2–4 cm. Disease incidence of nuts was assessed on a six-point scale of damage: 0 (= healthy) to 5 (= heavily infected, showing large necrotic areas around the injection point). The nuts injected with Xaj, in all treatments showed typical bacterial blight symptoms compared to controls. The bacterium was always reisolated on selective medium (brilliant cresyl blue-starch medium). The least susceptible cultivars were Serr, Iliana, Grand Jean and the selections EH28 and EK-1.

26.23 INVESTIGATION OF OCCURRENCE AND DISTRIBUTION OF BACTERIAL GRAIN ROT OF RICE IN KOREA AND DEVELOPMENT OF CONTROL METHODS. K.S. Han, B.R. Kim, Y.S. Choi, Y.H. Lee, T.H. Noh, D.K. Lee, E.S. Yang and J.E. Choi. *BioEnvironment Division, Chungnam Agricultural Research and Extension Services, Yesan, Chungnam 340-861, Republic of Korea. Email: hks081@lycos.co.kr*

Bacterial grain rot of rice caused by *Burkholderia glumae* was first reported in Korea in 1986. It has spread over the whole country since then, causing yield losses. However the symptoms seems to be different at the various rice growth stages and infection stages. Also it is very difficult to diagnose and control the disease, because the pathogenesis is not clear, and isolation and identification of the pathogen is very complicated. We have investigated the occurrence, distribution of the pathogen, and developed control methods. We found a 0.1-5.5% disease rate in Chungnam and 0.1-2.5% disease rate in Chungbuk. The bacterial population on the uppermost leaf sheaths and flag leaf sheaths was monitored periodically using the selective S-PG medium. Seven chemicals were selected for disease control. The results indicated that a leaf sheath pathogen population of more than 10^3 cfu/g fresh weight was the detection limit of the selective medium, and decreased greatly with the growth of internodes. For seed disinfection we developed hot–chilled water treatment.

26.24 CHARACTERIZATION OF ANTAGONISTIC SUBSTANCES PRODUCED BY *CLAVIBACTER MICHIGANENSIS* SSP. *MICHIGANENSIS*. I. Holtsmark, D. Mantzilas, V.G.H. Eijsink and M.B. Brurberg. *Bioforsk – Norwegian Institute of Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway. Email: may.brurberg@bioforsk.no*

The Gram-positive plant pathogen *Clavibacter michiganensis* subsp. *sepedonicus* is the causal agent of potato ring rot, a widespread disease that causes huge economical losses. One strategy that may aid in gaining control of the disease is the development of agents that specificially inhibit growth of the pathogen. In this

work, narrow-spectered antimicrobial substances that are secreted by the closely related tomato pathogen, *Clavibacter michiganensis* subsp. *michiganensis,* have been purified and studied. These antimicrobials exert growth inhibitory activity against *Clavibacter michiganensis* subsp. *sepedonicus*, and may become useful as control agents in the battle against the potato pathogen. The antimicrobial substances include a 14 kDa bacteriocin, a 2145 Da type B lantibiotic, and a series of non-proteinaceous antibiotics with molecular masses around 800-900 Da, putatively belonging to the tunicamycin family. The lantibiotic peptide, michiganin A, resembles actagardine, which is a type B lantibiotic produced by the actinomycete *Actinoplanes liguriae.* Both these peptides share traits with mersacidin, a well-known type B lantibiotic, including a conserved residue that is thought to be important for the antimicrobial activity of mersacidin.

26.25 GENETIC DIVERSITY AND EPIDEMIOLOGY OF *XANTHOMONAS AXONOPODIS* pv. GLYCINES ISOLATES IN KOREA. **S.J. Hong, B.C. Lee, Y.N. Yoon, J.B. Hwang, S.B. Song and S.T. Park.** *Yeongnam Agricultural Research Institute of NICS, RDA, Milyang 627-803, Republic of Korea. Email: hongsj7@rda.go.kr*

Bacterial pustule of soybean caused by *Xanthomonas axonopodis* pv. *glycines* (Xag) is one of the most prevalent bacterial disease in many areas where soybeans are grown. We used an amplified fragment length polymorphism (AFLP) analysis, a novel PCR based technique, to differentiate Korean isolates of Xag and to examine their genetic diversity. The 58 Xag isolates were collected in different regions of Korea. The isolates were tested with 32 AFLP primer combinations to identify the best selective primers and to study the genetic diversity. Some primer combinations could differentiate the isolates. Generally, it is known that Xag overwinters in seeds, in surface crop residues, and in the rhizosphere of wheat roots. We investigated to confirm the overwintering of Xag on soybean seed and in soil using dilution-plating assay and PCR assay. Examination of seeds of 36 cultivars harvested in 2005 and 2006 showed that the pathogen was detected from cvs. Pungsan-namulkong, Mallikong, Taekwangkong, Daemangkong and Ajukkarikong. This infected seed all came from the year 2006; the pathogen was not detected from any seed harvested in 2005. The pathogen may survive about 1 year in seed. From December 2006 to June 2007, we surveyed the pathogen populations in soil from six upland soybean crops. The pathogen was detected in all soils.

26.26 PCR-BASED SPECIFIC DETECTION OF POTATO BLACK LEG PATHOGEN *PECTOBACTERIUM CAROTOVORUM* subsp. *CAROTOVORUM*. **M. Horita and F. Tanaka.** *National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki 305-8604, Japan. Email: mhorita@affrc.go.jp*

Potato black leg is a seed-borne bacterial disease that primarily occurs in the cool temperate region (Hokkaido) in Japan and has been an important quarantine problem for seed potato production. *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc; synonym *Erwinia carotovora* subsp. *carotovora*), causes potato soft rot (Pcc-s) worldwide, but also includes a pathogen that causes potato black leg disease (Pcc-b) in Japan. These two pathogens within one species are almost identical in physiological and biochemical characteristics and are difficult to distinguish. We have developed a method to distinguish Pcc-b from Pcc-s strains by polymerase chain reaction (PCR). Comparison of small 16S-23S rDNA IGS sequences (354-453 bp) of fifteen Japanese and 19 foreign strains including three kinds of black leg pathogen (Pcc-b, *Pectobacterium atrosepticum* and *Dickeya dianthicola*) and one kind of soft rot pathogen (Pcc-s) clarified the inter- and intraspecies heterogeneity of *Pectobacterium* spp. Phylogenetic analysis of IGS sequence data showed that Pcc-b strains belonged to a cluster distinct from that of Pcc-s. Based on a conserved region of the IGS, a PCR primer set for Pcc-b was designed. A specific band (ca. 270 bp) was amplified from all Pcc-b strains but not from Pcc-s and two other kinds of black leg pathogen of potato. PCR-based detection of Pcc-b from contaminated potato tubers is now under investigation.

26.27 CHARACTERIZATION OF *CLAVIBACTER MICHIGANENSIS* subsp. *MICHIGANENSIS* STRAINS FROM ITALY. **G. Ialacci, G. Licciardello, V. Catara and P. Bella.** *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. Email: patrizia.bella@unict.it*

Clavibacter michiganensis subsp. *michiganensis* (CMM), the causal agent of bacterial tomato canker, is a quarantine pathogen that causes severe damage to tomato crops worldwide. We analyzed a collection of CMM from outbreaks of bacterial canker in six greenhouses in Sicily from 2004-2007 and CMM strains from other locations in Italy as well as reference strains. Different methods were used to study variability within the CMM strains. Phenotypic characterization included Gram reaction, oxidase, catalase, casein and aesculin hydrolysis, colony morphology on NBY and growth on TTC. Carbon substrate oxidation patterns (Biolog system) were also performed. On NBY, differences in morphology were observed after 4 days' incubation: a group of isolates showed convex, fluid colonies 1-4 mm in diameter whereas the remaining colonies were flat, 6 mm in diameter and more fluid. There was no growth on TTC medium. PCR response using two pairs of primers designed on the pat 1 gene (CMM5/CMM6) and on repetitive pat-1-rep motif (P1-rep/P3-rep) located on plasmid pCM2, depended on the strain tested. The size of amplicons obtained with P1-rep/P3-rep was variable. Sequencing showed that they differ in the number of repetitive sequences in the repetitive pat-1-rep motif. Some strains did not react with either primer set, but were nevertheless pathogenic on tomato and induced HR reaction on leaves of four o'clock plants. The lack of PCR products and the plasmid analysis suggest the absence of the pat 1 gene. The different populations of CMM from different geographical areas were analysed by fAFLP.

26.28 KEEPING THE SCOTTISH POTATO INDUSTRY FREE FROM *CLAVIBACTER MICHIGANENSIS* subsp. *SEPEDONICUS* (*POTATO RING ROT*). **E.M. Kerr and G.S. Saddler.** *Scottish Agricultural Science Agency, 1 Roddinglaw Road, Edinburgh, EH12 9FJ, UK. Email: ellen.kerr@sasa.gsi.gov.uk*

Clavibacter michiganensis subsp. *sepedonicus* (Cms) is a major concern of seed potato producing countries. A multi-disciplinary approach is underway to evaluate risks from Cms to the Scottish potato industry, encompassing: Cms epidemiology under Scottish conditions, detection and current control measures and stakeholder involvement. Glasshouse trials were conducted to assess the top 10 Scottish seed cultivars' susceptibility to Cms. In 2006, 30-day-old plants were stem-inoculated with Cms cells. Foliar symptoms were unclear except in two cultivars; however, real-time PCR confirmed that all cultivars were infected, highlighting the difficulty in providing agronomists with definitive cultivar-

specific symptoms and the necessity for post-harvest tuber testing. Effect of infection level on disease progression was studied in 2007 using daughter tubers confirmed to be disease-free or latently infected. Foliar symptoms expressed by the 10 cultivars, grown from infected daughter tubers, were categorised: symptomless (2), symptomatic (6) or symptoms masked (2). Improvement in Cms detection is also underway by development of a new monoclonal antibody. Additionally, weak points that may currently exist in current trading and agronomic practices have been assessed via a postal survey of 548 Scottish potato growers. Respondents (46%) were categorised into three business types: seed-only, seed and ware or ware-only. Results from the survey confirmed potential risks from: source of seed, inadequate on-farm hygiene and potential contact of seed and ware potatoes during storage and grading. Putting existing knowledge and experience in context coupled with finding practical solutions for the industry is essential if Scotland is to remain free of this disease.

26.29 INVESTIGATION OF ICE NUCLEATION ACTIVITY OF BACTERIA ON DIFFERENT GENOTYPES OF ALMOND TREES IN THE ZARGHAN AREA OF FARS PROVINCE. **S. Ketabchi.** *Department of Plant Pathology, Shiraz Islamic Azad University Iran, P.O. Box 19575-444, Tehran, Iran. Email: ketabchi@iaushiraz.ac.ir*

One factor which increases cold stress in plants is ice nucleation activity (INA) of bacteria that produce biological ice nuclei leading to frosting at higher temperatures. In this study four genotypes of almond trees were selected and examined in Zarghan agricultural research center, in Fars province. The four genotypes were 36 (early-flowering), 5 (flowering too early), 26 (mid-late flowering) and 8 (flowering too late). First, the INA^+ bacteria isolated from buds, blossoms, immature fruits and leaves were characterized according to standard bacteriological methods. Thereafter their population densities were determined separately during the months of February, March, April and May, 2002-2003. Strains of *P. syringae, Pseudomonas fluorescens, Pantoea agglomerans* and *Xanthomonas* spp. were identified as INA^+ bacteria. *P. syringae* was the most prevalent bacterium and *P. agglomerans*, *P. fluorescens* and *Xanthomonas* spp. were ranked second to fourth, respectively. Bacterial populations changed completely depending on the phenology of the plants and the temperature. Moderate temperatures in February and March induced early-flowering almond genotypes to blossom in the Zarghan area of Fars province. Since populations of epiphytic INA^+ bacteria on almond trees will be increased by moderate temperature and adequate nutrition, any decrease in temperature will intensify frost injury. It was concluded that almond genotype, weather conditions and populations of INA bacteria are the most important factors controlling frost damage in Zarghan area.

26.30 GENETIC CHARACTERIZATION OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE* STRAINS WITH SPECIFIC FINGERPRINTING. **S. Ketabchi.** *Department of Plant Pathology, Shiraz Islamic Azad University Iran, P.O. Box 19575-444, Tehran, Iran. Email: ketabchi@iaushiraz.ac.ir*

Pseudomonas syringae pv *syringae* is an important phytopathogenic bacterium with a wide host range. Isolates, strains and pathovars of *P. syringae* are not always distinguishable with previous methods and mistakes are made. Use of DNA primers corresponding to consensus motifs in bacterial repetitive elements for example repetitive extragenic palindromic sequence (REP) is a new technique for identify, genetically characterize and classify *P. syringae* strains below the level of species and subspecies. In this report, 24 strains of *P. syringae* pv *syringae* from different hosts and different areas in Iran were compared with standard strains using Rep primers and PCR. The DNA template was prepared with "freeze-boil", whole colony, boiling and directly from the leaf surface. Results showed that it was not necessary to use purified genomic DNA in the Rep-PCR. Analysis of Rep fingerprints from *P. syringae* pv. *syringae* strains showed that host specialization and habitant are correlated with genomic diversity. Strains isolated from stone fruits formed a distinct cluster, separate from strains isolated from wheat and sugar beet, and stone fruit strains isolated from same geographic area were very similar. The Rep-PCR technique appears to be rapid, simple and reproducible to identify and classify these important plant pathogens.

26.31 PATHOGENICITY OF *PANTOEA ANANATIS* CAUSING INTERNAL FRUIT ROT OF NETTED MELON. **K. Kido, T. Atsuchi and Y. Takikawa.** *Laboratory of Plant Pathology, Faculty of Agriculture, Graduate School of Science and Technology, Shizuoka University, 836 Ohya, Shizuoka 422-8529, Japan. Email: kido@yokohamaueki.co.jp*

An internal fruit rot of netted melon (*Cucumis melo* L.) was first observed in Kochi Prefecture, Japan, in 1998. The symptom is an internal rot with malodor, despite a healthy appearance without any water-soaking or brown spots on the surface. The causal agent was identified as *Pantoea ananatis* (=*Erwinia ananas*). To analyze the pathogenicity of the melon isolates of *P. ananatis*, a stabbing inoculation test using a pin smeared with bacterial growth was performed on ovaries (immature fruits) of melon flowers 5 to 7 days after artificial pollination. The symptom was confirmed on the melon fruits grown on for 40 to 60 days. When the melon isolates were compared with other *P. ananatis* strains isolated from various plants including rice, only the melon isolates showed specific pathogenicity to netted melon. The melon isolates also caused internal rot symptom on honeydew melon, oriental sweet melon and watermelon but not on cucumber. The melon isolates specifically harbored indole acetic acid (IAA) biosynthesis genes (iaaM and iaaH) and a cytokinin biosynthesis gene (etz). Some atypical *P. ananatis* isolates from melon that do not carry these genes could not evoke pathogenic reaction on melon fruits. From these results, we suggest that the melon pathogen constitute a distinct group in *P. ananatis* in terms of pathogenicity and genetic characteristics.

26.32 A NEW PSEUDOMONAS DISEASE OF KIWIFRUIT (*ACTINIDIA DELICIOSA*) CV. HAYWARD AND *ACTINIDIA CHINENSIS* CV. HORT 16A AND ITS CONTROL IN KOREA. **Y.J. Koh, G.H. Kim, G.S. Lee, K.Y. Jo, I.H. Jeong, M.T. Lim, Y.S. Lee, J.-S. Jung and J.-S. Hur.** *Department of Plant Medicine, Sunchon National University, Suncheon 540-742, Republic of Korea. Email: youngjin@sunchon.ac.kr*

Pseudomonas syringae pv. *actinidiae* and *P. syringae* pv. *syringae* are reported to cause bacterial canker and bacterial blossom blight of kiwifruit, respectively. A new bacterial leaf spot disease on kiwifruit (*Actinidia deliciosa*) 'Hayward' and *Actinidia chinensis* 'Hort 16A' was found in orchards in Jeonnam and Jeju Provinces, Korea, during the rainy seasons of 2006 and 2007. Typical symptoms were dark brown irregular spots without halos on the leaves of both cultivars. The symptoms were clearly different from those of bacterial canker and bacterial blossom blight

on 'Hayward'. Frequent rains rapidly spread the disease on all leaves of kiwifruit trees in a short period. Severely infected leaves were wilted and defoliated. Pathogenesis of the causal bacterium isolated from the infected leaves was confirmed on healthy leaves of kiwifruit by artificial inoculation. Based on its morphological, physiological and biochemical characteristics, the bacterium was identified as *Pseudomonas* sp. and further identification is now in progress using molecular biological techniques such as 16S rDNA sequence analysis and DNA-DNA hybridization. Oxolinic acid WP screened *in vitro* terminated the progress of the leaf spot disease on both cultivars.

26.33 BACTERIAL CANKER DISEASES ON KIWIFRUIT (*ACTINIDIA CHINENSIS*) CV. HORT 16 A CULTIVATED IN KOREA. Y.J. Koh, G.H. Kim, I.H. Jeong, M.T. Lim, H. S. Park, Y.S. Lee, J.-S. Jung and J.-S. Hur. *Department of Plant Medicine, Sunchon National University, Suncheon 540-742, Republic of Korea. Email: youngjin@sunchon.ac.kr*

An outbreak of bacterial canker on kiwifruit (*Actinidia deliciosa*) 'Hayward' was first reported at the southern coastal area, a major production area of Korea in the mid 1980s. The causal bacterium was found to be *Pseudomonas syringae* pv. *actinidiae.* The canker was severe in orchards exposed to strong wind and some of them were destroyed due to the epidemics. The disease was characterized by die-back or blight on young canes, often with red-rusty exudation on canes or trunks and dark brown irregular spots surrounded with yellowish halos on leaves. The same symptoms as shown on 'Hayward' were also observed on kiwifruit (*Actinidia chinensis*) 'Hort16A' in early spring of 2006 and 2007. The same bacterial pathogen, *P. syringae* pv. *actinidiae*, was isolated from the infected trees. On the other hand, a similar bacterial canker symptom without foliar spots was also found on 'Hort16A' in mid summer of 2006 and 2007. Another bacterial pathogen was isolated and identified as *Pectobacterium carotovorum* on the basis of its physiological and biochemical characteristics and 16S rDNA sequences, and further identification is now in progress. *P. carotovorum* was pathogenic on both kiwifruit cultivars. Optimal temperature ranges for the growth of *P. carotovorum* were 30-32 °C, clearly different from those (18-20 °C) of *P. syringae* pv. *actinidiae.* The bacterial canker diseases caused by *P. carotovorum* in mid summer as well as *P. syringae* pv. *actinidiae* in early spring resulted in death of severely infected trees of kiwifruit 'Hort16A' in Korea.

26.34 RELIABILITY OF DIAGNOSTIC TECHNIQUES FOR IDENTIFICATION OF FLUIDAL AND LESS FLUIDAL VARIANTS OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS*. B. Kokošková, I. Mráz and J. Fousek. *Department of Bacteriology, Plant Medicine Division, Crop Research Institute, 161 06 Prague 6, Czech Republic. Email: bkokoskova@vurv.cz*

Bacterial canker of tomato, caused by the quarantine organism *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), is a well-known disease resulting in serious losses to both greenhouse and field tomatoes. According to colony morphology, Cmm occurs mostly as fluidal, but sometime as less fluidal strains. Reliability of immunochemical (ELISA, IFAS), biochemical (BIOLOG) and molecular techniques (PCR, rep-PCR with BOX, ERIC, and REP primer sets, dot blot hybridisation with digoxigenin labelled probes) was compared for identification of fluidal and less fluidal variants of Cmm. Commercial polyclonal antibodies (NeogenEurope Ltd., UK) were used to identify more than 25 Cmm strains representing both forms. All Cmm strains were reliably identified by IFAS. The fluidal Cmm strains were more reliably identified using PTA-ELISA and BIOLOG GP MicroPlate System™ than less fluidal strains. Commercial primers Cmm5 and Cmm6 (Dreier *et al.,* 1995) and our primers (Cmm 1F and Cmm 1R) designed for detection of *Cmm* and used in PCR identified all Cmm strains regardless of morphological variability. The work was supported by the CR Ministry of Agriculture, project No. QH 71229 and by the Grant Agency of the Academy of Sciences of the Czech Republic, grant No. AV0Z50510513.

26.35 TEMPORAL PROGRESSION OF WALNUT BLIGHT, CAUSED BY *XANTHOMONAS ARBORICOLA* PV. *JUGLANDIS*, IN AUSTRALIA. M.D. Lang, K.J. Evans and S.J. Pethybridge. *Agronico Research Pty Ltd, 175 Allport St East, Leith, 7315, TAS, Australia. Email: mlang@agronico.com.au*

The temporal progression of walnut blight was monitored over three seasons in two commercial orchards of cultivar Vina, located in eastern and northern Tasmania, Australia. Progression of disease incidence was best described by the monomolecular growth model at both locations in 2004-05 and 2006-07, implying monocyclic disease. In contrast, the Gompertz and logistic growth models described polycyclic disease processes at one location each in 2005-06. Disease incidence and severity was greatest in 2005-06 with nearly all fruits developing blight within 80 days of bud-burst at both locations. The timing of disease onset was similar in all three seasons but increases in blight incidence were more gradual in 2006-07. The linearized disease progress curves were used to predict the time to 50% disease incidence, which was within 52 days of bud-burst in 2005-06 but was more than 84 days in 2004-05. A disease incidence of 50% was not reached in 2006-07 at either location. Average daily temperature and precipitation were used as independent variables in multiple linear regressions and accounted for 61% and 48% of the variation in the daily increase of disease incidence, for polycyclic and monocyclic epidemics, respectively. Disease severity at half full-size diameter of walnut fruits indicated the likelihood of nuts being present at harvest; lesion coverage greater than 8% of the fruit surface led to premature fruit drop. Temperature, precipitation and other environmental factors are being used to develop an empirical disease forecaster for timing applications of copper for the control of walnut blight in Tasmania.

26.36 SEQUENCE DIVERSITY OF 16S RDNA AND 16S-23S RDNA INTERNAL TRANSCRIBED SPACER (ITS) REGION AMONG *PSEUDOMONAS SYRINGAE* PV. *MORSPRUNORUM* ISOLATES. Y.S. Lee, S.Y. Park, Y.M. Kim and J.S. Jung. *Department of Biology, Sunchon National University, 540-742 Suncheon, Republic of Korea. Email: jjung@sunchon.ac.kr*

Pseudomonas syringae pv. *morsprunorum* is the causative agent of bacterial canker of Japanese apricot *(Prunus mume)*. A collection of 34 *P. syringae* pv. *morsprunorum* strains, including one type strain, 24 strains isolated from Korea, 9 strains from Japan Genebank (NIAS), was analyzed. Strains were divided into three groups on the basis of 16S rDNA sequences. Groups I, II and III differed at 15 positions in the 16S rDNA sequence. The reference strain and 19 Korean strains belonged to group I, while 5 Korean and 5 Japanese strains belonged to group II. Another 4 Japanese strains belonged to group III. When the three groups were compared with the 16S rDNA sequences of 21 *P. syringae* pathovars available in the GenBank database, they could be distinguished

on the phylogenetic tree. To establish intraspecies differences, the 16S-23S rRNA spacer regions of pv. *morsprunorum* strains were analyzed. Group I and III strains had a single ITS sequence 574 and 587 bp long respectively. Interestingly, group II had two types of ITS sequences, each 548 and 573 bp long. All ITS sequences contained $tRNA^{Ile}$ and $tRNA^{Ala}$ genes. Even though groups I and II were found together in Korea, the most prevalent Korean strain was group I. Korean strains belonged to groups I and II, whereas Japanese strains were in groups II and III, showing the geographic variation of *P. syringae* pv. *morsprunorum*.

26.37* BEHAVIOUR OF *DICKEYA DADANTII* IN THE PLANT APOPLAST. E. Lopez-Solanilla, M. Antunez, E. Cabrera, M. Sena, J. Larenas, R. Cuartas, C. Rojas, I. Toth, I. del Rio and P. Rodriguez Palenzuela. *CBGP; ETSI Agrónomos, Av. Complutense s/n, 28040 Madrid, Spain. Email: pablo.rpalenzuela@upm.es*

Dickeya dadantii (*Erwinia chrysanthemi*) is the causal agent of soft-rot disease in many important crops worldwide. It causes rapid necrosis of parenchymatic tissues, due to secretion of pectolytic enzymes. Our long-term goal is to understand the mechanisms that enable *D. dadantii* to survive in the harsh conditions of the plant apoplast, particularly in: 1) the role of bacterial resistance against antimicrobial peptides, 2) the role of acidic pH as a barrier to bacterial colonization, 3) the role of motility and chemotaxis in the pathogenicity of this bacterium. We are making a genome-wide analysis of the bacterial response to the above factors through microrray profiling, Ettan-DIGE and construction of a large series of specific mutants using a transposon grid. Genes differentially expressed at acidic pH include amino acid transporters, external membrane proteins and enzymes known to be involved in resistance to different kinds of stress, such as alcohol dehydrogenases and peroxidases. Genes differentially expressed in the presence of antimicrobial peptides include transcriptional regulators, ion channels, and enzymes involved in sugar and energy metabolism. This kind of global analysis may enable us to identify novel candidate genes and novel regulatory networks possibly involved in bacterial survival *in planta*. Also, mutants in the chemosensory pathway showed diminished ability to enter/colonize plant tissues.

26.38 DETECTION OF BACTERIAL SOFT-ROT OF CROWN IMPERIAL CAUSED BY *PECTOBACTERIUM CAROTOVORUM* SUBSP. *CAROTOVORUM* USING SPECIFIC PCR PRIMERS. E. Mahmoudi, M.J. Soleimani and M. Taghavi. *Department of Plant Protection, College of Agriculture, Islamic Azad University of Khorasgan, Isfahan, Iran. Email: esm_mah@yahoo.com*

Pectobacterium is one of the major destructive causal agents in most crop plants throughout the world. During a survey in spring of 2005 in the rangeland of Kermanshah and Isfahan provinces of Iran, samples of bulbs and stems of crown imperial with brown spot and soft rot were collected. Eight strains of pectolytic *Erwinia* were isolated and purified from these samples. Phenotypic tests indicated that the strains were gram-negative, facultative anaerobic, rod shaped, and motile with peritrichous flagella. They were oxidase-negative, catalase-positive and able to macerate potato slices. Pathogenicity of all the strains were confirmed on maize, philodendron and crown imperial by inoculation of these crops with a bacterial suspension and reisolation of the strain from symptomatic tissues. A pair of specific PCR primers was used to detect these bacterial strains. The primer set (EXPCCF/EXPCCR) amplified a single fragment of the expected size (0.55 kb) from genomic DNA of all strains used in this study. In nested PCR, the primer set (INPCCR/INPCCF) amplified the expected single fragment (0.4 kb) from the PCR product of first PCR amplification. On the basis of the biochemical and phenotypic characteristics and specific PCR amplification, these strains were identified as *Pectobacterium carotovorum* subsp. *carotovorum*. This is the first report of occurrence of crown imperial bacterial soft-rot in Iran.

26.39 CHARACTERIZATION OF STRAINS OF *PECTOBACTERIUM CHRYSANTHEMI* THE CAUSAL AGENT OF BACTERIAL STALK ROT OF MAIZE, AND EVALUATION OF SOME MAIZE HYBRIDS RESISTANT TO THESE STRAINS IN HAMADAN PROVINCE. E. Mahmoudi, M.J. Soleimani, M. Taghavi and A. Bagheri. *Department of Plant Protection, College of Agriculture, Islamic Azad University of Khorasgan, Isfahan, Iran. Email: esm_mah@yahoo.com*

Among pathogenic bacteria threatening maize, *Pectobacterium* is one of the most important. During summer and fall of 2005, maize samples with stalk rot symptoms from fields in Hamadan province were collected, and 20 bacterial strains, gram-negative, rod-shaped, facultative anaerobic, mobile with peritrichous flagella, were isolated. The strains were catalase-positive, oxidase-negative, pectolytic, and rotted potato tuber slices. Accordingly these strains were identified as *Pectobacterium chrysanthemi* biovar-3. In order to evaluate the resistance of maize hybrids to bacterial stalk rot, two methods were used, pseudostem injection in the greenhouse and leaf puncture in the laboratory. Among the 5 single-cross hybrids (108, 500, 647, 700 and 704), after pseudostem injection, 647 and 704 showed the highest disease rating index (DRI) and were placed in sensitive class (S), hybrid 700 had medium resistance (MR) and hybrids 108 and 500 with the least DRI were designated as resistant (R). the results from leaf puncture inoculation were highly correlated (r= 0.93) with pseudostem injection. Hybrids 647 and 704 as sensitive varieties and 108 as resistant variety were introduced in this method.

26.40 SUSCEPTIBLE HOSTS FOR TESTING PATHOGENICITY OF *AGROBACTERIUM TUMEFACIENS* AND *RHODOCOCCUS FASCIANS*. M.L. Miller and M.L. Putnam. *Department of Botany and Plant Pathology, Oregon State University, Corvallis OR, 97331-2902, USA. Email: millerml@science.oregonstate.edu*

Both *A. tumefaciens* and *R. fascians* have a wide host range and infect a large number of herbaceous perennial nursery crops. *R. fascians* infections result in shoot proliferation and leafy galls which are sometimes confused with tumors caused by *A. tumefaciens*. The only method known to prevent infection by *R. fascians* is good sanitation and removal of all diseased plants. The biocontrol agent *A. radiobacter* strain K1026 is helpful in preventing crown gall in some plants, but many *A. tumefaciens* strains are resistant to the agrocin produced by K1026. In order to test potential control products, reliably susceptible hosts typical of those propagated in nurseries were sought. Three plant species found naturally infected by both bacteria are *Argyranthemum*, *Erysimum* and *Leucanthemum* and all three show differential symptoms: tumors from *A. tumefaciens* and shoot proliferation from *R. fascians*. Ten strains of *A. tumefaciens* and ten of *R. fascians* were inoculated to plants and these were observed over a two-month period. *Argyranthemum* gave the most rapid and consistent symptom development of the three hosts. A combination of the four best strains of each bacterial species was chosen for

mixed inoculation of plants. *Argyranthemum* plants will be useful for confirming pathogenicity of new *A. tumefaciens* and *R. fascians* isolates and in testing control measures.

26.41* SUSCEPTIBILITY OF WALNUT CULTIVARS AND SPANISH SELECTIONS TO BACTERIAL BLIGHT (*XANTHOMONAS ARBORICOLA* PV. *JUGLANDIS*). **C. Moragrega, I. Llorente, E. Montesinos, M.Rovira and N. Aletà.** *Institute of Food and Agricultural Technology-CeRTA-CIDSAV, University of Girona, Av. Lluís Santaló s/n, 17071 Girona, Spain. Email: conceptcio.moragrega@udg.edu*

Bacterial blight of Persian (English) walnut (*Juglans regia* L.) caused by *Xanthomonas arboricola* pv. *juglandis* is a disease of economic importance in all walnut-producing areas. Most commercial walnut cultivars are susceptible. Walnut seedling trees from Spanish wild populations were pre-selected because of their apparent tolerance to *X. arboricola* pv. *juglandis* in their original area. They were planted in a field collection, located in an environment very favourable to bacterial disease development, together with five commercial cultivars used as reference (Adams-10, Amigo, Chandler, Franquette and Vina). The blight susceptibility of 22 selections and five reference cultivars was evaluated on the main vegetative organs affected. Disease severity on completely developed nuts and affected leaves was recorded in summer under field conditions, whereas blight susceptibility of young nuts was determined with artificial inoculation under environment controlled conditions on fruit collected 45 days after nut set. Field observations were made over eight years. Inoculation of immature nuts indicated nine local selections with low disease susceptibility. Five local selections showed little damage on fully developed nuts. Three local selections (MBLu-20, MBLu-21 and MBC-45) had low susceptibility to blight in nuts and the 'MBLu-20' clone always presented healthy foliage, a behaviour similar to 'Adams-10' during the growing season. The susceptibility of 'Franquette' did not differ significantly from that of 'Adams-10' in all vegetative organs studied under field conditions, whereas on immature nuts 'Franquette' was highly susceptible to blight.

26.42 DIVERSITY AND PATHOGENICITY OF *PSEUDOMONAS CICHORII* ISOLATES CAUSING MIDRIB ROT ON LETTUCE. **E. Pauwelyn, K. Vanhouteghem, B. Cottyn, J. Heyrman, M. Maes, P. De Vos, P. Bleyaert and M. Höfte.** *Laboratory for Phytopathology, Faculty of bioscience engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium. Email: ellen.pauwelyn@Ugent.be*

During the past 10 years, lettuce midrib rot, characterised by a dark brown rot of the midrib of one or more inner leaves, has become a serious threat of glasshouse butterhead lettuce (*Lactuca sativa* L. var. *capitata*). This disease causes severe losses and reduced market quality. *P. cichorii* is the causal organism of this disease, but is poorly investigated; better understanding of the epidemiology and pathogenicity is necessary to prevent and control the disease. The pathogenicity factors involved in the infection mechanism of *P. cichorii*, need to be studied. The type III secretion system is an important pathogenicity factor, required by many gram-negative bacteria. Presence of hypersensitive reaction and pathogenicity (hrp) genes in *P. cichorii* lettuce isolates has been established by PCR amplification of a specific DNA fragment of the hrc-RST sequence. Phylogenetic analysis showed that the lettuce isolates can be divided in 3 subgroups, according to the sequence of the hrc-RST fragment. Differences in intrinsic pathogenicity between the *P. cichorii* isolates, belonging to the different subgroups were observed. To ascertain if the type III secretion system is involved in the pathogenesis of *P. cichorii*, lettuce strain DGB54 was mutated by random transposon insertion. Of 1480 transconjugants, eight were altered in their ability to induce a hypersensitive reaction on tobacco and disease symptoms on lettuce. Further research will elucidate in which genes the transposon is inserted and how they contribute to the pathogenicity of *P. cichorii*.

26.43 EFFECTIVENESS OF PLANT ESSENTIAL OILS AGAINST *PSEUDOMONAS BACTERIA* CAUSING LEAF SPOT AND BLIGHT. **D. Pouvová, B. Kokošková, R. Pavela, P. Ryšánek.** *Faculty of Agrobiology, Food and Natural Resources, Czech Univerzity of Agriculture Prague, 165 21 Prague 6, Czech Republic. Email: dagmarpouvova@seznam.cz*

Pseudomonas bacteria cause mostly leaf spot and blight diseases on many plants. For chemical control, copper preparations are most often used but are not sufficiently effective. Antibiotics such as streptomycin are more effective than copper, but they are banned from use in plant protection in the majority of EU countries. In our tests, thirty four essential oils obtained from different aromatic plants were tested as potential inhibitors of plant pathogenic pseudomonads. The antimicrobial activity tests were carried out on agar plates seeded with the target bacterium. Essential oil as a crude extract was dropped on the surface of the plates. Efficiency was taken as directly proportional to the size of the inhibition zone. Streptomycin was used as the control. Essential oils from *Origanum vulgare*, *Thymus vulgaris*, *Mentha citrata* and *Mentha arvensis* were the most effective against *Pseudomonas savastanoi* pv. *glycinea.* Oils from *Origanum compactum, O. vulgare*, *Th. vulgaris* and *Citrus aurantifolia* were effective against *P. savastanoi* pv. *phaseolicola* and *O. compactum*, *Th. vulgaris*, *C. aurantifolia* and *Eugenia caryophyllata* were effective against *P. syringae* pv. *tomato.* Oils from *O. compactum, O. vulgare*, *Th. vulgaris* and *E. caryophyllata* were the most effective against *P. syringae* pv. *syringae*. Oils from *O. compactum, O. vulgare*, *Ocimum basilicum*, and *E. caryophyllata* were the most effective against *P. syringae* pv. *pisi*. The work was supported by the CR Ministry of Agriculture, project No. 320/5305 and by the Ministry of Education, project No. MSM6046070901.

26.44 A MINISATELLITE-BASED MLVA SCHEME FOR TYPING *XANTHOMONAS CITRI* PV. *MANGIFERAEINDICAE*. **O. Pruvost, K. Vital, N. Ah-You, C. Vernière, F. Chiroleu and L. Gagnevin.** *CIRAD, UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical (PVBMT), CIRAD-Université de la Réunion, 97410 Saint-Pierre, Réunion, France. Email: olivier.pruvost@cirad.fr*

Molecular typing techniques can be useful for improving our understanding on the epidemiology of bacterial plant pathogens. MLVA (multi-locus variable number of tandem repeats analysis) is suitable for typing bacterial species or pathovars with low overall genetic diversity. We evaluated the MLVA scheme developed for *Xanthomonas citri* pv. *citri* as a typing technique for phylogenetically related organisms, including *X. citri* pv. *mangiferaeindicae*, the causal agent of mango bacterial canker. For this bacterium, 12 out of 14 VNTRs produced amplification fragments. When a worldwide strain collection was typed, almost every strain had a unique fingerprint, leading to a discriminatory power index (≈ 1), higher than that derived from AFLP for the same collection. Trees of the genetic diversity showed a geographical correlation. MLVA was also used on a field scale and was useful for elucidating the

question of inoculum sources putatively associated with an outbreak. MLVA indicated that strains symptomlessly associated with nursery plants used for plot establishment contributed very little to the outbreak, suggesting a low biological significance for epiphytic populations of *X. citri* pv. *mangiferaeindicae.*

26.45 PHENOTYPIC AND GENETIC DIVERSITY IN *BRENNERIA NIGRIFLUENS*, THE CAUSAL AGENT OF WALNUT BARK CANKER IN KURDISTAN PROVINCE, IRAN. R. Roshangar and B. Harighi. *Plant protection department, College of Agriculture & Natural resources, University of Kurdistan, Sanandaj, Iran. Email: BHarighi@uok.ac.ir*

During the spring and summer of 2005-6, brown to black exudates oozing from cankers were observed on trunk and stems of walnut trees in some areas of Kurdistan province. Infected trees died 2 to 3 years after infection. Twelve bacteria were isolated from infected tissues by using EMB medium. According to phenotypic, biochemical and physiological properties, the isolates were identified as *Brenneria nigrifluens*. The isolates produced round, smooth and white colonies; all strains were gram negative, oxidase negative, catalase and urease positive and facultative anaerobic. No strains hydrolyzed gelatin, starch, lecithin, tween 80 or casein, nor reduce nitrate to nitrite or produce H_2S from peptone and cysteine. Reducing substances from sucrose, arginine dihydrolase, methyl red and indole production were negative. All strains grew on 4% NaCl and produced acetoin. All strains produced acid from glucose, cellobiose, arabinose, raffinose, mannitol, sucrose, fructose, maltose, salicin, sorbitol, adonitol and inositol, but not from lactose, trehalose, dulcitol, sorbose and rhamnose. The strains could not use xylose, galactose, propionate, malonate and tartrate as carbon sources. The 12 strains were assessed by means of repetitive PCR using ERIC, BOX and REP primers and analysis of whole cell protein extracts. From these studies *B. nigrifluens* strains were clustered into four and two groups by repetitive PCR and protein pattern analysis, respectively.

26.46 OIL FROM *CITRULLUS COLOCYNTHIS* SEEDS INHIBITS GROWTH OF SOME PHYTOPATHOGENIC BACTERIA. N. Sanadgol, M. Komijani, H. Ravan, M. Alahmoradi. *Sistan Biotechnology Research Institute, Zabol University, Zabol, Iran. Email: nima_bio_79@yahoo.com*

In a search of alternative ways to control plant disease, oils from seed of *Citrullus colocynthis* (L.) Schrad., Cucurbitaceae (colocynth, wild-gourd or bitter-apple) were examined for antibacterial properties. Colocynth is a non-hardy, herbaceous perennial vine, branched from the base. Originally from Tropical Asia and Africa, it is now widely distributed in the sistan phytogeographic region of Iran. The seeds are edible and have a high oil content (17-19%) with a large proportion of linoleic acid (C18:2) which is important for human nutrition and an essential Fatty acid. The oil contains very little linolenic acid (C18:3). Antibacterial activity of oils separated from the seeds was tested against *Xanthomonas campestris pv. Campestris* ATCC33913*, Agrobacterium tumefaciens* str. C58*, Burkholderia cenocepacia* HI2424 and *Pseudomonas syringae* pv. *syringae* B728a using three different concentrations (1, 2.5 and 5mg/ml). The agar disc diffusion method was used to assess inhibitory effect by measuring the inhibition zone against the test microorganisms. Antibacterial activity of the seed oil was confirmed for all concentrations, but with different ranges. This activity was observed to be dose-independent. *Xanthomonas campestris* was the most sensitive bacterium tested. A weak inhibitory effect was found against *Pseudomonas syringae.* The results offer a scientific basis for the use of *C. colocynthis* seed oil extracts to prevent diseases caused by these bacteria.

26.47 FIRST REPORT OF BACTERIAL SPECK OF TOMATO CAUSED BY *PSEUDOMONAS SYRINGAE* pv. TOMATO IN TANZANIA. K.C. Shenge, K. Wydra, D. Stephan, R.B. Mabagala and C.N. Mortensen. *Danish Seed Health Centre for Developing Countries, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, Frederiksberg C, DK-1871, Copenhagen, Denmark. Email: cnm@life.ku.dk*

A serious outbreak of a tomato leaf spot disease was observed during April 2004 in Mgeta, Mvomero District of Tanzania. The disease was characterized by small, sunken, and black lesions on green tomato fruits, surrounded by darker green haloes. Lesions on ripe fruits were dark brown to black, superficial, and measured 1 to 2 mm in diameter. On the leaves, lesions were small, black, and with chlorotic haloes; occasionally the specks coalesced to form large lesions on older leaves. Black lesions were also observed on stems and petioles. A disease survey of selected tomato-producing areas in Arusha, Dodoma, Iringa, and Morogoro in Tanzania during 2004 and 2005 revealed that the disease was widespread in all areas surveyed. Disease incidence was of approximately 80%, while severity ranged from moderate to severe. A bacterium that produced a greenish, diffusible fluorescent pigment was consistently recovered on King's medium B from lesions on tomato fruits collected from fields in all areas surveyed. All 56 isolates were gram-negative, oxidase-negative and induced symptoms in tomato seedlings 'Tanya'. The strains were further characterized by PCR and RFLP analysis, showing that the strains were genomically homogeneous. The bacterium was re-isolated from the infected plants and identified as *P. syringae* pv. *tomato* in accordance with Koch's postulates. To our knowledge, this is the first report of the occurrence of tomato bacterial speck in Tanzania.

26.48 TRANSCRIPTIONAL EXPRESSION PROFILING OF *XANTHOMONAS ORYZAE* PV. *OYZAE* ON HRP-INDUCING SIGNAL USING DNA MICROARRAY. B.H. So, J.W. Kim, B.M. Lee and H.W. Kang. *Graduate School of Biotechnology and Information Technology, Hankyong National University, Ansung 456-749, Republic of Korea. Email: kanghw2@hknu.ac.kr*

The whole genome sequence of *Xanthomonas oryzae* pv. *oryzae* (KACC10331) (Xoo), which incites bacterial blight disease on rice was determined. DNA chip technology promises to monitor the whole genome on a single chip so that researchers can have a better picture of the interactions among thousands of genes simultaneously. In this study, oligonucleotides were designed from 3,027 ORFs in the Xoo genome to have optimal specificity for target genes, and were synthesized on a Combimatricx microarray. Xoo cells of the wild type and hrpX mutant were grown on hrp-inducing media. The total RNA induced from the medium was isolated and used as probe. Genes up- and down-regulated by the inducing medium were profiled and characterized.

26.49 GENETIC DIVERSITY OF *STREPTOMYCES* SPP. CAUSING COMMON SCAB OF POTATO IN EASTERN CANADA. R. St-Onge, C. Goyer and M. Filion. *Université de Moncton, Department of Biology, Moncton, NB, E1A 3E9, Canada. Email: ers3453@umoncton.ca*

Common scab is an important disease of potato caused by *Streptomyces scabies* and by a few other *Streptomyces* species. Most strategies used to control common scab have not proved satisfactory, and scab prevalence has increased in Eastern Canada, the most important potato producing region in the country. In this study, the genetic diversity of scab-inducing *Streptomyces* present in Eastern Canada was investigated. Forty-one *Streptomyces* spp. were isolated from scab lesions on potatoes harvested from different regions of New-Brunswick (NB), Nova Scotia (NS) and Prince-Edward-Island (PEI). Rep-PCR banding pattern analysis revealed 10 different genetic groups: 3 in NB, 1 in NS, 4 in PEI and 2 found in more than one province. Results suggest that the genetic group distribution follows geographical patterns. However, there was no evidence of correlation between genetic groups and potato cultivars. For each isolate, sequence analysis of the partial *rpoB* gene and 16S rDNA revealed that thirty-nine isolates clustered closely with different *S. scabies* strains, while two isolates clustered with *S. acidiscabies* which, to our knowledge, has never before been isolated in Eastern Canada. Phylogenetic clustering of the *rpoB* gene and 16S rDNA was consistent with Rep-PCR banding patterns. Partial *txtA*, *txtC* and *tomA* genes associated with the pathogenicity island (PAI) were also amplified by PCR and sequenced, revealing different PAI gene profiles. While no variation in *tomA* sequences were observed, polymorphisms in *txtA* and *txtC* were found, suggesting a possible impact on thaxthomin production and virulence.

26.50 MOLECULAR MECHANISMS OF POTATO INFECTION BY *PECTOBACTERIUM ATROSEPTICUM*. G.W. Takle, T. Marthinsen, I.K. Toth and M.B. Brurberg. *Bioforsk – Norwegian Institute of Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway. Email: may.brurberg@bioforsk.no*

During the last years, Norway and several other countries have seen an increase in potato blackleg disease caused by the Gram-negative bacterium *Pectobacterium atrosepticum* (formerly known as *Erwinia carotovora* ssp. *atroseptica*). *P. atrosepticum* has a narrow host range, limited almost exclusively to potato plants in temperate regions, where it also causes soft rot of potato tubers. The pathogen spreads from infected seed tubers to progeny tubers either through the plant or over short distances in the soil. However, little is known about how seed potatoes are initially contaminated with the bacterium and what onsets the infection. Our overall aim is to understand the underlying molecular mechanisms behind potato infection by *P. atrosepticum* and to find possible means of controlling blackleg disease, as well as providing more knowledge of the overall lifestyle of the bacterium. The sequencing and annotation of the genome of *P. atrosepticum* strain SCRI1043 (Bell *et al.* 2004, Proc. Natl. Acad. Sci. 101, 11105-10) as well as a genome-wide mutant library (Holeva *et al.* 2004, Mol. Plant. Microbe Interact. 17, 943-50) provide valuable tools in examining the molecular aspects of both pathogenesis and environment related factors. Quorum-sensing seems to play an essential role in the pathogenesis of *P. athrosepticum*, and we are currently studying some of the many genes affected by this regulatory control as well as other putative virulence genes.

26.51 FUNCTIONAL GENOMICS TO STUDY THE EVOLUTION OF PATHOGENESIS IN THE POTATO PATHOGEN *PECTOBACTERIUM ATROSEPTICUM*. I. Toth, P. Birch, L. Pritchard, L. Moleleki, M. Ravensdale, C. Robert, H. Liu, S. Humphris, P. Hedley, E. Gilroy, N. Holden and E. Douglas. *SCRI, Invergowrie, Dundee DD5 2DA, UK. Email: ian.toth @scri.ac.uk*

Pectobacterium atrosepticum (Pba–formerly *Erwinia carotovora* subsp. *atroseptica*) is a pathogen of potato in temperate regions. However, as a member of the bacterial family Enterobacteriaceae it is also closely related to the animal pathogens, *E. coli*, *Salmonella*, *Shigella* and *Yersinia* spp. The genome of Pba SCRI1043 (Pba1043) was recently sequenced in a collaboration between SCRI and the Sanger Centre, allowing a number of new pathogenicity and life-style determinants to be identified. This work has led to the discovery of a resistance-related gene in potato, which has been used to generate Pba-resistant plants. In addition, the Pba sequence has allowed genome comparisons to be made between this and all other recently sequenced bacterial genomes. At SCRI we have development a software package called GenomeDiagram for the large-scale comparison of bacterial genome sequences. This, together with other bioinformatic analyses, has been used to determine the differences between Pba, the animal pathogenic enterobacteria and other plant-associated bacteria to discover for the first time what makes an enterobacterial plant pathogen.

26.52 EPIDEMIOLOGY AND CHARACTERIZATION OF *DICKEYA* STRAINS CAUSING SOFT ROT IN FLOWER BULBS. J. van Doorn, R.H.L. Dees, K.T.K. Pham, J.M. van der Wolf and M.J.D. de Kock. *Crop Protection, Applied Plant Research, P.O. Box 85, 2160 AB Lisse, The Netherlands. Email: joop.vandoorn@wur.nl*

In the last decade the flower bulb industry has suffered great losses due to *Dickeya* and *Pectobacterium* spp. of soft rot bacteria. Bulbous ornamentals such as *Hyacinthus*, *Muscari*, *Dahlia* and *Iris* are in most cases infected by *Dickeya* strains; *Calla* lily is infected almost exclusively by *Pectobacterium carotovorum* subsp. *carotovorum.* To evaluate the incidence and infection route of *Dickeya* spp. in the hyacinth bulb production chain, field plots were infected by planting diseased hyacinth bulbs, and crop rotation with different flower bulb species was applied over three growing seasons. Harvested bulbs were analyzed for soft rot during handling and storage by assessing symptoms as well as by plating and PCR techniques. Propagated hyacinth bulblets were also followed during two growing seasons. It was found that infection from the environment was of minor importance; the harvested flower bulbs showed no significant increase in incidence of soft rot bacteria. Latent infection of propagated flower bulbs with *Dickeya* spp. during hyacinth bulb handling seems to be the main cause of soft rot outbreaks. In order to correlate with pathogenicity the diversity and variation of strains of *Dickeya* spp. found in infected flower bulbs, several molecular typing methods like 16 S rDNA sequence alignment and variable number of tandem repeats were used. The application of VNTR has several advantages in strain identification and might be very useful to compare many *Dickeya* strains from different sources.

26.53 MOLECULAR CHARACTERIZATION AND REGULATION OF EXPRESSIONAL OF LIPOPOLYSACCHARIDE O-ANTIGEN BIOSYNTHESIS-RELATED GENES IN *XANTHOMONAS ORYZAE* pv. *ORYZAE*. J.-C. Wang, J.-W. Kim, J.-K. Kim, B.-M. Lee, Y.J, Park and H.-W. Kang. *Graduate School of Biotechnology and Information Technology, Hankyong National University, Ansung 456-749, Republic of Korea. Email: kanghw2@ hknu.ac.kr*

LPS biosynthesis-related gene clusters have been identified in the *X. oryzae* pv. *oryzae* genome. A gene cluster that is organized with xometC-xomtA-wxoABCD was analyzed for this study. Transposon mutations of the genes resulted in virulence-deficient phenotypes and colony morphologies with reduced mucoidy, indicating defects in EPS synthesis. An SDS-PAGE analysis using LPS extracts from the mutant strains confirmed that xometC, xomtA and wxoABC are involved in LPS O-antigen biosynthesis. A decrease in transcription of wxoABC was observed in the xomtA mutant, suggesting that xomtA transcriptionally regulates expression of wxo genes. XomtA was significantly homologous to FkbM methytransferase, SAM-dependent methyltransferase and RfbT related to lipopolysaccharide O-antigen protein. XomtA protein (29 kDa) and WxoC (49 kDa) were overexpressed in the vector pET 15b, purified and used to raise antibodies. Western-blot analysis using XomtA antibody showed that XomtA is a transmembrane protein spanning between membrane and cytoplasm. In Western-blot analysis using WxoC antiserum, xomtA mutant did not react with the antibody, suggesting the XomtA regulates the translational expression of wxoC.

26.54 A NEW CAUSAL AGENT OF HORSE CHESTNUT BLEEDING CANKER, *PSEUDOMONAS SYRIGAE* PV. *AESCULI*. J.F. Webber, J. Rose, N.M. Parkinson, H. Stanford and J.G. Elphinstone. *Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK. Email: joan.webber@forestry.gsi.gov.uk*

Stem bleeding on horse chestnut in the UK was first reported in the 1970s, when the cause was attributed to *Phytophthora*. Such *Phytophthora* bleeding cankers were considered to be uncommon and mainly seen in the south of England. However, over the past five years, reports of horse chestnut trees (*Aesculus hippocastanum*) with 'bleeding cankers' have increased markedly throughout the UK. Symptoms visible on affected trees include bleeding areas on their stems, which can extend into the branches, killing considerable areas of bark and causing branch dieback, sometimes killing entire trees. The increased incidence of stem bleeding on horse chestnut is not just limited to the UK: Belgium, France, Germany and the Netherlands are also experiencing a similar upsurge. Close investigation has revealed that *Phytophthora* is no longer the primary causal agent. The most frequently isolated agent is a species of gram-negative fluorescent bacterium, and inoculation of this agent into horse chestnut saplings reproduced the symptoms seen on mature trees. Fatty acid analysis and sequenceing of the gyrase B gene (*gyrB*) have indicated the presence of a single strain of *Pseudomonas syringae* in infected trees throughout the UK. The *gyrB* sequence of the isolates from bleeding canker was also identical to that of *Pseudomonas syringae* pv. *aesculi* originally isolated from *Aesculus indica* in India in the 1970's. The biology and possible origins of this pathogen which has only recently been found in Britain are discussed.

Plant pathology in industrialized and developing countries

46.1* ROLE OF PLANT PATHOLOGY IN INCREASING AGRICULTURAL PRODUCTIVITY AND FOOD SECURITY IN AFRICA. M. Besri. *Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco. Email: m.besri@iav.ac.ma*

Farmers in developing countries have substantial difficulties in managing plant diseases. Our knowledge on crop losses in Africa is very limited. Crop losses due to plant diseases have been estimated for some crops e.g. rice, wheat, potatoes and maize, but losses in some important African food crops e.g. cassava, millet and sorghum are not known, though in general are very high. Therefore, plant protection is a very important component in improving agricultural productivity and food security in Africa. Integrated pest management (IPM) has increasingly been promoted by major agriculture and development international institutions, governments and NGOs as a means of efficient pest control and also of reducing pesticide use. Opportunities for IPM among small-holder farmers in Africa are expanding because it is enabling poor farmers to maintain and sustain high agricultural productivity. Success stories in Africa abound.

46.2* THE PLANT PATHOLOGY OF A NATIVE PLANT RESTORATION EFFORT. A. Caesar, T.C. Caesar and D. Larson. *USDA, ARS Sidney, Montana 59270, USA. Email: caesara@sidney.ars.usda.gov*

Following successful biological control of the invasive plant *Euphorbia esula virgata*, in which stand density of this deep-rooted perennial was dramatically reduced in density, restoration of native species was undertaken at several locations within Theodore Roosevelt National Park in North Dakota, USA The synergistic interaction of root-feeding larvae of a flea beetle with soilborne plant pathogens was previously shown to be the essential mechanism through which infestations of *E. esula virgata* were controlled. For post-biocontrol restoration of native plant communities, several forbs and grasses were transplanted as seedlings into plots (15 replications per location) at five different locations within the park where *E. esula virgata* had been controlled. Surveys of the restoration plots were conducted in June, July and September of 2007. Plants with necrotic, wilted or chlorotic foliage were collected from plots and assessed for disease. In the June and July sampling, transplanted seedlings exhibited injured or dead top growth but root systems were generally healthy and there was a low disease incidence based on isolations from roots and crowns of sampled plants. Grasses exhibited little apparent disease. Based on the September survey, the forbs showed the most apparent disease, including crown and root necrosis, and among these, the natives species *Ratibida columnifera*, *Aster ericoides*, and *Helianthus pauciflorus* had the highest incidence of root and crown disease from which *Rhizoctonia*, *Fusarium* and *Pythium* spp. were typically isolated. Insect/pathogen interaction-driven stimulation of pathogen inoculum potential may affect the success of restoration efforts, particularly for certain desirable species.

46.3 IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF A NEW CUCURBIT-INFECTING TOBAMOVIRUS IN TAIWAN. Y.H. Cheng, T.Y. Chu, C.C. Chen and C.A. Chang. *Division of Plant Pathology, Agricultural Research Institute, Wufeng, Taichung 413, ROC. Email: yhcheng@wufeng.tari.gov.tw*

A watermelon plant with mild mosaic symptoms was found in central Taiwan. Rod-shaped tobamovirus-like virus particles were observed in the leaf tissue but they did not react with antiserum againt *Cucumber green mottle mosaic virus* (CGMMV), the only tobamovirus reported in Taiwan to infect cucurbits. This tobamovirus was isolated and designated WT2. Virions of WT2 were purified from inoculated squash by polyethylene glycol precipitation followed by Cs_2SO_4 isopycnic centrifugation. Purified virions were used for viral RNA extraction and antiserum preparation. The antiserum reacted strongly with it homologous antigen but not with

antigens of CGMMV and *Tobacco mosaic virus* (TMV), the two most commonly found tobamoviruses in Taiwan. A preliminary field survey using this antiserum showed that WT2-related viruses were widely distributed in watermelon, melon, wax gourd and bottle gourd. Poly(A)-tailed viral RNA of WT2 was used as template for cloning the 3' region of the viral genome using primer pair (poly(T) and WT23: 5'-AA(A/G)TTTTT(T/C) TTTTATACTAG-3') in an rt-PCR. The sequence of the amplified DNA fragment contained 2152 nucleotides including partial replicase, movement protein (MP), coat protein (CP) genes and 3' untranslated region (3'UTR). The sequence showed highest identities with *Cucumber mottle virus* (CMoV) (AB261167) when compared with known virus sequences in the GenBank. CmoV and WT2 shared 88% similarity in the MP, 87% similarity in the CP, and 82% identity in the 3'UTR. Based on these results, isolate of WT2 is probably a strain of CMoV.

46.4* *PSEUDOMONAS* ISOLATES FROM THE COCOYAM RHIZOSPHERE WITH QUORUM-SENSING MACHINERY HAVE ANTAGONISTIC PROPERTIES. **K. De Maeyer, J. D'aes and M. Höfte.** *Laboratory of Phytopathology, Faculty of Bioscience Engineering, Ghent University, Coupure Links, 653, B-9000 Ghent, Belgium. Email: katrien.demaeyer@ugent.be*

Among forty fluorescent pseudomonads randomly isolated from the rhizosphere of healthy cocoyam plants (*Xanthosoma sagitttifolium*) in Cameroon, eight isolates showed antagonism against the cocoyam root-rot pathogen *Pythium myriotylium.* Seven of the eight antagonistic isolates were identified as producers of phenazine antibiotics. In addition, whole-cell protein profiling clustered these seven strains together with above 85% similarity. These strains may represent a novel *Pseudomonas* species. Quorum-sensing is a cell density-dependent regulation mechanism in which acylated homoserine lactones (AHLs) are autoinducers. Streak assays with different biosensor strains demonstrated AHL production only in these seven antagonistic, phenazine-producing strains. The ability to accumulate quorum-sensing signals distinguished the 7 antagonistic strains again from the 33 other rhizosphere isolates. This supports the assumption that population density-mediated gene expression is widespread in bacteria involved in plant-pathogen interactions. AHLs from these seven strains were profiled by thin-layer chromatography and biodetected by *Agrobacterium tumefaciens* NT1. Most strains produced more than one AHL, among which were 3-unsubstituted AHLs such as 3-oxo- or 3-hydroxy-substituted AHLs. Currently we are using mass spectrometry to identify these AHL derivatives. In particular we are interested in the quite rare 3-N-hydroxy/oxo-dodecanoyl homoserine lactone that is being produced by two strains.

46.5* *DIPLODIA PINEA* IN FOREST PLANTATIONS OF *PINUS RADIATA* IN NORTHERN SPAIN: DIVERSITY AND ORIGIN OF THE SPANISH POPULATION. **I. Garcia-Serna, N. Mesanza and E. Iturritxa.** *Neiker Granja Modelo de Arkaute, Aptdo 46 Vitoria, Spain. Email: eiturritxa@neiker.net*

Pinus radiata is an exotic species first introduced in Spain at the end of the Nineteenth Century in private forests along with species like *P. pinaster* and *Cupressus macrocarpa.* An expansion of the *P. radiata* population took place during the forties and fifties. This rapid growth and desirable lumber and pulp qualities caused it to be the leading introduced species in the north of Spain. The domination of *P. radiata* is unquestionable, at altitudes lower than 600 m and in coastal areas, due to its susceptibility to frost. Thus *P. radiata* is an element of the landscape in the communities of Galicia, Asturias, Cantabria and the Basque Country, which latter grows 60 % of the *P. radiata* in Spain. *Diplodia pinea* is the most important pathogen of *Pinus* spp, causing serious problems in nurseries and specially in plantations in combination with two common meteorological factors in these regions: hail and strong wind. Here we report results of a hierarchical sampling in Spanish plantations of *P. radiata.* The diversity of the Spanish population of *D. pinea* was evaluated using the vegetative compatibility test, and compared with that of foreign isolates obtained from Chilean and New Zealand imported seed. The results showed high diversity in the *D. pinea* populations, due to the nature of the species and the introduction of new fungal isolates on pine seeds imported from other countries.

46.6 FARMERS PARTICIPATORY RESEARCH (FPR) FOR BIOLOGICAL MANAGEMENT OF BACTERIAL WILT OF TOMATO. **D.K. Hazarika and J.K. Choudhary.** *Krishi Vigyan Kendra, Napaam, Tezpur, Assam, India. Email: dkhazariak@yahoo.co.in*

Tomato is one of the important vegetables of Assam grow extensively as rabi crops. It suffers badly from bacterial wilt caused by *Ralstonia solanacearum* Yabuuchi. The disease causes considerable loss in yield, ranging from 10,8 to 92.62 per cent. In Assam, bacterial wilt is the most limiting factor for tomato as an early season crop. It is difficult to manage the disease with chemicals, and resistant cultivars fails to remain resistant for long. Bio control agents are the only solution to manage such soil borne pathogen and have several advantages over chemical control. Management strategy adopted in farmers field with active participation of farmers is a new approach for successful control of disease in sustainable manner. This new approach is based on learning by doing and believing by seeing. Keeping this in view, an experiment was conducted for three consecutive years in farmers field with commercial formulations of biocontrol agents containing *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescens* obtained from Assam Agricultural University, Assam and Karnataka Agrochemicals Pvt Ltd., Bangalore. All biocontrol agents used as seed treatment as well as soil application or combination of both significantly reduced plant mortally and increased fruit yield over farmers own management practice. This effect was more prominent with combination of seed treatment and soil application. Highest reduction of plant mortality and increase in yield was recorded with Sparsha, a commercial formulation of Karnataka Agrochemicals Pvt Ltd containing *P. fluorescens*. By conducting such type of Farmers Participatory research a sustainable technology can be transferred from laboratory to field.

51.1* DIVERSITY OF PLANT HEALTH ISSUES IN HOME GARDENS. **S. Helfer.** *Royal Botanic Garden Edinburgh, Scotland, UK. Email: s.helfer@rbge.ac.uk*

By their very nature, private home gardens are diverse places of individual taste, and the choice of planting materials, their cultivation and protection are equally individualistic. It is therefore not surprising to find a large diversity of pathogens and pests in these gardens. The main components of plant health for home gardens are discussed, including plant type (trees, fruit, ornamentals etc.), pests and pathogens involved, control measures used or recommended. The available data are currently restricted to home gardens in the British Isles but it is hoped to expand the research to other areas, especially to subsistence horticulture in

poor countries, where a substantial proportion of produce is grown in home garden environments. In Britain, the main plant health concerns are in connection with trees and the main groups of pathogens affecting crops are fungi. Often cultural or no control measures are available or needed to improve plant health. Occasionally, chemical pesticide use is recommended, in a climate of increasing problems in the availability to non-professionals of effective chemical treatments.

51.2* ANALYSIS OF RESEARCH, ANALYTICAL AND DIAGNOSTIC LABORATORIES IN SOUTHERN AFRICAN COUNTRIES FOR COMPLIANCE WITH INTERNATIONAL SANITARY AND PHYTOSANITARY REQUIREMENTS. **L. Korsten.** *Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002, South Africa. Email: lise.korsten@up.ac.za*

Rapid, accurate and internationally recognised diagnostic services for the detection of food-borne and plant pathogens form the basis of sanitary and phytosanitary (SPS) requirements for international trade in agricultural and food products. Surveillance systems were developed to track outbreaks of food-borne pathogens and identify sources of origin. Laboratory analysis of samples is often also required in dispute cases and for rejection of export consignments due to the presence of phytosanitary pathogens. For developing countries, it is often difficult to comply with new international trade standards to export to European or USA markets. All countries involved in international trade must be able to provide assurances (using diagnostic laboratories accredited to ISO 17025: 2005 and supportive research capacity) that export products are safe for human consumption and pose no phytosanitary risks. This paper deals with the status of diagnostic and research laboratories in Southern Africa and their ability to address international trade requirements. Laboratory capacity was assessed in terms of human capacity, accredited test methods, participation in ring-proficiency schemes and accreditation status. Several governmental, semi-governmental and private laboratories provide diagnostic services and microbiological tests of limited scope, and very few ring-proficiency schemes exist. Academic institutions do not train enough scientists in the area of agriculture and trade and do not cover SPS, risk assessment and food safety assurance systems in their curriculums. However, several international and regional initiatives have been established to address this critical need.

51.3 DEVELOPMENT OF PLANT HEALTH CLINICS IN INDIA -- STATUS, STRATEGIES AND CHALLENGES. **N. Mehta.** *Department of Plant Pathology, CCS Haryana Agricultural University, Hisar 125 004, India. Email: nareshmehta@hau.ernet.in*

The basic objective of plant clinics is to provide comprehensive diagnostic and advisory services encompassing all possible causes of ill health whether biotic or abiotic. The plant clinic will be useful in providing all the valuable qualitative and quantitative local information which will help the farmers to improve their decision making. By providing advice based on sound principles, plant clinics have an important role to play in reducing dependence on pesticides. The emphasis of plant clinics in broad terms should be on human resources and physical infrastructures. Clinics should have laboratories with diagnostic facilities for the whole range of plant diagnostic services integrated under one roof for the benefit of clients desiring simultaneously several diagnostic services. Plant pathologists working in plant clinics should be trained in the latest advances like information technology, or application of computer software for forecasting disease epidemics, and laboratories should be equipped with the audio, video and internet facilities for early dissemination of information to avoid losses. A clinic should be located within or near the campus of a University or Research Institute and easily approachable by the clients. There should be provision of electronic displays with scrolling text so that important messages regarding plant health care can be displayed. A mobile plant clinic with modest diagnostic facilities and trained professionals can do on-the-spot diagnosis. Clinics have to play a greater role by periodically organizing plant health camps, issuing handouts, anticipating disease outbreaks and providing solutions or options tuned to farmers' needs with the utmost clarity.

51.4 FIRST REPORT OF CONTROLLING STRIPE RUST (*PUCCINIA STRIIFORMIS* F. SP. *TRITICI*) BY NANO-SILICA. **A. Amirahmadi.** *NO. 73, Bagh Jannat Avenue Damghan, Semnan, Iran. Email:Alireza.amirahmadi@gmail.com*

Stripe rust was first described in Europe in 1777. First reports in the United States date from the early 20th century. Historically it has been a problem in the Pacific Northwest, California and the higher mountain valleys. Yield losses of up to 25% have been reported from Washington State. Over the last few years, stripe rust has become increasingly important in the central Great Plains, even in south-central states. This is probably due to the development of new strains which tolerate a much broader range of temperatures, and infect a broader range of wheat varieties. Stripe rust can attack wheat, barley, triticale, and many other related grasses. The disease is found in all highland and/or temperate areas where cereals are grown. No alternate host is known. I used Nano-silica particles to control stripe rust (yellow rust) in the laboratory and the field. Results of lab investigations showed 100 percent control on both agents at 100 and 250 ppm concentrations. The pustules of the *Puccinia striiformis* showed no growth or development from the beginning of the test even after 15 minutes, whereas all controls showed normal growth and development. The tests were then performed in the natural situation. In the field, concentrations of 25, 50, 75 and100 ppm were applied to control *P. striiformis* in wheat. Results showed 100 percent control with 100 ppm for 7 to 21 days. It could prevent using massive amount of toxicants and chemicals in the environment.

51.5 OBSERVATIONS ON THE CASSAVA ANTHRACNOSE DISEASE IN PARTS OF AKWA IBOM STATE OF NIGERIA. **E.N. Nneke, R.C. Wokocha and C.I. Umechuruba.** *Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia, Nigeria. Email: rocwokocha@yahoo.com*

Surveys of the cassava anthracnose disease incited by *Colletotrichum gloeosporiodes* f. sp *manihotis* were conducted in six major cassava-growing locations in Akwa Ibom State, in the humid tropics of southeastern Nigeria. The objective was to determine the incidence and severity of the disease in this agroecological zone. Samples were randomly taken during the wet season (May – October) and dry season (November – April) in 2006 and 2007, along two diagonal transects across each farm. Results indicated that the mean disease incidence for the study area was significantly ($P \leq 0.05$) higher (24.1%) in the wet season than in the dry season (5.9%). The highest disease prevalence occurred in Nsit Atai in both the wet season (36.6%) and dry season (9.3%),

while Essien Udim (13.6%) and Oruk Anam (4.1%) gave respectively the lowest disease prevalence in both seasons. Disease severity was assessed on a scale of 1-5. Mean disease severity for the area was higher (3.0) in the wet season than in the dry season (2.0). However, disease severity was fairly uniform across the locations in each season. Disease symptoms (lesions and cankers) appeared on the stems, leaves and fruits of cassava. In some cases, portions of stems riddled with cankers, showed marked inflammation and distortion, while infected shoots of young susceptible varieties dried up and wilted. Cassava anthracnose is thus an economically important disease in Akwa Ibom State of southeastern Nigeria.

51.6* SYNERGISTIC EFFECT OF EXTRACTS OF *AZADIRACHTA INDICA* AND *OCIMUM GRATISSIMUM* ON SUGARCANE RED ROT IN NIGERIA. **D.B. Olufolaji.** *Dept of Crop, Soil and Pest Management, Federal University of Technology, P.M.B. 70-4, Nigeria. Email: tundeolufolaji@yahoo.co.uk*

Crude aqueous and ethyl ether extracts of *Azadirachta indica* and *Ocimum gratissimum* were assayed *in vitro* and *in vivo* separately and in combination for their fungicidal attributes on *Colletotrichum falcatum* Went and the red rot disease of sugarcane. Extract concentrations of 40, 60, 80 and 100 % were used for laboratory and screenhouse studies using three replicates. Ridomil at 2.0 a.i.ml^{-1} was used as the standard. Fungicidal principles were used as cold aqueous extracts rather than organic solvent extracts since most of the aqueous extract treatments significantly inhibited mycelial growth and subsequent sporulation of the fungus better than in organic solvent. Cold extraction with *A. indica* and *O. gratissimum* at 100% and in combinations gave 41, 46, 63% and 36, 47, 57% respectively of inhibition of mycelial growth and sporulation of *C. falcatum* which was significantly the highest of all the extract treatments. In disease severity at 100% concentration, *A. indica* alone and when combined with *O. gratissimum* gave the least disease severity of 2.3 and 1.9 respectively. However, preventive treatments with the extracts were better than curative because the two plant extracts gave 2.4 and 3.2 disease severity ratings in preventive and curative treatments respectively. In terms of total soluble solids (TSS) of the sugarcane juice (which is the yield assessment), preventive control with *A. indica, A. indica* + *O. gratissimum* and Ridomil gave 11.0, 12.9 and 13.1% respectively while *O. gratissimum* alone gave 8.0%. Generally the combination of the two plants extracts was significantly better than individual usage.

51.7 PROTECTING WOUNDS FROM EUROPEAN CANKER (*NEONECTRIA GALLIGENA*) INFECTION OF APPLE IN WINTER AND AUTUMN. **R.W.A. Scheper, O.D. Stevenson and R.M. Beresford.** *The Horticulture and Food Research Institute of New Zealand Ltd, Private Bag 1401, Havelock North, Hastings 4157, New Zealand. Email: rscheper@hortresearch.co.nz*

In high rainfall apple-growing areas of New Zealand, fungicide protection of pruning wounds and leaf scars against infection by *Neonectria galligena* is essential. This study compared five treatments applied to simulated pruning wounds in winter, and two treatments applied to leaf scars in autumn, in two separate field trials, in 6-year-old Pacific Beauty™/Sciearly trees, using randomized block designs with four replicates. In winter, wound dressings containing 10g/l tebuconazole (as Bacseal® Super) with and without added carbendazim (25g active ingredient /l), and spray treatments of tolyfluanid (as Euparen® Multi, 1.25g a.i./l), captan (1.25g/l) and copper oxychloride (5g/l) were applied within 6h of wounding. Wounds were inoculated with ascospores of *N. galligena* (5×10^4/ml) 2 h after treatment. Nine months later, there were significantly ($P<0.01$) fewer symptoms on wounds treated with a dressing than on those sprayed with fungicide or water. The addition of carbendazim to the wound dressing significantly ($P<0.01$) reduced the percentage of wounds with symptoms, from 23% to 3%. No significant differences were detected among fungicide and water sprays, with 70-85% of wounds developing symptoms. In autumn, spray treatments of tolyfluanid (1.25g/l) and captan (1.25g/l) were applied to leaf scars 2 h prior to inoculation with conidia (10^5/ml). Twenty weeks later, there were significantly ($P<0.01$) more symptoms in the control (20%) compared with leaf scars treated with either fungicide application (0%). It is recommended to apply wound dressings amended with 25g/l carbendazim to all pruning wounds to minimize infection by *N. galligena* and to spray with fungicides in autumn to protect leaf scars.

PLANT PATHOGENS AND MICROBIAL INTERACTIONS IN SOIL

50.1* COMPARISON BETWEEN BACTERIA COLONIZING SCLEROTIA AND THOSE IN SOIL, USING CULTURE AND NON-CULTURE METHODS. **A. Adandonon, N. Momma, Y.T. Hoshino, N. Matsumoto and I. Okabe.** *National Institute for Agro-Environmental Sciences, 3-1-3 Kannondai, Tsukuba 305-8604, Ibaraki, Japan. Email: adanappo@yahoo.fr*

Bacterial communities concomitant with sclerotia of *Sclerotium rolfsii* and in soil adjacent to sclerotia were examined by using culture and non-culture methods. The fungal sclerotia were buried in soil amended with organic matter and incubated under flooded conditions for 30 days in the greenhouse. Recovered sclerotia (ground) and the adjacent soil were spread on R2A medium and incubated with or without Anaero-Pack. Bacterial populations (CFU/g) concomitant with sclerotia were higher than those from adjacent soil samples and the difference was more pronounced in amended flooded soil. The bacterial population recorded from the Anaero-Pack incubation was significantly lower than those without Anaero-Pack, indicating that few strictly anaerobic bacteria were present. The bacterial population in sclerotia was higher in flooded than unflooded soil. The sclerotia germinated on PDA (%) and southern blight disease incidence by the fungus in the greenhouse were negatively correlated with the amount of bacteria in the sclerotia. Polymerase chain reaction (PCR) – denaturing gradient gel electrophoresis (DGGE) patterns of 16S rDNA showed that the bacterial communities were more diverse in sclerotia than in soil samples. Examination of the nucleotide sequences of the DGGE bands revealed, in both sclerotial and soil samples, that the bacterial populations were dominated by clostridia.

50.2* SOILBORNE PATHOGENS ASSOCIATED WITH ROOT HERBIVORY OF THE INVASIVE SPECIES *LEPIDIUM DRABA*. **A. Caesar.** *USDA–ARS, 1500 N. Central Ave., Sidney, Montana, 59270, USA. Email: caesara@sidney.ars.usda.gov*

Isolation of fungi from insect-damaged roots of the invasive perennial *Lepidium draba* surveyed over 1999-2007 in Europe revealed that such roots of this species were often infected with one or more soilborne fungi. Plants with evident stunting and/or chlorosis and reddening of leaves nearly always exhibited root damage by one or more insect species. More than half of the plants with such symptoms were found to have galls typically

caused by *Ceutorhynchus* spp. larvae. Throughout the surveyed range of *L. draba*, the most prominent fungus isolated from root galls and adjacent root tissue was *Rhizoctonia solani*. The galls were often decayed at exit points of the larvae and adjacent root tissue was consistently infected with *Rhizoctonia solani*, *Fusarium* and *Pythium* spp. Eight of 11 isolates of *Rhizoctonia solani* anastomosed with tester isolates of AG-4, one was a binucleate and two isolates were unidentified to-date. Five isolates caused mortality of 9-10 plants when 5 wk-old seedlings were planted in soil mix infested with *Rhizoctonia* inoculum. In previous studies, a similar complex of soilborne fungi had been found in association with insect root herbivory of *Euphorbia esula/virgata*, *Centaurea maculosa* and *Centaurea diffusa*. The prevalence of such associations, especially in yet another instance involving a highly invasive herbaceous perennial weed may indicate that such highly destructive insect/pathogen combinations are a previously overlooked key to biological control success. A concept for utilizing a multitrophic approach to screening for new biocontrol agents of exotic, invasive herbaceous perennial weeds of rangelands will be discussed.

50.3 THE EFFECT OF RICE STRAW MULCHING ON THE COMPOSITION OF WHEAT RHIZOSPHERE ANTAGONISTIC BACTERIA AND WHEAT SHARP EYESPOT. **H.G. Chen, Q.G. Cao, G.L. Xiong, W. Li, A.X. Zhang and H.S. Yu.** *Institute of Plant Protection, Jiangsu Academy of Agriculture, Xiao Ling Wei, Nanjing 210014, P.R. China. Email: huaigu@jaas.ac.cn*

The wheat sharp eyespot caused by *Rhizoctonia cerealis* is one of main diseases in the lower reaches of the Yangtze River where the rice-wheat rotation is adopted. To assess the effect of rice straw mulching on the incidence of wheat sharp eyespot and on abundance of antagonistic bacteria, a multi-year field study was done to compare rice straw unmulched, mulched for two years, and mulched for three years. Analyses revealed that the disease index in the two mulched plots was similar but lower than in the unmulched control. Bacterial and fungal populations in wheat rhizosphere and bulk soil were detected based on cultivation-dependent analyses. The rice straw mulching treatments increased the bacteria and pseudomonad colony forming units (cfu) in wheat rhizosphere and bulk soil but decreased the fungal cfu. Bacteria isolates antagonistic to *R. cerealis* were identified by using an inhibition zone test, and a set of these isolates were typed by partial sequencing of the 16S rRNA gene. The proportion of fluorescent pseudomonads among total bacteria in straw-mulched soil was higher than that in no-straw-mulch soil. Above 80% of rhizosphere fluorescent pseudomonads were antagonistic to *R. cerealis*. The pseudomonads had higher antagonistic activity against *R. cerealis* than other species. The results suggest that rice straw mulching in rice-wheat rotation increased the fluorescent pseudomonads, which were antagonistic to *R. cerealis*, in wheat rhizosphere, and fluorescent pseudomonads may play an important role in the control of wheat sharp eyespot by rice straw mulching.

50.4 DEVELOPMENT OF QRT-PCR ASSAYS TO STUDY THE TRANSCRIPTIONAL ACTIVITY OF ANTIFUNGAL METABOLITE-CODING GENES PHLD AND HCNBC UNDER *IN VITRO* AND SOIL CONDITIONS. **N. DeCoste, M.M. Paulin and M. Filion.** *Université de Moncton, Department of Biology, Moncton, NB, E1A 3E9, Canada. Email: end3147@umoncton.ca*

Antifungal metabolites produced by *Pseudomonas* spp. have received increased attention as potential biocontrol agents able to control soilborne plant pathogens. However, very little is known about the impact that presence/absence of plant pathogens have on the transcriptional activity of genes encoding these metabolites. In this study, *Pseudomonas* sp. LBUM300, carrying genes for the production of 2,4-diacetylphloroglucinol (DAPG) and hydrogen cyanide (HCN) was isolated from the rhizosphere of strawberry and characterized for its biocontrol activity against the fungal plant pathogens *Phytophthora cactorum* and *Verticillium dahliae*. To assess the impact that pathogens might have on the transcriptional activity of DAPG and HCN-coding genes, specific real-time PCR primers and TaqMan probes were designed and used in qRT-PCR assays to monitor phlD (coding for DAPG) and hcnBC (coding for HCN) gene expression on synthetic media and under soil conditions. Direct RNA extraction from media cultures or soil followed by one-step qRT-PCR provided reproducible results and a high level of specificity. Expression of both genes remained detectable for at least 14 days either in the presence or absence of pathogens, but was differentially regulated in the presence of *V. dahliae* or *P. cactorum*. Higher levels of antagonism generally correlated with higher expression of *hcnBC* and *phlD*. Results suggest that the transcriptional activity of DAPG and HCN-coding genes is differentially regulated in time with respect to pathogen presence/absence.

50.5 SIGNALLING THROUGH THE STRESS ACTIVATED MAP KINASE PATHWAY IS REQUIRED TO MAINTAIN A MUTUALISTIC SYMBIOTIC INTERACTION BETWEEN *EPICHLOË FESTUCAE* and *PERENNIAL RYEGRASS*. **C. Eaton, B. Ambrose, J. Hyams and B. Scott.** *Molecular BioSciences, Private Bag 11 222, Massey University, New Zealand. Email: d.b.scott@massey.ac.nz*

Epichloë festucae is a biotrophic fungus that forms a mutualistic symbiotic association with perennial ryegrass. Growth of the fungus within the leaves is very tightly regulated; hyphae grow by tip growth within the meristematic tissue but after colonization of the leaf expansion zone, the hyphae further elongate by intercalary extension. This pattern of growth allows the fungus to synchronize its growth with that of the host. Fungal ROS produced by a specific NADPH oxidase isoform is critical for maintaining this mutualistic interaction (Tanaka *et al.* 2006; Takemoto *et al.* 2006). The aim of this project was to test whether the endophyte stress-activated MAP kinase pathway has a role in maintaining the symbiotic interaction. Deletion of *sakA* resulted in increased ROS production in culture, sensitivity to osmotic and temperature stress, and resistance to the phenyl-pyrrole fungicide fludioxonil. Expression analysis showed that deletion of *sakA* had no effect on transcript levels of either *noxA* or *noxR*. Deletion of *sakA* reduced the ability of *E. festucae* to colonize perennial ryegrass seedlings; where plants were infected they had a poorly developed root system, were severely stunted and underwent early senescence. The base of the tillers was bulbous and lacked anthocyanin pigments found in wild-type associations. Light and electron microscopy revealed that growth of the *sakA* mutant was unregulated, hyphae were frequently vacuolated and the extracellular matrix surrounding the cell wall was electron dense. Taken together these results demonstrate that signaling through the SakA pathway is critical for maintaining a mutualistic interaction between *E. festucae* and perennial ryegrass.

50.6 FUNGAL POPULATION PROFILES OF A *COLOPHOSPERMUM MOPANE ECOSYSTEM* IN THE KRUGER NATIONAL PARK, SOUTH AFRICA. **A. Jacobs, E.J. van der Linde**

and M. Truter. *Mycology Unit, Biosystematics Division, Plant Protection Research Institute, Agricultural Research Council, Private Bag X134, Queenswood, Pretoria, 0121, South Africa. Email: JacobsR@arc.agric.za*

The Kruger National Park (KNP) has conducted studies on different fire regimes for the past 51 years, but the effect of these fire regimes on fungal populations has not been studied. Fungal soil populations in the mopani ecosystem of the KNP were surveyed. Soil samples surrounding five mopani trees in each burn plot designated by park authorities, were collected. All soil samples were plated out directly and serial dilutions were also made. At present 1260 purified fungal cultures have been obtained. DNA extractions were completed for twenty six composite soil samples. Total DNA obtained from the samples was used to determine the population dynamics of all the soil samples. This was done by means of the denaturing gradient gel electrophoresis technique. Cluster analysis of the 18S gene resulted in a profile representing the prokaryotic populations present in the different samples. The profiles for all 26 samples showed no clear clustering among treatments. Cluster analysis of the internal spacer region (ITS) resulted in a profile representing the eukaryotic populations present in the different samples. The three main clusters represent no clear distinction among the exclosure plots, or the pre- and post-burning samples, although different statistical analyses were done. Dominant bands in this profile were further characterized and represent fungal species also /isolated via conventional mycological analysis.

50.7* MECHANISM OF INTERACTION BETWEEN *TRICHODERMA* SPP. AND THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*. **T. Khan, Y.P. Singh and U.S. Singh.** *Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture & Technology Pantnagar 263145, India. Email: taranakhan15@rediffmail.com*

Application to roots of spore suspensions of *Trichoderma harzianum* (PBAT 43) and *T. virens* (PBAP 27) suppressed root knot of *Abelmoschus moschatus* caused by *Meloidogyne incognita*. The mechanism of interaction between *Trichoderma* spp. and *Meloidogyne incognita* was explored by observing the direct interaction between *Trichoderma* spp. and egg sacs, eggs and second stage juveniles (J2) of *M. incognita*. The effect of culture filtrate of *T. harzianum* was studied on hatching and mortality of J2. The culture filtrate decreased the hatching and enhanced the mortality of J2. In a study of the direct interaction between the *Trichoderma* species (*T. harzianum & T. virens*) and *Meloidogyne* J2 using normal light and fluorescent microscopy, the hyphae of *T. harzianum* were found to colonize the egg masses and eggs and inhibited hatching. Hyphae of both *T. harzianum* and *T. virens* formed loops to trap the J2 juveniles. After trapping, fungal hyphae grew along the nematode body and formed appresorium-like structures (ALS) on the body surface. Penetration took place either directly by the hyphal tips or by forming an infection peg from ALS in both *T. harzianum* and *T. virens*. Penetrating hyphae extensively colonized the nematode body and digested the internal organs. At later stages all internal organs of the nematode body were replaced by the fungal hyphae and they attained the shape of the nematode body. No difference was observed between mechanisms of infection of *T. harzianum* and *T. virens* against *M. incognita*.

50.8 DAMAGE THRESHOLD OF *MELOIDOGYNE INCOGNITA* TO TOMATO IN RELATION TO SOME BIOTIC FACTORS. **A.M. Korayem.** *Plant pathology Dept., National Research Centre, Dokki Giza, Egypt. Email: kor_asm@yahoo.com*

The relation between population densities of *Meloidogyne incognita* and yield of the two susceptible tomato cultivars Super Strain B and Super Marmande was studied under field conditions during 2004 and 2005. There was a significant negative correlation between tomato yield and the initial nematode density for both cvs. Both cvs. were more tolerant to nematodes in 2004 than in 2005. 'Super Strain B' was more tolerant to nematode infection than 'Super Marmande' both in 2004 and in 2005. The first cultivar tolerated nematode infection up to 1600 nematodes per plant in 2004 and up to 1000 in 2005, while the second cultivar tolerated populations of up to 85 and 65 nematodes in 2004 and 2005, respectively. The tolerance limit (T) of 'Super Marmande' plants grown in soil amended with cattle manure was more than in non-amended soil, since T increased from 65 nematodes/plant (non-amended soil) to 120/plant (amended soil).

50.9* SAPROTROPHIC SOIL FUNGI FROM WHEAT FIELDS AND SCOTS PINE FORESTS ANTAGONISTIC TO ROOT PATHOGENS. **M. Mańka, P. Łakomy and M. Belka.** *Agricultural University, Department of Forest Pathology, Wojska Polskiego 71c, 60-625 Poznan, Poland. Email: mmanka@au.poznan.pl*

Four communities of saprotrophic soil fungi were isolated – two from Scots pine forests (age over 80 years) and two from winter wheat fields (in a conventional and an 'organic' system). From each community fungal components were tested against severe root and damping-off pathogens: *Fusarium oxysporum*, *Rhizoctonia solani* and *Rhizoctonia* sp. The tests were performed with the biotic series method. The most effective and promising species were also tested at various temperatures and pHs. They were: *Chrysosporium merdarium, Trichoderma koningii, Trichoderma viride, Cladosporium macrocarpum, Gliocladium viride and Penicillium atrovenetum*. Environmental conditions influenced the effect of the saprotrophic fungi to some extent. With increase of temperature *F. oxysporum* and *R. solani* were suppressed to a smaller extent.

50.10 ROOT-ASSOCIATED DISEASE-SUPPRESSIVE PSEUDOMONADS WITH INSECTICIDAL ACTIVITY. **M. Maurhofer, M. Péchy-Tarr, D. Bruck, E. Fischer, J. Grunder, J.E. Loper and C. Keel.** *Institute of Integrative Biology, ETH Zurich, Switzerland. Email: monika.maurhofer@agrl.ethz.ch*

Root diseases and pests are a major problem in agricultural crops. Their control with chemical pesticides is problematic. Disease and pest control by microbes has evolved as a promising alternative strategy. A prominent example is the biological control of root diseases with plant-beneficial pseudomonads that operate in the rhizosphere and protect crops from pathogenic fungi. Recently, we have made the exciting discovery that certain disease-suppressive *Pseudomonas fluorescens* strains also exhibit potent insecticidal activity. This is remarkable, as plant-colonizing pseudomonads have no known insect association. Anti-insect activity in these pseudomonads is linked to a genomic locus encoding a novel protein toxin that is related to the potent insect toxin Mcf (Makes caterpillars floppy) of the entomopathogen *Photorhabdus luminescens*, a mutualist of insect-invading nematodes. To assess the biological potential of the novel toxin, we created *P. fluorescens* mutants carrying a deletion in the toxin gene. We also cloned the toxin gene under the control of an inducible promoter

for controlled expression in a non-toxic *Escherichia coli* host. Injection of toxin-expressing cells of wild type *P. fluorescens* or transgenic *E. coli* into the hemocoel killed most larvae of the tobacco hornworm (*Manduca sexta*) and the greater wax moth (*Galleria mellonella*). Toxin-deficient *P. fluorescens* mutants were significantly less virulent to the insect larvae, illustrating that the Mcf-related gene products constitute potent insect toxins that define the anti-insect properties of these pseudomonads. The fact that insect toxins are produced by efficient crop-plant colonizers may provide clues to the development of novel pest and disease control strategies.

50.11 COLONIZATION OF MAIZE ROOTS BY *FUSARIUM* SPP. IN RELATION TO TRANSGENIC CORN ROOTWORM RESISTANCE. **G. Munkvold, L. Meinke, L. Lewis and A. Fessehaie.** *Iowa State University, 160 Seed Science Bldg., Ames, IA, 50011, USA. Email: munkvold@iastate.edu*

Larvae of the corn rootworm (CRW) (*Diabrotica* spp.) injure maize roots through their feeding activity, completely destroying some roots and leaving others with extensive epidermal and cortical damage. We hypothesized that the roots of plants with CRW injury will be more intensively colonized by soilborne fungi, including root and stalk rot pathogens. We planted maize hybrids in fields where high populations of CRW had been encouraged through the use of trap crops. Hybrids genetically engineered with different genes for CRW resistance were compared to their near-isogenic CRW-susceptible counterparts in replicated plots in each of three locations (Mead, NE; Ames, IA; Crawfordsville, IA, USA) in 2007. We measured CRW injury (0-3 nodal injury scale) and *Fusarium* colonization (by dilution plating and quantitative PCR) in mid to late July and again in mid September, and recorded the incidence of stalk rot symptoms in mid September to mid October for plants collected randomly from each plot. CRW injury was severe on susceptible hybrids, especially at the Mead location, with scores averaging ~2.0. Transgenic hybrids showed moderate to high levels of resistance, with average scores <1.0. Several *Fusarium* species were isolated from roots, including *F. verticillioides*, *F. proliferatum*, *F. semitectum*, and *F. graminearum*. Root colonization of CRW-susceptible hybrids exceeded that of their CRW-resistance counterparts in most cases, although results for individual plants were highly variable. Several of the observed *Fusarium* species are stalk rot pathogens and these results suggest that the risk of colonization by stalk-rot causing *Fusarium* fungi is reduced by protection from CRW injury.

50.12 BIOCONTROL OF CHARCOAL ROT OF SORGHUM BY USING ARBUSCULAR MYCORRHIZAL FUNGI. **C.R. Raghavender, A. Hindumathi and B.N. Reddy.** *Mycology and Plant Pathology Laboratory, Department of Botany, Osmania University, Hyderabad 500007, India. Email: raghav@osmania.ac.in*

Charcoal rot of sorghum caused by the fungus *Macrophomina phaseolina* is the most common (soilborne) root and stalk rot disease. It is widespread in winter and causes enormous yield losses due to drought stress during the grain filling stage. Biocontrol is gaining importance because pesticide control is not very effective. Thus the present study is aimed to explore the prospects of using the arbuscular mycorrhizal fungus *Glomus fasciculatum* in biological suppression of charcoal rot fungus. It is clear from our study that charcoal rot disease declined to a large extent when the sorghum plants were inoculated with the mycorrhizal fungi. No disease was observed in the varieties of RS-29 and E-36-1 when inoculated with mycorrhizae and pathogen. The disease incidence was reduced to 80% in the variety CSV-8R, which is considered as highly susceptible. The reduction in disease severity is due to competition of sites and also by strengthening the host plant where damage caused by the pathogen was offset by improved plant growth. Biocontrol certainly helps farmers growing sorghum particularly in winter. It is difficult to generalize the interactions of pathogen-host-mycorrhiza but AM fungi can be used in controlling disease biologically, and to obtain benefit it is essential to know the specificity of the AM fungus-host-pathogen combination. Exploitation of mycorrhizal fungi in commercial agriculture is possible only when the mycorrhizal inoculum is produced in large quantities, and appropriate strategies adopted for successful application of inoculum under field conditions.

50.13 RPB1-MEDIATED RESISTANCE TO *PLASMODIOPHORA BRASSICAE* IN *ARABIDOPSIS THALIANA*. **F. Rehn, A. Arbeiter, N. Galfe and J. Siemens.** *TU Dresden, Dept. Biology, Molecular Biotechnology, 01062 Dresden, Germany. Email: Johannes.Siemens@tu-dresden.de*

A monogenetic inherited phenotype resistant to *Plasmodiophora brassicae* (clubroot) has been found in *Arabidopsis thaliana* ecotypes Tsu-0, Ze-0, Ta-0, and RLD. The dominantly inherited *RPB1* gene mediates a hypersensitive-like response. Crossing experiments of Tsu-0 with mutant lines of salicylic acid, jasmonic acid and ethylene signaling revealed no influence of these hormones in the resistance reaction. Furthermore, mutations of *SGT1b* and *RAR1* showed no influence on *RPB1*-mediated resistance, but a mutation of *SGT1a* (At4g23570) was found to be epistatic to *RPB1*, indicating protein ubiquitination and protein degradation as important parts of the clubroot resistance mechanism. The *RPB1* locus has been mapped to a region on chromosome 1 with 3 putative coding sequences unknown in the sequence of ecotype Col-0. Transformation of susceptible ecotype Col-0 with these genes gives a resistance phenotype. In order to get further insights into the resistance mechanism, whole genome expression was compared between compatible and incompatible interactions. Corresponding to the genetic data, protein synthesis and degradation were highly regulated in resistant ecotypes. Furthermore the expression of major intrinsic transport proteins (PIP, TIP) was strongly changed in resistant ecotypes. AP2/EREBP and NAC domain transcription factors as well as heat shock factors appeared to be important for the signal transduction induced by *RPB1*.

50.14 EFFECT OF *VERTICILLIUM DAHLIAE* MICROSCLEROTIUM LEVELS IN SOIL ON *VERTICILLIUM* WILT OF SUNFLOWER IN ARGENTINA. **R. Rojo, F.J. Quiroz and A.R. Escande.** *Universidad Nacional de Mar del Plata - Facultad de Ciencias Agrarias, Ruta 226 km 73,5, Balcarce B7620ZAA, Argentina. Email: antagonistas@cnia.inta.gov.ar*

Verticillium dahliae is a soil invader pathogen and survives as microsclerotia (MS). *Verticillium* wilt is endemic and causes leaf mottle in Argentinian sunflower crops. We looked for the relation between MS in soil and leaf mottle. Five trials were run in the Buenos Aires province including three susceptible commercial hybrids: DK3920, PAIHUEN and PAN7009. A randomized complete block design with tree replications was used. The experimental unit was a plot three rows 0.7 m width and 5 m long with 60 plants. During the R6 sunflower stage a sample of 10 soil cores was removed from each plot. One hundred milligrams of soil were spread by hand on a 9-cm diameter Petri dish contain-

ing soil pectate tergitol agar medium with P3889 polygalacturonic acid as carbon source. Five dishes were used to estimate the number of CFUs. Cultures were incubated at 25±2°C and in darkness for 2 weeks. Plates were washed-off under running tap water. *V. dahliae* colonies were identified following a morphological key in a dissecting microscope at 40×, and counted. At the same sunflower-stage, severity of leaf mottle was recorded using a six-point scale. Disease severity increased with increments of MS in soil in all hybrids. All soil and hybrid points fitted a potential curve (disease=11.67*$ME^{0.29}$; P=0.0003; R^2=0.57). This is the first report of the effect of MS density in soil and *Verticillium* wilt of sunflower in Argentina.

50.15 A SURVEY OF INTERACTION BETWEEN PREDACEOUS FUNGI AND NEMATODES. A. Singh and U.P. Singh. *Institute of Bioengineering and Biological Sciences, Varuna Bridge, Varanasi, India. Email: amitabh_2k@rediffmail.com*

Thiry nine samples from different sources (compost, leaf litter, agricultural soil and rhizosphere soil) were collected from 13 different locations, and analysed for predaceous fungi using various media like water agar, maize meal agar, rabbit dung agar and rabbit dung + maize meal. We found that decomposed compost and leaf litter harboured the highest levels of these fungi, followed by agricultural soil and rhizosphere soil. The medim which proved best for culturing the fungi consisted of maize meal spread over 3/4 of the plate and rabbit dung agar spreasd over 1/4 of the plate. *Aphelenchus* sp. And *Aphelenchoides* sp. initially cultured on Czapek's medium on *Rhizoctonia* sp. fungus was used as bait for predaceous fungi. The typical predaceous fungi like *Arthrobotrys oligospora*, *Dactylaria brochopage*, *Dactylella phymatopage*, *Dactylella eudermata* and a non-sporulating fungus were grown in pure culture and then nematodes were added to the plates. The fungi developed hyphae with adhesive knobs which captured and killed the nematodes, though some nematodes turned away after sensing the danger. We conclude that these fungi might be used to control plant parasitic nematodes.

50.16 THE IMPACT OF SOIL MANAGEMENT PRACTICES ON SOIL FERTILITY AND DISEASE SUPPRESSIVENESS. L. Tamm, Chr. Bruns, C. Leifert, J. Cooper, J.G. Fuchs, B. Thürig, and A. Fliessbach. *Research Institute of Organic Agriculture, FiBL, CH-5070 Frick, Switzerland. Email: barbara.thuerig@fibl.org*

Crop rotation is widely used in organic and low-input farming systems to improve soil fertility. There is evidence that soil properties are affecting the occurrence of soil-borne diseases but also disease expression on foliar parts of the plant. The systematic use of soil fertility management techniques to reduce diseases is an intriguing concept in theory, but it is not yet widely used in practice, partly due to lack of understanding of the underlying principles. Within the framework of the EU-funded QualityLowInputFood project, research was started in 2004 to elucidate the influence of soil properties on soil suppressiveness and to quantify the relative importance of site-specific vs. cultivation-mediated soil properties. Four field sites were selected in Switzerland, Germany and the UK to identify site-specific differences, the impact of long-term farming practice, and the impact of short-term organic soil amendments. At each site, soil samples were taken from selected treatments in replicated field trials. All samples were submitted to in-depth soil analyses (chemical, physical, and microbiological) and studied for suppressive properties in four bio-assay systems (*Rhizoctonia*–basil, *Pythium*–cucumber, *Phytophthora*–tomato, and *Pseudoperonospora*–*Arabidopsis thaliana.* The results indicate that differences in suppressiveness may be quantified and related to soil properties and/or organic matter-based fertilisation regimes. Furthermore, site-specific factors, which cannot be influenced by agronomic practices, were found to have a greater impact than cultivation-specific effects within the same site.

50.17 THE FINE BALANCE BETWEEN MUTUALISM AND ANTAGONISM IN THE *EPICHLOË FESTUCAE*-PERENNIAL RYEGRASS SYMBIOSIS. A. Tanaka, D. Takemoto, S. Saikia and B. Scott. *Molecular BioSciences, Private Bag 11 222, Massey University, New Zealand. Email: d.b.scott@massey.ac.nz*

The growth of *Epichloë festucae* within its host perennial ryegrass is very tightly regulated; growth is very rapid in expanding leaves but ceases as elongation of the leaf stops. Such controlled growth is a unique and highly evolved ability of symbiotic endophtyes, and contrasts with the vigorous overgrowth in the host that characterizes most fungal pathogens. To identify the signaling mechanisms that are required to maintain this mutualistic interaction between *E. festucae* and perennial ryegrass we have adopted a forward genetics approach, using plasmid and T-DNA mutagenesis, to isolate endophyte mutants that are symbiotically defective. Using plasmid mutagenesis we have recently shown that disruption of a gene encoding a specific NADPH oxidase isoform (NoxA) in *E. festucae*, converts the fungal endophyte from a mutualist to an antagonist (Tanaka *et al*. 2006). Disruption of *noxA* results in an increase in hyphal branching and a dramatic increase in fungal biomass in all tissues. The host becomes severely stunted and shows precocious senescence. By comparison with the mammalian Nox complex we have used a candidate gene approach to isolate other fungal components of the Nox complex, including NoxR and RacA (Takemoto *et al*. 2006; Tanaka *et al*. 2008). Mutations in these genes had a similar host interaction phenotype as observed for the *noxA* mutant. To identify signaling components upstream and downstream of Nox we have screened for additional *E. festucae* mutants that give a stunted phenotype on the grass host. Molecular and functional analysis of these mutants is in progress.

50.18 BIOCHEMICAL AND PHYSICAL INTERACTIONS AMONG *FUSARIUM OXYSPORUM*, BIOCONTROL AGENTS AND TOMATO PLANT ROOTS. D. Vitullo, G. Lima, R. Castoria, F. De Curtis, L. Maiuro and V. De Cicco. *Department of Animal, Plant and Environmental Sciences, Plant Pathology, University of Molise, Italy. Email: lima@unimol.it*

Several bacterial strains were isolated from suppressive organic amendments and composts and tested for their ability to inhibit growth of *Fusarium oxysporum* f. sp. *lycopersici* (Fol). Four strains that proved to be very effective antagonists were identified as *Bacillus* spp. (strains BTd43, BO5A and BO7) and *Pseudomonas* spp. (strain BTd1). The possible physical and biochemical interactions of these selected bacterial strains with Fol hyphae and tomato roots were investigated. Scanning electron microscopy showed that bacterial cells apparently adhere to both pathogen hyphae and tomato roots. Fungal hyphae appeared to be frequently damaged at the fungus-bacterium interfaces. Glucanase and chitinase activities, which are known to degrade of fungal cell wall polymers, were investigated by growing bacterial strains in the presence of Fol cell walls. Enzyme assays of crude protein extracts as well as polyacrylamide gel electrophoresis analyses showed possible induction of proteins with glucanase and chitinase activity by fungal cell walls. The role of these lytic

enzymes in the mechanisms of action of the antagonist bacteria and the mutual interaction of biocontrol bacteria with pathogen and host plant are under study.

50.19 EFFECT OF OLIVE LEAF PHENOLIC EXTRACTS ON POTATO SOFT ROT CAUSED BY *PECTOBACTERIUM CAROTOVORUM* SSP. ATROSEPTICUM. **R. Yahiaoui-Zaidi, F. Zaidi and S. Bechar.** *University Abderrahmane Mira of Béjaia, Route Targa Ouzzemour, Béjaia 06000, Algeria. Email: rachida_zaidi@yahoo.fr*

The aim of this study was to assess the effect of plant phenolic extracts on the development of potato tuber soft rot. *P. carotovorum* ssp. *atrosepticum* isolates recovered from soft rots on potatoes in Algeria and previously characterised taxonomically, were assessed in a half-tuber test for ability to cause potato tuber rotting. A trial at 20°C, of *P. c.* ssp. *atrosepticum* (2×10^5 and 2×10^7 cfu×ml^{-1}) showed significant effects of inoculum dose on pathogenicity. Antimicrobial activity of phenolic plant extracts tested against *P. c.* ssp. *atrosepticum* was studied using the agar diffusion method and half-tuber test on potato. Two phenolic extracts were prepared with the following solvents: A, acetone:water (7:3) and B, methanol:water (8:2). The inhibition zone obtained for each extract against *P. c.* ssp. *atrosepticum*, increased whenever the total phenolic content was increased. A significant decrease of the amount of tuber rotting treated with the two phenolic extracts (A and B) was observed and varied from one extract to another.

Plant virus epidemiology

14.1 OCCURRENCE AND DISTRIBUTION OF "TORRAO" DISEASE INFECTING TOMATO CROPS IN SPAIN. **A. Alfaro-Fernández, C. Córdoba-Sellés, C. Cebrián, J.A. Herrera-Vásquez, I. Font, J.A. Sánchez-Navarro, M. Juárez and C. Jordá.** *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Email: analfer1@doctor.upv.es*

A new disease, known by the local name of "Torrao", is reported in Spanish tomato crops (*Lycopersicon esculentum* Mill.) since springtime of 2001. The main areas for greenhouse-grown tomato production in Spain were surveyed to evaluate the occurrence and distribution of this disease. Affected plants showed necrotic spots starting at the base of the leaflets. Later, the centres of these spots fell out producing little holes ("cribado"). Longitudinal stains appeared on the stems and the fruits were distorted with necrotic lines. Fruits finally cracked, becoming unmarketable. Affected plants presented a burned appearance. Tomato plants showing different necrotic virus-like symptoms were randomly collected during 2002–2007. Serological and molecular analysis of such plants revealed the presence of two viruses: *Pepino mosaic virus* (PepMV) and *Tomato torrado virus* (ToTV). Non-isotopic tissue-printing hybridization, with probes specific to both viruses, was developed to analyze some of the field samples collected. ToTV was detected in most samples analysed. Incidence of PepMV was also quite high in the samples showing "Torrao" symptoms. In conclusion, mixed infections of these two viruses are usually found in greenhouse tomato crops showing "Torrao" symptoms. The syndrome has been detected in the main tomato production areas in the country. In this study, the geographical distribution of the syndrome is presented.

14.2 OCCURRENCE OF SEVERAL VIRUSES IN WEEDS ASSOCIATED WITH TOMATO CROPS IN SPAIN. **A. Alfaro-Fernández, C. Córdoba-Sellés, C. Cebrián, J.A. Herrera-Vásquez, J.A. Sánchez-Navarro , M. Juárez , A. Espino , R. Martín and C. Jordá.** *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Email: analfer1@doctor.upv.es*

During 2007, a field survey of greenhouse-grown tomatoes was carried out in some of the main production areas in Spain. Surveys were conducted in tomato greenhouses that presented tomatoes with typical symptoms of "Torrao" disease or *Pepino mosaic virus* (PepMV) infection. Tomato samples were collected as well as different weed species found between rows of cultivated tomatoes. Serological and molecular analyses were performed in order to detect the presence of viruses such as *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV), *Potato virus Y* (PVY), PepMV and *Tomato torrado virus* (ToTV), that commonly caused the symptoms observed in tomato crops. Natural hosts surveyed that tested positive to some of those viruses belonged to several botanical families: Poaceae, Chenopodiaceae, Malvaceae, Brassicaceae, Solanaceae, Fabaceae and Asteraceae. Tomatoes collected from same greenhouses as the weeds, tested positive to the same viruses. More than one virus was sometimes detected in the same natural host. Arable weeds usually present in tomato greenhouses are known to be potential reservoirs of several viruses and could play a critical role in epidemiology as virus sources. Control measures such as elimination of these plants from inner and outer borders of greenhouses are required.

14.3 IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF *TOBACCO STREAK VIRUS* INFECTING *VICIA FABA* IN SUDAN. **M.A. Ali, S. Winter and G.A. Dafalla.** *Plant Pathology Centre, Faculty of Agricultural Sciences, Gezira University, Sudan. Email: maiadil@yahoo.com*

Unusual symptoms suggesting virus infection were observed in faba bean fields in the Gezira area and northern parts of Sudan coinciding with high infestations of *Aphis craccivora*. The chlorotic mottle and/or mosaic symptoms were accompanied by black streaks on petioles and stems. On some varieties a severe leaf chlorosis developed into browning and blackening of the entire leaves and death of the plant. The virus was mechanically inoculated to a number of host plants; *Nicotiana benthamiana* and *Chenopodium quinoa* were suitable propagation hosts. Electron microscopy revealed isometric virus-like particles about 30 nm in size. A specific antiserum was produced and Western blots showed that the putative coat protein was 29-30 kDa in size. Comparison with several viruses available in the DSMZ reference collection showed that the virus was related to *Tobacco streak virus* (TSV), a serious pathogen of groundnut, sunflower and okra in India. Seed transmission of the virus was confirmed by DAS-ELISA and Tissue print Immunoassay (TPIA). The identity of the virus was further confirmed by reverse transcription-polymerase chain reaction (RT-PCR) and sequence analysis. A fragment of 717 nt comprising the entire TSV coat protein was analysed. A comparison of the 237 aa coat protein residues revealed a high sequence similarity of the TSV Sudan virus with TSV isolates from Europe.

14.4 TWO PATHWAYS FOR TRANSLATION-ACTIVATION OF ENCAPSIDATED PVX RNA COULD BE COMMON TO ALL POTEXVIRUSES. **M.V Arhipenko, A.A. Mukhamedzhanova, O.V. Karpova, N.P. Rodionova and J.G. Atabekov.** *Depart-*

ment of Virology, Lomonossof State University, 119992 Moscow, Russia. Email: usinskma@mail.ru

Potato virus X (PVX), *Narcissus mosaic virus* (NMV) and *Papaya mosaic virus* (PapMV) belong to the *Potexvirus* genus of which PVX is the type member. The 5'-proximal gene codes for the 165 kDa replicase. The 3'-proximal gene for the coat protein (CP) is preceded by the three partially overlapping ORFs (2, 3, and 4) termed the triple gene block (TGB). The TGB-encoded proteins, referred to as TGBp1, TGBp2, and TGBp3, are essential for cell-to-cell movement of TGB-containing viruses. In addition to the TGB proteins, PVX also requires the CP for cell-to-cell transport. Previously we have reported that encapsidated PVX RNA is completely nontranslatable *in vitro*, but can be converted into a translatable form in two ways: 1) by selective binding of TGBp1 to virions and 2) *in situ* phosphorylation of the PVX CP. Here we demonstrate that PVX TGBp1 activates translation not only of the encapsidated virion PVX RNA, but also of the NMV virion RNA and the PapMV virion RNA. However, the NMV TGBp1 could activate only the homologous encapsidated NMV RNA, and could not act upon encapsidated PVX RNA. In situ phosphorylation of the NMV CP and PapMV CP also converted the encapsidated NMV RNA and PapMV RNA into translatable form. We presume that the two pathways of translation activation are common to all potexviruses.

14.5 ANALYSIS OF THE FITNESS COMPONENTS AND EVOLUTION OF *CUCUMBER MOSAIC VIRUS* AND ITS SATELLITE-RNAs IN DIFFERENT HOST PLANT SPECIES. M. Betancourt Vásquez, A. Fraile Pérez and F. García-Arenal Rodríguez. *Departamento de Biotecnología, E.T.S.I. Agrónomos, and Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid, 28040 Madrid, Spain. Email: monbeva@gmail.com*

Cucumber mosaic virus (CMV), a major pathogen of vegetable crops worldwide, is helper of a small, single-stranded satellite RNA (CMV-satRNA), which modulates CMV symptoms and accumulation. Two types of CMV-satRNA variants can be distinguished according to their phenotype in tomato, one causing systemic necrosis (N-satRNA) and one causing attenuation of CMV symptoms (A-satRNA). These two types have similar phenotypes on other host plant species. CMV+N-satRNA causes devastating epidemics of tomato necrosis, but these epidemics are infrequent and often of short duration. The conditions for the emergence, or disappearance, of N-satRNAs in CMV populations are poorly understood. An epidemic of tomato necrosis that occurred in Spain between 1988 and 1992 allowed the estimation of the fitness and virulence of different genotypes of CMV-satRNA, and model analyses revealed that a major factor in the emergency of N-satRNAs was the density of aphid vectors. These analyses did not consider the role of other hosts of CMV in the dynamics and evolution of CMV and CMV-satRNA populations. Now we have estimated in melon, a major host crop of CMV in Spain, the parameters of fitness components, of competition in mixed infections, of virulence and of transmission of N- and A-satRNAs. In spite of similar phenotypes in melon, these parameters differ for N- and A-satRNAs. We have built a model for CMV-satRNA evolution in a multihost system, which was run with the estimated parameters in melon and tomato. The predictions for N-satRNA emergency were analysed under realistic field assumptions.

14.6 COMBINATION OF REAL-TIME PCR AND CIM TECHNOLOGY ENABLES EFFICIENT DETECTION AND EPIDEMIOLOGY STUDIES OF VIRUSES. J. Boben, M. Banjac, D. Delic, I. Gutierrez-Aguirre, P. Kramberger, N. Mehle, M. Peterka, A. Strancar and M. Ravnikar. *National Institute of Biology, Department of Biotechnology and Systems Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia. Email: maja.ravnikar@nib.si*

Plant, animal and human viruses can be present in their hosts or in the environment at concentrations, which despite being too low as to be detected by conventional methods, can still constitute a risk of infection. We report on the development of new robust methods, which could quickly detect such low concentrations of viruses, improving the diagnostics and helping epidemiology studies by using a few model viruses. Real-time qPCR was used as it is the leading technology for molecular detection of pathogens, as it is sensitive, specific and quantitative. CIM (Convection Interactive Media) monolithic supports are chromatographic media that, being highly porous structure, can bind, purify and concentrate large biomolecules, such as viruses and nucleic acids. We have combined both technologies in order to concentrate and detect extremely low concentrations of different plant viruses from environmental waters. CIM allowed us to bind viruses present in samples of river and irrigation water, concentrate them and clean them from potential inhibitors which could compromise molecular detection. It was also used for quick preparation of purified viruses. Real-time qPCR allowed us to sensitively and specifically detect each target virus in the concentrated fraction. The combination of both, allowed us to improve the detection of several plant viruses (ToMV, CMV, PepMV) by several orders of magnitude in comparison with more classical methods.

14.7 MOLECULAR EVIDENCE FOR *TOBACCO NECROSIS VIRUS D* INFECTION OF *OLEA EUROPAEA*. J.M.S. Cardoso, M.R Félix, M.I.E Clara and S. Oliveira. *Institute of Mediterranean Agricultural Sciences, University of Évora, 7002-554 Évora, Portugal. Email: iclara@uevora.pt*

A number of viral isolates obtained from olive, *Olea europaea* L., in Portugal, were found to react with a mixture of antisera to eleven TNV species and isolates (Loewe Phytodiagnostica, Germany). Subsequent genome analysis of one such virus revealed it to be a new necrovirus species, Olive mild mosaic virus (OMMV), with coat protein (CP) gene having high sequence identity to TNV-D but the RNA-dependent RNA-polymerase (RdRp) gene having high sequence identity to *Olive latent virus 1*. To identify additional viral species, independent phylogenetic analysis were performed based on partial sequences of the RdRps and CPs from several isolates. Two degenerate primers were designed, based on published necrovirus genome sequences, for amplification of a single region that contains a significant part of the coding sequence of both RdRp and CP proteins. RT-PCR using these primers and RNA from an olive virus isolate previously selected through serial re-inoculation of a single lesion, resulted in a product that was cloned in pZerO2. Analysis of the clone identified the isolate as TNV-D. Further application of this strategy to viral populations recovered from 'Verdeal Alentejana' and 'Santulhana' olive cultivars, allowed the identification of all the twenty sequenced clones, as TNV-D. This is the first report on molecular data encompassing a large portion of the two main virus genes, RdRp and CP, and showing the presence of TNV-D in olive trees, clearly distinguishing it from OMMV.

14.8 THE POPULATION, VARIABILITY AND NOVELTY OF *POTATO VIRUS Y* IN SYRIA. M. Chikh Ali, T. Maoka, K. Katayama and K.T. Natsuaki. *National Agricultural Research*

Center for Hokkaido Region, Toyohira, Sapporo, Japan. Email: 70060002@nodai.ac.jp

Potato virus Y (PVY) is the main virus infecting potato in Syria. More than 55 PVY isolates collected from Syria from 2002 to 2006 were split into 6 groups according to their biological, serological and molecular characteristics. The majority were in Group 1 (PVY^{SYR} type, sharing properties of $PVY^{N}W$ and $PVY^{NTN)}$). Group 2 and 3 isolates were classified as $PVY^{N}W$ and PVY^{NTN} respectively. Group 4 isolates belonged to the recombinant PVY^{NTN} with regard to phenotype in potato and genomic identity. However, unlike PVY^{NTN} these isolates induced mosaic in tobacco although the HC-Pro of the representative isolate, namely PVY-12, contained the amino acid motifs K400/E419 that were previously reported as determinants of necrosis in tobacco. Moreover Group 4 isolates reacted to PVY^{N} and PVY^{O} monoclonal antibodies due to a point mutation in the CP gene as was found for PVY-12. Isolates of Group 5 and 6 seem to be variants of Group 1 and 2 respectively. Along with these groups, different combinations of mixed infection were detected. From our study we conclude: 1) PVY in Syria has a varied population with many novel isolates; 2) All PVY isolates studied had recombinant genomes; 3) The induction of tuber necrosis does not require the ability to induce necrosis in tobacco; 4) Determinants other than these previously reported are involved in the tobacco necrotic response to the necrotic isolates of PVY.

14.9 CHARACTERISATION OF AUSTRALIAN VITIVIRUSES NOT ASSOCIATED WITH DISEASES OF THE RUGOSE WOOD COMPLEX. **F.E. Constable, P. Nicholas, J. Connellan, T. Bass, N. Habili, and B.C. Rodoni.** *Department of Primary Industries, Knoxfield, Private Bag 15, Ferntree Gully Delivery Centre, VIC 3156, Australia. Email: fiona.constable@dpi.vic.gov.au*

Kober stem grooving disease, associated with *Grapevine virus A* (GVA), and corky bark disease, associated with *Grapevine virus B* (GVB), can be detected by biological indexing on the sensitive varieties Kober 5BB and LN 33 respectively. Although some GVB strains are present in Australia, corky bark disease has not been reported and remains a quarantinable disease. Previous studies showed that variation exists amongst Australian GVB isolates and between Australian and overseas isolates, particularly at the 5' end of ORF 5. Our results agree with this finding; however, the reason for the lack of symptom expression associated with some GVB isolates is not understood and we are investigating this phenomenon. Kober stem grooving disease occurs in Australia and is indexed for in Australian certification schemes. Using PCR we have identified a GVA isolate in grapevines that have undergone heat-treatment and meristem culture for virus eradication. GVA remained undetected when the treated vines were indexed using woody indicators, ELISA and PCR. Sequence analysis of an 868 bp region of the GVA genome, consisting of ORF4 and most of ORF5, indicated that this isolate had less than 90% sequence similarity with other GVA isolates. This GVA isolate was also detected in the original untreated material and there was 99% sequence similarity between the GVA isolates from the treated and untreated vines. These results suggest that this strain of GVA was not eradicated by an intensive heat treatment and meristem culture regime and remained undetected using standard diagnostic procedures for the detection of GVA.

14.10 BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF SEVERAL ISOLATES OF *PEPINO MOSAIC VIRUS*. **M.C. Córdoba-Sellés, A. Alfaro-Fernández, J.A. Herrera-Vásquez, C. Cebrián-Micó and C. Jordá.** *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Email: mcorsel@doctor.upv.es*

Pepino mosaic virus (PepMV) was first reported and characterized in *Solanum muricatum* (pepino) in 1980; for many years the virus was thought to be restricted to Peru and that it infected only pepino but no other solanaceous crop grown in the region. In 1999 PepMV was reported in tomato greenhouses in the Netherlands and the UK and has rapidly spread throughout the principal tomato production areas worldwide, causing severe epidemics in tomato. Since 1999, different PepMV strains have been detected, and it is common to find mixed infections between PepMV strains, with some interstrain recombinants being detected. In this study several PepMV isolates showing different symptoms in the field were chosen, and one symptomless PepMV isolate was included. Isolates from tomato plants were sap inoculated to some solanaceous test species and checked for symptoms in a growth chamber under controlled conditions. The results revealed diverse pathogenic behaviours in the test plants, and the isolates could be grouped according to different infection patterns. Analysis of a fragment from the RNA polymerase gene allowed us to classify the isolates at strain level. The biological diversity of the PepMV isolates observed was correlated with the nucleotide sequence diversity found among them.

14.11 EPIDEMIOLOGICAL STUDIES OF *ARABIS MOSAIC VIRUS* AND ITS VECTOR *XIPHINEMA DIVERSICAUDATUM* IN FLOWER BULBS. **M.J.D. de Kock, R.H.L. Dees, K.T.K. Pham and J. van Doorn.** *Wageningen UR, Crop Protection, Applied Plant Research, P.O. Box 85, 2160 AB Lisse, The Netherlands. Email: maarten.dekock@wur.nl*

Arabis mosaic nepovirus (ArMV) has a broad host range, including grapes, soybean and hosta. Bulbous ornamentals like tulip and lily can also be infected. In tulips ArMV can induce mild symptoms like mosaic spots and grey-necrotic ringspots that, if present, show only during first-year infections. However, this virus occurs mostly without any visible symptoms making diagnostics by eye very difficult. Plant-to-plant transmission of ArMV occurs mainly via free-living soil nematodes *Xiphinema* spp., probably *X. diversicaudatum*. The occurrence of both ArMV and its vector *X. diversicaudatum* in Dutch agricultural areas is rare. Although neither ArMV nor *X. diversicaudatum* are on international quarantine lists, their presence in Dutch bulbous crops might affect their value for export. The aim of this research is to evaluate the epidemiology of ArMV during the cultivation of host and non-host (bulbous) crops, and infection data will be correlated with the occurrence of *X. diversicaudatum*. Diagnostic tools such as ELISA and PCR for ArMV, and species-specific PCR for *Xiphinema*, will be applied to study this virus-vector interaction in detail. The knowledge gained will finally be transformed into applicable guidelines to minimize the risk of ArMV infection in agricultural practise.

14.12 OVER SEVENTY YEARS OF VIRUS DISEASES OF COCOA IN GHANA AND WEST AFRICA: SO WHICH WAY FORWARD? **H. Dzahini-Obiatey.** *Cocoa Research Institute of Ghana, P. O. Box 8, Akim Tafo, Ghana and School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AS2, UK. Email: h.k.dzahiniobiatey@reading.ac.uk*

Virus diseases have plagued cocoa production in West Africa for over six decades. Principal among them is that caused by the cocoa swollen virus (CSSV), which is endemic in Togo, Ghana, Nigeria, and Ivory Coast. The incidence of the disease in Ghana has led to the launch of the costliest and an over ambitious eradication control programme in the world. This paper highlights the research conducted mainly in Ghana that has influenced the various control strategies or may have the potential to influence future ones. Isolation of newly planted cocoa has been identified as an efficient method of reducing CSSV prevalence in the field, and thus the surest way of controlling the cocoa swollen virus disease (CSSVD). Identification of mealybugs as vectors, the role of alternative hosts in the spread of the disease, the need for an urgent review of eradication procedures, breeding specifically for resistance to CSSV as well as some biochemical and molecular studies are some of the points highlighted in the paper. The achievements and limitations made in these fields are noted. The future, however, will be to combine most of these strategies into one or two integrated approaches to control CSSVD. This will then be in tune with the suggestion that no single measure is adequate to solve the swollen shoot disease problem in Ghana and the rest of West Africa.

14.13 RESERVOIR WEED HOSTS OF *TOMATO YELLOW LEAF CURL SARDINIA VIRUS* AND *TOMATO YELLOW LEAF CURL VIRUS* IN SOUTHERN ITALY. A. Fanigliulo, R. Pacella, S. Comes and A. Crescenzi. *Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Italy. Email: aniello.crescenzi@unibas.it*

A survey to identify reservoir weed hosts of TYLCSV and TYLCS was made during summer from August 2004 to July 2007 in order to identify where the two viruses persist during the tomato-free period. The site was around a group of greenhouses in Calabria region, southern Italy, where tomato is grown hydroponically and a serious tomato yellow leaf curl epidemic is present. About 450 samples were collected from symptomless and symptomatic plants of different botanic families. Virus presence was evaluated by DAS-ELISA and confirmed by PCR/RFLP analysis performed according to Accotto *et al.* (2000), in order to differentiate between TYLCSV and TYLCV infection. TYLCSV was detected in four weed species: *Solanum nigrum*, *Datura stramonium, Sonchus asper* and *Cardaria draba*. Both TYLCSV and TYLCV were detected in *Abutilon theophrasti*. Similarity and phylogenetic analyses performed between the 580 bp fragment amplified from each isolate and the TYLCSV isolate recovered within the greenhouse and responsible for the epidemic in mixed infection with a TYLCV isolate resulted in a value of 100 % of identity, thus indicating that there was no variability in TYLCSV population in the surveyed area. The TYLCV isolate identified in *A. theophrasti* was closely related to a mild TYLCV isolate recovered in Spain. *S. nigrum*, *D. stramonium*, *C. draba, S. asper* and *A. theophrasti* were found to play an important role in virus ecology and epidemiology in the tomato ecosystem studied. To our knowledge this is the first report of *A. theophrasti* as TYLCSV and TYLCV host in natural infection.

14.14 DETECTION OF *CITRUS PSOROSIS VIRUS* IN FIELD TREES BY DAS-ELISA IN COMPARISON WITH BIOLOGICAL INDEXING. K. Felkai and H. Belkahla. *Institute of Agronomy, Virology Laboratory, P.O. Box 270, Blida University, Algerie. Email: inesarabelo@yahoo.fr*

Detection of citrus psorosis (CPsV) by double antibody sandwich (DAS) ELISA and by biological indexing was compared in samples from various citrus varieties growing in the field and glass-house. For DAS ELISA detection in young leaves, the calculated incidence of the different varieties was Palestinia 100%, Valencia late 96.29%, Hamlin 74%, Washington navel 72%, grapefruit 70%, Sanguina 51.51%, lemon 49.87%, Tarocco 44.82% and Satsuma 40%. Some trees showing psorosis bark and leaf symptoms in the field were shown to be psorosis by biological indexing and also to contain CPsV antigen by serological test. Other trees without bark-scaling were shown to be psorosis by biological indexing and to contain CPsV by serology. On the other hand trees showing psorosis leaf symptoms by biological indexing did not contain CPsV by serological test. However, in trees without leaf symptoms by biological indexing, CPsV was detected by DAS ELISA.

14.15 VIRUS-LIKE PARTICLES AND INCLUSIONS IN RED CLOVER PLANTS WITH DWARF DISEASE SYMPTOMS. J. Fránová, K. Petrzik, H. Jakešová. *Dept. Plant Virology, IPMB, ASCR, v.v.i., Branišovská 31, 370 05 České Budějovice, Czech Republic. Email: jana@umbr.cas.cz*

Clover plants with symptoms of compact dwarf growth habit, mosaic and gradual death have been repeatedly found in breeding nurseries in the Czech Republic. Plants examined by transmission electron microscopy (TEM) revealed the presence of virus-like particles and inclusions resembling those induced by viruses of different genera and families. Flexuous filamentous virions only were detected in negatively stained crude sap preparations from clovers. Rhabdovirus-like particles, aggregates of flexuous filaments typical for *Potexvirus*, cytoplasmic cylindrical inclusions characteristic of *Potyvirus* and unusual spiral-like structures were observed in ultrathin sections. Unexpectedly, occlusion-derived virions described previously for members of the *Baculoviridae* family were noticed. Mechanical inoculation of *Nicotiana occidentalis* 37B, gave symptoms from local chlorotic/necrotic lesions to severe systemic mosaic, necrosis and death of inoculated plants. TEM of tissues from symptomatic herbaceous hosts confirmed the transmission of viruses observed in clovers. Moreover, bacilliform particles (ca 213–533 nm by 44–58 nm) of the *Cytorhabdovirus* genus and rod-shaped particles resembling *Varicosavirus* but shorter and wider (cca 116–285 nm by 21–33 nm) in comparison with the size of *Lettuce big-vein associated virus* (320-360 nm by 18 nm) were found in negatively stained preparations from *N. occidentalis* 37B. *Red clover mosaic virus* (*Comovirus*), *Clover yellow vein virus* (*Potyvirus*), *Potato virus X* and *White clover mosaic virus* (*Potexvirus*) were identified by sequencing. The work is supported by the GA ASCR No. 1QS500510558, AV0Z50510513 and by the Ministry of Agriculture No. QH71145.

14.16 CHANGES IN POPULATION STRUCTURES OF *IRIS YELLOW SPOT VIRUS* IN ONION FIELDS AND LISIANTHUS GREENHOUSES IN JAPAN. S. Fuji, S. Zen, I. Sato, H. Kishi, H. Furuya, H. Naito and M. Okuda. *Faculty of Bioresource Sciences, Akita Prefectural University, Akita, Japan. Email: sfuji@akita-pu.ac.jp*

Iris yellow spot virus (IYSV, genus *Tospovirus*) mainly occurs on onion and lisianthus plants in Japan. The virus is highly diverse in its nucleocapsid protein and has been classified into two genotypes, IYSVNL from the Netherlands and IYSVBR from Brazil, based on amino acid sequences. Both genotypes have been

reported in Japan, where they sometimes occur simultaneously in the field. Furthermore, both genotypes were detected in a single necrotic lesion of an infected plant. Therefore, the population structures, and changes in the populations, of these two genotypes in onion fields and lisianthus greenhouses from 2004 to 2006, were analyzed using RT-PCR-RFLP. In onion fields the two genotypes were detected in all three years, and no significant population changes were observed. However in lisianthus greenhouses, although both genotypes were detected at the first outbreak each year, the IYSVBR population was generally larger and more widespread than that of IYSVNL. Only IYSVBR was isolated from each local region, even though both genotypes were detected in a whole leaf of *Chenopodium quinoa* that was inoculated with the sap from a lesion containing both genotypes. These results suggest that the two genotypes show differences in host adaptation. It is likely that incidence in lisianthus greenhouses occurs each year by invasion of viruliferous *Thrips tabaci* that have acquired one or both genotypes from nearby sources.

14.17 NUCLEOTIDE SEQUENCE OF COAT PROTEIN GENE OF *JAPANESE YAM MOSAIC VIRUS* ISOLATED FROM *DISCOREA OPPOSITA* CV. YAMATOIMO. **T. Fujita, K. Ogasawara, K. Fujita, R. Yoshida, Y. Ohtsuka and T. Sano.** *Plant Pathology Laboratory, Faculty of Agriculture and Life Sciences, Hirosaki University, Bunkyo-cho 3, Hirosaki 036-8561, Japan. Email: tafu@cc.hirosaki-u.ac.jp*

Four viruses are known to infect Japanese yam cultivars in Japan; i.e., *Japanese yam necrotic mosaic virus* (JYNMV), *Japanese yam mosaic virus* (JYMV), *Yam mild mottle virus* (YMMV) and *Broad bean wilt virus 2* (BBWV2). JYMV infects *Discorea japonica*, *Discorea opposita* and *Discorea alata*, which show mosaic, vein banding and distortion in the infected leaves. JYMV isolates from *D. opposita* (Yamatoimo) collected in Ajigasawa town in Aomori and Ohdate city in Akita was analyzed to compare the nucleotide sequences of the coat protein (CP) gene. Phylogenetic analysis on the nucleotide sequence and on the deduced amino acid sequence of the CP gene combined with the data from known JYMV isolates from *D. japonica* (Jinenjo) revealed that the two JYMV isolates from *D. opposita* were similar to those from *D. japonica* (Jinenjo). Amino acid substitutions characteristic to the isolates from *D. opposita* were detected near the N-terminus of the CP gene. The C-terminus was highly conserved.

14.18 EVALUATION OF *BANANA BUNCHY TOP VIRUS* RESISTANCE AND CHARACTERIZATION OF ENDOGENOUS BANANA VIRUSES IN BANANAS IN JAPAN. **N. Furuya, I. Nagashima, S. Kawano and K. T. Natsuaki.** *National Institute of Fruit Tree Science, Tsukuba, Ibaraki 305-8604, Japan. Email: nofuruya@affrc.go.jp*

The *Banana bunchy top virus* (BBTV) resistance of three main banana cultivars grown in Japan was evaluated in the field and by inoculation. The epidemic of banana bunchy top disease (BBT) was confirmed in many samples of two edible bananas, *Musa acuminata* cv. Sanjaku and *Musa* × *paradisiaca* cv. Shima in Okinawa Main Island. On the other hand, in 25 samples of fibre banana, *Musa balbisiana* var. *liukiuensis*, BBT was not observed and no BBTV was detected by ELISA, even though this banana species is a favorable host of the BBTV vector, *Pentalonia nigronervosa*. By inoculation test in the greenhouse using vectors, although high infectivity was observed in cv. Sanjaku (80%) and cv. Shima (75%), no seedlings of var. *liukiuensis* were infected by BBTV, and none showed bunchy top symptoms. These results show that var. *liukiuensis* has immunity for BBTV. We also tried to detect banana endogenous viruses (BEVs) in three kinds of Japanese bananas. A part of the sequences of *Banana streak OL virus* (BSOLV) was confirmed from var. *liukiuensis* (BB group) and cv. Shima (AAB/ABB? group). New and multicopies of BEV sequences, also found in both the bananas, means that this BEV could possibly propagate in the banana genome like an RNA-type transposon.

14.19 MOLECULAR CHARACTERIZATION OF AN INDIAN ISOLATE OF SUGARCANE YELLOW LEAF VIRUS. **R.K. Gaur, G.P. Rao and A. Lehrer.** *Deptartemnt of Bitechnology, Mody College of Arts, Science and Commerce, Lakshmangarh, Sikar 332 311, Rajasthan, India. Email: gaurrajarshi@hotmail.com*

A luteovirus (SCYLV-IND) was found to be associated with the midrib yellowing symptom of sugarcane in India. The full length viral genome was sequenced and compared with the open reading frames of the different SCYLV isolates reported worldwide. ORFs 0, 1, 2, 3, 4 and 5 of SCYLV-IND were analyzed and showed 100% homology with corresponding sequences of an Australian isolate. It was also found that ORFs 1 and 2 are most closely related to their *Polerovirus* counterparts, whereas ORFs 3 and 4 are most closely related to counterparts in the *Luteovirus* genome, and ORF 5 is most closely related to the read-through protein gene of the only known *Enamovirus*. These differences in affinity result from inter-species recombination.

14.20 EPIDEMIOLOGY OF *CHRYSANTHEMUM VIRUS B* BY *MACROSIPHONIELLA SANBORNI* (GILLETTE) IN NORTH EASTERN UTTAR PRADESH. **R.R. Gaur and R.K. Gaur.** *Aphid-Biocontrol, Laboratory, Department of Zoology, D.D.U University, Gorakhpur-273 009, UP, India. Email:richaraizada@hotmail.com*

Macrosiphoniella sanborni transmits five plant viruses including two viruses affecting chrysanthemums: *Chrysanthemum virus B* and *Chrysanthemum vein mottle virus*. During a survey of chrysanthemum-growing areas of North Eastern Uttar Pradesh, India leaf samples of chrysanthemum infested with *M. sanborni* were collected. The samples as well as *M. sanborni* were tested by DAC-ELISA against *Chrysanthemum virus B* antisera. Out of 100 leaf samples, 95 and almost all the *M. sanborni* tested positive. It is clear that the chrysanthemums were infected with *chrysanthemum virus B*. For further confirmation, samples are being subjected to molecular analysis in the lab.

14.21 REACTION OF CULTIVARS AND LINES OF BEAN (*PHASEOLUS VULGARIS*) TO *BEAN COMMON MOSAIC VIRUS* IN FIELD CONDITIONS. **S. Ghasemi, M.M. Kamelmanesh and M.R. Bihamta.** *Plant Protection Dept., College of Agriculture, Islamic Azad University, Shiraz Branch, Iran. Email: ssghasemi@yahoo.com*

Bean common mosaic virus is now distributed worldwide and causes great economic damage by reducing yield and quality of beans. To determine the reaction of cultivars and various bean lines to BCMV, 25 common bean genotypes were used in an RCB design with 3 replications. Plants were infected two weeks after cultivation and infection percent was recorded 3 weeks after cultivation. The trial showed that cultivars Akhtar (70%) and

Daneshjo (43%), and line WA4502-1 (65%) showed the highest BCMV rates of infection. Lines MCD4012, WA8563-2 and WA8563-4 remained uninfected. The results were confirmed by ELISA. Other cultivars and lines were infected at levels between 0 and 70 %. A further experiment showed that cultivars Akhtar and Daneshjo were susceptible to *Bean common mosaic necrosis virus* while lines MCD4012, WA8563-2, WA8563-4 and WA4502-1 were resistant.

14.22 IDENTIFICATION OF A POTYVIRUS IN TREE TOMATO (*SOLANUM BETACEUM*) IN A COLOMBIAN REGION. E.P. González Jaimes, J.F. Gil Ramírez, M.L. Ayala and M.A. Marin Montoya. *Politécnico Colombiano JIC, Carrera 48 No. 7-151 Medellin, Colombia. Email: epgonzalez@elpoli.edu.co*

Tree tomato (*Solanum betaceum)* is one of the most important fruits grown in Colombia, but since the 80s it has been attacked by a viral disease. Antioquia state, the principal producer, has more than 60% of the orchards affected by the disease, making it necessary to identify the causal agent, which presumably is a potyvirus. We used a commercial potyvirus DAS-ELISA kit, and electron microscopy. Twenty healthy plants of *S. betaceum* (3 months old), were inoculated with sap of diseased field plants, and collection were made of possible host plants of the virus in commercial orchards in 5 different producing regions around the Antioquia state. The inoculated plants were inspected every 8 days, and tested by ELISA every 15 days. After 15 days some of the inoculated plants showed symptoms and were also ELISA-positive. In addition, the symptoms were similar in all orchards sampled, and the electron microscopy showed potyvirus-like particles. The weeds did not show symptoms and none were positive in the serological tests. We conclude that the viral particle corresponds to a Potyvirus because of mechanical inoculation, and the field samples gave positive results with the kit.

14.23 DESIRABLE AND UNDESIRABLE VARIANTS OF GRAPEVINE LEAFROLL-ASSOCIATED VIRUS 3 IN CRIMSON SEEDLESS TABLE GRAPES IN WESTERN AUSTRALIA. N. Habili, I. Cameron and J.W. Randles. *School of Agriculture, Food & Wine, Waite Campus, University of Adelaide, Glen Osmond, SA 5064, Australia. Email: john.randles@adelaide.edu.au*

Grapevine leafroll disease is distributed world-wide and associated with loss of yield and changes in fruit and wine quality. At least 9 serologically distinct leafroll virus species (family *Closteroviridae*) have been isolated from grapevines. Of these, *Grapevine leafroll-associated virus 3* (GLRaV-3) is the most damaging. Most leafroll viruses occur as different sequence variants in the same plant. However, no particular sequence variant of GLRaV-3 has been assigned to any specific symptom in the host. We have detected two variants of GLRaV-3 in Western Australia. An undesirable variant which can specifically be detected by RT-PCR using primers on the p55 gene, as well as by ELISA. This variant is often associated with low yield and poorly coloured berries in Crimson Seedless. In contrast, infection with a desirable variant of the virus which is detectable by ELISA, but not by RT-PCR, is associated with high yields and outstanding fruit quality, superior to that produced on GLRaV-3 free vines. Both of these variants were variably mixed with *Grapevine virus A*, GLRaV-9 and *Rupestris stem pitting-associated virus* in the clones of Crimson Seedless. However, poor fruit quality was observed only in the presence of the undesirable GLRaV-3 variant.

14.24 DIFFERENTIAL SYMPTOM EXPRESSION IN TOMATO CAUSED BY DIFFERENT ISOLATES OF *PEPINO MOSAIC VIRUS*. I.M. Hanssen, L. Van Bergen, E. Vandewoestijne, A. Paeleman, L.P.F. Wittemans, K. Goen, C. Bragard, B. Lievens, A.C.R.C. Vanachter and B.P.H.J. Thomma. *Scientia Terrae Research Institute, Fortsesteenweg 30A, 2860 Sint-Katelijne-Waver, Belgium. Email: iha@scientiaterrae.org*

Pepino mosaic virus (PepMV), possessing a single-stranded RNA genome, was first detected in greenhouse tomatoes in 1999, and since then infection has spread quickly throughout tomato crops worldwide. The large variability in symptom expression and damage caused by PepMV in greenhouse tomatoes could thus far not unambiguously be related to PepMV genotypes, tomato varieties or environmental conditions. Recently, it was suggested that newly emerged genotypes, mixed infections and recombinant isolates could play a role in the symptom variability and the increasing losses seen in PepMV-infected tomato crops. However, their exact role has not yet been studied. Isolates differing significantly in symptom expression in commercial tomato greenhouses were selected to assess symptom reproducibility in field trials. Four PepMV isolates from different tomato production sites were inoculated to tomato plants grown in separate plastic tunnels. A fifth tunnel contained healthy tomato plants. Two of the isolates caused mild symptoms in the crop of origin and two others caused severe symptoms. Symptom development was assessed regularly and extensive sampling was followed by genotyping and sequencing to study the genetic characteristics of the isolates throughout the trial period. The entire genome sequences of all isolates were determined and compared, aiming to identify virulence factors in the PepMV genome. Cross-contamination between treatments did not occur and no external PepMV isolates invaded the plants during the trial. As symptom expression differed between treatments, minor differences in genome sequence could be responsible for the observed variability in symptom expression.

14.25* MOLECULAR ANALYSIS OF ISOLATES OF *CITRUS TRISTEZA VIRUS* THAT OVERCOME RESISTANCE EXPRESSED BY *PONCIRUS TRIFOLIATA* IN NEW ZEALAND AND THE SOUTH PACIFIC. S.J. Harper, T.E. Dawson and M.N. Pearson. *School of Biological Sciences, University of Auckland, P.O. Box 92019, Auckland, New Zealand Email: shar127@ec.auckland.ac.nz*

Nearly all citrus in New Zealand is grown on the *Citrus tristeza virus* (CTV) resistant rootstock *Poncirus trifoliata* or trifoliate orange hybrids. However, these are susceptible to the resistance-breaking (RB) strain of CTV present in New Zealand. The genomes of five isolates of this strain, obtained from field sources by graft and aphid transmission were completely sequenced. Phylogenetic analysis against other CTV genotypes revealed that the RB isolates are distinct from other extant CTV genomes with an average 83.7% homology at the nucleotide level, being most similar to T36 (90.4%) and least similar to VT (80.7%). Based on this sequence data the RB isolates comprise a previously unreported genotype. The sequences were utilised to develop markers to examine the incidence and spread of these isolates both in New Zealand and from sites across the Pacific. The RB genotype present in New Zealand is the dominant strain, and is also present in Western Samoa, Tahiti, and the Marianas. Sequence analysis of these isolates using a 700bp marker fragment show that the RB genotype is monophyletic, with nucleotide homology between isolates of approximately 96%. These data suggest that that the RB isolates comprise a single genotype that has remained stable

as it spread across the Pacific. The implications for the breakdown of resistance and impact of this strain are discussed.

14.26 SEED TRANSMISSION OF *MELON NECROTIC SPOT VIRUS* AND EFFICACY OF MELON SEED DISINFECTION TREATMENTS ASSESSED BY ELISA AND RT-PCR. J.A. Herrera-Vásquez, M.C. Cebrián, M.C. Córdoba-Sellés, A. Alfaro-Fernández and C. Jordá. *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Email: joshervs@doctor.upv.es*

Rates of transmission of *Melon necrotic spot virus* (MNSV) were estimated in seedlings grown from commercial melon seeds of cultivar 'Galia F1'. The seeds were planted and seedlings at the cotyledon and transplant stage were assayed in groups for MNSV by DAS-ELISA and RT-PCR. No group of seedlings gave positive results for MNSV by ELISA but the proportion of seedlings infected as estimated by RT-PCR was at least 7.33%. Twenty-two groups (10 seedling/group) of a total of 300 seedlings grown from infected seeds were MNSV-positive, corresponding to a seed-to-seedling transmission rate of 12.3%. Several seed disinfection treatments were evaluated for their ability to prevent seed transmission of the virus, which was only eradicated from seeds heated for 144 h at 70ºC. Germination was not reduced in seed treated in this way. The MNSV genome sequence obtained from seedlings grown from infected commercial melon seeds is published in the NCBI GenBank (MNSV-Seed, GenBank accession No. DQ443545).

14.27 MELON NECROTIC SPOT VIRUS: NEW IN MELON CROPS IN PANAMA. J.A. Herrera-Vásquez, M.C. Cebrián, M.C. Córdoba-Sellés, A. Alfaro-Fernández and C. Jordá. *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Email: joshervs@doctor.upv.es*

A new disease has recently been detected in melon crops in Panama, caused by the Carmovirus *Melon necrotic spot virus* (MNSV). During April 2005 and January 2006, several melon plants from commercial fields showed stem necrosis at the crown level, and less frequently, necrotic spots on leaves. In some cases, wilting and plant death were observed. The disease has been reported in melon fields in Cocle and Los Santos provinces, in the central and southeast region of Panama, respectively. Soil samples were collected from fields in these regions. In 91.6% of the soil samples analysed, the fungus *Olpidium bornovanus*, vector of MNSV, was found. Root samples were also analysed to detect the presence of MNSV by DAS-ELISA and the results were confirmed by RT-PCR. To establish the authenticity of this virus, RT-PCR products were purified and directly sequenced. MNSV sequences (GenBank accessions No. DQ443546 and No. DQ443547) were compared with other sequences in the NCBI GenBank. We present the distribution and molecular variability of MNSV in Panama.

14.28 FIRST REPORT OF VIRUS DISEASE ON GINSENG (*PANAX GINSENG* C. A. MEYER) IN KOREA. W.K. Jung, S.-H. Lee, J.-B. Lee, K.-C. Chung, C.-B. Kim, K.D. Gun and K.-W. Lee. *Youngsan-li 657. Anjeng-myun. Youngju-City 750-870 Republic of Korea. Email: go1961@yahoo.co.kr*

There have been no reports of virus disease on ginseng plants since an unknown virus was reported by Lee in 1982. Ginseng is a medicinal crop used to promote stamina. In the Gyeongbuk and Chungbuk provinces of Korea, where ginseng has been grown for 4-6 years, ginseng leaves with yellowing or mosaic symptoms were noted from 2005 to 2007. Filamentous virus particles were observed by negative-stain electron microscopy in the infected leaves. In ultrathin sections, cylindrical inclusions were seen in cytoplasm of the infected cells. Results of inoculaton to indicator plants were negative in *Nicotiana tabacum, Nicotiana glutinosa, Chenopodium amaranticolor, Chenopodium quinoa* and other test plants. The disease was transmitted by aphids. After piercing-sucking, virus infection rate were about 10%. In the future, through sequence analysis more detailed diagnosis should be possible.

14.29 DAMAGE CAUSED AND YIELD VARIATION IN BEAN (*PHASEOLUS VULGARIS*) INFECTED BY *BEAN COMMON MOSAIC VIRUS*. M.M. Kamelmanesh, S. Ghasemi and H.R. Dorri. *Plant Protection Dept., College of Agriculture, Islamic Azad University, Shiraz Branch, Iran. Email: kamelmanesh2000@yahoo.com*

Bean Common Mosaic Virus (BCMV) is a major yield reduction factor in bean production regions of Iran. In order to assay BCMV damage on grain yield and yield components in common bean, field trials were carried out during the 2006 cropping season. Two separate experiments (with and without inoculation) in the same conditions were conducted in an RCB design with 25 genotypes and 3 replications. The plants were mechanically inoculated at the 2 and 6 leaf stages. The results showed that virus stress had strongly increased empty pods and decreased grain yield and its components. Decrease in grain yield was estimated as about 43.74%. Among of yield components, number of seeds per pod and pod weight were more affected than others, 22.37% and 18.19% respectively. Harvest index (HI=grain yield / biological yield) indicated a loss about 28.57%. Our results suggest that, to produce varieties resistant to BCMV, specially attention should be given to these traits.

14.30 *CHERRY LEAF ROLL VIRUS* – GENETICS AND EPIDEMIOLOGY. J. Langer, S. von Bargen, J. Gentkow, A. Rumbou and C. Büttner. *Section Phytomedicine, Lentzeallee 55/57, Humboldt-Universität zu Berlin, 14195 Berlin, Germany. Email: phytomedizin@agrar.hu-berlin.de*

Cherry leaf roll virus (CLRV) is a member of the family *Comoviridae*, genus *Nepovirus*. The complete nucleotide sequence of the bipartite genome was determined from cDNA clones. RNA1 is 8250 and RNA2 6700 nucleotides (nt) in length. Each RNA contains a single open reading frame encoding one polyprotein, cleaved proteolytically into functional proteins. The genome organisation of CLRV is like that of other members of the genus, especially the long 3' non-coding region (3' NCR) of about 1600 nt on both RNAs, a typical feature of *Nepovirus* subgroup 3. CLRV is a world-wide pathogen occurring primarily on deciduous and fruit trees from at least 17 genera, but also on ornamentals and vegetables. The wide host range and geographical distribution of the virus indicates a rapid adaptability to different hosts and therefore a genetic heterogeneity among CLRV isolates of different origins. Sequence comparisons of the coat protein and the 3´ NCR of different CLRV isolates revealed nucleotide sequence diversities of up to 23%. Three CLRV-isolates from different hosts and phylogenetic groups were mechanically inoculated on five natural woody host plants to see if molecular variability

was correlated with biological characteristics. We found that a CLRV isolate from black elderberry could be transmitted to *Sambucus nigra*, *Juglans regia* and *Sorbus aucuparia*, but not to *Prunus avium* and *Betula pendula*, whereas a walnut isolate could only be detected in walnut plants (*Juglans regia*).

14.31 MOLECULAR ANALYSES OF DIFFERENT *BEET NECROTIC YELLOW VEIN VIRUS* ISOLATES FROM CHINA. **M. Li, T. Liu, C. Han, D. Li and J. Yu.** *Department of Plant Pathology, and State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing 100094, P.R. China. Email: hanchenggui@cau.edu.cn*

A survey was carried out to detect *Beet necrotic yellow vein virus* (BNYVV) across China sugarbeet-planting regions. Fifteen out of 36 sugar beet root samples were positive for BNYVV. The nucleotide sequences of CP, p25, p31 and p26 from the selected isolates were determined. Amino acid sequence analysis of the CP permitted us to distinguish between A-type and B-type CPs of Chinese isolates. A-type CP was distributed among Harbin, Hohhot, Baotou, Wuwei and Jiuquan, and B-type CP also in Hohhot and Changji. No Chinese CP clustered with European P-type CP in the phylogenetic tree. RT-PCR results showed that RNA 5 was present in most investigated regions and was associated with both A- and B-type CP. Multiple amino acid sequence alignments of Chinese p25 proteins showed that they were highly variable and had seven tetrad motifs. In particular, ASHG tetrad has not been reported previously. Chinese p25s were classified into three major groups and were closely related to the p25s of other countries. In contrast, p31s of Chinese isolates were more conserved and evolved into two groups. Seven out of nine Chinese p31s belonged in one cluster and separated from most European p31 proteins. Phylogenetic analyses of p25 and p31 from China displayed different evolutionary histories for the two proteins.

14.32 BLACKBERRY YELLOW VEIN DISEASE IS CAUSED BY MULTIPLE VIRUS COMPLEXES. **R.R. Martin, J. Susaimuthu, S. Sabanadzovic, R.C. Gergerich and I.E. Tzanetakis.** *USDA-ARS Horticulture Crops Res Lab, Corvallis, OR 97330, USA. Email: Bob.Martin@ars.usda.gov*

Blackberry yellow vein disease with symptoms of vein clearing, yellow mottling, ringspots and plant decline have been observed in blackberry in the southeastern USA since about 2000. At least six viruses have been identified by cloning and sequencing of double-stranded RNA from diseased plants. Blackberry yellow vein associated virus (BYVaV, a crinivirus) appears to be present in most if not all symptomatic plants along with at least one or more of the other five viruses. A novel potyvirus lacking the DAG triplet and containing an alkB domain, is found together with BYVaV in symptomatic plants from Arkansas but has not been detected in other areas. Blackberry virus X (Flexiviridae) has been detected in symptomatic plants along with BYVaV in North and South Carolina, Arkansas and in Mississippi. Beet pseudo yellows virus has been detected in blackberries from North and South Carolina. A novel putative allexivirus (BlVE) has been identified from symptomatic plants in Mississippi, though the extent of this virus is not yet known. Several symptomatic plants from Arkansas also contained an insect-like virus in addition to BYVaV and BVY. Tobacco ringspot and Raspberry bushy dwarf viruses have also been detected in the region and they may also be involved in disease symptoms when they occur in mixed infections with BYVAV. The current understanding of the blackberry yellow vein disease is that it is caused by BYVaV, in mixed infections with at least one or more of the above viruses.

14.33 MOLECULAR DETECTION OF *EUPHORBIA RINGSPOT VIRUS* IN VENEZUELA. **E. Marys and M. Romano.** *Instituto Venezolano de Investigaciones Científicas, Apartado 21827, Caracas 1020-A, Venezuela. Email: eemarys@ivic.ve*

Euphorbia milli is an ornamental plant widely grown in Venezuelan gardens. During April 2007, *E. milli* plants showing chlorotic spots, and leaf and flower malformation, indicative of viral infection were observed in several gardens in Caracas, Capital District. Leaf samples from nine symptomatic plants were brought to the lab at Instituto Venezolano de Investigaciones Científicas (IVIC) for further analysis. Electron microscopy of leaf-dip preparations from symptomatic samples revealed flexuous virus particles 750 nm long. Infected cells contained pinwheel inclusions and scrolls typical of infection by a potyvirus. Infection with *Euphorbia ringspot* potyvirus was confirmed by PCR amplification with degenerate primers. To our knowledge, this is the first report of EuRSV infecting ornamental crops in South America. Further studies are needed to clarify how EuRSV was introduced into Venezuela.

14.34 STUDY ON SYMPTOMS OF *JOHNSON GRASS CHLOROTICE STRIP MOSAIC VIRUS* (JCSMV) IN FIELD MAIZE AND THE BEST METHOD FOR INOCULATING THIS VIRUS. **E. Mohammadi, M. Koohi Habibi.** *Department of Plant Protection, College of Agriculture, University of Tehran, Karadj, Iran. Email: Elham54m@yahoo.com*

Leaves selected for making inoculum extracts were prepared from samples of field maize (*Zea mays* L.). To ensure the presence of JCSMV two serological tests, DAS-ELISA and TPIA, were performed. Rub-inoculation, injection to stem and VPI (vascular puncture inoculation) were used to obtain virus transmission. JCSMV was not transmitted to *Sorghum halepense* and *Zea mays* by rub-inoculation, but mechanical inoculation of virus on leaves of 7–10 day-old *Chenopodium amaranticolor* and *Ch. quinoa* gave distinctive necrotic lesions. Infected plant extracts were successfully assayed on seeds of maize cultivar 704 (a common cultivar in Iran) by VPI. Injected seeds were planted in degradable pots in the greenhouse. Virus symptoms on leaves included cream-colored or chlorotic stripes and crinkled edges of some leaves about a week post-VPI. Transmission of the virus was assessed in infected seedling by ELISA. Then 1000 pots were planted in the field in an isolated area. Subsequently developing leaves, before the ear stage, showed the same chlorotic stripe symptoms. No dwarfing or stunting was seen, but structural and physiological disorders were seen at the ear stage, including severe curling, reduced size, seed deformation, and poor seed fill. Ears were incompletely fertilized and matured late such that at harvest many plants were in transition between the milk and waxy stage or in the milk stage. The results show that VPI is the most efficient method to transmit JCSMV, and that the virus caused severe disease in maize, with potential to cause yield losses in commercial fields.

14.35 NATURAL OCCURRENCE IN IRAN OF *JOHNSON GRASS CHLOROTIC STRIPE MOSAIC VIRUS* IN MAIZE. **E. Mohammadi and M. Koohi Habibi.** *Department of Plant Protec-*

tion, College of Agriculture, University of Tehran, Karadj, Iran. Email: elham54m@yahoo.com

During a survey in Tehran Province of Iran of viruses infecting maize, 112 leaf samples with viral symptoms like mosaic, dwarfing and yellowing were collected from fields in different parts of the province. To ensure the detection of infection two serological tests, DAS-ELISA and TPIA, were performed with antisera to *Sugarcane mosaic virus*, *Maize dwarf mosaic virus, Sorghum mosaic virus* and *Johnson grass chlorotic stripe mosaic virus* (JCSMV). Five percent of the samples were infected with JCSMV. Immunoelectron microscopic techniques showed that the serum to JCSMV reacted only with particles of this virus. This is the first report of natural occurrence of JCSMV in maize fields in Iran.

14.36 DETECTION AND DISTRIBUTION OF *PRUNUS NECROTIC RING SPOT VIRUS* IN NORTHERN IRAN. S. Nasrollanejad, T. Fallah, M. Shahsavand and H. Delchosh. *Department of Plant Protection, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Email: snasrollanejad@yahoo.com*

*S*ymptoms of virus disease on stone fruit were observed widely in the North of Iran. Serological and molecular methods were used to identify the most important stone fruit viruses. Samples were taken from peach, nectarine, plum, cherry and apricot in different seasons during 2005-2006. Five polyclonal antisera [to *Prunus necrotic ring spot virus* (PNRSV), *Plum pox virus*, *Prune dwarf virus*, *Cherry leaf roll virus* and *Arabis mosaic virus*] were used in DAS-ELISA kits. The results showed that PNRSV was present in plum, peach and nectarine but there were no reactions on cherry and apricot. RT-PCR using two common and specific primers (NAS1 and NAS2, designed from the cp region of the genomic RNA) followed by agarose gel electrophoresis, gave a product of 356 bp with PNRSV. To study the infection rate of different cultivars to PNRSV, 17 treatments with twenty replications, in an RCBD trial, were conducted in a field test. The results of infection rates revealed that the treatments had significant effects on the response to disease. In general, the highest infection rate (15.5%) based on analysis of variance, (P>95%) was observed in peaches and the lowest rate was seen in plum (1.32%). Key word: PNRSV, cDNA, RNA, PCR, ELISA.

14.37 OCCURRENCE AND DISTRIBUTION OF *POTATO VIRUS Y STRAIN N* (PVYN) ON CELERY IN IRAN. K. Neda, M.H. Koohi and Gh. Mosahebi. *Department of Plant Pathology, Faculty of Horticulture Science and Plant Protection, University of Tehran, Karaj, Iran. Email: Neda2p@Gmail.com*

Celery (*Apium graveolens*) is an important crop grown in many countries. Different types of disease present a major constraint to celery production and can lead to significant reductions in yield. Some viruses damage this plant. *Potato Virus Y*, type member of the genus *Potyvirus* (family *Potyviridae*) is one of the viruses causing disease in celery, and is naturally transmitted by aphids in a non-persistent manner. During the 2006-2007 growing seasons, celery fields were visited throughout Tehran Province (Baghe daneshkade, Mohamadshahr, Varamin, Asgarabad, Hesarak and Savojbolagh). A total 172 samples, based on selection of plants expressing symptoms like mosaic, veinclearing and mottling, were collected. Using serological methods (ACP-ELISA and DAS-ELIZA) with specific *Potyvirus* antiserum (DSMZ-AS-0537.1) and PVY antiserum (DSMZ-AS-0137.403) 6.3% of the samples were found infected with PVY. The reactions of PVY-infected samples were positive in TAS-ELIZA with specific monoclonal antibodies to PVYN strain (DSMZ-AS-403.1). Some biological and molecular characters of the virus isolates were determined. PVYN strain isolated from celery was reported for the first time in Iran in this survey.

14.38 CHARACTERISATION OF NON-TRANSMISSIBLE MUTANTS OF TYLCSV IN THE WHITEFLY VECTOR *BEMISIA TABACI*. E. Noris, V. Medina, G. Mason, G.P. Accotto, D. Marian, M. Vecchiati, T. Falcioni and P. Caciagli. *Dept. Producció Vegetal i Ciència Forestal, Universitat de Lleida, Avda. A. Rovira Roure 191, 25198 Lleida, Spain. Email: medinap@pvcf.udl.es*

The monopartite *Begomovirus* (*Geminiviridae*) *Tomato yellow leaf curl Sardinia virus* (TYLCSV) has a capsid protein (CP) indispensable for plant infection and vector transmission. Four amino acids (aa) are essential for transmissibility: Q129, N130, Q134, and D152. In this study, three non-transmissible (NT) mutants were characterized: a single mutant N130D (named QDQD), a double mutant Q129P and Q134H (PNHD), and a triple mutant with a further D152E change (PNHE), in comparison with the wild-type virus (wt or QNQD). All these mutants formed virions in infected plants. To understand the reasons for their non-transmissibility, a detailed study on their relationship with whiteflies was undertaken. Using quantitative dot-blot hybridization and real-time PCR, the kinetics of these mutants was studied in the vector *Bemisia tabaci* and the non-vector *Trialeurodes vaporariorum* exposed to agroinoculated infected plants. The mutant QDQD was not detected by dot-blot in the insects, even at the end of the acquisition. PNHD was acquired and circulated in both *B. tabaci* and *T. vaporariorum* for at least 7 days, while the PNHE mutant circulated in *B. tabaci* only. We studied whether pre-acquisition of NT mutants would interfere with transmission of the wt virus. Transmission of the wt virus was inhibited when *B. tabaci* were pre-exposed to plants infected by the PNHE mutant, but not by other mutants. An immunolocalisation protocol was set up to detect the viral CP in *B. tabaci* previously exposed to infected plants. Data will be presented on the localization of the CP in the vector by immunoelectron microscopy.

14.39 STUDIES ON A VIRUS DISEASE OF *VERNONIA AMYGDALINA*. T.E. Owa and J.L. Ladipo. *Department of Plant Science, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria. Email: teaareg@yahoo.com*

A potyvirus was isolated from a naturally infected *Vernonia amygdalina* Del. plant that showed mosaic and green vein-banding symptoms. The virus was readily transmitted mechanically. It was also transmitted by the insect vector *Aphis craccivora* Koch. It induced diagnostic systemic mosaic, dark-green vein-banding, leaf curl and shoestringing symptoms in *Nicotiana benthamiana* Domin., systemic diffuse chlorotic spots in *Nicotiana clevelandii* and local chlorotic spots in *Chenopodium amaranticolor.* It has a narrow host range. The virus was maintained in *N. benthamiana*, also used as an indicator host. Attempts to graft-transmit the virus from *N. benthamiana* to *N. benthamiana* and from *N. benthamiana* to *V. amygdalina* were unsuccessful. In tests of physical properties using *N. benthamiana* extracts, the virus had a dilution end point of between 10^{-4} and 10^{-5}, a thermal inactivation point of between 55°C and 60°C when extract was heated for 10 mins. It had longevity *in vitro* of between 3 and 4 hours when kept at room temperature. Frozen infected leaves remained infective up

to 18 days. Indirect ELISA tests carried out showed that extracts from systemically infected *N. benthamiana* did not react with antisera to any of the potyviruses tested.

14.40 CHRONIC DEVELOPMENT OF RIO GRANDE GUMMOSIS IN FLORIDA GRAPEFRUIT. **C.A. Powell and P.J. Stoffella.** *IRREC, 2199 S. Rock Rd, Fort Pierce, FL, 34945, USA. Email: CAPowell@ufl.edu*

Rio Grande gummosis (RGG) is one of the most serious diseases of grapefruit in the Indian River Region of Florida. To determine the progression of disease symptoms over time, two grapefruit groves of three different tree ages (starting at 1, 11, and 21 years after planting) in each of three Florida counties (18 total groves) were rated for RGG symptoms over a period of 12 years. Only tree age (not county or location within county) significantly affected RGG symptom severity. At 7 years after planting, trees first started to develop symptoms which progressed to splitting, gumming and charcoal-like symptoms in many trees at 15 years after planting.

14.41 DISTRIBUTION OF APMV, ACLSV, ASGV AND ASPV IN APPLE AND PEAR ORCHARDS IN LATVIA. **N. Pupola, A. Kale and I. Morocko.** *Latvia State Institute of Fruit-Growing, Graudu Str.1, LV-3701 Dobele, Latvia. Email: neda.pupola@lvai.lv*

ApMV, ACLSV, ASGV and ASPV are widespread and economically important viruses in apple and pear orchards around the world, but since the 80s research has not been carried out on viruses of fruit trees. The aim of this study was to evaluate the distribution of ApMV, ASGV, ACLSV and ASPV in different fruit-tree varieties in commercial orchards and to collect virus isolates for further study. Leaf samples were collected over the country during early summer and autumn in 2007, and were tested by ELISA and multiplex RT-PCR. Totally 1097 samples were collected from 51 apple orchards and 240 samples from 34 pear orchards. Initial results show that all four common apple viruses are randomly distributed all over the country and the most common is *Apple chlorotic leaf spot virus* (ACLSV) in both apple and pear orchards, but *Apple stem grooving virus* (ASGV) is rarely present throughout the country. Several samples from apple trees showed mixed infections, e. g., ACLSV + ASPV, ApMV + ACLSV and ApMV + ASPV, but such mixed infections were not observed in pears. To limit the spread of viruses in orchards it is necessary to find virus-tolerant varieties and to introduce a certification program of planting material in the country.

14.42* GENETIC VARIABILITY AND MOLECULAR EPIDEMIOLOGY OF *WHEAT DWARF VIRUS*. **J.N.E. Ramsell, G. Köklü, A. Lemmetty, D.P. Martin, M.I. Boulton, J.P.T. Valkonen, R. Sigvald and A. Kvarnheden.** *Department of Plant Biology and Forest Genetics, SLU, Box 7080, SE-750 07 Uppsala, Sweden. Email: anders.kvarnheden@vbsg.slu.se*

Wheat dwarf virus (WDV) is a single-stranded DNA virus (family *Geminiviridae*, genus *Mastrevirus*) transmitted by the leafhopper *Psammotettix alienus*. WDV causes disease in wheat and barley in many parts of Europe, and has recently been detected in Africa and China. There are two strains of WDV, one infecting wheat and another infecting barley. To study the diversity of WDV, isolates from different hosts were sequenced. Two complete sequences of barley isolates from Hungary and Turkey showed 94% nucleotide identity to each other. Phylogenetic analyses showed that the barley strain could be divided into three subtypes correlating with geographic origin. Comparisons between barley and wheat isolates of WDV revealed an identity of only 84%. In a phylogenetic analysis, the WDV isolates from barley and wheat formed two distinct clades, confirming that they belong to two distinct strains. Among wheat isolates of WDV, the identity was high (>97%), including also an isolate from Finland. For plant and insect samples, a rapid detection method using polymerase chain reaction (PCR) was adopted. Partial sequences for Swedish WDV isolates from wheat, triticale, *P. alienus* and the grasses *Apera spica-venti*, *Poa pratensis* and *Avena fatua* were all closely related. The results suggest that the same WDV genotypes infect wheat and grasses in Sweden. Agroinfectious clones were constructed for a wheat and a barley isolate of WDV. Infectivity was confirmed for both clones by PCR and Southern blotting of inoculated plants. The barley clone was transmissible by *P. alienus*. This confirms that WDV causes the disease in barley.

14.43 MOLECULAR EVIDENCE THAT *SUGARCANE STREAK MOSAIC VIRUS* AND *SUGARCANE MOSAIC VIRUS* ARE RESPONSIBLE FOR *SUGARCANE MOSAIC DISEASE* IN INDIA. **G.P. Rao, M. Singh, A. Tewari and D. Singh.** *Division of Plant Pathology, Sugarcane Research Station, Gorakhpur 273008, UP, India. Email: gprao_gor@rediffmail.com*

Fifty three sugarcane leaf samples exhibiting mosaic symptoms from eight sugarcane growing states of India (Uttar Pradesh, Bihar, Haryana, Punjab, Andhra Pradesh, Kerala, Maharashtra and Tamil Nadu) were characterized through RT-PCR assays with primers designed to be specific for *Sugarcane mosaic virus*, SCMV (F3 and R3) and for *Sugarcane streak mosaic virus*, SCSMV(ST2 and P1), JGMV and SrMV. The expected 0.9 kbp SCMV fragment was amplified with the SCMV F3 (5'-TTTY-CACCAAGCTGGAA-3') and SCMV R3 (5'-AGCTGTGT-GTCTGTCTGTATTCTC-3') primers only with twelve mosaic samples from U.P., Bihar, Haryana, Maharashtra, Kerala and Punjab (CoC 671 and CoJ 85). However, a ca 0.5 kbp fragment was amplified from forty samples out of fifty three tested using primers SCSMV-ST2 (5'-CGTATCGYTACTATTCG-3') and SCSMV-P1(5'-CTGTAGGCACTGGGTCAATCCTCA-3'). Reactions were negative with both specific primers for *Sorghum mosaic virus*, *maize dwarf mosaic virus* and *Johnsongrass mosaic virus*. Our results suggest widespread existence of *SCSMV* in India followed by *SCMV*. Interestingly, mixed infection of *SCMV* and *SCSMV* was also detected in leaf samples showing sugarcane mosaic symptoms from U.P., Bihar, Kerala and Maharashtra. The Results indicates that the sugarcane mosaic symptoms in different regions of India are caused either by SCSMV or SCMV. No indication of the presence of MDMV, JGMV or SrMV was recorded in the study, which ssuggests that among the Sugarcane mosaic virus subgroup members only SCMV and SCSMV are causing mosaic disease in sugarcane in India.

14.44 THE DIVERSITY AND IDENTITY OF *APPLE FRUIT CRINKLE VIROID* ISOLATES IN APPLE AND HOP. **T. Sano, K. Matsuki, S. Isono, M. Tsuji, Y. Ito-Kawaguchi and K. Tanaka.** *Plant Pathology Laboratory, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan. Email: sano@cc.hirosaki-u.ac.jp*

Apple fruit crinkle viroid (AFCVd; *Pospiviroidae*, Apscaviroid

group) was first detected from apple (*Malus* × *domestica*) in 1976 (Koganezawa 1989) and is now observed sparsely in major apple producing areas in Japan (Ito et al 1993). Meanwhile, a new "strain" of AFCVd was discovered in 2004 from hops (*Humulus lupulus*) in Japan showing disease symptoms similar to hop stunt disease (Sano *et al.* 2004). To analyze the diversity and the relationship between the two AFCVd isolates, we have analysed more comprehensively the nucleotide sequence of AFCVd isolates from apples and hops. We found that the AFCVd isolates were divided into two clusters. One cluster included hop isolates from two different geographical regions; Yamagata and Akita. Yamagata isolates were more diverse than Akita isolates in sequence. The other cluster consisted of some of the Yamagata hop isolates and all the apple isolates from three different geographical regions; Iwate, Nagano and Aomori. It was noteworthy that the nucleotide sequence was completely identical in one of the cDNA clones of a Nagano apple isolate and two Yamagata hop isolates. In conclusion, the hop and apple isolates of AFCVd cannot be discriminated clearly by their nucleotide sequence. They are apparently the sequence variants of the same species; i.e., a "quasispecies".

14.45* DOES *LETTUCE BIG-VEIN ASSOCIATED VIRUS*, FORMERLY *LETTUCE BIG-VEIN VIRUS*, CAUSE BIG-VEIN DISEASE IN LETTUCE? T. Sasaya, H. Fujii, K. Ishikawa, and H. Koganezawa. *Team for Vector-Borne Diseases, National Agricultural Research Center, Tsukuba, Ibaraki 305-8666, Japan. Email: tsasaya@affrc.go.jp*

Two viruses, *Lettuce big-vein associated virus* (LBVaV) and *Mirafiori lettuce virus* (MiLV) are almost invariably detected in lettuce plants showing big-vein symptoms. Recently MiLV alone was shown to induce big-vein symptoms in lettuce plants. However, it remained unclear that LBVaV is really involved in the big-vein disease. Isolation of the two viruses is critical if we want to clarify the role of LBVaV in the etiology of big-vein disease. Using Western blotting specific for each virus and exploiting the differences between LBVaV and MiLV in terms of pathogenicity in cucumber and dependence of infection on low temperature, we separated the viruses from doubly infected lettuce plants and transferred them separately or together to virus-free fungal vectors. To confirm the separation, the viruses were transferred to fresh vectors twelve consecutive times. Lettuce seedlings infected at each transfer were infected with MiLV alone, with LBVaV alone, or with both viruses together, depending on the virus(es) carried by the vector. Lettuce seedlings infected with MiLV alone developed big-vein symptoms, while those infected with LBVaV alone developed no symptoms. In 126 fields of the 19 major winter-spring lettuce-producing regions of Japan, only MiLV was detected consistently in lettuce plants from big-vein-affected fields but was not from big-vein-free fields, whereas LBVaV was detected at high rates in lettuce plants not only from 15 big-vein-affected fields but also from 109 big-vein-free fields. Only two fields were free of LBVaV. These results indicate that LBVaV does not cause the disease but is distributed widely in lettuce-growing areas.

14.46 VIRUSES IN, AND SELECTION OF VIRUS-FREE PLANTS OF *RUBUS IDAEUS* IN BELARUS. S. Semenas, N. Valasevich and N. Kukharchyk. *Biotechnology Department, Institute for Fruit Growing, Samokhvalovichi, Belarus. Email: natavolosevich@yahoo.com*

Viruses cause pathological changes in small fruits and inhibit physiological processes, decreasing plant productivity sharply. It is necessary to carry out virus diagnosis in order to create virus-free foundation stock and to monitor the further propagation of certified planting stock. We studied the phytosanitary state of *Rubus idaeus* L. in nurseries. Virus diseases were monitored for the first time in 2007 in mother plantations of the Institute for Fruit Growing, Samokhvalovichi, Belarus. Cultivars 'Alyonushka', 'Balsam' and 'Meteor' were tested by ELISA. A high level of virus infection of raspberry plants in the field was detected: 54.5% of *Strawberry latent ringspot virus*, 42.4% of *Raspberry ringspot virus*, 36.4% both of *Raspberry bushy dwarf virus* and *Apple mosaic virus* ApMV, and 21.2% of *Arabis mosaic virus* (ArMV). *Cucumber mosaic virus*, *Tomato ringspot virus* and *Tomato black ring virus* were absent from all plants tested. Only 3.0% of samples were free from the viruses tested, 33.3% were infected by one virus, 51.5% had two viruses, and 12.1% contained three viruses. We also tested the regenerants propagated in vitro by meristem tip culture ('Alyonushka', 19 clones, 250 plants). We found that 89.5% of plantlets were free from all tested viruses and 10.5% had only ArMV. Virus-free clones were planted in the greenhouse and used as Nuclear stock, and other healthy material was planted in the orchards.

14.47 DIAGNOSIS OF PEPPER-INFECTING VIRUSES WITH RT-PCR IN PEPPER SEED INTRODUCED FROM ABROAD. C.K. Shim, H.I. Ahn and T.S. Kim. *Genetic Resources Division, National Institute of Agricultural Biotechnology, Rural Development Administration, Suwon 441-707, Republic of Korea. Email: ckshim@rda.go.kr*

Large quantities of seeds are traded among the nations. Nowadays, quarantine is the most important process in the exchange of plant genetic resources. In the Rural Development Administration genebank of Korea, we have detected pepper-infecting viruses by RT-PCR in some pepper seeds introduced from other countries. A total of 273 samples belonging to 20 cultivars were diagnosed for *Potyvirus*, *Tobamovirus* and *Cucumber mosaic virus* (CMV) infections, using RT-PCR with degenerate primers. The samples could be divided into 22 groups according to their infection patterns. Potyviruses were detected in all the groups, whereas CMV was detected only in four groups. *Tobamovirus*-specific cDNA fragments were detected in seven groups. Of tobamoviruses, among sixty-three samples tested, 13 were infected with *Tobacco mosaic virus*, 5 with *Tomato mosaic virus* and 14 with *Pepper mild mottle virus*. Of 24 samples tested for potyviruses, 20 were found to be infected.

14.48 NON-TRANSMISSIBILITY OF *ZUCCHINI YELLOW MOSAIC VIRUS* (ZYMV) BY SEEDS OF INFECTED CUCURBITACEOUS VEGETABLES. J. Svoboda. *Crop Research Institute, Drnovská 507, 161 06 Prague 6 - Ruzyne, Czech Rebublic. Email: jiri.svo@vurv.cz*

ZYMV has caused considerable losses of cucurbits grown in the Czech Republic and has spread gradually from South Moravia to northern areas during last ten years. The spread might be caused by seed transmission of the virus. Commonly grown cultivars are highly susceptible to ZYMV. Characteristic symptoms of cucurbitaceous plants are yellow mosaic on leaves and deformed fruits. The virus is very variable, and three ZYMV strains are known in the Czech Republic that differ in their pathogenicity. Seeds for the trial were obtained from malformed fruits of cucumbers, summer squashes and pumpkins from plants inoculated with each of the three Czech ZYMV strains. The plants

were inoculated mechanically at the first true leaf stage; the presence of ZYMV in parent plants and their fruits was proved by ELISA and symptoms. Seeds were neither washed nor fermented. Seeds of each cultivar, from plants infected with each ZYMV strain, were sown in equal amounts, and at intervals from one to six months after harvesting. The germinated plants were evaluated at four to eight weeks for symptoms and the presence of ZYMV. None of 1206 seedlings was ZYMV-positive. Regardless of some authors who refer to ZYMV seed transmissibility, it can be assumed that ZYMV is not seed transmissible, and their observations could derive from other causes. Work was supported by the Project QH 71229 and the Funding No. 10/2006-2199St of the Ministry of Agriculture, Czech Republic.

14.49 STRATEGIES TO CONTROL *PAPAYA RINGSPOT VIRUS* IN PERU. **J. Tenorio, J. Salazar, S. Fuentes, C. Aguilar, C. Malpartida, G. Müller, A. Cabrera, C. De La Torre, J. Marín, E.V. Campoverde, I. Barker and L.F. Salazar.** *International Potato Center (CIP), Lima, Peru. Email: j.tenorio@cgiar.org*

Papaya (*Carica papaya*) is an important cash crop in the jungle region of Peru, traditionally planted in small field lots. The disease caused by PRSV has affected approximately 90% of the main papaya growing areas in Peru. Farmers as a common practice invade new areas of the rainforest to escape from the disease, a process that causes deforestation of the jungle. When the disease arrives to these new areas farmers continue to move to virgin rainforest, a cycle that never ends. Integrated management was initiated with the participation of CIP, INIA, and SENASA to reduce disease incidence in the main papaya-producing areas, where up to 100% PRSV incidence in papaya fields was recorded. An antiserum specific for PRSV was produced. Five species of aphids capable of transmitting PRSV were found in traps placed in papaya fields. A technological package to control PRSV consisting in the use of ground cover crops, manure application, establishing definitive crops by transplanting 3-month old, virus-free papaya seedlings, natural barriers, isolation –200 m– from other papaya fields was tested in pilot areas (Shincayacu, San Ramon, and Pichanaki in the Junin Department). Results have shown that using this technological package has delayed virus infection by over 6 months, allowing plants to reach maturity to resist the devastating effect of the disease (mature plant resistance) and therefore yield better commercial production. Inclusion of mild strains of PRSV (for cross-protection) and use of tolerant varieties in the technological package is now under evaluation.

14.50 BEGOMOVIRUSES ARE THE MAJOR VIRUSES AFFECTING CUCURBITACEOUS CROPS OF EASTERN UTTAR PRADESH, INDIA. **A.K. Tiwari, S.K. Snehi, G.P. Rao, P.K. Sharma and S.K. Raj.** *Dept. Genetics & Plant Breeding, Ch.C.S. University, Meerut, UP, India. Email: ajay_biotech2005@rediffmail.com*

Begomoviruses (family *Geminiviridae*) are transmitted by whiteflies and have a bipartite genome referred to as DNA-A and DNA-B. Begomoviruses cause a number of serious diseases of cultivated crops, like cotton, cucurbits, bean, pepper and tomato, worldwide. During a rainy season survey in 2007, different types of symptom such as yellow mosaic, leaf curling, puckering and vein bending were observed on five cucurbitaceous species viz. *Luffa cylindrica, Luffa acutangula, Momordica charantia, Cucurbita pepo* and *Coccinia grandiflora* growing in and around Gorakhpur and Kushinagar districts of Uttar Pradesh. White flies (*Bemisia tabaci*) on these cucurbits were also noticed in the area, and *Begomovirus* infection was suspected. Total DNA was extracted from infected as well as healthy leaf samples of these species and PCR was performed with primers specific to the *Begomovirus* coat protein gene. The expected ~800 bp fragment was amplified from all five infected plant species while no such amplicon was observed in healthy samples. These results indicate the presence of Begomoviruses on all these cucurbitaceous crops. The wide spread of Begomoviruses may be due to the high population of *B. tabaci* feeding on cucurbitaceous crops in eastern U. P. Cloning and sequencing of PCR amplicons is in progress for further identification of the Begomoviruses and to establish the phylogenetic relationships among them and with the other Begomoviruses known in India.

14.51 POTYVIRUS DISEASES OF MELON IN SOUTHERN ITALY. **L. Tomassoli, M. Meneghini, A. Tiberini, T. Colella, D. Battaglia and M. Barba.** *CRA-Plant Pathology Research Center, Via C.G. Bertero 22, 00156 Rome, Italy. Email: l.tomassoli@ispave.it*

Among cucurbits viruses, the most threatening to open air crops are non-persistently aphid transmitted ones, and most of these are potyviruses, which cause worldwide economic damage. In several areas of southern Italy, melon (*Cucumis melo* L. var. *cantalupensis*, var. *inodorus*, and var. *saccharinus*) is an important open air crop which, in the last decade, has been seriously affected by mosaic-inducing viruses. During recent surveys, in addition to *Cucumber mosaic virus*, the presence of three potyviruses in commercial fields was assessed: *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Zucchini yellow fleck virus* (ZYFV). In cases of single infections (90% of symptomatic samples), specific leaf symptoms for each potyvirus were observed whereas more severe and unspecific symptoms were seen on doubly infected plants (10%). WMV was the most frequently detected virus accounting for 75% average of the infected plants in every region visited. In contrast, ZYMV was detected in fewer sites and at lower and variable incidence (1-30%) according to year. The presence of ZYFV was noted in two Sicilian provinces with increasing incidence year after year. Melon aphid (*Aphis gossypii* Glover) was the main vector colonizing the crops. *Papaya ringspot virus* and *Moroccan watermelon mosaic virus* were also included in the disease monitoring but were not found.

14.52 CHARACTERIZATION OF THE CP GENE AND 3'UTR OF *CHILLI VEINAL MOTTLE VIRUS* ISOLATES FROM CHINA, INDIA, INDONESIA, TAIWAN AND THAILAND. **W.S. Tsai, Y.C. Huang, D.Y. Zhang, K. Reddy, S.H. Hidayat, W. Srithongchai, S.K. Green and F.J. Jan.** *AVRDC-The World Vegetable Center, Shanhua, Tainan, 741 ROC. Email: wenshi@netra.avrdc.org.tw*

The genetic relatedness of twenty-four isolates of *Chilli veinal mottle virus* (ChiVMV) from South and Southeast Asian countries was determined. Pathogenicity of virus isolates was confirmed by induction of systemic mosaic and/or necrotic ringspot symptoms on *Capsicum annuum* plants after mechanical inoculation. The 3' terminal sequences of the viral genomic RNA were determined. The coat protein (CP) coding regions ranged from 858 to 864 nt and the 3' untranslated regions (3'UTR) from 275 to 289 nt. All isolates had the inverted repeat sequence GUGGNNNCCAC in the 3'UTR. The DAG motif, conserved in aphid-transmitted potyviruses, was observed in all isolates. All the isolates were considered to belong to ChiVMV because of

their high CP amino acid and nucleotide identities (more than 94.8% and 89.5% respectively) with known ChiVMV isolates including pepper vein banding virus (PVBV), chilli vein-banding mottle virus (CVbMV), and the CVbMV Chiengmai isolate (CVbMV-CM1). Phylogenetic analysis indicates that ChiVMV isolates including all 24 tested by us, PVBV, CVbMV and CVbMV-CM1 can be placed in three groups. In addition, a conserved region of 204 amino acids with more than 90.2% identities was identified in the C terminal of the CP gene of ChiVMV and *Pepper veinal mottle virus* (PVMV) and may explain the serological cross-reaction between these two viruses. This conserved region may also provide useful information for developing transgenic resistance to both ChiVMV and PVMV.

14.53 PHYLOGENETIC ANALYSIS OF *TOMATO YELLOW LEAF CURL VIRUS* ISOLATES OCCURRING IN CENTRAL JAPAN. **T. Ueno, M. Suzuki and M. Ugaki.** *Laboratory of Bioresource Technology, Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, 202 Bioscience Building, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8562, Japan. Email: 76509@ib.k.u-tokyo.ac.jp*

Tomato yellow leaf curl virus (TYLCV) is a whitefly-transmitted geminivirus in the genus *Begomovirus*, and is one of the causal agents of tomato yellow leaf curl diseases in tropical, subtropical and temperate regions. TYLCV has been raging in Japan since 1996, and four representative isolates have been identified, each from four southern and west-central Prefectures, Kochi (TYLCV-Tosa), Nagasaki (TYLCV-Ng), Shizuoka (TYLCV-Mld-Sz) and Aichi (TYLCV-Mld-Aic). The latter two isolates belong to the TYLCV-Mild strain. TYLCV has recently been reported also in the central Prefectures, Japan's largest tomato producing and consuming area, including metropolitan Tokyo. To study the lineage of the emerging viruses, we obtained 26 isolates from the area. We sequenced approximately 800-nucleotides around the intergenic region of the viral genomic DNA, and compared these sequences with those in databases. As a result, one isolate was located in the TYLCV-Mild cluster, another was related to TYLCV-Ng, and all the others were related to TYLCV-Tosa. The cluster of 24 isolates related to TYLCV-Tosa had diverged into three subgroups. Interestingly, isolates of one subgroup were each distributed locally in specific places, and those of the other were distributed throughout the area. Therefore the virus should have established infection in the area more than once. Because some isolates relative to TYLCV-Tosa have also been reported in China, Mexico and the USA, their epidemiology is of interest. To get a clear view, construction of infectious clones is underway for analyzing their pathogenicity.

14.54 dsRNA ANALYSIS AND RT-PCR ASSAYS TO EVALUATE VIRAL INFECTIONS: THE CASE OF NECROVIRUSES IN OLIVE. **C.M.R. Varanda, M.R.F. Félix and M.I.E. Clara.** *Laboratório de Virologia Vegetal, Instituto de Ciências Agrárias Mediterrânicas, Universidade de Évora, Apartado 94, 7002-774 Évora, Portugal. Email: carlavaranda@uevora.pt*

Olive is host to fourteen known viruses, often symptomless, and thus diagnosis is indispensable to assist in plant improvement programmes. In this study, 67 genotypes of cv 'Galega vulgar' were tested for the presence of dsRNA and subjected to RT-PCR assays for the detection of olive necroviruses (*Olive latent virus 1* (OLV-1), *Tobacco necrosis virus D* (TNV-D) and Olive mild mosaic virus (OMMV)) which are considerably widespread in Portugal. Fruit and stem samples were repeatedly tested over 3 years. A pair of primers specific for OLV-1 detection and a pair of primers that detect both TNV-D and OMMV were used in separate RT-PCR analyses. Results showed that 24 trees contained dsRNA, suggesting viral infection, but none of the dsRNA gel electrophoretic patterns were those expected of necrovirus replication. Yet, RT-PCR showed that 8 of these 24 trees were necrovirus-infected. Out of the 67 olive genotypes tested, 26 revealed the presence of a necrovirus by RT-PCR analysis: TNV-D and/or OMMV were found in 22, OLV-1 in 13 and several trees were multiply infected. Results also showed that dsRNA species were more readily detected in stems than in fruits, whereas RT-PCR analysis showed TNV-D and/or OMMV more frequently in fruits and OLV-1 in stems. This study clearly shows that necroviruses in olive are not always detected by dsRNA analysis, most probably due to their low titres in the tissues. Therefore, a highly sensitive method, PCR-based, is essential for the successful detection of necroviruses in olive. This work was financed by funds from Research Project Agro 683.

14.55 *CYMBIDIUM MOSAIC VIRUS* STRAINS IN TAIWAN ORCHIDS. **P.H. Wang, H.H. Wu and Y.T. Wang.** *Department of Life Science, Tunghai University, 40704, ROC. Email: phwang@thu.edu.tw*

During 2003 and 2004, 218 phalaenopsis plants of six cultivars from three orchid gardens in Taiwan were examined for *Cymbidium mosaic virus* (CyMV) using one-step RT-PCR. CyMV was detected in 100%, 96%, 50%, 10%, 10%, and 0% plants of cultivars RedSky, Wedding, LittleBoy, Alisan, Salagold and Taisuco Firebird, respectively. The PCR products of the CyMV coat protein (CP) gene fell into three *Hba*I RFLP subgroups, and some plants were doubly infected by subgroup A/C or B/C. The sequences were compared with those in GenBank of 40 CyMV strains from different countries, by the neighbor-joining method. The phylogenetic tree had 7 clades. CyMV strains from Taiwan were represented in 5 clades. Some strains may have been imported with orchids from abroad, increasing the diversity of CyMV strains in Taiwan. There were 11 strains from 4 cultivars from three orchid gardens in Taiwan in the third clade. The strains in this clade might represent the indigenous Taiwan strain of CyMV.

14.56 INSIGHT INTO THE MOLECULAR EVOLUTION AND DIVERSIFICATION OF A SINGLE-STRANDED DNA VIRUS, *WHEAT DWARF VIRUS*. **B. Wu, X. Wang and G. Zhou.** *Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Yuan Ming Yuan West Road, No.2, Beijing, P.R. China. Email: xfwang@ippcaas.cn*

As one of the most successful plant pathogens, *Wheat dwarf virus* (WDV) can cause severe economic losses to wheat, barley and oat. The genetic diversity of WDV has provided important insight into the evolution and biology of the geminiviruses, a family of DNA viruses. The entire genomes of 28 representative WDV isolates, collected from different regions of China and sequenced in this study were compared with genome sequences of other isolates in the world from GenBank and used to analyze the diversification and molecular evolution of WDV populations. In the phylogenetic analysis, for consistency of the WDV isolates in the Rep A-LIR-MP region, 57 isolates hosted on wheat, barley and oat from China, Europe and USA respectively were analyzed. HBSJZ06-7 was the oldest isolate, suggesting China might be one of the origins of WDV in the world. Estimation of genetic diver-

sity disclosed the possible existence of diversification and recombination, which truly reflected the characteristics of the evolution of WDV. The variability of Rep A, SIR, LIR, and Intron regions suggested that diversification mirrored the trace of coevolution among the hosts, vectors and viruses.

14.57* BEAN YELLOW MOSAIC VIRUS: ITS PHYLOGENY, BIOLOGICAL PROPERTIES AND EVOLUTION IN RESPONSE TO PLANT DOMESTICATION. S.J. Wylie, B.A. Coutts, M.G.K. Jones and R.A.C. Jones. *State Agricultural Biotechnology Centre, Murdoch University, Perth, WA 6150, Australia. Email: wylie21_98@yahoo.com*

The genetic diversity of Bean yellow mosaic virus (BYMV) was studied by comparing sequences of complete coat protein (CP) genes of 63 isolates. Phylogenetic analysis of CPs showed that isolates fell into eight distinct groups, with an overall nucleotide diversity of 19%. The largest and most genetically diverse group was from natural infections in both wild and domesticated host plant species in both monocotyledonous and dicotyledonous plant families, indicating a generalized host strategy. The other seven groups had a narrower genetic base and in each case, isolates were from a single or limited number of domesticated species, indicating a specialized host strategy. We propose that the generalized group represents the ancestral type from which specialist groups evolved within domesticated plants after the advent of agriculture, since approximately ten millennia BP. The centre of origin of the ancestral group is unclear from its present distribution, but the specialized groups probably originated where their principal hosts were first domesticated, in most cases Eurasia. Specialist groups have adapted to domesticated hosts by often becoming seed-borne, facilitating their world-wide spread. Inter- and intra-specific recombination is a strategy used to increase genetic diversity in BYMV. Recombination was found in CPs and complete genomes and occurred between the generalized group and a specialized group, and between different specialized groups. Phylogenetic analysis of all genes within six complete BYMV genomes and 14 VPg gene sequences showed that branch topologies were sometimes incongruous. This indicates that its different genes evolved independently.

14.58 MOLECULAR IDENTIFICATION OF VIRUSES THAT INFECT *PANAX NOTOGINSENG* IN CHINA. T. Zhou, Z. L. Yan, H.F. Li and Z.F. Fan. *Department of Plant Pathology, China Agricultural University, No. 2 Yunmingyuan West Road, Beijing, P.R. China. Email: taozhoucau@cau.edu.cn*

Sanqi [*Panax notoginseng* (Burk) F. H. Chen, PN] is a famous Chinese traditional medicinal herb which grows specifically in Yunnan and Guangxi provinces. Since the 1990s, virus infections have caused increasingly destructive disease in Sanqi crops and the infected plants showed severe leaf mosaic and/or crinkle. In this study, samples showing this symptom were collected from Sanqi plantations. Seventeen indicator plants from 5 families (Chenopodiaceae, Cucurbitaceae, Leguminosae, Solanaceae and Umbelliferae) were mechanically inoculated with the crude leaf sap,but no plants showed symptoms in 40 days. Filamentous and spherical particles were found in the leaf sap by Electron microscopy. ELISA revealed that the viruses in *P. notoginseng* did not serologically react to any of the 29 antisera of 14 genera stored in our lab. Double-strand RNA was isolated for construction of a cDNA library, from which virus genome segments were cloned randomly. BLAST results showed that there was one potyvirus. Using potyvirus degenerate primers, the sequences covering regions encoding the coat protein (CP), HC-P3 and CI were obtained. Sequence comparison of the CP amino acids showed that this virus was most closely related to *Carrot virus Y*, *Apium virus Y* and *Celery mosaic virus* with 75% identities. Phylogenetic analyses of the sequences indicated that this virus isolate is a distinct potyvirus.

POST-HARVEST PATHOLOGY

3.1 BIOLOGICAL CONTROL OF GREY MOULD OF APPLE BY YEAST ISOLATES. F. Alavifard, H.R. Etebarian, N. Sahebany and H. Aminian. *Department of Plant Protection,Aburayhan campus,University of Tehran, P.O. Box 11365/4117, Tehran, Iran. Email: alavi_6180@yahoo.com*

Yeast isolates A2, A4 and A5 (*Candida membranifaciens*), A1 and A3 (*Rhodotorula mucilaginosa*) and A6 (*Pichia guilliermondii*) obtained from the surface of healthy apples were evaluated as potential biological control agents for apple grey mould caused by *Botrytis mali*. The isolates inhibited *B. mali* mycelial growth in dual culture tests by 17.45 to 35.16 %. Volatile metabolites emitted from all isolates inhibited growth of *B. mali*, with A3 (*R. mucilaginosa*) and A6 (*P. guilliermondii*) being the most inhibitory. Cell-free metabolites of A1 (*R. mucilaginosa*), A2 and A5 (*C. membranifaciens*) and A6 (*P. guilliermondii*) inhibited the pathogen 3.23, 5.68, 3.23 and 3.8%, respectively. *In vivo* the antagonists significantly reduced decay area caused by *B. mali* at 4°C and 20°C. Isolate A2 (*C. membranifaciens*) at 20°C and isolate A5 (*C. membranifaciens*) at 4°C had the most effect on decay suppression. The population of A2 (*C. membranifaciens*) and A6 (*P. guilliermondii*) increased in wounds of apples during the experiment at 4°C.

3.2 2,4-D, AN IMPORTANT CHEMICAL FOR CONTROL OF POST-HARVEST DECAY IN LISBON LEMON. K. Ayazpour and A. Aboutalebi. *Plant protection department, Islamic Azad University of Jahrom, Iran. Email: Kayazpour@yahoo.com*

To determine the effect of different chemical compounds on post harvest decay in Lisbon lemon under cold (5°C) and ambient (25°C) storage an experiment was conducted in the form of factorial in C.R.D. with 3 replications. Fruits were harvested at maturity and then treated with different concentrations of calcium carbonate, sodium bicarbonate, thiabendazole, benomyl, 2,4,-D and sodium hypochlorite. They were packed in plastic bags and held in storage for 105 days. Results indicated that temperature of storage and different treatments had an effect on fruit decay. Among different treatments, 2,4-D and sodium bicarbonate showed the best effects. Cold storage had a significant effect in controlling post-harvest decay in comparison to ambient storage.

3.3 TRANSCRIPTOMIC AND METABOLOMIC ANALYSES OF INDUCED RESISTANCE IN CITRUS FRUIT AGAINST *PENICILLIUM DIGITATUM*. A.R. Ballester, M.T. Lafuente, J. Gadea, J. Forment and L. González-Candelas. *Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC). P.O. Box 73, Burjassot, 46100 Valencia, Spain. Email: lgonzalez@iata.csic.es*

Penicillium digitatum is one of the major postharvest pathogenic fungi of citrus fruit. Although control is achieved by chemical fungicides, new and safer alternatives, such as induced resistance, are being developed. When citrus fruits are inoculated with the

pathogen and then subjected to heat treatment, disease does not progress and fruits become more resistant to a subsequent pathogen attack. Using this system as a model we have analyzed global changes in gene expression at different times during the induction of resistance. To examine transcript accumulation in the flavedo and albedo tissues (outer coloured and inner white part of the rind, respectively), a cDNA microarray containing 12,000 unigenes generated by the Spanish 'Citrus Functional Genomics Project' was used. Analysis of three independent biological replicates for each time point revealed major changes in expression of genes related to the metabolism of aromatic compounds and phenylpropanoids; these were more noticeable in the inner issue. To gain a deeper insight into this latter pathway we studied changes in the expression of genes coding for PAL, 4CL, F3H, IRL, CADs, SAD and OMTs by Northern blot hybridization. Most of these genes are up-regulated by the induction treatment, but to a higher level in the albedo. We have also performed a metabolic profile analysis of phenolic compounds by HPLC. Scoparone, a hydroxycinnamic acid, showed the highest induction. However, the largest number of changes was observed in benzoic acid derivatives.

3.4 RESISTANCE INDUCED BY ELICITORS IN MELONS FOR CONTROL OF POSTHARVEST DISEASE IN CHINA. Y. Bi. *College of Food Science and Engineering, Gansu Agricultural Universty, Lanzhou 730070, P.R. China. Email: beyang62@163.com*

Melons (*Cucumis melo* L.) are the most important crop grown in the northwestern provinces of China but are highly perishable after harvest. Decay is caused mainly by *Alternaria alternata, Fusarium* spp*., Rhizopus stolonifer* and *Trichothecium roseum.* Postharvest treatment with imazalil and iprodione has been successful in controlling decay of melons. However, concerns about potential impact on public health and environment, as well as development of pathogen resistance to the fungicides, have stimulated the search for alternative control methods. Resistance induced by elicitors, such as acibenzolar-S-methyl (ASM), harpin and soluble silicon, can be part of postharvest disease control of melons. Latent infection and decay of fruit were decreased significantly by pre- or postharvest treatment with these chemicals. Treated fruit developed resistance to pathogen infection. Resistance induced was systemic, broad-spectrum and long-lasting, but rarely provided complete control. The mechanism of induced resistance involved is the accumulation of PR proteins, defence enzymes, antifungal compounds, increasing of activated oxygen, and lignification of epidermal cells. In order to maximize the efficacy of resistance elicitors, a greater understanding of the effect of maturity, postharvest environmental factors, and their interactions is required. Changes in quality induced in fruit also need to be evaluated.

3.5 CHITOSAN REDUCES DRY ROT CAUSED BY *FUSARIUM SULPHUREUM* ON POTATO TUBERS: FUNGISTATIC AND INDUCED-RESISTANCE EFFECTS. Y. Bi. *College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, P.R. China. Email: beyang62@163.com*

Dry rot, caused by *Fusarium* spp. is reported as the most important postharvest disease of potato in China. *F. sulphureum* is one of the principal pathogens. The ability of postharvest application of chitosan to control dry rot was studied on potato cv. Atlantic. The results showed that lesion diameter after inoculation with *F. sulphureum* was significantly reduced in tubers and slices treated with 0.25% chitosan. In vitro tests showed that chitosan decreased mycelium growth, dry weight of mycelium and spore germination. Changes in hyphal morphology were observed such as intertwining, distortion and swelling; inflated mycelium became blasted, wizened and cupped. Alteration of hyphal structure included abnormal distribution of cytoplasm, non-membranous inclusion bodies assembling in cytoplasm, considerable thickening of hyphal cell walls and very frequent septation with malformed septa. New hyphae (daughter hyphae) inside the collapsed hyphal cells were often found in the cytoplasm. Protection by chitosan was also associated with activation of peroxidase (POD), polyphenoloxidase (PPO), phenylalanine ammonia-lyase (PAL), the accumulation of total phenolics and flavanoids, and the production of H_2O_2 and $O_2^{-\cdot}$. This suggests chitosan has dual effects: fungistasis and induced resistance, and is promising as a potential natural compound to control dry rot on potato tubers.

3.6 COMMERCIAL APPLICATIONS OF "SHEMER" FOR THE CONTROL OF PRE- AND POST-HARVEST DISEASES. D. Blachinsky, J. Antonov, A. Bercovitz, B. El-ad, K. Feldman, A. Husid, M. Lazare and M. Keren-Zur. *Agrogreen Minrav Group - Kiryat Minrav Hi-Tech Park, P.O.Box 153, Ashdod 77101, Israel. Email: Email: motik@agrogreen.co.il*

"Shemer" is a biofungicide based on the yeast *Metschnikowia fructicola*. Its mode of action is believed to be mainly through competition. The commercial product is stable under ambient storage, and can be applied by spray or drench application in the field or in packing houses. "Shemer" treatments in commercial packing houses significantly reduced the development of *Penicillium digitatum* on oranges, and of *Rhizopus stolonifer* and *Fusarium* sp. on sweet potatoes. Similarly, significant reduction was achieved in the development of *R. stolonifer* on peaches, *Botrytis cinerea* on pepper and *Sclerotinia sclerotium* on carrots in pilot-scale tests. Application of "Shemer" in the field also proved useful in protecting fruits after harvest, where post-harvest treatments are not practiced. Weekly "Shemer" application on strawberry reduced rot development in the field and also the incidence of grey mould (*B. cinerea*) and Rhizopus fruit rot (*R. stolonifer*) during storage. In table grapes, application of "Shemer" 24 h before harvest, significantly reduced the number of decayed berries caused by *B. cinerea*, *R. stolonifer* or *Aspergillus* after storage. In most of the trials, the level of decay control was comparable to that of the most common chemical fungicides currently used by the industry. These results demonstrate the suitability of "Shemer" to a wide range of crop-pathogen, agricultural practices and climatic conditions.

3.7 *COLLETOTRICHUM ACUTATUM*, A POST-HARVEST PATHOGEN ON APPLE IN NORWAY. J. Børve, R.T. Djønne and A. Stensvand. *Norwegian Institute for Agricultural and Environmental Research, Norway. Email: jorunn.borve@bioforsk.no*

Bitter rot on apple, caused by *Colletotrichum actutatum*, develops symptoms during summer in warm, humid apple-growing regions of southeastern USA, Brazil and New Zealand. In Norway, the disease is severe on the main late ripening cultivar 'Aroma', but symptoms normally develop after some time in cold store. Summer epidemics on apple have never been observed in Norway, and only occasionally have symptoms developed at harvest. In a series of experiments with organically grown 'Aroma' apples picked over five weeks, 6, 14, 35, 33, and 35% (mean of three experiments (in different years) of the apples developed bitter rot if harvested 2 or 1 wk prior to normal harvest time, at normal harvest, or 1 or 2 wk after normal harvest time, respectively. Foliar applications of calcium during summer reduced bitter rot in cold store. In conventionally grown apples, dithianon either applied

once 3 wk prior to harvest or 6 times earlier in the season (against apple scab, *Venturia inaequalis*), reduced the incidence of bitter rot after storage from about 50 to 25%. Various alternative means to reduce bitter rot in organic and conventional apple orchards (including harvest time and applications of calcium salts) and fungicide programmes are currently being developed in Norway.

3.8 USE OF OZONE IN SEED DECONTAMINATION. F. Ciccarese, T. Ziadi, A. Ambrico, A. Ciccarese, M. Sciacovelli and M. Gallo. *Department of Biology and Plant Pathology, University of Bari, Via Amendola 165/A, 70126 Bari, Italy. Email: fciccare@agr.uniba.it*

Surface contamination of seed by pathogenic and/or saprophytes fungi is considered a serious problem because disease may be transmitted or because dangerous mycotoxins may be carried. The mycotoxines (aflatoxin B1, B2, G1, G2, M1, ocratoxin A, fumonisin, zearalenone, etc.) are produced by *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., etc. Ozone is a biocide used widely for microbial decontamination. It is classified as a safe substance "GRAS" (Generally recognized as safe) by the U.S. Food and Drug Administration. We report the results of a trial of ozone treatments in seeds decontamination. A local variety of pea seed collected in Southern Italy from a grower producing the seed himself for sowing, a 1-year old sample of stored wheat grain, cv. Cappelli, and barley grain, cv. Jiaidor, were tested. Ozone at a concentration of 4 ppm was applied as a mixture of ozone + air for 1, 1.5 and 3 minutes. Untreated seeds were used as control. Treated and untreated seeds were analyzed and the fungi developed were identified and counted. *Fusarium* spp., *Alternaria* spp. *Penicillium* spp. and *Aspergillus* spp. were observed in most cases. Exposure to ozone for 1 min was partially effective, whereas treatment for 3 min gave good seed decontamination without any influence on germination.

3.9 CONTROL OF *PENICILLIUM DIGITATUM* IN CITRUS FRUIT BY BERGAMOT ESSENTIAL OIL VAPOURS. S. D'Aquino, L. A. Iuliano, A. Palma and M. Schirra. *CNR - Institute of Sciences of Food Production, Via dei Mille 48, 07100 Sassari, Italy. E-mail:salvatore.daquino@ispa.cnr.it*

The effect of bergamot essential oil on postharvest decay incited by *Penicillium digitatum* Sacc. was evaluated on 'Marsh' grapefruit and 'Tardivo di Ciaculli' mandarin. Wound-inoculated fruits were left to incubate at 20 °C and 90-95% RH for 24 h before treatments. Treatments were carried out at 20 °C in air-tight 200-litre plexiglas cabinets equipped with two fans. For 'Marsh' grapefruits 2 ml of bergamot oil were placed on a strip of Watman paper in a dish and allowed to volatilize (method A) or vaporized by an aerosol device (method B). With 'Tardivo di Ciaculli' mandarins, 2.5 or 5 ml essential oil were supplied by method B. Alfter 24 h of vapour exposure fruit were stored at 20 °C and 90-95% RH and checked for decay after 7 (grapefruit) and 5 or 8 days (mandarin). At the end of storage the incidence of decay in untreated fruit of 'Marsh' grapefruit averaged 16.8%, against 15.6% and 9.8% of those treated by method A and B, respectively. In 'Tardivo di Ciaculli' mandarins after 5 days, decay in untreated fruit was 50%; bergamot oil at 2.5% reduced decay by 48% at 2 ml and by 70% at 5 ml. After 8 days, untreated fruit decay increased to 84.4%, whereas bergamot oil reduced the losses to 59.4 and 44.4%, at 2.5 and 5 ml, respectively. Bergamot oil thus showed good activity against *P. digitatum*, but due to its low volatility it needs to be actively vaporized in the environment.

3.10 PHENOTYPIC AND MOLECULAR VARIATIONS OF *COLLETOTRICHUM* ISOLATES CAUSING POSTHARVEST DISEASES OF DESSERT BANANA. D.M. De Costa and M.D. Kalpage. *Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya, Peradeniya, 20400, Sri Lanka. Email: devikacos@yahoo.com*

Colletotrichum musae causes latent and wound infections of dessert banana grown worldwide leading to severe postharvest losses. In-depth investigations on the pathosystem would facilitate the designing of effective management programmes. This study was conducted to determine the diversity of phenotypic and molecular signatures of different *Colletotrichum* isolates associated with anthracnose, blossom-end rot and crown rot, the major postharvest diseases of banana. Twenty *Colletotrichum* isolates representing the three postharvest diseases infecting six different dessert banana cultivars collected from three different locations in Sri Lanka were used to determine phenotypic and molecular variations. Colony morphology and growth rate on PDA, growth inhibition by the fungicide Daconil (chlorothalonil) and spore morphology were investigated as phenotypic features. Molecular signatures of different isolates was determined based on PCR-RFLP of the rRNA gene cluster. PCR products were digested by four different restriction endonucleases namely, *Rsa*I, *Hha*I, *Hae*III and *Msp*I. All the isolates tested were morphologically different in colony morphology and showed significant variation in spore dimensions and rate of growth. Different fungal isolates showed slight variation in fungicide response. PCR-RFLP of the rDNA product using MspI showed genomic variations among the isolates. The present study revealed the association of morphologically, behaviourally and genomically different *Colletotrichum* isolates with different banana cultivars and postharvest infections, in addition to the commonly existing isolate of *C. musae*.

3.11* MICROBIAL ECOLOGY OF LITCHI FRUIT SURFACE. B.T. Demoz and L. Korsten. *Department of Microbiology and Plant Pathology, University of Pretoria 0002, Pretoria, South Africa. Email: lkorsten@fabi.up.ac.za*

Microbial phyllosphere communities are diverse and include bacterial, fungal and yeast genera, which are pathogenic or non-pathogenic to the host plants. Investigating the dynamics between these communities is crucial in understanding the relationship between epiphytic microorganisms and plant diseases. In view of this, the microbial dynamics of the litchi fruit were studied from flowering till harvest. The study was carried out on cultivar 'Mauritius' in Malelane, South Africa (2004 and 2005 seasons) and 'McLean's Red' in Tzaneen, South Africa (2005 and 2006 seasons). Samples were collected from flowering till harvest from three and four orchards respectively every second week. Total fungal, yeast and bacterial counts were taken of all samples. Results indicate that population density gradually increased from flower bud stage to full bloom and to senescence stage. A population density decrease was observed for both cultivars during both seasons. Although the total *Penicillium* counts fluctuated between samplings, a relatively higher number was observed between flowering and fruit set and at maturity. This could possibly be attributed to the amount of nutrients released from the flowers and the developing fruit and the change in weather conditions during the seasons.

3.12 *IN VITRO* PRODUCTION OF ORGANIC ACIDS BY *PENICILLIUM EXPANSUM*. I. Donati, D. Mazzoni, M. Mari

and P. Bertolini. *CRIOF- DIPROVAL, Alma Mater Studiorum, University of Bologna, Italy. Email: idonati@agrsci.unibo.it*

Twenty isolates of *Penicillium expansum,* recovered from rotten pome fruits, were tested in vitro for ability to produce acid. To measure acid production, isolates were grown on creatine sucrose agar for 7 days at 20°C, the halo round the colony revealing a change of pH. Three isolates out of 20 did not produce the halo, while the others showed wide halos, revealing abundant acid production. The same isolates were also grown in yeast sucrose liquid medium (YSM) at different pH levels (7.0, 5.0, 3.0) for 3 days at 20°C. In general, the greatest reduction of pH was observed in isolates grown at pH 7. Four isolates maintained the pH of the medium close to 7, but the others significantly decreased the pH, raging from 5.5 to 4.1. To determine the prevalent organic acids produced, isolates were grown on liquid YSM for 7 days at 20°C, and the medium was analyzed by HPLC. Results showed abundant production of galacturonic (GA), malic, and citric acids and some unknown organic acids in smaller amounts. A high concentration of GA was recorded in the isolates that most reduced the pH of the medium. These isolates produced GA within 48 h of inoculation, while the concentration of citric and malic acid initially increased, remained constant up to 72 h and then increased again. More work is needed to understand whether organic acid production and the aggressiveness of *P. expansum* isolates are correlated.

3.13 INVOLVEMENT CITRUS FRUIT VOLATILES IN GERMINATION AND GROWTH OF *PENICILLIUM DIGITATUM AND PENICILLIUM ITALICUM*. A. Eick, D. Macarisin, L. Cohen, G. Rafael, M. Wisniewski and S. Droby. *Dept. Postharvest Science, ARO, the Volcani Center, P.O. Box 6, Bet Dagan 5250, Israel. Email: samird@volcani.agri.gov.il*

Volatiles emitted from wounded peel tissue of various citrus cultivars stimulated germination and germ tube elongation of both *Penicillium digitatum* and *Penicillium italicum*, but the effect on *P. digitatum* appeared stronger. When exposed to volatiles from grapefruit, the percentage of germinated spores of *P. digitatum* and *P. italicum* was 10 and 5 fold, respectively as compared to the control. In contrast, *Botrytis cinerea* and *Penicillium expansum* were either not affected or were inhibited by the peel volatiles. GS-MS analysis of volatiles present in the peel of various citrus fruit cultivars revealed that limonene is the major fruit peel volatile. Its percentage ranged from 89% to 95% at the early stages of fruit development throughout the harvest season. Myrcene and α-pinene made up the second and third greatest amounts among the volatiles. All four monoterpenes, limonene α-pinene, β-pinene and myrcene stimulated *P. digitatum* and *P. italicum* but inhibited or had no effect on *P. expansum* and *B. cinerea*. Germ tube elongation in *P. digitatum* responded most strongly to limonene and less strongly to α-pinene and β-pinene while myrcene had little effect. In *P. italicum*, myrcene stimulated germ tube elongation the most followed by limonene, with α-pinene, and β-pinene being about equal. Germination of *P. italicum* conidia was highest in response to myrecene with the effect of the other compounds being about equal at concentrations of 5 µl or more per plate.

3.14 REDUCED RISK STRATEGIES FOR CONTROL OF BLUE MOULD AND GREY MOULD OF APPLES AND PEARS. D. Errampalli and L.I. Wainman. *Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 4902 Victoria Ave. N., Vineland Station, ON L0R 2E0, Canada. Email: errampallid@agr.gc.ca*

Blue mould caused by *Penicillium expansum* Link and grey mould caused by *Botrytis cinerea* Pers.:Fr., are the two most important postharvest diseases of apples and pears in Canada. Intensive and exclusive use of postharvest fungicide, thiabendazole (TBZ), has resulted in selection of TBZ-resistant pathogens in most packinghouses in North America. The effectiveness of reduced-risk postharvest fungicides, fludioxonil, and pyrimethanil, was evaluated against blue mould and grey mould in apples and pears in cold and controlled-atmosphere storages. In post-inoculation treatments, wounded apples (cv. Empire, Gala, McIntosh and red delicious) or pears (cv. Bosc) were inoculated with 1×10^4 conidia/ml of either TBZ-resistant or -sensitive *P. expansum* or *B. cinerea* and incubated for 18-20 h at 13°C, and then drenched with appropriate concentrations of fungicides. Treated fruits were incubated at 2-4°C for 3 months, in controlled atmosphere storages for 4.5 months and at 20°C for 6 days. Fludioxonil at a concentration of 300 µg a.i./ml and pyrimethanil at a concentration of 500 µg a.i./ml controlled blue mould and grey mould in apples and pears. The fungicides were effective in the co-treatment and in the post-inoculation treatment. Fludioxonil and pyrimethanil can provide an alternative to TBZ in postharvest control of blue mould and grey mould of apples and pears, where any TBZ-resistant spores are present. These two new reduced-risk fungicides can be incorporated in postharevest disease management strategies for the control of blue mould and grey mould of apples and pears.

3.15 REDUCTION OF ANTHRACNOSE INFECTIONS BY A PROTEIN INHIBITOR EXTRACTED FROM 'PINK LADY' APPLE. R. Gregori, M. Mari, P. Bertolini, J.A. Sañudo Barajas, J.B. Tian and J.M. Labavitch. *CRIOF - Department of Agri-Food Protection and Improvement, University of Bologna, Italy. Email: rgregori@agrsci.unibo.it*

The majority of fungi gain access to plant cells by breaking the cell wall barrier and secreting enzymes able to degrade cell wall polymers. Cell wall enzymes (polygalacturonase–PG, protease, pectin lyase, etc.) are a wide group which mainly catalyse the hydrolysis of proteins; they are relevant in the "break down" of host cells and are regarded as important pathogenicity factors for soft-rot fungi. Evidence shows that the PGs produced by *Colletotrichum acutatum*, a fungal pathogen of fruit, are involved in the hydrolysis of cell walls when grown on pectin or apple cell walls. No data are available on the ability of proteins from stored apple to inhibit the activity of *C. acutatum* hydrolases. An enzymatic inhibitor complex was extracted from healthy apples, cv Pink Lady®, after storage, and evaluated against *C. acutatum* endo-polygalacturonase (EC 3.2.1.15) in *in vitro* and *in vivo* trials. In *in vitro* trials the inhibition determined by radial diffusion assay was over 62% after 24 h while in inoculated fruit the inhibition ranged from 33.9% to 54.4% after 4 days at 20°C. The PG inhibitor extracted from healthy apple skin was a heat-denaturable protein since the halo produced by protein extracted from *C. acutatum* and added to boiled protein extracted from healthy apple skin tissue was 246 mm^2, significantly higher than the halo produced by protein extracted from *C. acutatum* diluted with fresh protein extracted from healthy tissue (93.6 mm^2). More investigations are required to evaluated the possibility of reducing antrachnose infections by manipulating PGIP levels in fruit.

3.16 MYCOFLORA OF POMEGRANATE FRUITS. K. Grigoryan, H. Lusine and B. Gohar. *Charents 4-117,0025, Yerevan, Armenia. Email: foodlab@inbox.ru*

Juice made from raw materials highly contaminated by filamentous fungi are a risk for consumer health. The basic purpose of this research was to study the mycobiota of pomegranate fruits in the post-harvest period and determine the specific and dominating species of filamentous fungi. During the years 2005-2007 we studied more than 300 samples of pomegranate fruits from Iran, Afghanistan, Azerbaijan and Armenia. Pomegranate often is contaminated by *Penicillium* species from the sections *Monoverticillata* and *Biverticillata*, among which *P. implicatum* Biourge and *P. variabile* Sopp are the most typical pathogenic species. The basic route of contamination of fruits with *P. implicatum* and other species are the stamens, with further penetration of micromycetes into the fruit. This species is characterised by high growth rate on a substrate, and in most cases in a fruit there is monopoly development of this species with contamination index =1. Contamination of pomegranates with this fungus was not found to depend on the variety, climato-geographical conditions or country of origin.

3.17 *PENICILLIUM* INOCULUM IN THE CITRUS COLD CHAIN: AN INDICATOR OF HYGIENE STANDARDS. R. Jacobs and L. Korsten. *Dept. Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002, South Africa. Email: rene.jacobs@fabi.up.ac.za*

Penicillium species are responsible for excessive post-harvest decay of fresh fruit during cold storage and export. The evolutionary function of many *Penicillium* species is the decay of dead or dying organic material. Once harvested, fruits shift their metabolism to senescence, which creates an ideal environment for colonization by opportunistic decay fungi such as *Pencillium* spp. Fruit storage environments are therefore favourable for these pathogens. Since most species are soil-borne, the presence and inoculum load of these species in various indoor environments is indicative of the level of hygiene being enforced. The aim of this study was to follow the citrus cold chain from local packhouses in South Africa to various European destinations, and sample environments such as packhouses, coldrooms, re-pack facilities, distribution centres and retail outlets that the fruit moves through. Swabs were taken from walls and floors, packlines, rollers, brushes etc and processed by dilution plating. All *Penicillium* spp. were isolated, purified, preserved and morphologically identified, and identity confirmed using ITS and β-tubulin sequence data. The dominant species isolated were *P. glabrum, P. crysogenum, P. polonicum, P. paneum, P. corylohilum, P. crustosum, P. brevicompactum, P. biourgeianum, P. commune* and *P. citrinum.* Inoculum levels were relatively high for walls and floors of repacking and storage facilities. The level and type of *Penicillium* spp present in these environments provided evidence of the hygiene standards enforced and can represent a baseline for international hygiene standards.

3.18 IDENTIFICATION OF AIRBORNE *PENICILLIUM* SPECIES IN THE SOUTH AFRICAN LITCHI EXPORT CHAIN. C.L. Johnston, R. Jacobs and L. Korsten. *Plant Pathology Department, University of Pretoria, Pretoria 0002, South Africa. Email: candice@student.up.ac.za*

Penicillium is one of the most common fungal genera encountered in the environment. Being a natural soil inhabitant, it's presence on litchi fruit may indicate cross-contamination. This can cause major losses to the fruit export industry, as postharvest decay of litchi fruit by *Penicillium* species dramatically reduces the shelf-life of the fruit. This study was aimed at identifying 58 dominant *Penicillium* groups throughout the South African litchi export chain. Morphological, as well as molecular methods such as DNA sequencing and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) were used in the identification. The Internally Transcribed Spacer (ITS) and beta tubulin (β-tubulin) gene regions were analysed in this study. Eighteen of the most dominant *Penicillium* species isolated during the 2004-2005, 2005-2006 and 2006-2007 seasons were identified as *P. glabrum*, *P. biourgeianum*, *P. citreonigrum*, *P. paneum*, *P. solitum*, *P. crustosum*, *P. expansum*, *P. brevicompactum*, *P. polonicum*, *P. citrinum*, *P. chrysogenum*, *P. bialowiezense*, *P. echinulatum*, *P. corylophilum*, *P. commune*, *P. piscarium*, *P. sumatrense* and *P. italicum*. Fourteen of these 18 species could be differentiated from one another through PCR-RFLP of the β-tubulin gene region. The remaining four groups (*P. solitum*, *P. crustosum*, *P. commune* and *P. echinulatum*) showed little variation in the banding patterns of the ITS region, while the β-tubulin region appeared to be highly variable. The ITS gene region is highly conserved and serves as a taxonomic indicator for *Penicillium* species while β-tubulin is more variable and can be used to differentiate between closely related species.

3.19 EFFECTIVENESS OF *TRICHODERMA VIRENS* IN CONTROLLING MANGO ANTHRACNOSE. J. Jomduang, S. Aemprapa and T. Manond. *Lampang Agricultural Research and Training Center, Rajamangala University of Technology Lanna, P.O. Box 89, Muang District, Lampang 52000, Thailand. Email: jinantanaj2@yahoo.com*

Chemical fungicides have been used for controlling mango anthracnose, caused by *Colletotrichum gloeosporioides,* for a long time in Thailand. However, the disease still causes serious damage on mango produce. Therefore, antagonistic microorganisms are proposed as alternatives. The present study was done to evaluate the effectiveness of *Trichoderma virens* to control anthracnose by spraying on mango trees in an orchard, 3 or 5 times starting from bearing thumb-sized fruits. Three sprays were applied at 30-day intervals while five sprays were applied at 15-day intervals. Air-dried PDB-biomass of *T. virens* mixed with rice bran (1:1 w/w) was used at a concentration of 100 g per 20 litre of water. Anthracnose development on mango fruits was determined, using 0-6 disease scoring, at two weeks after ripening. The result showed that 5 sprays of *T. virens* could reduce anthracnose development as effectively as 3 sprays of the chemical fungicide mancozeb. This was indicated by disease scores of 1.09 and 1.00, respectively. Five sprays of mancozeb provided the best control (disease score of 0.6). Meanwhile, three *T. virens* sprays provided satisfactory control (disease score of 1.58) even though less effective than the fore-mentioned treatments. All treatments significantly reduced anthracnose compared to the non-spraying (control) treatment which showed a disease score of 2.49.

3.20 FACTORS WITHIN THE FRUIT CHAIN THAT IMPACT ON POSTHARVEST FRUIT QUALITY. L. Korsten. *University of Pretoria, Department of Microbiology and Plant Pathology, South Africa. Email: lise.korsten@up.ac.za*

Postharvest fruit quality and product safety were monitored throughout the citrus and litchi postharvest production chains. The impact of preharvest production practices on product quality were assessed by monitoring litchi fruit development, microbial population dynamics, wax composition and presence of *Pencillium* spp. from flowering till harvest. Comparing production practices, temperature, humidity, rainfall, irrigation schedules,

fertiliser programmes and quality parameters i.e. weight, Hunter colour values, titratable acidity, soluble solids concentration etc. provided a best practices profile using Multivariate Canonical Variate Analysis (CVA). Different integrated treatments in combination with modified atmosphere packaging (MAP) low-density polyethylene (LDPE) packaging or biorientated polypropylene (BOPP) was evaluated as alternatives to replace commercial sulphur dioxide litchi treatments. Quality and sensory analysis were performed to determine the colour retention of the pericarp and aril, flavor qualities, taste, odour, juiciness and overall acceptability. Dip treatments alone or in combinations with EDTA or 4-Hexylresorcinol, prochloraz or *Bacillus subtilus* (10^7 cfu ml^{-1}) effectively reduced *Pencillium* decay while MAP could retain the colour up to 32 days after harvest without compromizing taste and flavour. Survival of *B. subtilus* and of total bacterial populations were higher, with low yeast populations in BOPP. *Candida, Cryptococcus* and *Zygosaccharomyces* were predominant yeasts in all LDPE treatments. The presence of *Pencillium* spp. and foodborne pathogens throughout the litchi and citrus fruit chains were monitored using standard microbiological assays on selective media. The diversity of *Pencillium* spp. increased significantly towards the end of the chain as the products became exposed to different fruit types and increased temperature deviations. *Staphylocccous aureus* was prevalent throughout the chain.

3.21 LONG-TERM STORAGE AND SEED SOURCE VERSUS FUNGI COMMUNITIES ON *PINUS SYLVESTRIS* SEEDS. A. Kowalczyk, M. Mańka and M. Kacprzak. *Agricultural University, Department of Forest Pathology, Wojska Polskiego 71c, 60-625 Poznan, Poland. Email: mmanka@au.poznan.pl*

Scots pine (*Pinus sylvestris*) is one of the main tree species in Polish forests (regenerated mainly artificially). Production of healthy seedlings from stored seeds is a priority but pathogenic fungi on seeds and seedlings contribute to losses in forest nursery production. Thus, it is of utmost importance to produce clean, healthy seeds and store them free of pathogens. The effects of storage time and location of stands from which seeds were collected, on seed inhabiting-fungi were investigated. The number of fungal pathogens on seeds increased with storage time. The fungal communities varied depending on storage time and seed source. After a few years their structure in seed lots depended more on storage time than on seed source. With increasing storage time, the structure of fungal communities became more and more similar. The method of seed extraction from cones was of no importance to the structure of the fungal communities. The most common seed lot-inhabiting fungi in 2003-2006 were the following non-specific species/genera: *Alternaria* spp., *Aspergillus* spp., *Cladosporium cladosporioides, Fusarium* spp., *Penicillium* spp., *Sclerotinia sclerotiorum, Trichoderma* spp., with prevalence of the following: *S. sclerotiorum, C. cladosporioides, Aspergillus niger, Alternaria alternata, Fusarium solani, Penicillium commune* and *P. expansum.*

3.22 IDENTIFICATION OF *PENICILLIUM DIGITATUM* GENES PUTATIVELY INVOLVED IN VIRULENCE/PATHOGENICITY TOWARDS CITRUS FRUIT. M. López-Pérez and L. González-Candelas. *Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), P.O. Box 73, Burjassot, 46100 Valencia, Spain. Email: mlopez@iata.csic.es*

Penicillium digitatum is the major post harvest pathogen of citrus fruit, being responsible for up to 80% of the losses due to decay. This necrotrophic fungus shows a very restricted host range, infecting only citrus fruit. Despite this fact, our knoweldege of the factors involved in pathogenicity is very scarce; therefore we aimed to study at the molecular level the mechanisms involved in pathogenicity and virulence of this fungus. We have applied the Suppression Subtractive Hybridization (SSH) technique to obtain a cDNA library enriched in *P. digitatum* genes that are upregulated during infection using a mixture of RNAs from uninfected fruit and *in vitro* grown fungus as a "driver" and RNA from infected fruit tissue as "tester". After two rounds of PCR amplification subtracted cDNA fragments were ligated into plasmid pCRII and cloned into *E. coli* competent cells. DNA inserts from 1440 clones together with positive and negative controls were PCR-amplified and spotted onto replicate nylon membranes. Using this macroarray we have identified *P. digitatum* genes that are upregulated during infection as compared to *in vitro* growth conditions and thus are good candidates to be involved in pathogenicity. The results of these hybridizations together with sequence analysis of a number of clones constitute a first step to elucidate the virulence/pathogenicity determinants of this important citrus pathogen. We will also present a more detailed Northern blot analysis of selected *P. digitatum* genes.

3.23* SUPPRESSION OF THE DEFENCE-RELATED HYDROGEN PEROXIDE BURST BY *PENICILLIUM DIGITATUM* DURING INFECTION OF CITRUS FRUIT. D. Macarisin, M. Wisniewski and S. Droby. *USDA-ARS, 2217 Wiltshire Rd, Kearneysville, WV, 25430, USA. Email: michael.wisniewski@ars.usda.gov*

Current knowledge of plant-fungal interactions postulates that a plant's basal immune system can detect microbe-associated-molecular patterns (MAMPs), activating a strong defence response. Pathogenic fungi, however, can counteract these defenses by suppressing signal transduction or gene expression in plant cells, or by producing enzymes that neutralize antifungal compounds. The present research shows that the postharvest pathogen, *Penicillium digitatum,* the causal agent of green mould, actively suppresses a defence-related hydrogen peroxide (H_2O_2) burst in citrus fruit. In contrast, inoculation of citrus fruit with a non-pathogenic fungus, *P. expansum,* triggers massive production of H_2O_2 by flavedo tissue. Both fungi induce an elevation in H_2O_2 levels in citrus fruit exocarp from 8 to 17 h after inoculation. Thereafter, *P. digitatum* suppresses H_2O_2 production by host cells and by 66 h the H_2O_2 level was three-fold below that in uninoculated controls. In wound sites inoculated with *P. expansum,* the level of H_2O_2 was 11-fold above the control value at this time. Enzymatic removal of H_2O_2 by exogenous catalase, or specific suppression of H_2O_2 production in flavedo tissue by exogenous citric acid, significantly ($P \leq 0.05$) enhanced pathogenicity of *P. digitatum* and even allowed non-pathogenic *P. expansum* to develop lesions on lemon, orange and grapefruit. Our results, together with recent reports suggesting the potential involvement of citric acid and catalase in green mould pathogenesis, indicate that in suppressing the defence-related hydrogen peroxide burst in citrus fruit, these compounds could act as pathogenicity factors for *P. digitatum.*

3.24 PRE- AND POST-HARVEST MANAGEMENT TO CONTROL *BOTRYTIS* STORAGE ROT IN NEW ZEALAND KIWIFRUIT. M.A. Manning and R.M. Beresford. *HortResearch, Mt Albert Research Centre, Private Bag 92169 Auckland 1142, Australia. Email: rberesford@hortresearch.co.nz*

Storage rot (*Botrytis cinerea)* of 'Hayward' kiwifruit became a serious problem in New Zealand during the 1980s, costing the

NZ$200m industry up to NZ$10m per year. Disease symptoms develop during 4-8 weeks storage at 0°C. A single rotting fruit in a tray can cause the whole tray to soften prematurely. Control attempts with pre-harvest fungicides led to resistance in *B. cinerea* to dicarboximide and benzimidazole fungicides. *B. cinerea* is only visible in the orchard on flower petals, although it occurs on all plant surfaces, in understory weeds and in necrotic kiwifruit leaves. Infection occurs through the picking wound at harvest. Research into storage rot risk factors revealed a relationship between rot incidence and the incidence of *B. cinerea* on discs of necrotic kiwifruit leaf tissue sampled late in the growing season. From this, a predictive system has been developed that can identify high-risk orchards. The botrytis problem has largely been solved by vine management that avoids dense leaf canopies and thereby avoids necrotic leaf tissue on which *B. cinerea* multiplies. It was also found that the picking wound can be "cured" by storing fruit for 48 hours at ambient temperature before cooling, which greatly reduces rot incidence. Incidence is also reduced by harvesting fruit when they are more mature (>6º Brix), as riper fruit are much less susceptible to botrytis than immature fruit. The botrytis storage rot problem has thus been avoided by a combination of pre-harvest orchard management and post-harvest handling practices, without the need for intervention with fungicides.

9.1 FOOD INDUSTRY BY-PRODUCTS AS RAW MATERIAL FOR PRODUCTION OF THE BIOCONTROL AGENT *PANTOEA AGGLOMERANS* PBC-1. **T. Manso, C. Nunes, D. Neto, J. Pardão, S. Raposo and E. Lima-Costa.** *Univerdiade do Algarve, Fern Campus de Gambelas, 8000 Faro, Portugal. Email: temanso@gmail.com*

Pantoea agglomerans PBC-1 was originally isolated from the surface of oranges and can control postharvest pathogens in pome and citrus fruits. The use of a biocontrol agent depends on developing a culture medium which can produce large amounts at low cost. In this study, food industry by-products were investigated to assess their potential as a raw material for production of the biocontrol agent *Pantoea agglomerans* PBC-1. Carob extract, sugar beet molasses, orange by-products, and commercial sugar were used as carbon source. Viable populations were determined at the end of 24 h incubation at 30ºC under orbital agitation at 150 rev min^{-1}. No differences were observed between the various media studied when compared with the standard media; the viable populations ranged between 1×10^9 an 3×10^9 $cfu.ml^{-1}$. The biomass produced in the different media was then evaluated against blue mould of apple fruit. Fruits were wounded artificially, treated with a *P. agglomerans* suspension at 1×10^8 $cfu.ml^{-1}$ and infected with *Penicillium expansum* at 1×10^4 $spores.ml^{-1}$. The greatest reduction in lesion diameter (99%) and incidence (95%) were obtained by treating with *P. agglomerans* produced with carob extract. The biomass productivity and efficacy against the pathogen obtained with the different by-products show that these industrial by-products can be used efficiently to produce biocontrol agents.

9.2 NEW APPROACHES TO POSTHARVEST DISEASE CONTROL IN EUROPE. **M. Mari, F. Neri and P. Bertolini.** *Universityof Bologna, Italy. Email: marta.mari@unibo.it*

Alternatives to fungicide treatment to prevent postharvest fruit losses have been studied. Applications of (a), biological control agents (BCAs), (b) plant bioactive compounds and (c) elicitors of resistance showed results that were interesting but still far from a practical application in Europe. So far, despite the substantial progress obtained with BCAs, no biofungicide has been registered in Europe to control postharvest pathogens, also because of insufficient and inconsistent performance. The use of plant bioactive compounds has shown that the treatment conditions (concentration, form of application, formulation, exposure time, time of treatment, etc.) can strongly influence their efficacy. The different responses found in many studies indicate a cultivar specificity in the product-pathogen-volatile interaction. Apart from efficacy, a barrier to the use of plant bioactive compounds may be the off-odours caused in fruits and vegetables, and/or phytotoxicity. Elicitors (acibenzolar, chitosan, jasmonate, salicyilic acid, heat, etc.) showed sometimes inconsistent fungicidal activity, limited to fungistatic effects and related to treatment timing and the developmental stage of the plant. Heat treatments by hot water dips, hot dry air, vapour heat or very short water rinse and brushing appear more promising. To overcome the drawbacks that have arisen with the these methods, integration of the antagonist with other treatments such as low toxic substances, heat, etc. has been proposed; this strategy could produce an additive or synergic effect on disease control and obtain satisfactory levels of disease reduction.

9.3 *CHALARA THIELAVIOIDES* CAUSES BLACK ROOT ROT OF CARROTS DURING LONG-TERM COLD STORAGE. **M. Mayama.** *Food Microbiology Lab., Shikoku university, Tokushima, Japan. Email: mayama-mari@shikoku-u.ac.jp*

Black root rot of carrots has been one of the most common post-harvest diseases in supermarkets as well as domestic refrigerators in Japan. It is especially noted that root rot occurs in spite of storage at low temperatures. In carrot fields, such as in Hokkaido in the northern part of Japan, two species, *Chalara elegans* and *Chalara thielavioides* have been detected as soilborne pathogens of black root rot. However, the pathogen isolated from black lesions on carrots, that were stored at low temperature and distributed to the market, was exclusively *C. thielavioides*. Thus, the effects of temperature on the growth of two *Chalara* species on potato-dextrose agar and the symptom development on inoculated carrot roots were examined. The data showed that mycelial growth and the formation of chlamydospores and phialospores of *C. thielavioides* occurred even at 2.5°C and 5°C, but not with *C. elegans*. In fact, black rot lesions developed on *C. thielaviodes*-inoculated carrots, but not on *C. elegans*-inoculated carrots, even if stored for two months. Conversely, the growth of *C. elegans* was better than that of *C. thielaviodes* at 20-30°C. The present study indicates that *C. thielavioides* is highly tolerant of low temperatures and can cause black root rot on carrots as a serious postharvest disease during long-term cold storage.

9.4 INACTIVATION OF *PENICILLIUM EXPANSUM* POLYGALACTURONASE USING ANTIBODY TECHNOLOGY. **J.L. McEvoy, W.J. Janisiewicz and W.S. Conway.** *USDA-ARS, Appalachian Fruit Research Station, Kearneysville, W V, USA. Email: Wojciech.Janisiewicz@ars.usda.gov*

The blue mould pathogen, *Penicillium expansum*, produces polygalacturonase (PG) during decay of apples in storage. PG, believed to be a major virulence factor, is produced in a medium-dependent fashion. Four or more isozymes are produced in culture depending on the medium but only one major and one minor isozyme are produced in apple tissue. The in *vivo*-produced isozymes were purified using ammonium sulfate precipitation, gel filtration and ion exchange techniques. Fractions from the two

peaks of activity separated by gel filtration were used in generating anti-PG polyclonal antibodies in mice. Antibodies raised against the major PG peak were found to inhibit enzyme activity by up to 60% in *in vitro* assays. Current work, which includes enzyme inactivation on fruit (and its effect on virulence) and the generation and use of recombinant antibodies to determine the role of individual isozymes in disease development, will be discussed.

9.5 THE PEACH STORY. **P. Melgarejo.** *INIA-Department of Plant Protection, Crtra. de la Coruña km. 7, 28040 Madrid, Spain. Email: melgar@inia.es*

One of the most important postharvest disease of peaches is brown rot caused by different species of the fungus *Monilinia.* Anamorphs dominate as inoculum sources especially in the Mediterranean areas of Europe, where brown rot in peaches is caused by *M. laxa* and *M. fructigena*. A third species, *M. fructicola* causes brown rot in other parts of the world and is included in the A2 list of quarantine organisms for Europe (organisms present in the EPPO region, but contained, under official control) because its broad dissemination in Europe would be devastating especially for peach and nectarine. These fungi overwinter and produce mycelium in fruit mummies and infected wood. This produces conidia under favorable conditions or from stromata that produce ascospores in the case of *M. fructicola*. Fruit infection by conidia of *Monilinia* spp. can occur secondarily from any infected tissue in which the moisture content is sufficient for sporulation. When the microclimate is unfavourable, infections may remain latent until conditions favour disease expression, which finally leads to fruit rot. Correlation between the incidence of rotting and latent infection caused by *Monilinia* spp. has been reported. Management of orchards focused to decrease postharvest brown rot will be treated here. Detection and identification methods, inoculum sources, and epidemiological factors affecting latent fruit infections and postharvest brown rot will be described, together with the models available to predict disease risk. Different integrated control strategies will be presented.

9.6 EVALUATION OF A NEW DISINFECTANT FOR CONTROL OF CITRUS POSTHARVEST DISEASES. **M. Mitidieri, V. Saliva, V. Brambilla, E. Piris, A. Miranda, N. Mancuso and A. Valenzuela.** *INTA San Pedro, CC 43, CP 2930, San Pedro, Bs. As., Argentina. Email: mmariel@correo.inta.gov.ar*

The development of safe postharvest treatment alternatives is a human health and environment protection concern and a key for fresh citrus fruit export. The objective of this experiment was the evaluation of a new disinfectant: PHMG poly-hexametilen guanidine, provided by Diransa San Luis S.A. under two formulations (hygisoft pH and hygisoft V-20). Treatments were applied by dipping orange fruits (cv Washington Navel) for two minutes in one of the following: 200 ppm sodium hypochlorite, 0.5% or 1% hygisoft pH, 1% hygisoft V-20, 3% sodium bicarbonate, 1 or 4 % peracetic acid, 0.5% or 1% hygisoft pH in combination with 3% sodium bicarbonate. Fruits were conditioned in cages of 20 units and stored for 45 days in a packinghouse storage chamber at 4°C. A completely randomized design was used with four repetitions. The *in vitro* effect of Hygisoft pH and Hygisoft V-20 on a suspension of the bacterium *Xanthomonas axonopodis* pv *citri,* the causal agent of citrus canker, was also assayed. After 45 days of storage, 1% hygisoft V-20 and 0.5% or 1% hygisoft pH in combination with 3% sodium bicarbonate, significantly decreased green mould incidence caused by *Penicillium digitatum*. The values registered were 1.25%, 5% and 6.25% for these treatments respectively whereas Green mould incidence for the untreated control was 28.8%. Hygisoft pH and Hygisoft V-20 (1 or 2%) inhibited *Xanthomonas axonopodis* pv *citri in vitro* growth in three assays. The experiment will be repeated in order to assess the potential of these disinfectants for control of citrus postharvest decay.

9.7 BIODIVERSITY OF *FUSARIUM* SPECIES IN MEXICO ASSOCIATED WITH EAR ROT IN MAIZE. **I. Morales-Rodríguez, M. de J. Yáñez-Morales, H.V. Silva-Rojas, G. García-de-los-Santos and D.A. Guzmán-de-Peña.** *Colegio de Postgraduados, Campus Montecillo, Seed Production Program and Plant Pathology Dept., km 36.5 Carr. Mexico-Texcoco, Montecillo-Texcoco, Edo. de México, CP 56230, Mexico. Email: imorales@colpos.mx*

Fusarium proliferatum, *Fusarium subglutinans*, and *Fusarium verticillioides* are known causes of ear and kernel rot in maize worldwide. In Mexico, only *F. verticillioides* and *F. subglutinans*, have been reported previously as causal agents of this disease. However, *Fusarium* isolates with different morphological characteristics were obtained in the Highland-Valley region of this country from symptomatic and symptomless ears of native and commercial maize genotypes. Moreover, besides the morphological studies, analyses based on the Internal Transcribed Spacer region and the Nuclear Large Subunit Ribosomal partial sequences led to the identification of identification of *F. subglutinans*, *F. solani*, and *F. verticillioides*, as well as four species (*F. chlamydosporum*, *F. napiforme*, *F. poae*, and *F. pseudonygamai*) that had not previously been reported to be associated with ear rot. In addition, *F. napiforme* and *F. solani* were absent from symptomless kernels. Phylogenetic analysis showed genetic changes in *F. napiforme* and *F. pseudonygamai* isolates. Our results suggest that the biodiversity of *Fusarium* species involved in ear rot in Mexico is greater than reported previously in other places in the world. This new knowledge will permit a better understanding of the relationship between all the species involved in ear rot disease and their relationship with maize.

9.8 MOLECULAR APPROACH TO MONITOR THE POSTHARVEST BIOCONTROL AGENT *PANTOEA AGGLOMERANS* CPA-2. **C. Nunes, V. Stepien, T. Manso, R. Torres, J. Usall and H. Jijakli.** *Centro de Desenvolvimento de Ciências e Técnicas de Produção Vegetal, University of Algarve, Faro, Portugal. Email: canunes@ualg.pt*

A monitoring technique was developed to identify and quantify the population of biocontrol agent *Pantoea agglomerans* CPA-2. To identify molecular markers the RAPD technique was applied to a collection of 13 strains of *P. agglomerans*, including CPA-2. The primer OPL-11 amplified a fragment (about 720 bp) specific to strain CPA-2 and on the basis of this fragment, two SCAR markers were amplified. A first SCAR marker of 720 bp was specifically amplified for the strain CPA-2 and a second one of 270 bp was obtained for all *P. agglomerans* strains tested, including CPA-2. To quantify the population, formulations of *P. agglomerans* CPA-2 in commercial trials were determined on fruit surfaces and in the environment using both the classical plating technique and PCR with SCAR primers. Regarding population level, the two methods, in general, gave similar results. On fruit surfaces, one day after CPA-2 application its population estimated by classical methods was 4.37×10^6 cfu wound^{-1} and at the end of the experiment the population increased to 5.8×10^5 cfu

wound^{-1}. The percentages of colonies identified as *P. agglomerans* CPA-2 at these sampling times using SCAR primers were 90% and 95% respectively. Population dynamics studies of *P. agglomerans* CPA-2 showed it has a limited persistence and limited capacity for dispersion. Commercial trials demonstrated a significant reduction of decay on fruit after treatment with formulated cells of *P. agglomerans* CPA-2.

9.9 RESISTANCE TO CHEMICAL SEED AND POST-HARVEST TREATMENTS IN ISOLATES OF *FUSARIUM* SPP. CAUSING POTATO SEED-PIECE DECAY AND TUBER DRY ROT IN ATLANTIC CANADA. R.D. Peters, K.A. Seifert and H.W. (Bud) Platt. *Agriculture and Agri-Food Canada, 440 University Ave., Charlottetown, PE C1A 4N6, Canada. Email: petersr@agr.gc.ca*

Various *Fusarium* spp. can cause potato seed-piece decay prior to planting and tuber dry rot in storage, resulting in tuber yield and quality losses. In Atlantic Canada, *F. sambucinum*, *F. coeruleum* and *F. avenaceum* are the species most commonly isolated from diseased tuber tissue. Testing of isolates of *Fusarium* spp. collected in Atlantic Canada from 2000-2007 for resistance to thiophanate-methyl and thiabendazole using amended agar assays has revealed the association of resistance with a particular species. All isolates of *F. sambucinum* recovered during this time period were resistant to both thiophanate-methyl and thiabendazole, whereas all isolates of *F. coeruleum* and *F. avenaceum* were sensitive to both compounds. In field trials where potato seed pieces were inoculated with either *F. sambucinum* or *F. coeruleum* and then treated with either thiophanate-methyl, fludioxonil or water (control), significant yield increases (compared to the inoculated control plots) were achieved with both chemical seed treatments when seed pieces were inoculated with *F. coeruleum*. However, only plants in plots grown from fludioxonil-treated seed yielded significantly more than plants in inoculated control plots when *F. sambucinum* was used for inoculation. More recently (spring 2007), isolates of *F. sambucinum* and *F. coeruleum* resistant to fludioxonil in amended agar assays have been recovered in Atlantic Canada. Since the isolates of *F. sambucinum* were also resistant to thiophanate-methyl and thiabendazole, multi-class (benzimidazole and pyrrole) resistance was also documented. Resistance of *Fusarium* spp. to chemical products is increasing the challenge of managing potato seed-piece decay and tuber dry rot in Atlantic Canada.

9.10 ENZYMATIC ACTIVITIES OF INFECTED AND HEALTHY POST-HARVEST TUBERS OF CASSAVA (*MANIHOT ESCULENTA*). A.O. Salami and A.K. Akintokun. *Department of Plant Science, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. Email: sola1salami@yahoo.com*

Post-harvest rots of three cultivars, namely TMS 4 (2) 1425 (hybrid cultivar) and two local cultivars Oko-Iyawo and Odongbo, of cassava (*Manihot esculenta* Crantz) were surveyed in southwestern Nigeria. A total of ten fungi were isolated from rotted cassava tubers collected from eight towns in south western Nigeria. The most frequently isolated and pathogenic ones used in this study as test pathogens were *Lasiodiplodia theobromae*, *Macrophomina phaseolina*, *Rhizopus stolonifer* and *Fusarium pallidoroseum*. In all the three cultivars inoculated with each of the test pathogens, enzyme activities were found to increase with incubation period between 6 and 8 days of inoculation and declined at day 10. PME and PG activities were found highest in cultivar TMS 4(2) 1425 and least in Odongbo. The test pathogens also behaved differently when inoculated with the culture filtrates of the different enzyme activities. *M. phaseolina* was found highest while *F. pallidoroseum* was the least in all the enzyme activities cultured except in pectin methyl esterase where *L. theobromae* had the highest activity. Mycelium dry weight of the test pathogens were also found to increase with incubation period with *F. pallidoroseum* having the highest weight of mycelial mats while *L. theobromae* had the least. Enzyme production and activities of the test pathogens in the utilization of carbon sources varied with the different carbon sources and the test pathogens. Generally, enzyme activities reflected the rate of maceration of tissues and the extent of rot disease of cassava tubers as well as the aggressiveness and pathogenic abilities of the test pathogens.

9.11 CHARACTERISATION OF *PENICILLIUM DIGITATUM* ISOLATES FOR FUNGICIDE RESISTANCE. P. Sánchez-Torres and J.J. Tuset. *Instituto Valenciano de Investigaciones Agrarias, Ctra Moncada-Náquera km 4,5, 46113 Moncada-Valencia, Spain. Email: palomas@ivia.es*

Citrus green mould, caused by *Penicillium digitatum* is one of the most important postharvest diseases of citrus. Fungicide treatments are a key component in the integrated management of many plant diseases, therefore the appearance of resistance has become an important factor in limiting the efficacy. The most recent thought for fungicide resistance is based on active eflux of these toxic compounds due to transporters like ABC (ATP binding cassette) or MFS (Major Facilitator Superfamily) that have a remarkably broad susbstrate specificity although they can also transport specific compounds. Despite the size of the economic losses caused by this fungus, another important aspect is that some of these resistant genes may be involved in pathogenicty/virulence. To uncover these factors relevant to fungicide resistance we analysed at the molecular level the differences between resistant and sensitive *P. digitatum* strains. A single point mutation in the β-tubulin gene was found to be reponsible for thiabendazol resistance while different mutations were identified in two ABC transporters, PMR1 and PMR5. The method involved detection of the presence of the tandem repeat of five copies of a 126 bp transcriptional in the promoter region of PdCYP51, leading to overexpression of this gene and conferment of DMI resistance to the fungus. The absence of this repeat does not imply that *P. digitatum* is DMI-sensisitive. In fact, many isolates with a high degree of resistance to DMIs only exhibited one repeat, supporting the hypotheis that many factors could be involved in fungicide resistance.

9.12* RESISTANCE OF MANGO FRUITS AND SEEDLING LEAVES TO *COLLETOTRICHUM GLOEOSPORIOIDES*, CAUSAL PATHOGEN OF ANTHRACNOSE. S. Sangchote. *Dept. of Plant pathology, Kasetsart Uni., 50 Jatuchuk, Bangkok 10900, Thailand. Email: agrsrs@ku.ac.th*

Anthracnose severity on mango fruits of five cultivars including Nam Dorkmai, Nang Klang Wan, Chok Anan, Khew, and Rad, naturally infected with *Colletotrichum gloeosporioides*, was assessed for 2 years. Khew was rather resistant whereas Nang Klang Wan was susceptible. Fruits of these five cultivars were also evaluated by inoculating with *C. gloeosporioides* at 48 h before and after harvest. In both periods, disease severity on the fruit was in line with cultivar susceptibility. Fruits inoculated 6 h after harvest showed lower disease severity than at 24 h. Seedling

leaves starting from the unfolded leaf up to 15 days were tested for their susceptibility to *C. gloeosporioides* infection. Young leaves (5-7 days after unfolding) were susceptible, but resistant at mature stage (13 days after unfolding). Fruits of these cultivars were inoculated with *C. gloeosporioides* and disease severity evaluated at ripening stage. Seed was then separated from each infected fruit and grown to obtain a seedling. Young leaves of each seedling were inoculated and assessed for disease severity. Disease severity on the infected fruit and on leaves of the derived seedlings was not correlated.

9.13 EFFECT OF β-CYCLODEXTRIN-THIABENDAZOLE-PIPERONYL BUTOXIDE SUPRAMOLECULAR COMPLEX ON CONTROL OF POSTHARVEST BLUE AND GREEN MOULD DECAY INOCULATED CITRUS FRUIT. **M. Schirra, S. D'Aquino, G. Delogu, V. Borzatta.** *CNR – Istituto di Scienze delle Produzioni Alimentari, Via dei Mille, 07100 Sassari, Italy. Email:mario.schirra@ispa.cnr.it*

Piperonyl butoxide (PBO), widely known as an insecticide and herbicide synergist, blocks metabolic detoxification mediated by cytochrome P450 monooxygenases. PBO also increased the effectiveness of the anilinopyrimidine cyprodinil but had controversial effects on triazoles by either increasing or decreasing their antifungal properties. The influence of postharvest 2-min dip treatments at 20 or 50 °C with a supramolecular complex of β-cyclodextrin-thiabendazole-piperonyl butoxide (βCD-TBZ-PBO) or Thiabendazole (TBZ) was compared for controlling postharvest decay on artificially inoculated 'Okitsu' Satsuma fruits caused by blue mould *(Penicillium italicum*) and green mould *(P. digitatum*). Water dip at 20 °C did not affect the incidence of lesions caused by blue mould but favoured the development of green mould during 4–8 days of storage at 20 °C with respect to untreated fruit. While water at 50 °C effectively reduced the incidence of blue mould and totally suppressed green mould during the first 4 days, its efficacy notably declined or was lost afterwards. By contrast both TBZ and βCD-TBZ/PBO had a lasting effect and were equally effective in controlling green and blue mould decay when applied at 20 °C and 60 mg/l active ingredient (a.i.); whereas at 50 °C and 20 mg/l a.i. the activity of βCD-TBZ/PBO against blue mould was lower than that of TBZ. It is possible that the co-application of a proper adjuvant may enhance the activity and/or performance of βCD-TBZ/PBO by lowering the surface tension and allowing better spreading of the mixture.

9.14* DO AGRONOMIC FACTORS INFLUENCE SKIN PITTING ON KIWIFRUIT CAUSED BY *PHIALOPHORA* SP.? **D. Spadaro, A. Galliano, G. Gilardi, A. Garibaldi and M.L. Gullino.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: davide.spadaro@unito.it*

In recent years a postharvest disease of kiwifruit, characterized by skin pitting appearing after 3 or more months of storage, and caused by *Phialophora* sp., has been reported in most Italian packinghouses. The damage, often only minor lesions when the storage rooms are opened, becomes economically significant by the time fruit reaches the distribution channels and consumers, after transport. Nineteen kiwifruit orchards were chosen for survey in 2002-03. The two samples showing highest disease incidence (15.33 and 15.04%) had significantly lower dry matter content than the other samples (10.61% on average). The nitrogen content, by contrast, increased with increased *Phialophora* incidence. A lower calcium content also contributed to create favourable conditions for the growth of *Phialophora*, although less significantly. Further evidence of these relationships were provided during the seasons 2004-05 and 2005-06. Healthy batches had dry matter significantly higher and nitrogen content significantly lower than diseased batches. A low level of dry matter was related to the use of excessive irrigation aimed at obtaining higher yield. High nitrogen levels, directly related to excessive fertilization, also contributed to lower the storability of the fruit and its resistance to *Phialophora*. Accurate estimation of the susceptibility to skin pitting of kiwifruit entering the packinghouse is necessary. In particular, batches with predisposing factors, such as low dry matter and high nitrogen content, should be more carefully handled, and their time in storage reduced.

9.15 CULTURABLE BACTERIAL AND *TRICHODERMA* BIOFILMS ISOLATED FROM SWEET POTATO AS IT RELATES TO BIOLOGICAL CONTROL OF RHIZOPUS SOFT ROT. **C. Stevens, A. Fyffe, V.A. Khan, C. Wilson, J. Williams and M. Wisniewski.** *207 Milbank Hall Tuskegee University, Tuskegee Institute, AL, 36088, USA. Email: cstevenstu@yahoo.com*

Culturable biofilms were isolated on nutrient yeast dextrose agar (NYDA) and potato dextrose agar (PDA) by direct impression (storage root impression culture plate method) and washings from (storage roots disk washate method) from 'Jewel' sweetpotato. Root impressions revealed the presence of amorphous biofilm-like particles, and culturable bacteria, and *Trichoderma* biofilms, within 24 h on PDA and NYDA; within 12 days different colony morphologies were identified using stereo microscope images digitized by a Kodak Microscopy Documentation System. The colony morphotypes termed culturable biofilm morphotypes, when streaked on NYDA contained a mixture of solitary and biofilm bacteria, and grew on both media. Bacteral biofilms from washings were identified mainly as *Bacillus cereus*. Antagonists of the microflora present on the root surfaces, played an important role in suppression the growth of *Rhizopus stolonifer*. For example, simultaneous growth of *R. stolonifer* and bacterial biofilms in vitro, suppressed and deteriorated aerial hyphal growth of *R. stolonifer* on NYDA. Suppression of aerial hyphal growth and disintegration of *R. stolonifer* by *Trichoderma* isolates were also apparent on PDA. Results revealed that suppression of aerial hyphal growth of *R. stolonifer* occurred as a result of mycoparasitism of *Trichoderma* biofilm isolates. Antagonistic biological control microorganisms involved in reducing the inoculum potential of *R. stolonifer*, appeared to achieve better control with a mixture of several antagonists or types of antagonist, than with a single one.

9.16 IMPROVEMENT OF BIOCONTROL ACTIVITY OF *PANTOEA AGGLOMERANS* CPA-2 TO CONTROL POSTHARVEST DISEASES IN CITRUS BY PREHARVEST APPLICATIONS. **N. Teixido, T. P. Cañamás, R. Torres, M . Anguera, J. Usall and I. Viñas.** *IRTA, UdL-IRTA Ccentre, XaRTA-Postharvest, 191, Rovira Roure Av., 25198 Lleida, Catalonia, Spain. Email: rosario.torres@irta.cat*

Postharvest pathogens such as *Penicillium digitatum* often occur in the field prior to harvest. It would be better to apply biocontrol agents before harvest, to reduce initial infection and then maintain active control of pathogens in storage and/or commercial conditions, but biological control in the field is usually limited by fluctuating environment and by the narrow range of environmental conditions. The main goal of this research was to determine the influence of different formulation strategies on the survival and effi-

cacy of *Pantoea agglomerans* cells under field conditions, including lyophilised cells, osmotic adaptation of *P. agglomerans* by NaCl treatments, and additives. Different additives, such as summer oils, alginate, glycerol and food additives were tested mixed with *P. agglomerans* in laboratory studies. The additive providing the highest bacterial viability on oranges was the food film Fungicover (FC) at 50 g/l. In general, osmotically adapted and lyophilised *P. agglomerans* cells survived better than non-adapted or fresh cells when sprayed in field conditions. However, this superiority was only found when Fungicover was also added to the suspensions. These results show that it is possible to improve environmental stress tolerance and ecological competence of *P. agglomerans* cells using certain formulation strategies.

9.17 IMPROVEMENT OF BIOCONTROL ACTIVITY OF PANTOEA AGGLOMERANS CPA-2 TO CONTROL POSTHARVEST DISEASES IN CITRUS BY PREHARVEST APPLICATIONS. N. Teixido, T. P. Cañamás, R. Torres, M. Anguera, J. Usall and I. Viñas. *IRTA, UdL-IRTA centre, XaRTA-Postharvest, 191, Rovira Roure Av., 25198-Lleida, Catalonia, Spain. Email: rosario.torres@irta.cat*

Postharvest pathogens such as *Penicillium digitatum* often occur in the field prior to harvest. It would be better to apply biocontrol agents before harvest, to reduce initial infection and then maintain active control of pathogens in storage and/or commercial conditions, but biological control in the field is usually limited by fluctuating environment and by the narrow range of environmental conditions. The main goal of this research was to determine the influence of different formulation strategies on the survival and efficacy of *P. agglomerans* cells under field conditions, including lyophilised cells, osmotic adaptation of *P. agglomerans* by NaCl treatments, and additives. Different additives, such as summer oils, alginate, glycerol and food additives were tested mixed with *P. agglomerans* in laboratory studies. The additive providing the highest bacterial viability on oranges was the food film Fungicover (FC) at 50 g/l. In general, osmotically adapted and lyophilised *P. agglomerans* cells survived better than non-adapted or fresh cells when sprayed in field conditions. However, this superiority was only found when Fungicover was also added to the suspensions. These results show that it is possible to improve environmental stress tolerance and ecological competence of *P. agglomerans* cells using certain formulation strategies.

9.18* ROLE OF HYDROGEN PEROXIDE IN THE DEVELOPMENT OF POSTHARVEST DISEASE IN ORANGES CAUSED BY *PENICILLIUM DIGITATUM*. R. Torres, N. Mir, N. Teixidó, J. Usall, M. Abadias, C. Larrigaudiere and I. Viñas. *IRTA, UdL-IRTA Centre, XaRTA-Postharvest, 191, Rovira Roure Av., 25198 Lleida, Catalonia, Spain. Email: rosario.torres@irta.cat*

Plant cell strategies against pathogens include mechanisms directed at weakening or killing the pathogen. These include the accumulation of reactive oxygen species (ROSs) such as H_2O_2 and the superoxide anion, in a process known as the oxidative burst. The ROSs generated during these reactions have direct antimicrobial activity that inhibits fungal spore germination, and could be involved in other processes such as induction of systemic responses. Our objective was to understand the role of H_2O_2 in the response of oranges infected with *Penicillium digitatum* or treated with the biocontrol agent *Pantoea agglomerans* CPA-2. The participation of antioxidant enzymes such as superoxide dismutase and catalase, and the peroxidases was also investigated. H_2O_2 levels and enzymatic activities were evaluated in Valencia oranges, after infection with the pathogen or treatment with the antagonist. We used fruit just at harvest and after postharvest storage. Similar levels of H_2O_2 were observed after 72 h in all fruits. However, a decrease was observed in fruits with visible symptoms of decay, and high levels of H_2O_2 were found in fruits without the pathogen. Changes in enzyme activities were also observed due to the presence of *P. digitatum* or *P. agglomerans*. The different behaviour observed in oranges in the presence of pathogen or antagonist might be explained in terms of a suppression or induction of hydrogen peroxide metabolism.

9.19 CONTROL OF BROWN ROT OF PEACH AND NECTARINE BY COMBINING HOT WATER, ANTAGONISTS AND SODIUM BICARBONATE. J. Usall, C. Casals, N. Lamarca, J. Segarra, J. Cambray, I. Viñas. *IRTA, UdL-IRTA Centre, XaRTA-Postharvest, 191, Rovira Roure Av., 25198 Lleida, Catalonia, Spain. Email: josep.usall@irta.cat*

Monilinia spp. is the most important postharvest disease in peaches and nectarines in the Ebro Valley (Spain). Nowadays, chemical postharvest treatments are not allowed for control of *Monilinia* spp. So it has been necessary to develop other control methods. The aim of this study was to evaluate the effect of hot water dip, sodium bicarbonate solutions or biocontrol agents alone or combined in order to control *M. laxa* in peach and nectarine fruits. The first year, we evaluated separately, eight biocontrol agents, three hot water temperatures and three sodium bicarbonate concentrations per three different times. The treatments 60ºC during 40 seconds and 2% during 40 seconds for hot water and sodium bicarbonate, respectively, were the most efficient, without affecting fruit quality. Moreover, from the eight biological agents evaluated, three were selected. The second year, the most efficient treatments were evaluated alone and combined using five varieties of peaches and nectarines. Two different storage temperatures were studied (20ºC and 0ºC). Generally, when the storage period was at 20ºC, the combination of hot water (60ºC during 40 s) and sodium bicarbonate (2%) showed no significant additional effect against *M. laxa*. In contrast, when the combination was hot water and biocontrol agents (10^7 cells per ml) there was a significant additional effect. Also, when the combination was triple: hot water, biocontrol agents and sodium bicarbonate there was a significant additional effect. These significant differences between single or combined treatments were reduced when the fruits were stored for 21 days at 0ºC more 5 days at 20ºC.

9.20 *COLLETOTRICHUM* SPECIES ASSOCIATED WITH CHERIMOYA AND OTHER HOSTS. R. Villanueva-Arce, M. J. Yáñez-Morales, A. M. Hernández-Anguiano. *Unidad Profesional Interdisciplinaria de Biotecnología del Instituto Politécnico Nacional, Av. Acueducto s/n, Ticomán, México, D F, C. P. 07340, Mexico. Email: rarce@ipn.mx*

Colletotrichum species on chirimoya (*Annona cherimola* Mill.), ilama (*Annona diversifolia* Saff.), and blackberry (*Rubus* sp.) were identified at several sites from Guerrero, Mexico, and Michoacan states, and studied by PCR analysis of the ITS region of rRNA genes. In potato-dextrose-agar and potato-carrot-agar growth media, eight *Colletotrichum* isolates were selected and identified by asexual and sexual structures, and cultural characteristics. Four species were identified, *C. acutatum* (EU016517) on mummified blackberry fruits, *C. fragariae* (AY841137, AY605089) on ilama fruits with anthracnose; this specie plus *C.*

gloeosporioides (AY841132, AY841134, AY841135, AY841136) and its teleomorf *Glomerella cingulata*, and *C. orbiculare* (AY841133) on fruits and leaves of cherimoya with anthracnose, stem-end rot, and black spot.

9.21* RELATIONSHIP BETWEEN FRUIT SURFACE CONIDIA, INCIDENCE OF LATENT INFECTIONS CAUSED BY *MONILINIA* SPP. AND BROWN ROT OF PEACH FRUIT IN SPAIN. M. Villarino, I. Gell, C. Casals, N. Lamarca, J. Usall, J. Segarra, A. De Cal and P. Melgarejo. *Department of Plant Production and Forest Science, University of Lleida, 191 Rovira Roure Av., 25198 Lleida, Catalonia, Spain. Email: segarra@pvcf.udl.es*

Monilinia spp. are the most important cause of post-harvest brown rot in peaches and nectarines in Spain. Conidia produced in overwintered fruit mummies, and necrotic twigs infected by *Monilinia* spp. act as primary inoculum sources, causing blossom blight occasionally, and brown fruit rot frequently. Post-harvest losses are typically more severe, especially when conditions are favourable for disease development, in some cases reaching losses of 80-85%. When microclimatic conditions are unfavourable, infections may remain latent until conditions become favourable for disease expression, leading to fruit rot. To evaluate the effect of surface concentration of conidia on the incidence of latent infection and brown rot of peaches, 17 field experiments were done in commercial orchards located in Lleida, Spain, over six growing seasons from 2002 to 2007. A positive relationship between the numbers of conidia of *Monilinia* spp. on the fruit surface and the percentage of latent infections caused by *Monilinia* spp. in stone fruit was observed. Regression analyses indicated that the number of conidia on peach surfaces explained 68% of the incidence of latent infection variation caused by these fungi, and the correlation coefficient between the variables was 0.82. A positive relationship between the incidence of latent and postharvest brown rot was observed. Mean of latent infection over the crop season contributed to 55-75% of the post-harvest brown rot. The importance of conidia on fruit surfaces and latent infections on brown rot development will be discussed.

9.22 HYDROGEN PEROXIDE INDUCTION IN CITRUS FRUIT BY ANTAGONISTIC YEASTS USED FOR POSTHARVEST BIOCONTROL: A NEW MECHANISM OF ACTION? M. Wisniewski, D. Macarisin and S. Droby. *USDA-ARS, 2217 Wiltshire Rd, Kearneysville, WV 25430, USA.Email: michael.wisniewski@ars.usda.gov*

There is evidence that some biocontrol agents transiently induce ROS production in host plants, triggering local or systemic pathogen resistance. An oxidative response is triggered in a host plant by a microbe by either recognition of microbe-associated molecular patterns (MAMPs) or by specific, effector-triggered mechanisms. The ability of postharvest biocontrol agents to induce defence-related, oxidative responses in fruits, has not been investigated. Using laser-scanning-confocal microscopy, we showed that the antagonist yeast, *Metschnikowia fructicola* induces a rapid increase in H_2O_2 in host tissue when applied to citrus fruit wounds. By 18 hours after inoculation, the level of H_2O_2 around inoculated wounds increased by 3-fold compared to levels in controls. H_2O_2 levels in yeast-inoculated fruit were still significantly ($P < 0.01$) greater than controls 66 h post-inoculation. At this time, living yeast cells were detected in fruit wounds, indicating the ability of *M. fructicola* to tolerate high levels of ROS, which has been suggested to be an intrinsic characteristic of effective yeast antagonists. This data, together with our earlier observations of the importance of H_2O_2 production by flavedo tissue in citrus fruit resistance to postharvest pathogens, indicate that the ability of antagonist yeasts to induce an oxidative response in host tissue could be an important aspect of biocontrol activity. Whether the induction of an oxidative response in citrus fruit by yeast is due to an MAMP response or a more complex interaction involving specific yeast effectors needs to be determined.

9.23 PRE- AND POSTHARVEST STRATEGIES FOR CONTROL OF *PHACIDIOPYCNIS* ROT IN D'ANJOU PEARS. C.L. Xiao and R.J. Boal. *Washington State University, Tree Fruit Research and Extension Center, 1100 North Western Avenue, Wenatchee, WA 98801, USA. Email: clxiao@wsu.edu*

Phacidiopycnis rot caused by *Potebniamyces pyri* (anamorph *Phacidiopycnis pyri*) is a common postharvest fruit rot disease in d'Anjou pears grown in Washington State, USA. *P. pyri* is associated with dead bark and twig dieback of pear trees in the orchards. Infection of pear fruit by the fungus occurs in the orchards, but symptoms develop in storage. In this study, selected pre- and postharvest fungicide programs were evaluated in 2005 and 2006 for control of *Phacidiopycnis* rot. Fruit was inoculated in the orchard with a conidial suspension of the fungus at 3 weeks before harvest. Part of the fruit was sprayed with a premixed formulation of pyraclostrobin and boscalid (Pristine) or thiophanate-methyl at 7 days before harvest, and the remaining fruit was either not treated as controls or dipped in fludioxonil, pyrimethanil or thiabendazole solutions after harvest. Fruit was stored at 0°C for 7 months and *Phacidiopycnis* rot (stem-end and calyx-end rot) was monitored periodically. Pristine and thiophanate-methyl reduced *Phacidiopycnis* rot by 41-44% and 77-86%, respectively, in comparison with the nontreated control. Thiophanate-methyl was significantly more effective than Pristine. Fludioxonil, pyrimethanil and thiabendazole alone provided similar levels of control and reduced *Phacidiopycnis* rot by 88-100%. The results indicate that although infection of pear fruit by *P. pyri* occurs before harvest, a preharvest fungicide applied within 7 days before harvest or a postharvest fungicide applied prior to storage can be effective for control of *Phacidiopycnis* rot in d'Anjou pears.

9.24 EFFECT OF ETHYLENE ON THE GROWTH AND PATHOGENICITY OF *BOTRYTIS CINEREA*. L. Xu, L.J. Zhang, Y. Liu, W. Zhang, S.Y. Zhang and H. Toyoda. *School of Life Science, P.R China, Normal University, Shanghai 200062, P.R. China. Email: lxu@bio.ecnu.edu.cn*

During the storage and transportation of fruit and vegetable crops, ethylene promotes not only their senescence, but also the outbreak of disease in the crops. Many pathogenic fungi can produce ethylene, but its role in expression of pathogenicity has remained obscure. To give some clues to these problems, the present research was conducted using *Botrytis cinerea* isolated from harvested grapes. All isolates produced ethylene on methionine-containing media (M-media). The highest production was on M-medium supplemented with grape juice, suggesting that ethylene production by the pathogen is largely promoted by host-derived nutrients. Ethylene production varied among the isolates, and solid medium was best for ethylene production, with an average 9.5 times higher than in liquid M-medium. *B. cinerea* can synthesize ethylene between pH 2 and 5, and the optimum is pH 3.0, corresponding to the physiological pH of grape juice. Exogenous

ethylene did not affect spore germination, but significantly promoted *B. cinerea* hyphal growth. Germ tube elongation was promoted to various extents, depending on concentrations of ethylene given. In addition, ethylene treatment enhanced the pathogenicity of *B. cinerea* to post-harvest berries in a bell-shaped curve to concentrations. The disease index reached a maximum when the inoculated berries were exposed to ethylene at 50 µg/ml. No significant effect of ethylene was detected on the activity of cell wall degrading enzymes secreted by *B. cinerea*. Thus, the present study suggested that host nutrients induce ethylene production by *B. cinerea* to promote the hyphal growth and pathogenicity of the pathogen.

Precision agriculture and plant pathology

35.1 OCCURENCE AND PATHOGENICITY OF *ALTERNARIA* SP. IN FLOWERS, FRUITS AND LEAVES OF THE MAIN CROPS OF KAZAKHSTAN. N. Aitkhozhina and N. Aitkhozhina. *86 Dosmukhamedov str.050012 Almaty, Kazakhstan. Email: nazira_aitkhozhina@yahoo.com*

Apple, pear, cherry, tomato, potato, aubergine, zuchini and wheat are the most popular edible crops in Kazakhstan. They are subject to different diseases at all stages of their development. In some apple, cherry and pear orchards there is considerable damage to flowers, leaves and fruits. Infection by *Alternaria* sp. also causes fruit rot of many vegetables and wheat during vegetation and in storage. About 70 isolates were obtained from infected plant tissues. Some of them were identified as *A. alternata*, *A. mali*, *A. tenuis*, *A. tenuissima*, *A. gossipi* and *A. consorcialae.* All *Alternaria* species isolated from plant tissues were pathogenic to most of the plants and to a range of related plants after artificial inoculation with a conidial suspension. Moreover *Alternaria* sp. obtained from apple was highly pathogenic to vegetables and young wheat seedlings. The fungal culture liquid was pathogenic to all tested plant organs owing to the secretion of several toxic products. One of these products was a cell wall degrading enzyme, polygalacturonase.

35.2* QUANTITATIVE PCR-DETECTION FOR MAPPING IN-FIELD VARIATION OF *PLASMODIOPHORA BRASSICAE AND APHANOMYCES EUTEICHES*. C. Almquist, A.C. Wallenhammar and A. Jonsson. *Detartment of Soil Sciences, Section of Precision Agriculture, Swedish University of Agricutural Sciences P.O. Box 232, SE 532-23 Skara, Sweden. Email: anders.jonsson@mv.slu.se*

Spatial variability within fields and variations between fields in the occurrence of *Plasmodiophora brassicae* and of *Aphanomyces euteiches* were determined on farms in south and central Sweden using quantitative PCR. The molecular methods were validated by traditional bioassays. Soil samples were mapped using GPS from fields where the disease occurred and the results presented as an interpolated disease map. Relations between the occurrence of pathogens and soil parameters such as pH, soil type, clay content, plant available macro- and micro nutrients were evaluated. Species-specific primers and TaqMan fluorogenic probes were designed to amplify small regions of *P. brassicae* and *A. euteiches* ribosomal DNA. Total genomic DNA was extracted and purified from soil samples using commercial kits. The amount of pathogen DNA was quantified using a standard curve generated by including reactions containing different amounts of a plasmid carrying the *P. brassicae* or *A. euteiches* target sequence. Regression analysis showed that the assays were linear over at least 6-7 orders of magnitude (R^2>0.99) and that the amplification efficiency was >95%. A considerable (100-1000 times) variation in DNA content was observed in the fields sampled for the two pathogens. Molecular methods for routine diagnosis will enable producers to respond to market opportunities. The results will provide basic data for evaluating the benefit of systematic detection of soil-borne pathogens by quantitative PCR, aiming at efficient application in Precision Agriculture.

35.3 QUANTITATIVE DETECTION METHODS FOR MAPPING IN-FIELD VARIATION OF *PLASMODIOPHORA BRASSICAE* AND *APHANOMYCES EUTEICHES*. C. Almqvist, A-C. Wallenhammar and A. Jonsson. *Detartment of Soil Sciences, Section of Precision Agriculture, Swedish University of Agricutural Sciences P.O. Box 232, SE 532-23 Skara, Sweden. Email: charlotta.almqvist@lantmannen.se*

Spatial variability within fields and variations between fields in the occurrence of *Plasmodiophora brassicae* and of *Aphanomyces euteiches* were determined on farms in south and central Sweden using quantitative PCR assays. These were validated by traditional bioassays. Soil was sampled using GPS location in fields where the disease occurred, and the results are presented as an interpolated disease map. Relations between pathogen occurrence and soil parameters such as pH, soil type, clay content, and available macro- and micro-nutrients were evaluated. Species-specific primers and TaqMan fluorogenic probes were designed to amplify small regions of *P. brassicae* and *A. euteiches* ribosomal DNA. Total genomic DNA was extracted and purified from soil samples using commercial kits. The amount of pathogen DNA was quantified using a standard curve generated by including reactions containing different amounts of a plasmid carrying the *P. brassicae* or *A. euteiches* target sequence. Regression analysis showed that the assays were linear over at least 6-7 orders of magnitude (R^2>0.99) and that amplification efficiency was >95%. A considerable (100-1000 times) variation in DNA-content was observed for *P. brassicae*, while the variation was less pronounced for *A. euteiches*. Molecular methods for routine diagnosis will enable producers to respond to market opportunities. The results will constitute basic data for evaluating the benefit of systematic detection of soil-borne pathogens by quantitative PCR, aiming at efficient application in Precision Agriculture.

35.4* EVALUATION OF *SALIX* GENOTYPES FOR INCIDENCE OF *MELAMPSORA* SPECIES USING INFRA RED SPECTROSCOPY, THERMOGRAVIMETRY AND SCANNING ELECTRON MICROSCOPY. D. Begley, H.S. Shekhar Sharma, A. McCracken and D. McCall. *Agri-Food and Biosciences Institute, Newforge Lane, Belfast, BT9 5PX, UK. Email: shekhar.sharma@afbini.gov.uk*

Increasing areas in Northern Ireland and Great Britain are being planted with willow (*Salix* spp.) for the production of biomass as a renewable source of energy. Leaf rust caused by *Melampsora* species is by far the commonest, most serious, and widely distributed disease of *Salix*. Leaves of three *Salix* genotypes with varying rust susceptibilities were evaluated for changes in the infrared spectra and composition. The key components are carbohydrates, cellulose, thermal stable lignin fractions and inorganic residue using thermogravimetric analysis (TA). The evaluation of leaf samples inoculated with rust spores showed varying levels of susceptibility both inter-specifically and within a single

plant. The infrared spectra of the samples was able to show consistent differences between top, middle and bottom leaves of the genotypes and a relationship between spectra and leaf position was established using multivariate regression analysis. The incidence of rust on the genotypes also correlated with spectra of the samples especially in the 900–2000 cm^{-1} segment indicating significant differences in fundamental and rotational vibrations of single and double bonds including C=O, C=N and C=C linkages. TA was able to show compositional differences in leaves between genotypes and with increasing distance from the shoot tip. Scanning electron microscopy was employed to characterise, the leaf surface morphology of *Salix* leaves detached from several regions of the shoot. Generally, leaves detached from the top of the shoot had more hairs and lower leaves tended to have more surface wax which may form a barrier to penetration by spore germ tubes.

35.5 SYNERGISTIC EFFECT OF PGPRS ON ROOT COLONIZATION BY *GLOMUS FASCICULATUM* AND ITS FEASIBILITY FOR MASS PRODUCTION OF ARBUSCULAR MYCORHIZA INOCULUM. **S. Dutta, J. Chakraborty, S. Chatterjee and N.C. Chatterjee.** *Department of Botany, The University of Burdwan, Burdwan 713104, West Bengal, India. Email: sikha_bu_bot@yahoo.com*

Standardization of technique for rapid culture of arbuscular mycorhiza inoculum is certainly an important challenge for its mass production. The present investigation has been conducted with a view to explore the possible use of PGPRs to maximize root colonization by AM fungi and their sporulation in a relatively short period on roots of two grasses such as Rhodesgrass (*Chloris gayana*) and Lemongrass (*Citronella flexuosa*) and subsequently to search for suitable hosts other than maize where AM inocula can be built up, multiplied and maintained efficiently. The efficacy of PGPRs like *Pseudomonas fluorescens*, *Azospirillum* sp., *Rhizobium japonicum* and *Bacillus subtilis* was investigated for maximizing the root colonization by the vesicular AM fungus, *Glomus fasciculatum*. Root colonization and spore frequency of *Glomus* in the root regions of Rhodesgrass and Lemongrass were studied in terms of percent root infection. Pot culture experiments were conducted under polyhouse conditions and observations were recorded at intervals (15, 30, 45, 60 and 75 days). Volume of roots and dry weight of roots and shoots per pot were determined at each harvest. Almost all the PGPRs considerably enhanced root colonization by *Glomus* and enhanced root growth except *Bacillus*. *Azospirillum* appeared to be the most efficient. PGPRs thus revealed their potential role in mass production of AM inoculum. Both the grasses responded well for establishing mycorrhizal symbiosis as evident from root colonization percentage (45.0-89.0%). Their possible role as suitable hosts for mass production of AM inocula was confirmed.

35.6 EFFECT OF NITROGEN-FIXING AND PHOSPHATE-SOLUBILIZING BACTERIA ON WATER HYACINTH VERMICOMPOST. **A. Rakshit.** *Institute of Agricultural Science, Banaras Hindu University, UP, India. Email: amitavabhu@gmail.com*

The effect of inoculating water hyacinth-vermicompost with nitrogen-fixing *Azotobacter chroococcum* strains, *Azospirillum lipoferum* and the phosphate-solubilizing *Pseudomonas striata* on N and P contents of the vermicompost was assessed. Inoculation of N_2-fixing bacteria into vermicompost increased contents of N and P. Enriching vermicompost with rock phosphate significantly improved the available P when inoculated with *P. striata*. During the incubation period, the inoculated bacterial strains proliferated rapidly, fixed N and solubilized added and native phosphate. It is evident from this experiment that *Azotobacter*, *Azospirillum* and *Pseudomonas* inoculation helped to increase the N and P contents of vermicompost, and rock phosphate was solubilized during composting.

35.7 STRATEGIES TO AVOID FUNGAL DISEASES OF CASSAVA IN MADAGASCAR. **S. Ranomenjanahary.** *Laboratory of Plant Pathology, P.O. Box 1444, Ambatobe, 101 Antananarivo, Madagascar. Email: fofifa-atobe@wanadoo.mg*

Leaves, stems and tubers of cassava plants are attacked by the fungal pathogens *Cercospora cassavae*, *C. henningsii*, *C. vicosae*, *and C. caribae*, causing leaf spot mostly in the winter season. Their proliferation may be dangerous for young cassava plants as they involve leaf fall and yield loss. All cassava varieties are sensitive to these diseases. The only control measure is to collect infected leaves and incinerate them. *Colletotrichum gloeosporioides* and *Diplodia theobromae* are also pathogens of cassava stems. The first provokes stem canker and the second brown rot which leads to drying out of the stem, then death. Seventy-five percent of cuttings are attacked by diplodiosis in farmers' fields, inducing very high yield losses and a deficiency of cuttings. An alternative strategy is to soak selected healthy cuttings in warm water (47 °C) for 30 minutes and cultivate in soil prepared with plant rubbish as fertilizer, made from banana stem and leaf for potassium, wild sunflower for phosphorus, rice straw for silicon and calcium, water hyacinth (*Eichornia crassipes*) for nitrogen and *Melia azedarach* for insect repellent. With this fertilizer, the cassava growth is very healthy and can give very high yields which can reach 50 kg of tubers per foot. *Lasidiplodia theobromae*, *Fusarium* spp. (sect. *Martiella*), *Corticium rolfsii*, *Phaeolus manihotis* and *Clitocybe tabescens* cause tuber rot. These pathogens are favoured by excess of soil humidity, so stagnation of water in the cassava fields must be avoided.

35.8 SURVEY FOR CEREAL DISEASES IN GEORGIA IN 2004-2007. **Z. Sikharulidze, L. Gorgiladze, L. Mgeladze, K. Natsarishvili, M. Gabaidze, T. Tsetskhladze and S. Mepharishvili.** *Ministry of Education and Science, Institute of Plant Immunity, 90, Tavisupleba St., Kobuleti, 6200, Georgia. Email: zoia_sikharulidze@yahoo.com*

The climate of Georgia (Transcaucasus region) is ideal for growing cereals but also for the development of diverse diseases. A survey of wheat and barley diseases was conducted in 2004-2007 in different geographical zones (Colkhis lowland, Imereti hill, Inner Kartli plain, Lower Kartli plain, Inner Kakheti valley, External Kakheti valley, Meskheti, Djavakheti) of Georgia. In 17 regions of Georgia 386 wheat and 205 barley fields were monitored. Winter wheat and barley varieties were the most common. Spot blotch (*Cochliobolus sativus*), *Alternaria* leaf blight (*Alternaria triticina*), tan spot (*Pyrenophora tritici-repentis*), *Septoria* glume blotch (*Stagospora nodorum*) and *Septoria* leaf blotch (*Septoria tritici*) were the most widely distributed. Wheat rusts (*Puccinia triticina*, *P. graminis*, *P. striiformis*) and wheat powdery mildew (*Blumeria graminis*) incidence levels were low, with the exception of fields surveyed in 2004 in which powdery mildew was rated as severe. Covered smut (*Tilletia tritici*) and scab (*Fusarium graminearum*) were moderately distributed. Loose smut (*Ustilago tritici*) and root rot (*Fusarium gibbosum*, *F. oxysporum*, *F. avenaceum*) were found occasionally. Incidence of viral and bacterial diseases of wheat was the lowest. Significant levels

of the spot blotch (*Cochliobolus sativus*) and powdery mildew (*Blumeria hordei*) on barley were present over the entire survey area. Net blotch (*Pyrenophora teres*), scald (*Rhynchosporium secales*), barley stripe (*Pyrenophora graminea*) and loose smut (*Ustilago nuda*) have been found annually with moderate incidence in some regions. Leaf rust (*Puccinia hordei*) and speckled leaf blotch (*Septoria passerinii*) were rarely observed. *Barley yellow dwarf virus* was found only in Inner Kakheti valley.

35.9 IMPROVEMENT OF CZECH HOPS. P. Svoboda. *Hop Research Institute Co. Ltd, Czech Republic. Email: p.svoboda@telecom.cz*

Hop as a perennial and vegetatively propagated crop is endangered by virus diseases. They cause economic losses showing in lower yield and lower contents of alpha bitter acids, which are very important for beer production. To cope with this problem an improvement programme has been started in the Czech Republic. Meristem tips cultures are used to obtain virus-free basic material. Tissue cultures serve as an origin for propagation of planting material. Micropagation *in vitro* is used for multiplication of basic virus-free material. At every step of the process, testing for the individual pathogens is very important. Planting material is produced in accordance with EPPO guidelines [PM 4/16(1)] and Act no. 316/2006. The Central Institute for Supervising and Testing in Agriculture must certify planting material. Using ELISA, the viruses ApMV, HLV and HMV are tested. Dot-blot hybridisation is used for HLVd determination. Health status is assessed at the level of *in vitro* cultures, technical and space isolation, high quality mother plants and propagated material in glasshouses. Plants in hop gardens, which are applied for admitting process of exploitation of planting material and material in rootstock nurseries, are tested for ApMV. Acknowledgements: The results were obtained with the help of financial support from the project of MSM 1486434701 granted by Ministry of Education, Youth and Sport Czech Republic.

Soil disinfestation

22.1 PROGRESS ON ALTERNATIVES TO METHYL BROMIDE SOIL FUMIGATION IN CHINA. A. Cao, X. Duan, H. Yuan, M. Guo and W. Zhang. *Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China. Email: caoac@vip.sina.com.cn*

Twenty-nine alternatives to methyl bromide, for soil fumigation, have been tested in five major methyl bromide consumption provinces in China since 1997. The alternatives tested included chemical and non-chemical technologies, e.g. floating seed-tray, dazomet, metham sodium and sulfuryl fluoride in tobacco; bio-fumigation, resistant cultivars, grafting, substrate solarization, deep digging with organic amendment, avermectine, dazomet, metham sodium, 1,3-dichloropropene, sulfuryl fluoride, DMDS in tomato and cucumber; bio-fumigation, resistant cultivars, steaming pasteurization of substrate, dazomet, metham sodium with solarization, chloropicrin capsules, calcium cyanamide, chlorine dioxide, deep digging with organic amendment in strawberry. After laboratory and field tests, some potential and promising alternatives were recommended. Floating seed tray is a good alternative to MB in tobacco seed-beds and is widely used in China currently. Metham sodium combined with solarization, chloropicrin capsules, DMDS and fresh manure amendment are promising alternatives for strawberries. Metham sodium, dazomet with solarization, 1,3-dichloropropene, sulfuryl fluoride, DMDS, avermectins, grafting, substrate are potential alternatives in protected vegetables.

22.2* SUSCEPTIBILITY TO *FUSARIUM OXYSPORUM* OF DIFFERENT BRASSICAS USED IN BIOFUMIGATION TREATMENT. P. Lu, G. Gilardi, A. Garibaldi and M.L. Gullino. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco(TO), Italy. Email: lvpingxiang@yahoo.com.cn*

Biofumigation refers to the suppression of soil-borne pests and pathogens by biocidal compounds released by brassicaceous green manure and rotation crops when glucosinolates (GSLs) in their tissues are hydrolyzed. We investigated the susceptibility of 17 brassicaceous species to different strains of *Fusarium oxysporum* including *F. o.* f. sp *raphani*, *F. o.* f. sp *conglutinans*, *F. o.* f.sp *matthioli* and *F. o.* from lamb's lettuce. The roots of 14-20-day-old plants were dipped in spore suspension of 1×10^6 CFU ml^{-1} before transplanting into sterilized soil. The control plants of each species and cultivar were prepared similarly but not inoculated. Three replicates were used for each species, arranged in a randomized complete block design. All trials were carried out in a glasshouse at temperatures of 25 to 33°C. Symptoms of infected plants were expressed as disease index (0-100). The results indicated that different *Brassica* species reacted differently to different *F. oxyporum* forms. *B. juncea* (ISCI 99 and ISCI 20) and *Rapistrum rugosum* were resistant to *F. o.* f.sp *raphani*. Different species including *B. juncea* (ISCI 61 and ISCI 99), *B. nigra*, *Crambe abyssinica*, *B. carinata*, *Lepidium campestre*, *L. sativum*, *Diplotaxis tenuifolia*, *Barbarea verna*, *R. rugosum*, *B. juncea* and *Sinapis arvensis* were resistant to *F. o.* f.sp *matthioli*, and all the species except *C. abyssinica* were resistant to *F. o.* from lamb's lettuce. High susceptibility to *F. o.* f.sp *conglutinans* was observed for all species tested except *Barbarea verna*, which was resistant.

22.3 EFFECT OF GREEN BRASSICA MANURE AGAINST *VERTICILLIUM* WILT ON EGGPLANT. A. Minuto, M.L. Gullino and A. Garibaldi. *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco, Italy. Email: angelo.garibaldi@unito.it*

The biocidal activity of green Brassica manure is due to presence of the glucosinolate-myrosinase system in the plant and to its capacity to produce a number of biologically active compounds, including isothiocyanates, nitriles, epithionitriles and thiocyanates. In recent years, several *in vitro* tests have shown a potentially wide biocidal effect against soilborne pests and diseases. During 2004, 2005 and 2006 three field trials were carried out in Liguria (northern Italy), to test the efficacy of applying green manure obtained after a winter cycle of *Brassica juncea* ISCI20 (Cerealtoscana-Italy) sown (10-14 kg/ha) in November 2003, 2004 and 2005. The trials soil was naturally infested with *Verticillium dahliae*. In May 2004, 2005 and 2006 *B. juncea* was cut and tilled into the soil. Some plots were then mulched with a clear plastic film. Unsown plots, mulched and not mulched, served as controls. After 25-30 days of mulching, mulched plots were uncovered and eggplant (cv. Prosperosa) was transplanted at 2 plants/m^2. Severe infections of *V. dahliae* were recorded in both years in control plots (63-56-85% infected plants), without plastic mulch (66-65-55% infected plants). In contrast, treated and mulched plots showed a significant reduction of disease incidence (52-49-12% infected plants), particularly in 2005 and 2006. During the third trial, yield in untreated and non-mulched plots was significantly lower (0.4 kg/plant) than in treated and mulched plots (1.4 kg/plant).

SOILBORNE PLANT DISEASES AND THEIR CONTROL

38.1 ANTIFUNGAL ACTIVITY OF FURFURAL ON *SCLEROTINIA SCLEROTIORUM*. **M.A. Al-Hamdany.** *Agricultural Researche Office, Ministry of Science and Technology, P.O. Box 765, Baghdad, Iraq. Email: ma_alhamdany@yahoo.com*

Effective control of *Sclerotinia sclerotiorum*, the causal pathogen of white and stem rot diseases of vegetables could be successfully achieved by using certain concentrations of furfural in growth medium or heavily infested soil. Culturing mycelia discs or sclerotia of *S. sclerotiorum* on PDA amended with 500, 1000, or 1500 ppm of furfural caused significant reduction in fungal growth and dry weight of sclerotia at 500 ppm with complete growth inhibitions when 1000 ppm was used on mycelial discs and 1500 ppm when sclerotia were cultured respectively. In soil, high levels of soil infestation with *S. sclerotiorum* (fungal growth suspension or sclerotia) were completely suppressed when 1500 ppm of furfural was incorporated in the soil 48 h prior to transplanting eggplant seedlings. Disease incidence recorded on eggplant plants grown in untreated soil was 45%. These results confirmed the antifungal activity of furfural obtained with *Rhizoctonia solani*, *Fusarium* spp. and *Pythium* along with the root knot nematode *Meloidogyne javanica* in previous studies.

38.2 SELECTION AND EVALUATION OF BACTERIA ANTAGONISTIC AGAINST *PYTHIUM APHANIDERMATUM*. **A. Al-Hinai, A.M. Al-Sa'di, M.L. Deadman, A. Mothershaw and S. Al-Bahri.** *P.O. Box 34, Al Khoud 123, Oman. Email: aalhinai@squ.edu.om*

Twenty one isolates of *Pseudomonas aeruginosa* were evaluated for their efficacy to inhibit *Pythium aphanidermatum*, which causes damping-off in cucumber. Paper disk diffusion assays were used to assess bacterial antagonism *in vitro*. Bacterial development and activity were evaluated under different temperatures and salinity levels. Bioassays to monitor the suppression of damping-off of cucumber seedlings were performed under greenhouse conditions. Seven isolates were superior in inhibiting radial growth of *P. aphanidermatum* on PDA. Bacterial isolates showed optimal growth at 30ºC; whereas a temperature of 37ºC favored bacterial antagonistic activity. Growth of all seven bacterial isolates was inhibited at a salinity of 12.03 ds/m. All the selected isolates enhanced the growth of cucumber seedlings (height, fresh weight, root length, root weight and number of leaves). Isolates 7, 38, 48, 49, 94 and 95 appeared to have potential to control damping-off.

38.3 GENETIC DIVERSITY OF *PYTHIUM APHANIDERMATUM* AND *PYTHIUM SPINOSUM* IN OMAN. **A.M. Al-Sa'di, A. Drenth, M.L. Deadman and E.A.B. Aitken.** *P.O. Box 34, AlKhoud 123, Oman. Email: alsadi@squ.edu.om*

Using AFLP fingerprinting, we studied the genetic diversity among 89 isolates of *Pythium aphanidermatum* obtained from 73 cucumber and 16 muskmelon samples, and among 24 isolates of *Pythium spinosum* obtained from cucumber from five different regions in Oman. Using four primer-pair combinations, AFLP analysis of *P. aphanidermatum* isolates produced 45 different phenotypes with a high level of genetic similarity (> 90 %). Analysis of molecular variance indicated that most of the variation was associated with geographical regions (F_{ST} = 0.118; $P < 0.0001$), not hosts (F_{ST} = -0.004; P = 0.4323), which was supported by the high (24%) rate of recovery of identical phenotypes between cucumber and muskmelon fields in the same region as compared to the low (10%) recovery across regions in Oman. These data suggest more frequent movement of *Pythium* inoculum among muskmelon and cucumber fields in the same region as compared to movement across geographically separated regions. On the other hand, AFLP fingerprinting of 24 isolates of *P. spinosum* using six-primer-pair combinations produced seven different phenotypes with a very high level of genetic similarity (≥99%), two of them contained 79% of the isolates and one was recovered from all regions in Oman. These data suggest a recent introduction of *P. spinosum* in Oman via a common source compared to a longer history of *P. aphanidermatum* in Oman and introduction via multiple sources.

38.4 INTEGRATED SOIL TREATMENTS FOR MANAGEMENT OF SOILBORNE STRAWBERRY DISEASES. **M.A. Awad, E.Z. Khalifa, Y.S. Khafagi and S.S. Ragab.** *Plant Pathology Department, Faculty of Agriculture, Minufiya University, Egypt. Email: moahawad@yahoo.com*

The long-term effectiveness of integrated pest management (IPM) i.e. soil solarization integrated with a biological control agent (*Trichoderma* spp.), chemical fungicide (Topsin M-70), drenching with antioxidant (salicylic acid) or physical method (black agriplastic mulch) to reduce soilborne fungal infection (wilt and/or root rot diseases) were evaluated on strawberry production. Results showed that the long-term effectiveness of IPM plus soil solarization reduced the soilborne diseases. The interaction between black mulch with antagonistic *T. harzianum* grown on sugarcane bagasse and different antioxidants tested on strawberry plants infected with root rot and wilt pathogens showed decreased infection and disease severity. Salicylic acid was superior in this respect. The interaction between black mulch with combination fungicides was the best treatment for reducing root rot, wilt disease incidence and severity, and increasing yield compared with other treatments. This was true for both of two growing seasons. Ten treatments (black mulch, Topsin M-70, *T. hamatum* and salicylic acid with their combinations) were used to study their effect on controlling root rot and wilt diseases in two successive seasons (2003-2004) & (2004-2005). Black mulch + Topsin M-70 + *T. hamatum* + salicylic acid as complex was the most effective treatment on 'Chandler' strawberry, and increased crop yield compared with other treatments and the control.

38.5* DETECTION AND EVIDENCE OF BIOLOGICALLY-CAUSED *RHIZOCTONIA SOLANI* SUPPRESSION. **J.O. Becker, M. Matsui and R. Fukui.** *Department of Nematology, University of California, Riverside, CA 92521, USA. Email: obecker @ucr.edu*

Seedling diseases cause by *Rhizoctonia solani* are surprisingly rare in the Tochigi Prefecture, Japan, considering the intensity of agricultural production, the nearly ubiquitous presence of the pathogen and disease-conducive environmental conditions. Six soil samples were collected from various locations and cropping systems on the Utsunomiya University Research Farm and Campus Garden. The soils were classified as volcanic ash, Haplic Andosol. They were tested in a bioassay for their potential to suppress sugar beet damping-off caused by *R. solani* AG 2.2. Disease incidence exceeded 80% in all pasteurized *Rhizoctonia*-infested soils, while in two untreated infested soils Rhizoctonia damping-off was less than 10%. This suppressiveness was transferred when 1% of untreated soil was mixed into the respective pasteur-

ized, or *Rhizoctonia*-infested soil. In both soils suppressiveness was eliminated by chloroform fumigation. Furthermore, in one soil the suppression was diminished after 30 minutes exposure to 55°C in a water bath while complete loss of this potential occurred at 65°C. In the other soil, the suppression potential remained unchanged at 55°C but was eliminated at 65°C. The results indicate a biological component of the suppression but suggest potentially different causal agents.

38.6 NEW DEVELOPMENTS WITH TOMATO GRAFTING AS ALTERNATIVE TO METHYL BROMIDE IN MOROCCO. **M. Besri.** *Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco. Email: m.besri@iav.ac.ma*

Soil-borne disease problems were relatively uncomplicated and of lesser importance in the early years, but increased in importance as intensive cultivation continued. Soil fumigation with methyl bromide to control soil-borne pathogens was until recently considered a major factor for successful production in plastic greenhouses. With consumer demand for untreated and organic products, the withdrawal of methyl bromide and its unavailability on the market, growers are increasingly looking for alternative approaches. Grafting, which was considered at one time too expensive, is now widely used on a commercial level. Resistant rootstocks provide excellent control of many soil-borne pathogens and have many other advantages. In addition, grafting does not require major adaptations in farming practice. When grafted plants are used, higher yield and quality are obtained. In 2006-2007, 95% of the protected tomato-producing areas were planted with grafted plants. A wide range of rootstocks with multiple resistances to infectious and non-infectious diseases are available and their number is increasing every year, driven by high demand and by the interest of seed companies.

38.7 BIOLOGICAL AND CHEMICAL CONTROL OF *RHIZOCTONIA* DRY ROOT ROT AND LEAF BLIGHT OF SOYBEAN CAUSED BY *RHIZOCTONIA SOLANI*. **M.K.A. Bhuiyan and M. Haider.** *Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh. Email: kabhuiyan55@yahoo.com*

An attempt was made to control dry root rot and leaf blight of soybean caused by *Rhizoctonia solani* in a pot culture experiment followed by a field experiment in which soybean seedlings inoculated with *R. solani* were treated with *Trichoderma harzianum* isolate HT7 integrated with Vitavax-200. Before setting up the integration experiment, a series of preliminary tests was made including a pathogenicity test of *R. solani* isolates against soybean seedlings to select a virulent isolate, and a screening test of the selected *T. harzianum* isolates against the growth of the test pathogen following dual culture. To select a suitable fungicide against the growth of *R. solani*, several fungicides were also evaluated *in vitro* at different concentrations. Compatibility of the growth of *T. harzianum* with fungicides was also evaluated. In the pot culture and field trial, integration of *Trichoderma* with Vitavax-200 appeared to be the most effective in controlling seedling mortality, dry root rot and leaf blight of soybean. Individual components of integrated measures significantly reduced the infection of soybean seedlings both in the pot culture and field trial but efficacy of the individual components was much improved when they were applied in the integrated approach. In conclusion, integration of wheat grain colonized with *Trichoderma* and seeds treated with Vitavax-200 appeared to be best in controlling seedling mortality and dry root rot, and also increased germination.

38.8 INTEGRATED MANAGEMENT OF *FUSARIUM EQUISETI* AND *SCLEROTIUM ROLFSII* OF TUBEROSE (*POLIANTHES TUBEROSA*). **M.K.A. Bhuiyan, M.T.Rahman and J.A. Begum.** *Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh. Email: kabhuiyan55@gmail.com*

An attempt was made to control the most prevalent pathogens *Fusarium equiseti* causing blossom blight and *Sclerotium rolfsii* causing stem rot of tuberose through an integrated approach including fungicides, *Trichoderma* and organic amendments in pot culture. The experiment was carried out at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh during March 2005 to July 2006. Altogether six fungi were found to be associated with the 21 tuberose plant samples tested, collected from different locations of Chuadanga district. Among the fungi isolated, *F. equiseti* and *S. rolfsii* were predominant. A total of 18 isolates of *Trichoderma harzianum* were screened against the virulent isolates *F. equiseti* F15 and *S. rolfsii* SR4 following the dual plate culture technique. *T. harzianum* TH6 was found to be the most effective against the test pathogens. In a preliminary laboratory trial Aimcozim and Mustard oilcake appeared to be the most effective in inhibiting the radial growth of the test pathogens and also compatible with the antagonist *T. harzianum* TH6. In pot culture, integration of *T. harzianum* TH6 with Aimcozim and Mustard oilcake appeared to be best in controlling leaf blight and stem rot of tuberose in comparison to any individual component of the integration, although individual components also significantly reduced leaf blight and stem rot.

38.9 SOIL-BORNE VIRUSES OF SUGAR BEET: A MORE COMPLEX PICTURE THAN PREVIOUSLY THOUGHT. **C. Bragard, M. Merhvar, C. Nagy, F. Crutzen and A. Legrève.** *Unité de Phytopathologie, UCL-FYMY, Croix du Sud, 2bte 3, B-1348 Louvain-la-Neuve, Belgium. Email: claude.bragard@uclouvain.be*

Sugarbeet is affected by numerous soil-borne viruses transmitted by *Polymyxa betae*, like the Benyviruses *Beet necrotic yellow vein virus*, and *Beet soil-borne mosaic virus*, the Pomoviruses *Beet soil-borne virus* and *Beet virus Q*, or like the Necrovirus *Beet black scorch virus* transmitted by *Olpidium brassicae*. Multiple infections are often recorded, raising questions about potential synergism or competion between the viruses and their respective vectors. Recent data gathered both in Europe and in Asia will be presented.

38.10 EFFECTIVENESS OF *STREPTOMYCES* SPP. ISOLATES TO CONTROL *COLLETOTRICHUM SUBLINEOLUM* IN SORGHUM. **W. Bressan and J.E. Figueiredo.** *Embrapa – Maize and Sorgum Research Center, P.O. Box, 151, 35701-970 Sete Lagoas, MG, Brazil. Email: bressan@cnpms.embrapa.br*

Anthracnose of sorghum, *Sorghum bicolor* (L.) Moench, caused by the fungus *Colletotrichum sublineolum* Ces. Wils. has been reported in most sorghum-growing regions of Brazil. The high genetic variability of the fungus is one of the most limiting factors for its control by genetic resistance. The effectiveness of four *Streptomyces* spp. isolates (DAUFPE 14632, DAUFPE 11470, CMS2A,

CMS4A) were evaluated to control nine sorghum physiologic races of *C. sublineolum* (13A, 15A, 31B, 30B, 15B, 13B, 31C, 30C, 29E) and four additional isolates (51, 57, 126, 148) from different locations in Brazil. Evaluations *in vitro*, were made by measuring the diameter of the inhibition halo formed by *Streptomyces* spp. isolates. Sorghum seeds were also inoculated with *Streptomyces* spp. isolates for evaluating the incidence of the pathogen *C. sublineolum* and coleorhizal protrusion during germination. The resistance of *C. sublineolum* to control by *Streptomyces* spp. isolates varied among races and isolates of *Streptomyces* spp. Isolate DAUPFE 11470 was the most efficient in controlling all *C. sublineolum* races and isolates both *in vitro* and in seed inoculation experiments. Fungus incidence in seeds inoculated with this isolate ranged from 88.5% to 99.1%, respectively for the most virulent race (31A) and the less virulent isolate (148). No control was detected by the *Streptomyces* spp. isolates CMS4A and CMS2A *in vitro* or with sorghum seed inoculation. These results indicate that *Streptomyces* spp. isolates are potential control agents against *C. sublineolum.*

38.11 SUPPRESSION OF *FUSARIUM MONIFORME* BY *STREPTOMYCES* SPP. ISOLATES IN RELATION TO DOSE-RESPONSE RELATIONSHIP. W. Bressan and J.E. Figueiredo. *Embrapa – Maize and Sorgum Research Center, P.O. Box, 151, 35701-970 Sete Lagoas, MG, Brazil. Email: bressan@cnpms.embrapa.br*

Fusarium moniliforme J. Sheldon (*Giberella fujikuroi* Sawasa Wollen) commonly infects a wide range of crops. On maize (*Zea mays* L.) the fungus causes seedling blight as well as root, stalk, ear and kernel rot. Two isolates of *Streptomyces* spp. DAUFPE 11470 and DAUFPE 14632, were evaluated to determine the suppression of *Fusarium* disease through antagonist-pathogen relationship under greenhouse conditions. Control plants were grown in soil without antagonist *Streptomyces* spp. isolates. Pathogen (10^3, 10^4, 10^5, 10^6 Chl/ml) and antagonist (10^3, 10^4, 10^5, 10^6 Cfu/ml) concentrations, significantly affected the development of *Fusarium* disease with a significant interaction between pathogen and antagonist concentration. Both *Streptomyces* spp. isolates demonstrated effective control of *Fusarium* disease, regardless of pathogen concentration. The hightest disease suppression for both isolates occurred at low pathogen concentration (10^3 Chl/g soil) and high antagonist concentration (10^6 Cfu/ml). The isolate DAUFPE 11470, provided the most effective control for all antagonist-pathogen inoculum concentration. Disease supression by isolate DAUFPE 11470 (10^6 Cfu/ml) did not differ significantly ($p \leq 0.05$) when the pathogen inoculum concentration ranged from 10^3 to 10^5 Chl/g soil. In relation to control plants, the highest disease supression, for both antagonist isolates, ocurred at the highest antagonist-pathogen concentrations. These values were 62% and 55% for DAUFPE 11470 and DAUFPE 14632, respectively. The results indicated that the effectiveness of suppression of *Fusarium* disease by *Streptomyces* spp. isolates depends on antagonist-pathogen concentration.

38.12 SURVIVAL AND INFECTION POTENTIAL OF *VERTICILLIUM DAHLIAE* IN WOOD CHIP MULCH FROM INFECTED OLIVE TREES. E. Cabeza-Fernández and J. Bejarano-Alcázar. *IFAPA. Centro Alameda del Obispo, Apartado 3092, 14080 Córdoba, Spain. Email: jose.bejarano@juntadeandalucia.es*

Mulching with wood chips from olive trees could serve as a source of inoculum in olive orchards if these materials come from *Verticillium dahliae* (Vd)-infected trees. Two experiments were conducted in two olive orchards infested with Vd in southern Spain in 2003 and 2004. Eleven trees seriously affected by *Verticillium* wilt were selected in each field. Single-spored Vd isolates from selected trees were characterized as defoliating (D) or non-defoliating (ND) pathotypes using a pathogenicity test on cotton cultivar Acala SJ-2. Pruned wood of each tree was chipped, placed in mesh bags and kept on the soil under field conditions. The chips without leaves were analyzed monthly for Vd, and the potential of D-infected chips to cause disease was determined in bioassays on Acala SJ-2. Seeds of Acala SJ-2 were sown in pots filled with a mixture of ground chips and sterile soil. All trees were infected by the D pathotype, except two trees in 2003 that were infected by the ND pathotype. Vd was isolated from 0.4 and 7.3% of D-infected chips 150 and 120 days after the start of experiments in 2003 and 2004, respectively. On the contrary, the fungus only survived in ND-infected chips for 30 days. Cotton plants showed severe *Verticillium* wilt symptoms with variable incidence, even when the soil was infested with D-infected chips maintained in the field for 120 days. These results suggest that chipped wood from infected olive trees used as mulch is a potential source of Vd inoculum in olive orchards.

38.13 EFFECT OF CROPPING SYSTEMS ON THE DYNAMICS OF BEAN WEB BLIGHT EPIDEMICS. A.C. Café-Filho, G.R. Costa and M. Lobo-Júnior. *Departamento de Fitopatologia, Universidade de Brasília, 70910-900, Brasília, DF, Brazil. Email: cafefilh@unb.br*

Web blight, caused by *Thanatephorus cucumeris* (anamorph *Rhizoctonia solani*) is one of the main tropical diseases of common bean (*Phaseolus vulgaris*). Grain yield losses may reach 100% in favourable conditions and high inoculum levels. Even at lower severity levels, grain from affected fields is unfit for use as seed or human consumption. *T. cucumeris* survives in cultivated or wild plants, crop debris, infested soil and seeds. No commercial bean cultivar is highly resistant, and control relies mainly on seed treatment and fungicide application. Despite its importance in several microclimates, especially during the rainy season, there has been very little study on the bean-*Thanatephorus* pathosystem, particularly in Brazil. We report the results of three years of field trials using different planting systems on the dynamics of web blight epidemics. Experiments followed a randomized complete block design with four replicates, with treatment units composed of four 5-meter lines planted with cv. Pérola. Three crop systems were compared: (1) No-till, over *Brachiaria* grass mulch; (2) Minimum till; and (3) Conventional tillage. Disease severity was rated several times during each cropping season and disease progress curves were drawn. Results indicated that the no-till system gave the lowest disease levels and lowest disease progress rates, possibly due to the presence of the grass mulch over the soil, which may have served as a physical barrier against basidiospore dispersion. Choice of cropping system has a significant impact on the severity of bean web blight.

38.14* GLOBAL POPULATION GENETICS AND PHYLOGEOGRAPHY OF THE MAIZE, RICE, AND SOYBEAN PATHOGEN *RHIZOCTONIA SOLANI* AG-1 IA. P.C. Ceresini, J. Bernardes de Assis, M.B. Ciampi, A.D. Gonzalez-Vera, M. Zala and B.A. McDonald. *ETH Zurich, Institute of Integrative Biology (IBZ), Plant Pathology, Universitaetstrasse 2, LFW B28, 8092, Zurich, Switzerland. Email: paulo.ceresini@agrl.ethz.ch*

One of the most important groups within the Basidiomycete

fungus *Rhizoctonia solani* species complex is AG-1, a major pathogen infecting maize, rice, soybean, and other important crops worldwide. Increased knowledge of the population biology of the pathogen is needed to implement more effective sustainable management strategies and to identify sources of infection. As part of this goal, our first objective was to determine the worldwide genetic structure of geographically distinct Poaceae-infecting (rice/maize) and Fabaceae-infecting (soybean) populations of *R. solani* AG-1 IA. We have analyzed populations from Asia (China and India), South-Central America (Brazil, Colombia, Panama and Venezuela) and North America (Texas and Louisiana, USA), totaling around 1500 samples. A second objective was to determine the importance of recombination in populations of *R. solani* AG-1 IA originating from different hosts. These populations were analyzed using a set of ten microsatellite loci recently developed in our lab as novel molecular markers for *Rhizoctonia*. Our study provided evidence for: i) Speciation, driven by host-specialization, between Poaceae-infecting (rice/maize) and Fabaceae-infecting (soybean) populations; ii) Mixed reproductive mode, consistent with clonal expansion with random mating episodes, for maize-, rice- and soybean-infecting populations; iii) Some genetic exchange between certain groups of populations over the spatial scales tested but not between other groups of populations. To answer questions about the origins of the Poaceae- and Fabaceae-infecting populations of *R. solani* AG-1 IA, we will present information on the historical and contemporary patterns of gene flow detected among these populations.

38.15 SELECTION OF ISOLATES IS IMPORTANT IN SCREENING WHEAT GERMPLASM FOR CROWN ROT RESISTANCE. **S. Chakraborty, C.Liu, J.B, Scott and K. Abeywickrama.** *CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, Brisbane, QLD 4067, Australia. Email: sukumar.chakraborty@csiro.au*

Crown rot (CR) and head blight of wheat, caused predominantly by *Fusarium pseudograminearum* and *F. graminearum* have linked epidemiology. Head blight has emerged as a disease of global significance and new knowledge has been generated from targeted research in many countries. CR is poorly understood despite being present in most cereal-growing regions. It causes serious loss of yield and grain quality in Australia and the Pacific Northwest of the USA. Variability in CR reactions has plagued germplasm screening, and research has not produced resistant varieties. Recently, a high throughput bioassay has reduced variability by standardising environmental and other conditions. In this work we examine potential pathogenic specialization within *F. pseudograminearum* and *F.graminearum* to further improve germplasm screening. Initially 60 wheat germplasms were screened with two isolates of different aggressiveness for each *Fusarium* species to identify a subset of 24 putative wheat differentials that discriminated the isolates. In repeat experiments the 24 differentials were further screened with five isolates from each of the two *Fusarium* species. CR severity was analysed using multivariate and other approaches. Results from principal component analysis show a differential reaction of the isolates, indicating potential specialization in the pathogen towards some wheat genotypes. Further work must include screening an expanded collection of field isolates and creating an inventory of dominant groups in the major wheat growing areas. Confirmation of pathogenic specialization will mean that representatives of all major pathogen groups may need to be used in developing wheat germplasm with broad-based CR resistance.

38.16 CULTIVAR RESPONSE TO AND CHEMICAL CONTROL OF *FUSARIUM* ROOT ROT OF LUPIN. **K.F. Chang.** *Alberta Agriculture and Food, Field Crop Development Centre, 5030 - 50 Street, Lacombe, AB T4L 1W8, Canada. Email: kan.fa.chang@gov.ab.ca*

Root rot of narrow-leaved lupin (*Lupinus angustifolius* L.) is primarily caused by *Fusarium* spp. in central Alberta, Canada. *F. avenaceum* (Corda ex Fries) Sacc. is the predominant species in our culture collections. Nineteen narrow-leaved lupin cultivars and lines were tested for their resistance to *F. avenaceum* under greenhouse and field conditions in 2006 and 2007. Reduction in emergence, plant height, root length and weight were observed in the inoculated plants of all genotypes under greenhouse conditions, and were associated with high root rot severity. Mean yield reduction ranged from 1-56 % among field-tested lines in 2006. The late-maturing lines MLU-324 and MLU-320 had the lowest yield losses under inoculated field conditions. Arabella and MLU-317 showed the lowest losses in emergence due to seedling blight. Field trials to assess the impact of six fungicide seed treatments [thiram; metalaxyl-M + fludioxonil (Apron Maxx); carbathiin + thiram (Vitaflo 280); carbathiin + thiabendazole (Crown); diazinon + captan (Agrox CD); diazinon + captan + thiophanate methyl (DCT)] on seedling blight were established at Lacombe, AB, in 2006 and 2007. Apron Maxx, Crown, and Vitaflo 280 significantly improved seedling establishment over thiram, Agrox CD and control treatments. In 2006, Apron Maxx and Vitaflo 280 also significantly improved yields in inoculated treatments relative to other fungicides and the untreated control. The use of resistant cultivars or lines with fungicide seed treatment appears to be an effective strategy to control the disease.

38.17 THE PRESENCE OF dsRNA ELEMENTS AND VIRUS-LIKE PARTICLES IN *RHIZOCTONIA SOLANI* AG-1 IA COLLECTED FROM TAIWAN. **L.C. Chen, Y.J. Lin, J. Cian. Tu and S.H. Chen.** *Department of Plant Pathology, National Chung Hsing University, Taichung 402, ROC. Email: lcchen@dragon.nchu.edu.tw*

Double-stranded RNA (dsRNA) genetic elements and mycoviruses are frequently found associated with fungi. In *Rhizoctonia solani* AG-1 IA isolates collected from Taiwan we detected four dsRNAs 2.0 kb, 2.5 kb, 3.0 kb and 14.0 kb in size. These dsRNAs were present in the cell debris and microsomal fractions of isolates Rs1M-3 and PR-06. The dsRNAs appeared to be stable in cells of isolates Rs17 and Rs1M-3 serially transferred up to four generations on PDA. In anastomosis tests, the 14.0 kb dsRNA in Rs17 and the 2.0 kb dsRNA in Rs1M-3 could be transmitted through hyphal fusion. The amount of pigment and the number of sclerotia produced by six isolates carrying the 2.0 kb dsRNA were less than with the other two isolates without the 2.0 kb dsRNA, but there were no differences in growth rate among those isolates. We conclude that presence of the 2.0 kb dsRNA in *R. solani* AG-1 IA may effect the production of pigment and sclerotia. Virus-like particles 20-25 nm in diameter, were purified from dsRNA-containing Rs1M-3. The size of its coat protein was 60 to 70 kDa. The dsRNA segments isolated from purified particles had the same molecular weight as those isolated from Rs1M-3mycelial tissue. From this study, we found that dsRNAs very commonly occur in *R. solani* AG-1 IA, and we showed that the dsRNA elements could be transmitted by hyphal fusion, and that virus-like particles are present in *R. solani* AG-1 IA.

38.18 IDENTIFICATION OF A SIMPLE SEQUENCE REPEAT (SSR) MARKER IN CULTIVATED PEANUT (*ARACHIS HYPOGAEA* L.) ASSOCIATED WITH *SCLEROTINIA* BLIGHT RESISTANCE. **K.D. Chenault, H.A. Melouk and A. Maas.** *USDA-ARS, Stillwater, OK 74075, USA. Email: Hassan.melouk@ars.usda.gov*

The production of cultivated peanut, an important agronomic crop throughout the United States and the world, is consistently threatened by various diseases and pests. Although information on the variability of morphological traits associated with disease resistance is plentiful, few molecular markers associated with such resistance have been reported. The identification of such markers would greatly assist peanut geneticists in selecting genotypes to be used in breeding programs. The objective of this work was to use simple sequence repeat (SSR) primers previously reported for peanut to identify a molecular marker associated with resistance to the fungus *Sclerotinia minor* Jagger. Total peanut genomic DNA was extracted from cultivated peanut genotypes, advanced breeding lines and plant introductions and subsequently subjected to PCR using different SSR primer pairs. As expected, most primer pairs revealed little or no polymorphism among the genotypes tested. However, one primer pair consistently produced a banding pattern distinctly different for those genotypes with demonstrated resistance to *S. minor* compared to that generated for genotypes with demonstrated susceptibility. The identification of a potential marker for *S. minor* resistance in peanut may prove to be extremely useful for screening germplasm collections.

38.19 INOCULATION OF SOILS WITH DIFFERENT AMOUNTS OF *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI* TO DETECT TAKE-ALL SUPPRESSIVENESS. **S.F. Chng, M.G. Cromey, A. Stewart, S. Dodd and M.V. Jaspers.** *New Zealand Institute for Crop & Food Research Limited, Private Bag 4704, Christchurch, New Zealand. Email: chngs@crop.cri.nz*

The practice of introducing the pathogen *Gaeumannomyces graminis* var. *tritici* (Ggt) into soils to screen for take-all suppressiveness has been widely used in field or laboratory trials, but the amounts of Ggt inoculum reported have varied greatly. In our study, the effects of adding sand/maizemeal inoculum to soil at five different levels of Ggt (0, 0.2, 0.5, 1 and 4% w:w) were investigated in a pot assay using wheat plants. Three soils with different cropping histories (5 years ryegrass, 8 and 2 years wheat) had different Ggt DNA concentrations (0, 200 and 1126 pg g^{-1} soil), and therefore represented different take-all suppressiveness. After 4 weeks growth at 19 °C, plant assessments showed that introducing 4% w:w of Ggt inoculum effectively differentiated the suppressiveness among the soils ($P<0.01$). The take-all root incidences were, respectively, 83, 69 and 81% for the three soils. The 4% w:w inoculum rate did not reduce root growth of the wheat plants, and is therefore an appropriate level for investigating take-all suppressiveness in different soils.

38.20 EVALUATION OF INTEGRATED STRATEGIES FOR THE CONTROL OF TOMATO CORKY ROOT. **M. Cirulli, M. Amenduni, C. Colella and M. D'Amico.** *Department of Biology and Plant Pathology, University of Bari, Via G. Amendola 165/A, 70126 Bari, Italy. Email: cirullim@agr.uniba.it*

Corky root caused by the soil-borne fungus *Pyrenochaeta lycopersici* has been known as a greenhouse tomato disease since the 1960s; subsequently it has spread epidemically almost everywhere in field-grown tomato in California, Israel and Italy, where it is nowadays considered as the major cause of tomato root rot. We studied the effectiveness of different control strategies in reducing corky root severity in different experiments under field and greenhouse conditions on soil naturally infested by *P. lycopersici*. In the field, in two distinct experiments, each of which was repeated for three years, soil solarization was used singly or in combination with 1- Brassicaceae green manure and 2- calcium cyanamide. Soil solarization in combination with Brassicaceae green manure or calcium cyanamide reduced the disease by 55 to 70% and by 40 to 70% respectively, in all years of testing. Calcium cyanamide and Brassicaceae green manure applied singly also provided significant control of tomato corky root, but not in all years. In further five-year field trials, yearly applications of manure (horse and cow mixture) significantly reduced corky root severity by 20 to 50%. In greenhouse, the effects of calcium cyanamide and soil solarization applied singly or in combination were evaluated in three-year trials. In these experiments all treatments, applied singly and in combination, significantly reduced corky root severity over the three years. The results have proved that integrated strategies are effective in controlling tomato corky root both in field and in greenhouse conditions.

38.21 DIVERSITY OF *SCLEROTINIA SCLEROTIORUM* FROM AGRICULTURAL CROPS AND MEADOW BUTTERCUP IN THE UK. **J. Clarkson, E. Clewes and J. Whipps.** *Warwick HRI, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK. Email: john.clarkson@warwick.ac.uk*

Sclerotinia sclerotiorum is a major pathogen of horticultural and agricultural crops worldwide and has a host range of more than 400 plants. Previous work suggested that sclerotia from different isolates varied in their ability to produce apothecia, which has implications for the temporal dynamics of ascospore release and management of the disease. Isolates of *S. sclerotiorum* collected in 2005 from carrot, lettuce, and oilseed rape crops were characterised using mycelial compatibility tests, microsatellite markers and by sequencing a section of the intergenic spacer (IGS) region of the ribosomal RNA (rRNA) gene repeat. Within the 96 isolates tested (32 from each crop), there were 65 mycelial compatibility groups (MCGs), with a group of 13 compatible isolates being the most frequently found MCG in each crop (5 each from carrot and lettuce and 3 from oilseed rape). Isolates from the same MCG often had the same IGS sequence or microsatellite marker profile. Sclerotia from *S. sclerotiorum* isolates from both the same and different MCGs were also assessed for their ability to produce apothecia. Overall there was a wide range of germination times, and isolates within the same MCG sometimes responded differently. In 2007, *S. sclerotiorum* isolates were also collected from meadow buttercup (*Ranuncuclus acris*) and are currently being characterised in the same way. The implications of the results will be discussed in relation to population structure and diversity of *S. sclerotiorum* in the UK and elsewhere.

38.22 EFFECTS OF CROP ROTATION ON LEVELS OF *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI* INOCULUM IN SOIL. **M.G. Cromey, S.L. Bithell and A. McKay.** *New Zealand Institute for Crop & Food Research Ltd, Private Bag 4704, Christchurch, New Zealand. Email: cromeym@crop.cri.nz*

Crop rotation has a strong effect on the severity of the root disease take-all of wheat, caused by *Gaumannomyces graminis* var. *tritici*, (Ggt). Inoculum survives between susceptible crops

on infected crop residues, on volunteer wheat in break crops, and on alternative hosts. In this study, we monitored 87 fields on 16 farms over five years to determine the effect of crop rotation on levels of inoculum in soil. Each field was sown in wheat in 2003. Ggt DNA levels in the soil were measured prior to sowing, and then after each crop was harvested (February–April each year). Rotations were those chosen by the farmers involved in the study. Ggt inoculum levels after a susceptible cereal (wheat or barley) averaged 644 pg/g soil, but ranged between 0 and 2988 pg/g soil. Where fields were sown again in cereal the following year, Ggt levels remained high (mean of 630 pg/g soil). Where break crops (usually brassicas, peas, ryegrass or white clover) were sown, inoculum levels usually dropped dramatically after a single year (mean of less than 20 pg/g). Inoculum levels increased again if a wheat crop was then planted, but this increase differed according to the break crop. The increase in Ggt inoculum on wheat or barley following pea or brassica crops was substantially greater than after ryegrass or white clover. Implications of these findings will be discussed.

38.23 VEGETATIVE COMPATIBILITY GROUPINGS AND VIRULENCE OF *FUSARIUM OXYSPORUM* IN BITTER GOURD AND BOTTLE GOURD IN THE PHILIPPINES. **C.J.R. Cumagun, Z.C. Oribiana, J. A. Aguirre, M.S. Tolentino, C. H. Balatero and C. A. Relevante.** *Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna 4031, Philippines. Email: christian_cumagun@yahoo.com*

Vegetative compatibility groups (VCGs) were determined for 57 *Fusarium oxysporum* isolates from *Momordica charantia* L. (bitter gourd) and *Lagenaria siceraria* (Mol.) Standley (bottle gourd) in two provinces in the Philippines namely Batangas and Bulacan using nitrate-nonutilizing (nit) mutants. Sixty one and 25 nit mutants were generated from *F. oxysporum* f.sp. *momordicae* and *F. oxysporum* f. sp. *lagenariae* isolates, respectively. Frequency of reversion to wild type was higher in *F. oxysporum* f. sp. *momordicae* than in the *lagenariae* populations. Two and four VCGs were found in bitter gourd from Batangas and Bulacan, respectively with a ratio of VCGs to isolates (VCG_{div}) of 0. 20 - 0.33. Two and three VCGs were found in bottle gourd from the two locations with VCG_{div} of 0.40 - 0.50. VCG was not correlated with radial growth rates of *F. oxysporum* in either hosts or locations. The two *formae speciales* of *F. oxysporum* were not vegetatively compatible. Low VCG diversity of *F. oxysporum* populations in both hosts and locations could be attributed to clonal reproduction, parasexual recombination and limited gene flow. Bottle gourd isolates from Bulacan were not compatible with those from Batangas whereas some bitter gourd isolates from both locations were compatible. incompatibility of bitter gourd and bottle gourd isolates suggests high host specificity of *F. oxysporum*. Virulence of some bitter gourd isolates tested was not associated with a particular VCG. Cross-inoculation tests showed that bitter gourd isolates were not pathogenic to bottle gourd and vice versa.

38.24 REACTIONS OF DURUM WHEATS TO *FUSARIUM PSEUDOGRAMINEARUM*, IN THE NORTHERN GRAIN GROWING REGION OF AUSTRALIA. **M. Davis, S. Simpfendorfer, T. Walters and L. Page.** *Queensland Department of Primary Industries and Fisheries, 13 Holberton Street Toowoomba, Queensland 4350, Australia. Email: Mattley.Davis@dpi.qld.gov.au*

To identify potential sources of resistance to *Fusarium pseudograminearum* (Fp), the causal agent of crown rot, a range of tetraploid germplasm was evaluated for incorporation into the durum-breeding program in the northern grain-growing region of Australia. Annually, Australia produces around 500,000 tonnes of durum wheat, of which 40% is exported. It's estimated that crown rot costs the Australian grains industry $56 million per year in yield and quality losses. Current durum varieties are highly susceptible to crown rot, with favourable disease conditions causing yield losses up to 50% with losses frequently between 20-30%. Partial resistance has been identified in hexaploid wheats at seedling and adult growth stages. To increase the disease resistance of Australian durum varieties, it is thought that pyramiding these sources with tetraploid resistance sources will capture multigenic resistance traits with loci on the A and B genomes. In Toowoomba, Queensland, seedling evaluations were conducted under glasshouse conditions with leaf-sheath lesioning assessed three weeks after inoculation with Fp. Field trials were conducted to determine adult plant resistance, on artificially inoculated black earth soils. Mature plants were assessed for disease incidence and severity by rating the internode lesioning and premature head death, expressed as the number of white or deadheads. Valuable resistance was identified in diverse origins including *T. monococcum, T. timopheevii, T. dicoccum* and *T. carthlicum*. Single plant selections in our crown rot nurseries; have produced homozygous lines with high levels of resistance whilst retaining important agronomic characteristics, beneficial to durum breeding and the Australian durum industry.

38.25 INTEGRATED CONTROL OF *SCLEROTINIA SCLEROTIORUM* IN SUNFLOWER. **W. Dercks, A. Keuck, F. Meißner, C. Seyler, M.L. Kreller and K. Binder.** *Fachhochschule Erfurt - University of Applied Sciences, Fachbereich Landschaftsarchitektur, Gartenbau und Forst, Leipziger Straße 77, D-99085 Erfurt, Germany. Email: dercks@fh-erfurt.de*

Soft rot caused by *Sclerotinia sclerotiorum* is a major threat to many crops. From 2001 to 2003 a field trial was carried out in which all legal control measures available to German growers were tested. Sunflower was used as a model plant because it is infected by *S. sclerotiorum* mycelium in soil, not by airborne ascospores. Thus, airborne infections can be excluded when efficacy of soil treatments is compared. The following treatments were tested according to the manufacturer's recommendations: 1 - untreated control; 2 - Contans WG (6 kg/ha before sowing); 3 - Basamid-Granulate (500 kg/ha before sowing); 4 - lime-nitrogen (600 kg/ha before sowing); 5 - Rovral (0.1%); 6 - Contans WG (6 kg/ha before sowing) plus Rovral (0.05%). Treatments 2, 3, and 4 were worked 15 cm deep into soil, 5 was sprayed on the plants at occurrence of first disease symptoms and again 14 days later. Treatment 6 was done as in 2 and 5, respectively. Contans WG (a product based on the biocontrol agent *Coniothyrium minitans*) exhibited good activity against *S. sclerotiorum* if applied to soil early. Under wet conditions, efficacy was better than that of Basamid-Granulate (dazomet, a soil-applied biocide and MITC generator; banned in Germany since 1 January 2007) and lime-nitrogen (calcium cyanamide, a fertilizer with biocidal activity). Rovral (iprodione, a fungicide) was almost ineffective when applied alone. However, the combination of Contans WG before sowing and Rovral at occurrence of first symptoms was consistently among the top two treatments every year, thus pointing towards a promising strategy for integrated control of soft rot.

38.26 SPATIAL INVASION THRESHOLDS: FROM THEORY TOWARDS EXPLOITATION IN PLANT PROPAGATION. V. Deytreux, W. Otten, A. Bates, C.A. Gilligan and D.J. Bailey. *INRA-Agrocampus Rennes, UMR BiO3P BP 35327 F-35653 Le Rheu Cedex, France. Email: djb21@cam.ac.uk*

Field vegetable production routinely involves the propagation of seedlings in trays composed of units of varying size and planting density. Recent epidemiological (percolation) theory for the spread of soil-borne disease demonstrates a clear link between fungal growth from an individual host, the density of hosts, and invasive spread of the pathogen in a population of hosts. For predicting disease risk in vegetable seedlings this theory suggests a switch from restricted spread of the pathogen at low planting density to invasive spread at higher planting densities. The main risks to these systems are (i) invasive spread of soil-borne pathogens and (ii) hidden infestation without visible symptoms of disease. The relevance of this theory for the spread and infestation of damping-off disease caused by *Rhizoctonia solani* was examined in commercial propagation trays. Epidemic behaviour of *R. solani* was strikingly similar to predictions from percolation theory. For propagation systems with the highest planting densities, disease was invasive for all replicates. At intermediate planting density some disease patches were invasive whilst other were not, and at low planting densities no patches were invasive. An infestation-front was detected (using ELISA) extending one or two plants beyond the disease front. We conclude that percolation theory can be adapted and used as the basis for analysis and management of disease risk in plant propagation. The implications for disease control are discussed.

38.27 MANAGING PLASMODIOPHORID PATHOGENS ON AUSTRALIAN VEGETABLE FARMS. E.C. Donald, I.J. Porter, R. Faggian, N.S. Crump, R.F. de Boer, T.J. Wiechel, C.S. Scoble and A.K. O'Toole. *Department of Primary Industries Victoria, Private Bag 15, Ferntree Gully Delivery Centre, VIC 3156, Australia. Email: caroline.donald@dpi.vic.gov.au*

Plasmodiophora brassicae and *Spongospora subterranea* are two important horticultural pathogens causing clubroot of vegetable brassicas and powdery scab of potatoes respectively. An integrated program to control and prevent the spread of *P. brassicae* is widely used on Australian vegetable brassica farms. The program uses a 'whole of production' approach and incorporates pathogen detection, eradicant and preventative methods to manage inoculum below the threshold required for disease. In commercial nurseries a PCR assay has been used to identify sources of inoculum contamination. In the field, real-time PCR has been used to quantify the inoculum load in soils and predict expected yield loss. Manipulation of soil pH (by application of burnt lime, CaO), application of calcium and boron, and strategic placement of the fungicide fluazinam (1.5 l a.i. /ha in 500 l water/ha) have been used alone (on low risk sites), or together, as part of an integrated clubroot control strategy (on moderate-high risk sites). A similar strategy is now being developed for *S. subterranea*. In field trials, fluazinam has consistently caused a significant reduction in the incidence and severity of powdery scab on tubers. In spite of their taxonomic similarities, there are key differences between these pathogen/host interactions, including the duration of cropping and infection sites (root and tuber) and this influences the effectiveness of different management strategies. The implication of these differences is discussed in relation to the interpretation of predictive soil DNA tests and the method and timing of application of fluazinam.

38.28 BIOLOGICAL CONTROL OF *FUSARIUM* WILT OF BANANA USING NON-PATHOGENIC F. OXYSPORUM ENDOPHYTES. A. Faber, C. Steinberg and A. Viljoen. *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa. Email: AneenF@arc.agric.za*

Fusarium oxysporum f.sp. *cubense* (Foc) is responsible for *Fusarium* wilt, a disease that caused serious losses to export bananas in Central America in the mid-1900's. Non-pathogenic isolates of *F. oxysporum*, found in disease-suppressive soils, were reported to suppress *Fusarium* wilt in greenhouse pathogenicity trials. To study their interaction on banana roots, a non-pathogenic *F. oxysporum* endophtye from banana roots in Kiepersol, South Africa that reduced *Fusarium* wilt incidence by 67% were modified with a red fluorescent protein gene (DsRed-Express), while a pathogenic isolate of Foc was modified with a green counterpart (GFP). The modified and unmodified isolates did not differ in growth and morphological characteristics, or in their virulence to banana plantlets. By using a split-root inoculation system, we showed that the non-pathogenic *F. oxysporum* isolate induced significantly higher levels of cell wall-bound phenolics in non-inoculated banana roots than the water control. Competition is unlikely to be the primary mode of action when the pathogen and non-pathogen were inoculated on the same roots at equal concentrations. Confocal laser scanning microscope observations were unable to prove that the reduced infection of banana roots was the result of induced resistance, as neither the pathogen nor the non-pathogen was able to infect banana roots in the absence of wounds. Whether the non-pathogenic *F. oxysporum* isolates would protect bananas by means of induced resistance after wounding and following abiotic stress needs to be further investigated.

38.29 EFFECT OF SOWING DATES ON NATURAL EPIDEMICS OF TAKE-ALL IN SOUTHERN IRAN: A STUDY OF THREE SUCCESSIVE WHEAT CROPS. A. Fassihiani. *Fars Agricultural and Natual Resource Center, P.O. Box 73415-111, Fars, Zargan, Iran. Email: a_fassihiani@yahoo.com*

Take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* has recently been reported in Fars province, Iran. The effect of sowing dates on take-all was studied in a naturally infested field grown with wheat for three consecutive years. The disease was most intense in the first year and subsided in the 2nd and the 3rd years. The disease incidence for combined sowing date data during three years for the sowing dates 20th October, 5th and 20th November, 5th and 20th December were 34, 25, 27, 25, and 21%, respectively. While take-all index varied from 1.7-2.4 (maximum 4). In each year the disease levels among sowing date treatments were varied. In the first wheat crop, disease incidence and severity were higher in the early-sown crop compared to the rest of sowing dates (P=0.05). In the 2nd and 3rd year, no significant differences were found among sowing date treatments. However disease was lower as the sowing dates were more delayed. In the first year, where the disease was most intense, delayed sowing led to yield increase in some treatments. In the 2nd and 3rd wheat crop where the disease was slight, delayed sowing had no effect on yield. A linear regression between yield and take-all index for pooled sowing dates data for the first wheat crop showed a decreased of 0.775 t ha^{-1} for each take-all index unit. This suggests a 3 t ha^{-1} yield loss in fields severely infected with take-all.

38.30 IMPACT OF INPUT LEVELS ON *FUSARIUM* INFECTION OF WHEAT CROWNS IN SASKATCHEWAN, CANADA. **M.R. Fernandez, D. Ulrich, S. A. Brandt, A.G. Thomas, O. Olfert, R.P. Zentner and P. Basnyat.** *Semiarid Prairie Agricultural Research Centre, Agriculture and Agri-Food Canada, P.O. Box 1030, Swift Current, Saskatchewan S9H 3X2, Canada. Email: fernandezm@agr.gc.ca*

Fusarium head blight (FHB) pathogens are known to infect crowns and roots of wheat. In 2004-06, crown rot was examined in a cropping system experiment in west-central Saskatchewan, Canada. There were three input levels: High (HI) with conventional tillage and recommended herbicide and fertilizer rates; Reduced (RI) with tillage reduced, herbicide and fertilizer use; and Organic (OI) with intensive tillage and no chemicals, and three cropping diversity levels (fallow-based, diversified annual grains and diversified grain/forage). Crowns were scored for discoloration and plated for fungal identification. Input level had a greater impact on disease severity and fungal frequency than cropping diversity. Severity of crown discoloration was greatest for HI and lowest for OI. Frequency of isolation of *Cochliobolus sativus*, the most common fungus, was highest in OI followed by HI, and lowest in RI. *Fusarium* isolates were most common in RI where they were as frequent as *C. sativus*. However, individual species varied with input system. *F. avenaceum* was most associated with RI. In OI, *F. avenaceum*, *F. culmorum* and *F. oxysporum* were at the lowest frequency, whereas the weak pathogen/saprophyte *F. equiseti* was at the highest frequency and accounted for most of the *Fusarium* isolates. We concluded that management systems that decrease tillage frequency, and rely on herbicides for weed control, would help to reduce crown rot caused by *C. sativus*, but would increase infections by *F. avenaceum*. In contrast, OI would help to reduce populations of *F. avenaceum* and *F. culmorum*, two of the most important FHB pathogens.

38.31 RESURGENCE OF BACTERIAL WILT IN THE SOUTHEASTERN USA AND A REFORMULATION OF OUR MANAGEMENT SYSTEM. **B.A. Fortnum and P. Peterson.** *Clemson University, Pee Dee REC, 2200 Pocket Road, Florence, SC, 29506, USA. Email: ppeters@clemson.edu*

The systems approach developed by North Carolina, experiment station and USDA (United States Department of Agriculture) scientists provided effective bacterial wilt control on tobacco for nearly a half-century. Sudden outbreaks of bacterial wilt in previously uninfested areas as well as a general increase in disease intensity across the Carolina since the 1980s provides evidence of a breakdown in the management system used to control this disease. Generally, infection of tobacco by *Ralstonia solanacearum* occurs through the root system. Epidemics of bacterial wilt are so common within the Carolina that the organism must be spread in a more rapid and efficient manner than the movement of soil on equipment. Contamination of steel cutter blades with the bacterium during mechanical flower removal (topping) or stalk cutting can spread *R. solanacearum* from diseased to healthy tobacco plants both within and between flue-cured tobacco fields. *R. solanacearum* contaminated steel blades can easily move the bacterium from a diseased plant to the next 50 or more healthy plants resulting in near total field collapse by the end of the growing season. Likewise contaminated defoliator blades on tobacco harvesters can move the bacterium from disease plants to healthy plants. Redesigned tobacco toppers (flower removal) and leaf harvesters were evaluated to reduce mechanical transmission of *R. solanacearum*. The development of a revised management system including redesigned topping and harvesting equipment will be discussed.

38.32 INFLUENCE OF THE QUALITY OF COMPOSTS ON THEIR POTENTIAL FOR DISEASE SUPPRESSION. **J.G. Fuchs, A. Berner, J. Mayer, K. Schleiss and L. Tamm.** *Research Institute of Organic Agriculture, FiBL, CH-5070 Frick, Switzerland. Email: jacques.fuchs@fibl.org*

In order to estimate the potential of Swiss composts and digestates to improve soil fertility and plant health, a representative sample of one hundred composts was analyzed. The influence of physical, chemical and biological quality on soilborne diseases was investigated under controlled conditions. Two pathosystems were used: cucumber–*Pythium ultimum* and basil–*Rhizoctonia solani*. While the majority of composts protected cucumber plants against *P. ultimum*, only a few composts suppressed *R. solani* in basil. Management of the maturation process seems to play a major role in disease suppression. Some of the composts were also tested in the field with maize. At maize harvest, biological activity of the soils was increased by the composts. However, no significant difference on disease receptivity of the soils was observed. The possibility to improve the capacity of composts to protect plants against disease will be discussed.

38.33 HIGHLY SENSITIVE DETECTION OF *PHOMOPSIS SCLEROTIOIDES* FROM SOIL BY NESTED TIME-RELEASE PCR. **H. Furuya, E. Sato and S. Fuji.** *Department of Biological Production Science, Akita Prefectural University, Shimo-shinnjo, Nakano, Japan. Email: furuya@akita-pu.ac.jp*

Black root rot of cucumber is caused by *Phomopsis sclerotioides* and was first reported in 1983 in Japan. Since then the disease has spread to many cucurbit-producing regions, causing severe damage. The pathogen is a typical soil-borne fungus that can increase its inoculum potential in the soil before causing the typical plant symptom of wilting. It is therefore necessary to detect the pathogen in soil before the symptom appears, in order to prevent further spreading. Soils of volcanic ash origin are widely distributed in Japan. In order to prevent DNA absorption by this type of soil matrix, skim milk was added to soil samples (usually 40 mg/0.5 g). DNA was then extracted using a commercial kit. Detection of *P. sclerotioides* was performed by time-release PCR with 50 cycles, using fungus-specific primers (CPs1 and CPs2) and two polymerases (r Taq and Taq Gold). By this technique it was possible to detect 50 fg of pathogen DNA extracted from mycelia grown in nutrient broth, and to detect the fungus in sandy soil which had been artificially inoculated at 10 cfu/g. Furthermore, the fungus could be detected with more sensitivity and from a wider range of soil types when a pre-PCR step was conducted using fungal universal primers ITS1 and ITS4, before the time release PCR. The pathogen could be detected in soil samples collected from commercial fields, not only in regions where the disease was recognized, but also in regions where the disease had not been found.

38.34 EFFECT OF TILLAGE AND RESIDUE MANAGEMENT ON MAIZE AND WHEAT ROOT-ROT IN DIFFERENT AGRO-ENVIRONMENTS. **B. Govaerts, M. Mezzalama and K.D. Sayre.** *CIMMYT Apdo. Postal 6-641, 06600 México D.F., Mexico. Email: m.mezzalama@cgiar.org*

Conservation agriculture –the use of reduced or zero-tillage, retention of rational amounts of crop residues, and use of viable crop rotations– can reduce production costs and help meet the need for more economically sound, environmentally friendly, sus-

tainable agricultural production systems for rainfed and irrigated areas. Little is known about the mid-to-long-term effects of tillage and residue management on wheat and maize root-rot. CIMMYT long-term sustainability trials begun in the early 1990s compare various tillage and residue management practices in semi-arid (2,240 m asl; 19.31°N; 98.50°W; Cumulic Phaeozem) and humid (2,640 m asl; 19.17°N, 99.33°W silty clay loam soils of volcanic origin) central highlands of Mexico under rainfed conditions and in the arid, irrigated systems of northern Mexico (38 m asl; 27.33°N; 109.09°W; Calcic Vertisol). This paper describes long-term effects of the tested practices on root rot and the disease's possible detrimental effects on yield. Under zero-tillage with residue retention, root rot was moderate in maize, and yield was higher than under alternative practices. In wheat zero-tillage with residue retention also gave the highest yields, with intermediate root rot incidence. Zero-tillage with residue retention and rotations enhanced water availability, soil structure, and nutrient availability more than conventional tillage. Soil microbial diversity increased under zero-tillage with residue retention, facilitating integrated pest management.

38.35 A BANANA AMENDMENT TRIAL: DO AMENDMENTS ALTER *FUSARIUM* WILT SEVERITY AND SOIL MICROBIOLOGY? **L.M. Gulino and A.B. Pattison.** *Department of Primary Industries and Fisheries, 80 Meiers Rd, Indooroopilly,* QLD *4068, Australia. Email: lisa-maree.gulino@dpi.qld.gov.au*

A banana amendment glasshouse trial was conducted using naturally and artificially infested soil, containing sub-tropical race 4 of the pathogen *Fusarium oxysporum* f.sp. *cubense* (Foc). Soil was mixed with nine amendment treatments and tested for capacity to alter disease severity, plant growth, soil microbiology, and to determine interactions. In the naturally infested soil, addition of mill ash significantly reduced wilting caused by Foc, while addition of calcium silicate resulted in the lack of development of internal disease symptoms. In artificially inoculated soil, the addition of amendments did not significantly alter wilt symptoms when compared to the control. However, there was an increase in disease severity with the addition of banana trash and grass hay. The addition of amendments in both the natural and artificially inoculated soil resulted in soil microbiological changes, and in some instances changes in plant growth. It has been proposed that disease severity may be related to changes in soil microbial diversity or activity. In naturally infested soil, it was observed that when *Fusarium* disease severity was significantly lower than the untreated control (no amendment), the number of green leaves and the soil microbial activity were significantly higher. This however, did not occur with the artificially inoculated soil. There may be a link between *Fusarium* disease severity on bananas, plant growth and soil microbial activity; however, further work is required to elucidate the relationship.

38.36 THE BRASSICA STEM CANKER DISEASE COMPLEX. **C. Hitch, B. Hall and T. Wicks.** *South Australian Research and Development Institute, P.O. Box 397, Adelaide, SA 5001, Australia. Email: hall.barbara@saugov.sa.gov.au*

Stem-girdling cankers with varying degrees of severity have been observed on many Brassica crops in Australia. In recent years growers in South Australia have reported losses of up to 80% as a result of stem canker. Disease surveys in 2005/06 showed that a complex of fungi including *Rhizoctonia* sp., *Leptosphaeria maculans*, *Phoma* sp., *Pythium* sp., *Sclerotinia sclerotiorum*, *Fusarium* sp. and *Verticillium alboatrum* were associated with such cankers. *R. solani* and *L. maculans* were the dominant pathogens of the disease complex, and 3 anastomosis groups (AG2.1, AG2.2 and AG4) of *Rhizoctonia* were identified in association with the cankers. Seedlings are commonly grown in a nursery for 6-8 weeks before field planting. No infected plants were found in the nursery, whereas high levels of both *R. solani* and *L. maculans* were detected in soil pre-planting. Management strategies are targeted to pre-planting and planting soil drenches of fungicides and biological agents, as infection occurs 2–4 weeks after planting. Greenhouse trials have shown that some fungicides registered for control of *Rhizoctonia* in other crops are not consistently effective against all the anastomosis groups when applied as planting drenches. For example Amistar® (250g/l azoxystrobin) was not effective against AG2.1 in 2 out of 3 experiments, but was effective against AG4 in all 3 experiments. Greenhouse and field trials are continuing to determine the most effective treatment regime to manage Brassica stem canker.

38.37 DOES WATERLOGGING INFLUENCE PHOSPHITE PROTECTION OF BANKSIA SPECIES AGAINST *PHYTOPHTHORA CINNAMOMI*? **D. Hüberli, T. Paap, K. Gower, N. Long, B. Dell and G.E.StJ. Hardy.** *Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Perth, WA 6150, Australia. Email: d.huberli@murdoch.edu.au*

Parts of the southwest of Western Australia are subject to periodic flooding in areas that are also devastated by *Phytophthora* dieback disease caused by *P. cinnamomi*. Phosphite has been shown to be effective in controlling this pathogen. Waterlogging induces multiple physiological dysfunctions in plants, but it is unknown whether waterlogging alters the uptake, distribution and efficacy of phosphite in controlling *P. cinnamomi*. Waterlogging trials were conducted in the greenhouse using *Banksia attenuata* and *B. baxteri*. The response of these plants and subsequent recovery from waterlogging was examined. A phosphite spray treatment was applied pre- and post- waterlogging of either 3 or 14 days duration. Leaf gas exchange, leaf water potentials, lesion development and phosphite concentrations in leaf, stem and root tissue were monitored 1 week, 1 month and 4 months after the phosphite treatment. For the 1 week harvest when phosphite was applied pre-waterlogging, phosphite in plant tissue was at similar levels for each species and was not affected by waterlogging. But lesions on *B. baxteri* stems were not reduced in treated plants as they were for *B. attenuata*. Photosynthesis and water potentials were reduced for waterlogged *B. attenuata*, but had no impact on waterlogged *B. baxteri*. Leaf water potentials, leaf gas exchange, lesion lengths on inoculated stems, and phosphite concentration in leaves, stems, and roots measured at different time periods after waterlogging will be presented.

38.38 DOES FIRE INFLUENCE PHOSPHITE PROTECTION OF WESTERN AUSTRALIAN INDIGENOUS PLANT SPECIES AGAINST *PHYTOPHTHORA CINNAMOMI*? **D. Hüberli, T. Paap, N.A. Moore, S. Barrett, G. Freebury, B. Dell and G.E.St.J. Hardy.** *Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Perth, WA 6150, Australia. Email: D.Huberli@murdoch.edu.au*

Large areas of indigenous forests, and *Banksia* woodlands and heathlands in Australia are devastated by *Phytophthora* dieback caused by *P. cinnamomi*. Phosphite has been shown to be effec-

tive in controlling this pathogen on a wide range of plant species across different families. Although fire is a regular event in the Australian landscape and plays key roles in the ecosystem, nothing is known about the relative uptake of phosphite by shoots pre- and post-fire or how fire may alter the redistribution and persistence of phosphite within woody plants. *Adenanthos cuneatus* (re-sprouter), *Banksia attenuata* (re-sprouter) and *B. baueri* (re-seeder) are all susceptible to *P. cinnamomi* and are responsive to phosphite treatment. These species were selected within four plots in an area of the Stirling Range National Park that was scheduled for a fuel-reduction burn in November 2006. Treatments of the plots were: 1) phosphite spray without fire, 2) phosphite spray with fire, 3) no phosphite spray without fire, and 4) no phosphite spray with fire. A phosphite treatment was applied either 6 weeks pre-fire or 9 months post-fire when all re-sprouter species had sufficient foliage. Leaf water potentials, leaf gas exchange, lesion lengths on inoculated stems, and phosphite concentration in leaves, stems, lignotubers and roots measured periodically throughout the experiment will be presented.

38.39 SOIL TREATMENTS AND AMENDMENTS FOR MANAGEMENT OF CLUBROOT ON CANOLA IN ALBERTA, CANADA. **S.F. Hwang, S.E. Strelkov, G.D. Turnbull, V. Manolii, R.J. Howard, M. Hartman and P. Laflamme.** *Alberta Agriculture and Food, 17507 Fort Road, Edmonton, AB, Canada. Email: sheau-fang.hwang@gov.ab.ca*

Clubroot, caused by *Plasmodiophora brassicae* Woronin, has appeared in many canola crops near Edmonton, Canada. With a half-life of four years, this pathogen represents a long-term challenge to canola production in central Alberta. Field plots were established in infested soils near Leduc and St. Albert, Alberta, to determine the effects of soil amendments and chemical soil treatments on crop damage due to clubroot. Clubroot severity was significantly lower compared to the untreated control in soils treated with Terraclor 75% WP. This treatment also resulted in reduced seedling mortality, increased plant cover and increased plant height in severely infested soils. Yield increased with dosage level of Terrachlor, but was unaffected by the other chemical treatments. Percentage plant cover and height also responded positively to treatment with Ranman at 7.5 l/ha in less severely infested soils. Amendment of infested soils with calcium carbonate, wood ash, or calcium cyanamide did not result in changes in clubroot severity, compared to the untreated control. In severely infested soils, amendment with wood ash at 7.5 t/ha or with calcium carbonate at 5.0 or 7.5 t/ha resulted in greater plant height and crop cover compared to the untreated control. Yield was greater in plots treated with the high rate of wood ash, and the two highest rates of calcium carbonate, compared to all other soil amendments. Results indicate that Terraclor 75% WP and treatment with high levels of calcium carbonate or wood ash have the potential to reduce the effect of *P. brassicae* on canola.

38.40 PATHOGENIC STRAINS OF *VERTICILLIUM ALBO-ATRUM* AND THE REACTION OF HOPS. **J. Hýsek and P. Svoboda.** *Crop Research Institute, v.v.i., Drnovská 507, 16106 Praha 6 - Ruzyn, Czech Republic. Email: hysek@vurv.cz*

Strains of *Verticillium albo-atrum* pathogenic for hops were tested on young and old plants of Czech hop varieties. The fungal pathogenic strains came from England, Holland, Slovenia and Germany. The most resistant hop varieties were Sládek and Premiant. Varieties Agnes and new selections OK-72 and OK-114 had medium resistance. The new breeding line OK-31 was weakly resistant (susceptible). They had only medium susceptibility on biological treatment with biopreparations (Supresivit, Polyversum and Ibefungin) and medium susceptibility on chemical preparations Ridomil Gold Plus 42,5 WP, Aliette 80 WP and Kuprikol.

38.41 IMPACT OF SOIL TEXTURE ON THE REPRODUCTION AND SURVIVAL OF *MELOIDOGYNE INCOGNITA* AND *THIELAVIOPSIS BASICOLA* ON COTTON. **J. Jaraba, C. Rothrock and T. Kirkpatrick.** *217 Plant Science Building. Fayetteville, AR, 72701, USA. Email: jaraba@uark.edu*

Meloidogyne incognita (Mi) and *Thielaviopsis basicola* (Tb) are two important pathogens on cotton in the USA, and interact in a synergistic manner. The role of soil texture on cotton growth and pathogen reproduction and survival was studied in microplot experiments at Fayetteville (in 2006 and 2007) and Hope (in 2006), Arkansas. Sandy loam soils (48% sand) were used, and artificial soil textures were produced by mixing these soils with sand (texture range 54 to 91% sand). Soils were pasteurized and six treatments were applied: 1) noninfested, 2) Mi - 4 eggs/cc, 3) Mi - 8 eggs/cc, 4) Tb (always at 100 chlamydospores/g), 5) Mi - 4 eggs/cc plus Tb, and 6) Mi - 8 eggs/cc plus Tb. Soil water was controlled by watering each soil mix to saturation at -10 or -30 joules/kg daily (early and late season, respectively). Plant height was reduced by both pathogens, with greater reduction on the sandiest soil when both pathogens were present. Tb decreased plant nodes, dry weight and root weight over all soil textures. Root discoloration was higher on fine soil textures. Galling and Mi reproduction was reduced by Tb in all soil textures. Overwinter populations of Mi were reduced by Tb in fine soil textures, and Mi survival was greater in sandy soils. Tb reproduction and survival was greater in fine textured soils. Soil texture influenced Mi and Tb survival and reproduction in this study, in which soil water content was controlled.

38.42 ETIOLOGY AND MANAGEMENT OF CORM ROT OF SAFFRON (*CROCUS SATIVUS*) IN KISHTWAR DISTRICT OF JAMMU & KASHMIR, INDIA. **C.S. Kalha, V.K.Razdan and V. Gupta.** *Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Udheywalla Campus 180002, J&K, India. Email: kalhacs@yahoo.co.in*

Saffron (*Crocus sativus* L.) is an important low-volume, high-value perennial crop known for its medicinal properties. In Jammu province of the state of Jammu and Kashmir, Kishtwar is a place known for high quality saffron. However, corm rot was observed as the major biotic factor limiting cultivation in the area. The highest incidence of corm rot was recorded in Upper Pochhal (35.7%) and lowest in Naghani (8.7%). Pathogens viz., *Fusarium oxysporum* f.sp. *gladioli*, *F. solani*, *Penicillium* sp. and *Sclerotium rolfsii* (first report from India) were found associated with diseased corms. All the pathogens were found highly sensitive to carbendazim 50% W.P. under laboratory conditions. In field conditions corm treatment with carbendazim 50% w.p @ 0.2% for 20 minutes reduced the disease incidence (12.15%) and promoted germination percentage (88.95%). Integration of cultural practices i.e. optimum seed rate @ 60q/ha, planting corms at 10 cm depth with row spacing of 15 cm, corm treatment with carbendazim @ 0.2% and soil application of *Trichoderma harzianum* @ 1 kg in 50 kg of well decomposed farm yard manure

for one acre enhanced the production. These practices have encouraged the farmers to go in for the cultivation of saffron, which otherwise had take a severe set back due to corm rot. This also helped in increasing the area of saffron cultivation in Kishtwar due to production of healthier daughter corms.

38.43 DEVELOPMENT OF BACTERIAL FORMULATIONS FOR CONTROLLING SHEATH BLIGHT DISEASE OF RICE. **M. Kanjanamaneesathian, R. Wiwattanapatapee, A. Pengnoo and A. Chumthong.** *Plant Production Technology Program, Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi IT campus, Cha-Am, Phetchaburi, 76120, Thailand. Email: kmana@su.ac.th*

Sheath blight caused by *Rhizoctonia solani* Kühn is one of the more damaging rice diseases. The following control measures have some shortcomings. Fungicide application is not compatible with the production of organic rice which is in demand by certain groups of consumers. Implementation of crop rotation is limited or not applicable due to the lack of alternative economic crops. Burning crop residues has been considered as contributing to the deterioration of air quality. Our laboratory has developed formulations, using endospores of *Bacillus megaterium* as an active ingredient, to control sheath blight disease of rice. Three types of formulation have been produced with pharmaceutical technology. Floating pellets composed of hydrogenated vegetable oil, lactose, microcrystalline cellulose and cross-linked sodium carboxymethyl cellulose are prepared by extrusion-spheronization. Water-soluble granules composed of lactose, polyvinyl pyrrolidone (PVP) and sodium alginate and effervescent granules composed of lactose, PVP and effervescence base are prepared by a wet granulation method. Greenhouse and field tests have shown promising results in suppressing sheath blight. The final formulations contain high numbers of *B. megaterium* endospores (10^9 CFU/g after 21 months storage at 26–34°C). Physical characteristics (such as friability and dissolution time) of these formulations have been determined and data indicate that % friability is low and the dissolution time is short, showing that the formulation is suitable for transportation and application. These products are being tested for controlling diseases in hydroponically grown plants. Augmented applicability would enhance the chance of commercializing these novel bacterial formulations.

38.44 EFFECT OF COMPOSTED ANIMAL MANURE ON DAMPING-OFF OF VEGETABLE SEEDLINGS. **M. Kena and W.J. Swart.** *National University of Lesotho, P.O. Roma 180, Lesotho. Email: am.kena@nul.ls*

Composted animal manure is traditionally used by small-scale farmers in Lesotho to produce seedlings, but there have been no investigations in the country on the effect of these composts on soil-borne diseases, especially damping-off. The objectives of this study were to evaluate the suppressiveness of cattle, sheep, poultry and pig manure towards damping-off caused by *Fusarium oxysporum*, *Rhizoctonia solani* and *Pythium ultimum* and to determine their effect on seedling biomass. The four composts were each inoculated with isolates of these fungi. Compost prepared from cattle, poultry or pig manure were most suppressive and also significantly increased the biomass. Pig manure compost showed highest disease suppressiveness and significantly higher seedling biomass. Disease severity in both cattle and poultry manure composts were not significantly different at (15%) and (19%) respectively. The same trend was observed with seedling biomass in these composts. In sheep manure compost, few seeds germinated and total biomass was consequently very low (0.05 g). This compost did not suppress disease severity or increase plant biomass. These results show that composted animal manure can be useful as an alternative to chemical control in the management of damping-off of seedlings. However, materials used for compost preparation and curing time should be taken into consideration to avoid compost toxicity.

38.45 *MONOSPOROASCUS CANNONBALLUS*; THE CAUSAL ORGANISM OF CUCUMBER SUDDEN WILT DISEASE AND ITS CONTROL. **E.Z. Khalifa, M.A. Awad, M.M. Mostafa and A.M. El-Saidy.** *Plant Pathology Department, Faculty of Agriculture, Minufiya University, Shibin El- Kom 32516, Egypt. Email: khalifasz@yahoo.com*

Monosporoascus cannonballus was isolated for the first time in Egypt from Sadat district, Minufiya governorate from cucumber plants showing sudden wilt symptoms, grown under greenhouse conditions in newly reclaimed soil. Later two other isolates of *M. cannonballus* were isolated from cantaloupe and watermelon. The three isolates, when tested for pathogenicity, proved their agressiveness against cucumber and caused typical symptoms of sudden wilt (root rot and vine decline). It was found that PDA was the best medium for fungal growth and perithecium production of the three isolates. Superior linear growth of all three was obtained on arabinose as a carbon source, whereas galactose gave the greatest number of perithecia. Peptone as a nitrogen source gave the best radial growth and the greatest number of perithcia for all the isolates. 26 °C was the best for fungal growth, while 29 °C was the best for production of perithecia. Different cucurbitaceous plants were found to be susceptible to *M. Cannonballus*, and pumpkin was the least susceptible. Disease severity was increased by increasing inoculum density. Cucumber 'Dallah' was the most resistant of the varieties tested. Biological control of *M. cannonballus* was tested in the laboratory and under greenhouse conditions, using four *Trichoderma* species. All were effective, but *T. viride* followed by *T. harzianum* gave the best results.

38.46 THE POSSIBILITY OF USING THIN-LAYER CHROMATOGRAPHY FOR SIMPLE IDENTIFICATION OF THE POTATO SCAB PATHOGEN. **K. Kikuchi, T. Tateno, H. Negishi, K. Suyama and H. Yoshida.** *Faculty of Bioproduction, Tokyo university of Agriculture, Yasaka 192, Abashiri, Hokkaido, 099-2493, Japan. Email: h-yoshid@bioindustry.nodai.ac.jp*

We tested the possibility of identifying potato scab pathogens such as *Streptomyces scabies, Streptomyces turgidiscabies* and *Streptomyces acidiscaies,* by thin-layer chromatography (TLC) using a secondary metabolic product of the *Actinomyces* genus. We used potato scab-diseased tubers from Hokkaido district which is the main potato production area in Japan. 93 *Actinomyces* isolates from scab-diseased tubers were separated by the TLC method into 48 groups; *S. turgidiscabies* formed 8 groups and *S. acidiscabies* formed 3 groups. The geographical distribution of each potato scab pathogen isolate distinguished by TLC was similar to that of the potato scab pathogen species in Hokkaido, as identified by genetic analysis. Our results suggest that this TLC identification method, which is simpler and cheaper, could possibly be used at the farm-field site level to identify the main potato scab disease pathogens.

38.47 CONTROL STRATEGIES FOR SOILBORNE SCLEROTIAL RICE DISEASE IN LOWLAND RAIN-FED KHARIF RICE. **J. Konthoujam and G.K.N. Chhetry.** *Department of Agriculture, Government of Manipur, India. Email: konthoujam@yahoo.com*

Sclerotia of both varieties of *Sclerotium oryzae* Catt., incitant of stem rot of rice, viz. var. *sigmoidea* and var. *irregularae* occurred together at 4.25:2.43 (wt/wt) in field scum, floating on recently ploughed and levelled stem rot-endemic, monocropped rice fields in the Manipur valley (India). The two varieties also occurred together in diseased plants. Forty two percent of the floating sclerotial biomass adhering to transplanted rice stems were viable in August and incited the disease on all cultivars grown. *In vitro* sclerotial growth and development of the two varieties were affected to the same extent, and specific to the fungicide treatment. Propiconazole 75% EC, hexaconazole 50% WP and (carbendazim 50% WP + mancozeb 20% WP) showed similar *in vitro* efficacy at 50 ppm. However, propiconazole 75% EC was superior to the other two fungicides under field conditions @ 0.25% (a.i)/ha. It reduced disease incidence by 51.42%, disease severity by 52.80% and increased yield by 16.91%. N-P fertilization above an optimum dose (60-40 kg/ha) to (120-80 kg/ha), enhanced disease incidence over 37.53%, severity over 37.50% and reduced yield by 15.07%. For controlling stem rot of rice, flooding and draining off the field scum in stem rot-infected fields prior to transplanting in July, N-P fertilization restricted to 60-40 kg/ha, and split-dose spraying of selective fungicides at late tillering and booting stages of the crop are needed.

38.48 ROLES OF BACTERIA COLONIZING CROP RESIDUES IN SUPPRESSION OF DAMPING-OFF DISEASES. **M. Kusuya, Y. Ota, J.O. Becker and R. Fukui.** *Utsunomiya University, Japan. Email: ryo@cc.utsunomiya-u.ac.jp*

Effects of soil amendment with air-dried plant residues on damping-off of sugar beet by *Rhizoctonia solani* and/or *Pythium ultimum* were examined in pasteurized paddy field and upland soils. Twelve cultivars of *Brassica rapa* plants plus peanut 'Chibahandachi' and red clover 'Harukaze' were examined for their effects on suppression of pericarp colonization by either or both pathogens and subsequent damping-off in the infested soils. The incidences of pericarp colonization and damping-off by *R. solani* were both reduced significantly by incorporating the residue of komatsuna (*B. rapa* ssp. *rapifera*) 'Saori' or peanut into paddy field and upland soils (1% w/w) maintained at 17-22 °C. At higher temperature (27-32 °C), however, only the peanut residue was effective in suppressing *R. solani*. In contrast, neither residue suppressed pericarp colonization or damping-off by *P. ultimum* at both temperatures. The numbers of culturable bacteria in the residue-amended soils peaked in two days, at 8.0–8.5 log CFU g^{-1} soil, whereas those from the residues incubated on water agar for two days were >10.2 log CFU g^{-1}, suggesting that most bacteria multiplying in the residue-amended soils originated from the plant residues. In addition, damping-off by *R. solani* was suppressed when the residues were incorporated into infested river sand, and the suppressive effects were diminished drastically or nullified when the residue-amended soils were treated with streptomycin and chloramphenicol. There results suggest that phyllosphere bacteria were responsible for suppression of *R. solani* in the residue-amended soils, but additional disease-controlling agents are required to suppress *P. ultimum*.

38.49 SOILBORNE DISEASES CAUSE YIELD DEPRESSION OF MAIZE IN SOUTH AFRICA. **S.C. Lamprecht, M.P.W. Farina, G.R. Thibaud, M. Marais, J.H. Habig, J.F. Bloem and A. Swart.** *ARC-Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7599, South Africa. Email: lamprechts@arc.agric.za*

The effects of soilborne pathogens on growth and yield of maize following winter wheat in no-till systems at two localities (Bergville and Winterton) in KwaZulu-Natal were quantified. Fumigation with methyl bromide (MB) was used as an experimental tool to eliminate soilborne pathogens, and the effects of two biocontrol products on growth and yield were also assessed. Assessments were made three times at one-month intervals starting 6 weeks after planting. Soil fumigation increased growth and grain yield, and reduced root and crown rot severity at both sites. This was particularly evident at Winterton**,** where measurements from MB plots differed significantly from the other treatments. The control and two biocontrol treatments did not differ significantly with regard to crown and root rot severity. MB had no influence on plant nutrition, and growth and yield responses were the result of improved root health. Incidences of fungi were affected by sampling time, locality and treatments. Predominant fungi isolated from crowns and roots were *Trichoderma* spp. followed by *Fusarium oxysporum*, *Fusarium graminearum* and *Pyrenochaeta terrestris.* Carbon utilization profiles, conducted to evaluate microbial diversity in soil, separated MB-treated soil from soil subjected to the other treatments. Plant parasitic nematodes were also present, and MB fumigation appeared to reduce the incidence of these nematodes. This investigation highlighted the need to establish integrated management strategies able to equal the effects of MB on plant health and to determine the relative importance of fungi and nematodes associated with diseased maize crowns and roots.

38.50 EFFECT OF NITROGEN FERTILIZATION ON *FUSARIUM* CROWN ROT AND TAKE-ALL OF WHEAT IN SOUTH AFRICA. **S.C. Lamprecht, J.P.C. Tolmay and G.A. Agenbag.** *ARC-Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7599, South Africa. Email: lamprechts@arc.agric.za*

Fertilizer source and placement effects on the incidences and severity of *Fusarium* crown rot (FCR) and take-all (TA) of wheat in the Western Cape province of South Africa were evaluated. Trials were conducted on canola/wheat and pasture/wheat rotations in the southern (Caledon) and western (Moorreesburg) Cape, respectively. Limestone ammonia nitrate with single superphosphate (LAN+SSP) or urea with monoammonium nitrate (urea+MAP) were placed in different proportions with relation to the seed. The incidence and severity of FCR and TA were assessed three times during the growth season. Additional parameters evaluated included plant height (second sampling) and number of tillers (third sampling). Incidences and severity of the diseases were relatively low at both localities. The placement of LAN+SSP with the seed significantly increased the incidence and severity of TA compared with placement of urea+MAP with the seed at Moorreesburg. Conversely, urea+MAP placed with the seed significantly increased the incidence and severity of FCR compared to LAN+SSP at both localities. Plant height was not affected by fertilizer source; urea+MAP significantly increased the number of tillers at Moorreesburg, but not Caledon. Placement of different concentrations of the fertilizers with the seed did not affect any parameter measured at Caledon, but higher proportions of urea+MAP increased the incidence of FCR at Moorreesburg, compared with lower proportions. This study showed that the source of fertilizer and proportion placed with seed at planting can significantly affect the in-

cidence and severity of FCR and TA in wheat production in the Western Cape province.

38.51 GENETIC CHARACTERISATION OF *FUSARIUM OXYSPORUM* FROM NATURAL SOILS IN AUSTRALIA. M.H. Laurence, B.A. Summerell, L.W. Burgess and E.C.Y. Liew. *Royal Botanic Gardens Sydney, Botanic Gardens Trust, Mrs Macquaries Rd, Sydney, NSW 2000, Australia. Email: matthew.laurence@rbgsyd.nsw.gov.au*

Fusarium oxysporum is a ubiquitous fungal species complex that includes both non-pathogenic and pathogenic strains, the latter being responsible for disease in over one hundred cultivated plant species. The origin of many of these strains is poorly understood but recent studies on the cotton wilt pathogen, *F. oxysporum* f.sp. *vasinfectum* in Australia, indicate an indigenous origin from populations associated with native cotton relatives. Research to date has focused on isolates from agricultural environments but these are unlikely to represent the natural underlying diversity of the species complex in Australia, with anthropogenic distribution of pathogens and selection pressures that favour clonality. Given the broad host range of *F. oxysporum* and its potential threat to agriculture, there is a pressing need to characterise the native species complex. We have addressed this need by sampling isolates associated with native vegetation geographically isolated from cultivation throughout the continent. DNA fingerprints were obtained using various genetic markers to indicate the extent and distribution of genetic diversity. In addition the phylogenetic position and lineage composition of the native soil populations were investigated on the basis of DNA sequences of the β-tubulin, EF-1α, NIR, CAL and mtSSU rDNA regions. The evolutionary potential of native *F. oxysporum* populations in Australia is discussed.

38.52 DEVELOPMENT OF TECHNIQUES FOR ASSESSING SPORE VIABILITY OF *PLASMODIOPHORA BRASSICAE*. M.C. Lewis, E. Clewes and R. Kennedy. *University of Warwick, Warwick HRI, Wellesbourne, CV35 9EF, UK. Email: M.C.Lewis@warwick.ac.uk*

Clubroot of vegetable *Brassica,* caused by the pathogen *Plasmodiophora brassicae*, is an important problem within the UK due to the crops' high economic value and reduction in quality/yield that results from infection. Spores of *P. brassicae* can remain dormant in the soil for many years, and there are currently no reliable methods to accurately determine spore viability. DNA-based techniques can detect spores in soil, but the most robust test of viability to date is a traditional bait test. In this new project we investigate factors affecting the viability of *P. brassicae* spores, and develop methods for determining spore viability in soil.

38.53 OCCURRENCE OF RACES OF *PHYTOPHTHORA CLANDESTINA* – CAUSAL AGENT OF SUBTERRANEAN CLOVER ROOT ROT IN THE RAINFALL ZONES OF THE AGRICULTURAL BELT OF WESTERN AUSTRALIA. H. Li, X. Ma, P.H. Nichols, M.P. You, M.J. Barbetti and K. Sivasithamparam. *School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, Crawley, WA 6009, Australia. Email: xma@cyllene.uwa.edu.au*

Phytophthora clandestina is an important pathogen of annual pasture legumes across southern Australia, especially subterranean clover (*Trifolium subterraneum*) on which it is the most important root rot pathogen. *P. clandestina* had been found to only occur in Australia, with the majority of its distribution and most diversity of races found in the 700–1000 mm rainfall zones in the south-west of Western Australia. The most promising and economic approach to manage this disease is through host resistance. However, this appeared to break down and/or was lost over time, suggesting the rapid development of new races of the pathogen in response to field deployment of various host resistances. Our recent work, screening isolates of *P. clandestina* across subterranean clover host differentials to characterize races, identified 10 races with varying degrees of pathogenicity on the differential host plant cultivars. Races 173 and 177 were found to be widely distributed and were the most common in Western Australia, together constituting 80% of the isolates characterized. While resistance in subterranean clover against some races was easy to identify, it was less readily found against other races. One race in particular, race 177, was the most virulent of the races across the subterranean clover genotypes tested and no resistance to this race has been identified to date. Hence, studies were undertaken to locate sources of tolerance/resistance to race 177, among newly available subterranean clover germplasm.

38.54 *FUSARIUM* PATHOGENS OF CULTIVATED CROPS FROM NATURAL ECOSYSTEMS IN AUSTRALIA. E.C.Y. Liew, A.R. Bentley, H.T. Phan, T. Petrovic, J.L. Walsh, B.A. Summerell and L.W. Burgess. *Royal Botanic Gardens Sydney, Botanic Gardens Trust, DECC, Mrs Macquaries Rd, Sydney, NSW 2000, Australia. Email: edward.liew@rbgsyd.nsw.gov.au*

In Australia the majority of our agricultural crops are species and cultivars introduced into the continent. Until recently *Fusarium* species associated with various diseases of these crops were generally thought to have been introduced, concurrent with or subsequent to the host introduction. The cotton wilt pathogen, *F. oxysporum* f.sp. *vasinfectum*, however, has been shown to be genetically more closely affiliated with native populations and is believed to have a local origin. We have been investigating *Fusarium* endophytes in non-cultivated hosts, in both natural ecosystems and agricultural environments. The focus of our research has been on grasses (native and introduced) in natural ecosystems geographically isolated from the agricultural environment. A wide range of plant pathogenic species have been commonly isolated from these grasses. Some of these, e.g. *F. thapsinum* and *F. pseudograminearum,* were shown to differ little genetically from strains obtained from their respective cultivated hosts, sorghum and wheat. Furthermore, some were shown to be pathogenic on the cultivated host in the greenhouse. Implications of these findings and future research directions are discussed.

38.55 EFFECTS OF METALAXYL AND PHOSPHONATE ON *PHYTOPHTHORA* ROOT ROT OF WOLLEMI PINE. E.C.Y. Liew, C.A. Offord, A. Pinaria, C. Pavich and B.A. Summerell. *Royal Botanic Gardens Sydney, Botanic Gardens Trust, DECC, Mrs Macquaries Rd, Sydney, NSW 2000, Australia. Email: edward.liew@rbgsyd.nsw.gov.au*

The Wollemi pine (*Wollemia nobilis*) was first shown to be susceptible to *Phytophthora cinnamomi* in pot trials in 1999, and since then precautions have been taken to prevent the inadvertent introduction of the pathogen into the wild population within Wollemi National Park, Australia. However, in 2005 *P. cinnamomi* was detected in soil surveys following observations of disease

symptoms at the site. On the basis of systematic transects, subsequent surveys established the precise location of the pathogen, which appeared to be confined to two areas within one of the Wollemi pine stands. Concurrent to the on-going monitoring of pathogen spread, there was an urgent need to establish control strategies for managing this problem. A study was initiated to investigate the effects of metalaxyl and potassium phosphonate on *Phytopthhora* root rot of Wollemi pine. Post-infection soil drenching of potassium phosphonate was shown to be effective in controlling this disease in a greenhouse trial. No phytotoxicity was observed on plants treated with phosphonate. However, neither foliar spray of phosphonate nor soil drenching with metalaxyl effectively controlled the disease. Implications of these results in relation to management strategies are discussed.

38.56 VANILLA STEM ROT PATHOGEN CAN SURVIVE AS AN ENDOPHYTE WITHIN HEALTHY VINES. E.C.Y. Liew, A. Pinaria, F. Rondonuwu, J. Paath, D.T. Sembel and L.W. Burgess. *Royal Botanic Gardens Sydney, Botanic Gardens Trust, DECC, Mrs Macquaries Rd, Sydney, NSW 2000, Australia. Email: edward.liew@rbgsyd.nsw.gov.au*

Vanilla is an important and popular cash crop offering high economic returns to smallholding farmers in North Sulawesi, Indonesia. However, vanilla production in this region is greatly constrained by *Fusarium* stem rot. Although the disease is most severe on the stems, it is also found on the leaves and roots. On the stem internode, small brown water-soaked spots or lesions initially appear, which enlarge and become necrotic, eventually girdling and shrivelling the stem. Etiological studies confirmed the causal agent to be *Fusarium oxysporum* f.sp. *vanillae.* Interestingly, although this is a soilborne vascular pathogen, disease lesions are often observed between healthy internodes in the absence of any apparent wounds, raising questions as to the pathogen's mode of entry. We showed in a greenhouse trial that *F. oxysporum* isolates obtained from healthy stems without any external or internal symptoms were pathogenic on vanilla vines, indicating the possibility of this pathogen surviving as an endophyte within healthy vines. This finding has significant implications on disease management as vanilla is vegetatively propagated and most planting material is obtained from existing farms with various levels of disease incidence.

38.57 *SPONGOSPORA SUBTERRANEA* DAMAGES POTATO PLANT GROWTH AND YIELD. R.A. Lister, R.E. Falloon and D. Curtin. *New Zealand Institute for Crop & Food Research Limited, PB 4704, Christchurch, New Zealand. Email: ListerR@crop.cri.nz*

Spongospora subterranea f.sp. *subterranea* causes powdery scab on potato tubers (*Solanum tuberosum*), which is the well recognised quality-limiting effect of this pathogen. Root infections by *S. subterranea* (zoosporangia and root galls) are common but rarely observed, and their significance has not been documented. We have completed several experiments indicating that this pathogen can adversely affect plant growth and yield parameters. A field trial, where powdery scab was severe, measured a mean total tuber yield increase of 28% due to soil-applied pesticides that effectively controlled the disease. A second field trial measured a 42% reduction in mean tuber yield following *S. subterranea* inoculation of uninfested soil. Several glasshouse experiments have tested whether inoculation with *S. subterranea* sporosori affected plant growth. Relative to uninoculated plants, inoculated plants yielded 21% less total dry matter, were 17% shorter and had 7% fewer leaves. Shoots from inoculated plants had reduced content of the elements P, K, S, Mn, Cu, and Zn, and increased amounts of N, Mg, and Na, and their roots were discoloured, indicating that the pathogen damaged roots and disrupted root membrane function. Cultivar resistance to powdery scab is generally assessed as low tuber infection. However, inoculation reduced total plant dry weight of cv. Iwa (very susceptible to powdery scab on tubers) by 27%, and of cv. Gladiator (resistant) by 28%. These results are strong evidence that *S. subterranea* has effects that could harm crop yields as well as quality, in cultivars that are resistant or susceptible to powdery scab on tubers.

38.58 UNDERESTANDING AND MANAGING EPIDEMICS CAUSED BY *PHYTOPHTHORA CAPSICI* IN CHILE-PEPPER. J. Luna-Ruiz and O. Moreno-Rico. *Universidad Autónoma de Aguascalientes, Av. Universidad 940, Cd. Universitaria, C.P. 20100, Aguascalientes, Ags., Mexico. Email: jjluna@correo.uaa.mx*

Phytophthora capsici causes severe infection of roots, crowns, stems, leaves and fruits of chile-pepper (*Capsicum annum* L.) in commercial fields worldwide including Mexico. Our objectives are to present (1) advances related to the understanding of epidemics caused by *P. capsici* in Central Mexico, and (2) some recommendations for integrated disease management based on experimental results. As no genetic resistance to *P. capsici* is currently available in commercial chile-pepper cultivars, the initial infection and development of epidemics seem to be caused by three main factors: (a) high concentration of initial inoculum (oospores) in soil, (b) presence of summer rains, and (c) frequent and prolonged crop flooding (excess soil moisture). Factors b and c have been well documented, but even where natural sexual recombination of *P. capsici* has been demonstrated, the role of oospores as the primary/initial inoculum for infection has not been proved in Central Mexico. Experimental results and field observations indicate that early transplanting (March 15-31) on raised mulching beds, followed by drip irrigation, reduce the impact of rain and disease risk. Good crop-plant nutrition and soil applications of beneficial microorganisms (*Trichoderma harzianum*, *Bacillus subtilis*, etc.) improve plant vigor, health and strength, therefore reducing the vulnerability of susceptible chile-peppers to *P. capsici*. Crop genetic resistance is a major component of integrated disease management. Screening traditional local varieties and landraces of chile-pepper against regional aggressive strains of *P. capsici* has led to identifying excellent levels of genetic resistance for crop improvement, a major component of integrated disease management and sustainable agriculture.

38.59 ROLE OF PLANT GROWTH-PROMOTING RHIZOBACTERIA IN THE SUPPRESSION OF SOIL-BORNE DISEASES OF TWO MEDICINAL CROPS, *COLEUS FORSKOHLII* AND *WITHANIA SOMNIFERA*. S.B. Mallesh and S. Lingaraju. *Department of Plant Pathology, U.A.S., Dharwad 580005, Karnataka, India. Email: lingaraju_s@rediffmail.com*

Fusarium chlamydosporium, *Ralstonia solanacearum* and *Meloidogyne incognita* were found to be the predominant pathogens affecting the medicinal crops, *Coleus forskohlii* and *Withania somnifera* in a survey done in Karnataka, southern India. We investigated the ability of 50 rhizobacterial strains isolated from healthy rhizoplanes and rhizospheres of these crops to suppress the activity *in vitro* of *F. chlamydosporium* or *R solanacearum*, using the dual culture technique. Cell-free filtrates of the same strains were tested for *M. incognita* juvenile mortality and inhibition of egg

hatch. Twelve, eleven and fifteen strains were the most efficacious, respectively inhibiting the said fungus, bacterium and nematode. These results led to selection of seven rhizobacterial strains highly inhibitory to all three pathogens. The plant growth-promoting (PGP) ability of these strains was ascertained: The roll towel method was employed to measure the vigour index of bacterized *W. somnifera* seeds and seedling bacterization to assess the growth promotion of *C. forskohlii* cuttings. Both showed significant growth promotion. Phenotypic characterization and physiological tests showed that these seven potent PGP isolates to be fluorescent pseudomonads and *Bacillus* spp. Talc-based formulations of these were used to treat *W. somnifera* seeds and *C. forskohlii* cuttings at 50 g l^{-1} water for 2 h to evaluate their efficacy in multilocational field experiments with natural incidences of fungus/bacterium/nematode and also in greenhouse experiments involving different combinations. Progress on these studies will be reported. PGP rhizobacteria capable of suppressing multiple pathogens hold promise in the management of soil-borne diseases.

38.60 CONSIDERATION OF SOIL BIOLOGY IN THE *BRASSICA* SEED MEAL-INDUCED CONTROL OF *RHIZOCTONIA SOLANI*. **M. Mazzola.** *USDA-ARS, 1104 N. Western Ave., Wenatchee, WA, 98801, USA. Email: mazzola@trfl.ars.usda.gov*

Efficacy of brassicaceae amendments for soilborne disease control is typically attributed to the generation and activity of biologically active chemistries, including ITCs. However, suppression of root rot incited by *Rhizoctonia solani* in response to several different brassicaceae seed meal amendments requires an active soil microbial community. All seed meals tested suppressed root infection by native *Rhizoctonia* spp. and an introduced isolate of *R. solani* AG-5. When introduction of the pathogen was delayed until 4 to 8 weeks post seed meal amendment, disease suppression was associated with proliferation of resident *Streptomyces* spp. and not qualitative or quantitative attributes of seed meal glucosinolate content. Using the same experimental system, pasteurization of seed meal amended soils prior to pathogen infestation abolished control of *R. solani* regardless of seed meal type. For *B. juncea* seed meal amendment, the mechanism of *R. solani* suppression varied in a temporal manner, which initially was associated with generation of allylisothiocyanate and was not affected by soil pasteurization. No disease control was observed when introduction of *R. solani* into *B. juncea* amended soils was deferred until 48 h post-amendment. This corresponded with AITC emission from soils, which was completed within 24 h. Incubation of these same soils for 4 weeks led to a restoration of soil suppressiveness toward *R. solani*, which again was associated with proliferation of resident *Streptomyces* spp. These studies demonstrate the multiplicity of mechanisms involved in brassica amendment-induced disease control and the fundamental role of resident soil microbial communities in eliciting the disease control response.

38.61 TEMPERATURE INFLUENCES THE INCIDENCE AND SEVERITY OF CLUBROOT ON ASIAN LEAFY BRASSICA VEGETABLES. **M.R. McDonald, S.M. Westerveld and B.D. Gossen.** *Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada, N1G 2W1, Canada. Email: mrmcdona@uoguelph.ca*

Clubroot of crucifers (*Plasmodiophora brassicae* Woronin) is a limiting factor in the production of leafy Brassica vegetables in the muck soil of the Holland /Bradford Marsh region of Canada (44º 5' N, 75º 35' W), but only at certain times of the year. To determine the effects of soil and air temperatures on the development of clubroot on these crops, Shanghai pak choy [*Brassica rapa* L. subsp. *chinensis* (Rupr.) var. *communis* Tsen and Lee] and flowering [*B. rapa* L. subsp. *chinensis* (Rupr.) var. *utilis* Tsen and Lee] were seeded into organic soil, naturally infested with the clubroot pathogen in May, June, July, and August of 1999, 2000, 2001, and 2007. Data from 22 trial years were used to compare disease incidence and disease severity index (DSI) with weather conditions during crop development. Clubroot was highest on crops seeded in June and July and lowest for the August seedings. In general, air temperatures were a better predictor than soil temperatures recorded by a nearby weather station. Mean daily air temperatures during the 10 days before assessment ranged from 13 to 27 ºC and were positively correlated with disease incidence and DSI ($r = 0.70$ to 0.84). There is a potential to predict levels of clubroot based on daily air temperatures in the crop. Crop management methods to keep soil temperatures cool may suppress clubroot.

38.62 NEW SOURCES OF RESISTANCE IN PEANUT TO *SCLEROTINIA MINOR*. **H. Melouk, K. Chenault and R. Pittman.** *USDA–ARS, Plant Science Research Laboratory, Oklahoma State University, Stillwater, OK 74078, USA. Email: hassan.melouk@okstate.edu*

A 4-year study (2003–2006) in field plots at the Caddo Research Station, Ft. Cobb, Oklahoma was conducted to evaluate the reaction of new peanut introductions to *Sclerotinia minor.* Seventeen genotypes including two cultivars (Okrun and Southwest Runner; *Sclerotinia*-susceptible and *Sclerotinia*-resistant cultivars, respectively) were planted in four replications in a complete randomized block design. Each plot consisted of two 6-m rows spaced at 0.91 m. Sclerotial density of *S. minor* was 2-3/100 g of soil (Tremona loamy fine sand). Over years, average incidence of *Sclerotinia* blight in thirteen genotypes (PI's 476016, 501288, 501983, 501996, 502006, 502009, 502034, 502046, 502068, 502093, 502133, 502154, and Grif 13838) ranged from 7 to 17% which was lower than the 24% in Southwest Runner, and 69% in Okrun. The least significant difference ($p=0.05$) of *Sclerotinia* incidence between the genotypes was 6%. Based on acceptable agronomic characteristics (pod yield, grade, and kernel weight), PI 476016 and Grif 13838 were selected for inclusion in a crossing program to enhance the *Sclerotinia* resistance of high oleic peanut cultivars.

38.63 COMPARATIVE EFFICACY OF VARIOUS ISOLATES OF *TRICHODERMA* AGAINST *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY CAUSING WHITE STEM ROT OF MUSTARD. **N. Mehta, N.T. Hieu and S.K. Gandhi.** *Department of Plant Pathology, CCS Haryana Agricultural University, Hisar 125 004, India. Email: nareshmehta@hau.ernet.in*

Eight *Trichoderma* isolates from four species showed their antagonism using the dual culture technique. *Trichoderma harzianum*-3 and *T. harzianum*-4 were most potent in inhibiting the growth by 89.7 and 89.2%, respectively. *T. harzianum*-4, *T. harzianum*-3 and *T. virens* inhibited sclerotial formation, with 1.2, 2.0 and 2.7 as compared to 36.2 sclerotia per plate in controls. Mycelial growth inhibition was negatively correlated with number of sclerotia formed. Three isolates found most potent in in vitro studies were multiplied on wheat bran and mixed in sandy loam soil @ 5, 10, 15g/kg soil in clay pots. The antagonists were inoculated either 7 days prior or simultaneously or 7 days after the pathogen applica-

tion @ 2g/ kg soil. After seven days of inoculation, fifty seeds of Indian mustard were sown in each pot. After recording observations on pre- and post-emergence damping off up to 21 days, ten seedlings were maintained in each pot. In the sets where antagonists and pathogen were applied simultaneously, or *T. viride* was added 7 days after the pathogen, minimum pre-emergence damping off (15.74%) was recorded. However, *T. harzianum* was better in checking lesion length and post-emergence damping-off, applied 7 days after or simultaneously with the pathogen. Soil application of *T. harzianum* resulted in less disease intensity as compared to control (54%) when applied simultaneously or 7 days prior to the pathogen. The antagonists @ 15 g/kg soil irrespective of time of application were better, compared to lower doses.

38.64* CULTURAL METHODS TO CONTROL *VERTICILLIUM* WILT AND CORKY ROOT, TWO IMPORTANT SOILBORNE DISEASES. **V.V. Michel.** *Agroscope Changins-Wädenswil ACW, Centre des Fougères, CH-3960 Switzerland. Email: vincent.michel@acw.admin.ch*

Cultural methods, such as biofumigation with plants and pellets, green manures, and organic amendments were tested to control *Verticillium* wilt caused by *Verticillium dahlia*e and corky root caused by *Pyrenochaeta lycopersici* in pot and field trials. The best control was achieved with biofumigation using brown mustard (*Brassica juncea*) cultivars ISCI-20 and ISCI-99. This method is based on transformation of glucosinulates in brown mustard tissue into isothio- and thiocyanates after incorporation of the plants in soil. Isothio- and thiocyanates are volatile molecules toxic to a number of soil-borne pathogens. With biofumigation, the number of viable microsclerotia of *V. dahliae* was reduced in pot trials by 85%. On corky root, the effect of biofumigation using brown mustard was, measured with a biotest, the same as in a steam-sterilised soil. Intermediate results in the control of both diseases were obtained with canola green manure. Furthermore, Agrobiosol (a chitin-containing organic fertilizer) and Biofence (biofumigation pellets) had an intermediate effect on corky root; and mature windrow compost on *V. dahliae* microsclerotia. Field trials partly confirmed the results obtained in pot trials, especially for the effect of biofumigation with brown mustard on *V. dahliae*. However, the efficiency of biofumigation was influenced by soil type. In a sandy soil, the incorporation of brown mustard had no effect on numbers of viable microsclerotia in the soil.

38.65 CULTURAL CONTROL METHODS AGAINST *PHYTOPHTHORA* ROOT ROT OF RED RASPBERRY. **V.V. Michel and A.Ançay.** *Agroscope Changins-Wädenswil ACW, Centre des Fougères, CH-3960 Switzerland. Email: vincent.michel@acw.admin.ch*

The combination of several cultural control methods to control root rot caused by *Phytophthora fragariae* var. *rubi* and other *Phytophthora* spp. was tested in a naturally contaminated field. The effect of raised bed planting, windrow compost and plastic cover in different combinations was studied using two red raspberry cultivars, 'Zewa 2' (highly susceptible) and 'Tulameen' (susceptible), over four years. The number of fruiting canes and the cumulative yield was significantly higher when raspberries were planted on raised compared to flat beds. Adding compost to the raised beds or covering them with a water-proof plastic film resulted in an additional significant increase of the cumulative yield. However, all three factors combined did not result in a third increase of the cumulative yield. The efficacy of the cultural methods can be explained by the reduction of water logging conditions, a prerequisite for the infection of the roots by *Phytophthora* spp., through the improvement in drainage (raised beds, compost) or the shelter from rainfall (plastic cover). With no method, single or combined, was the optimal number of ten fruiting canes per linear meter at harvest obtained. Mortality caused by *Phytophthora* spp. continued after pruning in spring and the root growth could explain this ongoing mortality. Observations four years after planting revealed that most roots were located in the raised beds. However, a proportion of the roots were growing into the soil below the raised beds where conditions for a infection by *Phytophthora* spp. were still favorable.

38.66 EMERGING SOILBORNE DISEASES IN GRAFTED TOMATOES IN ITALY. **A. Minuto, G. Gilardi, D. Bertetti, G. Causarano, S. Longombardo and A. Garibaldi.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: andrea.minuto@unito.it*

Use of grafted tomatoes has significantly increased during the last seven years, particularly in southern Italy where methyl bromide (MB) was largely adopted for pre-plant soil fumigation. In Italy around 10 million tomato plants are estimated to be grafted and further increases are expected. Because grafted plants may be infested by root knot nematodes, using them is generally recommended in combination with chemical nematicides. On the contrary, fungicides applied before or after transplant are not commonly used on grafted tomatoes. Within the period 2000–2007, some outbreaks caused by soilborne fungi have been reported on grafted tomatoes grown in unfumigated soils. Root rots caused by *Colletotrichum coccodes* were observed on interspecific and intraspecific hybrids of tomato rootstocks. In northern Italy the disease was reported to depress yield when grafted tomatoes were transplanted to fields not fumigated for at least 3 previous years. *Phytophthora nicotianae* and *Rhizoctonia solani* were occasionally observed from 2000 to 2003. More recently *P. nicotianae* caused severe outbreaks particularly in spring and summer. *C. coccodes* can be controlled through the adoption of soil fumigants alternative to MB; moreover soil biofumigation with *Brassica* meal and soil solarizarion are promising techniques. Against *R. solani* and *P. nicotianae* specific fungicides may be applied at the nursery stage or immediately after transplant. In conclusion the use of grafted plants has to be considered as a component of a more complex strategy to control soilborne tomato diseases.

38.67 ANALYSIS OF GENETIC VARIATION OF IRANIAN OF *PHYTOPHTHORA SOJAE* USING RAPD AND ISSR. **A. Mohammadi, A. Alizadeh, N. Safaie, J. Mozafari and N. Nooras Mofrad.** *Dept. Plant Pathology, College of Agriculture, Tarbiat Modares University, Tehran, Iran. Email: abbas_mohammadi229@yahoo.com*

The genetic diversity of four geographic populations of *Phytophthora sojae* from Iran (Golestan, Lorestan, Mazandaran and Ardabil provinces) was determined using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) techniques. A total of 68 reproducible RAPD and ISSR fragments were scored among 40 individuals form race1 and race3. Based on RAPD and ISSR markers, the isolates clustered into three distinct groups. Analysis of genetic variation showed that there existed 15% genetic variation in the Iranian population of *P. sojae*. We did not find any race-specific RAPD and ISSR fragments. These data support the hypothesis that *P. sojae* in Iran has been introduced from another country such as the USA.

38.68 PHYLOGENETIC ANALYSIS OF THE SEQUENCES OF rDNA INTERNAL TRANSCRIBED SPACER (ITS) OF *PHYTOPHTHORA SOJAE*. **A. Mohammadi, A. Alizadeh, M. Mirabolfathy and N. Nooras Mofrad.** *Dept. Plant Pathology, College of Agriculture, Tarbiat Modares University, Tehran, Iran. Email: Abbas_mohammadi229@yahoo.com*

The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) was amplified by PCR in 23 different isolates of races 1 and 3 of *Phytophthora sojae* using the primers ITS4 and ITS5. Fragments of around 800–1000 bp were obtained. Based on the sequences of the PCR products a phylogenetic tree was constructed using maximum parsimony analysis, and the evolution among isolates was analyzed. The results showed that isolates can be classified into three groups. There was no correlation between ITS sequences, geographical distribution and race reaction of isolates.

38.69 EFFECT OF THREE MEDIA ON GROWTH RATE, BIOMASS PRODUCTION AND VIRULENCE OF *PHYTOPHTHORA SOJAE*. **A. Mohammadi and N. Nooras Mofrad.** *Islamic Azad University of Birjand, Birjand, Iran. Email: Nasrin229@yahoo.co.in*

Effect of three media, oat meal agar (OM), corn meal agar (CM) and anasazi bean agar (AB) on growth, biomass production and pathogenecity of races 1 and 3 of *Phytophthora sojae* on differentials. Races were identified by hypocotyl inoculation with 2 × 2 mm plaques of fungal culture. Results showed that growth rate of *P. sojae* on all media was similar but biomass and oospore production on AB was greater than on OM and CM. There was no differences between OM and CM in oospore and biomass production. Results of race identification tests with OM were similar to other documented results but results on CM and AB were different from those of standard tests. Results of this research showed that LBA is the best medium for growth and biomass production but OM is the best for race identification tests.

38.70 COMBINING LIGNOSULPHONATE WITH ORGANIC SUBSTRATES TO INDUCE SUPPRESSIVENESS IN SOIL. **M. Montanari and G. Innocenti.** *Department Protezione Valorizzazione Agroalimentare, University of Bologna, Viale Fanin 46, 40127 Bologna, Italy. Email: matteo@agrsci.unibo.it*

Lignosulphonates (Ls) are low-cost by products of the acid sulfite pulping process. They have a lignin-like structure, containing mono- and polysaccharides and are widely used in the building industry as components of cement and concrete and in the production of animal food and pesticides as a co-formulant. Ls introduced alone into agricultural soils showed several beneficial properties on plant nutrition as carriers of macro- and micro-elements. Furthermore recent studies carried out by the Lazarovitis group in Canada, demonstrated the suppressive effect of direct application to the soil of Al-lignosulphonate against scab disease and *Verticillium* wilt in potato crops. Nevertheless Ls may be toxic to plants, depending on the type of soil, crop and time of application. Phytotoxicity may be due to the large amount of carbon introduced in N-deficient soil with lignosulphonate application, resulting in C/N increase and subsequent plant starvation by microbial competition for nitrogen. Combining Ls with N-rich organic materials as composts before field application, may have several positive effects on compost quality and on lignosulphonate suppressive activity. This combination has been investigated by our group in the last four years through laboratory, greenhouse and field experiments, with different crops, target pathogens and Ls-compost combinations. Previous results showed that Ls-compost combinations were suitable for plant growth and biocontrol activity, enhancing antagonistic fungi naturally present or artificially introduced into the substrate.

38.71 ROOT DISEASE OF MILKVETCH (*ASTRAGALUS ADSURGENS*) IN CHINA. **N.Z. Nan, Y.Z. Li and Y.L. Yin.** *College of Pastoral Agriculrure Science and Technology, Lanzhou University, Lanzhou 730020, Gansu, P.R. China. Email: zhibiao @lzu.edu.cn*

Milkvetch (*Astragalus adsurgens*) is one of the most important forage legumes in northern China. Poor persistence is the main limiting factor of milkvetch pasture production and utilization. A field survey showed, for instance, the percentage of number of plant dead is 26.0%, 68.5%, 85.0% and 99.6% for 3, 4, 6, and 8 year-old pastures, respectively, in Huanxian county, Gansu province, where the mean annual rainfall is 350 mm. Research was undertaken during the last 10 years to study seed-, soil- and air-borne diseases of milkvetch grown in the nine provinces (Autonomous Regions) in northern China. In total 37 fungal species belonging to 34 genera were isolated from various milkvetch parts. Among those, 25 species were found in the roots. A new fungal species named *Embellisia astragali* Li and Nan was identified and reported. Yellow stunt and root rot caused by *E. astragali* is the most important disease. It is widely distributed and is strongly pathogenic to milkvetch. Another 8 root-invading fungal species showed significant reduction of plant growth and biomass accumulation in pathogenicity tests. They are *Cladosporium herbarum*, *Fusarium solani*, *F. oxysporum*, *F. verticilloides*, *F. semitectum*, *F. avenaceum*, *F. chlamydosporum*, and *Verticillium dahliae*. Seeds treated with fungicides can improve the seed germination and field emergence rate by up to 30% to 40%.

38.72* TAKE-ALL BIOCONTROL BY *PSEUDOMONAS* BACTERIA ON WHEAT: PLANT-BACTERIAL GENE EXPRESSION. **M. Nayudu, T. Murphy, M. Koeck, A. Franklin, Y. Zhang, C. Samundsett and H. Millar.** *School of Botany and Zoology, Faculty of Science, Australian National University, ACT 0200, Australia. Email: Murali.Nayudu@anu.edu.au*

Effective control of soil-borne fungal pathogens such as *Gaeumannomyces graminis* var. *tritici* (Ggt) by antagonistic micro-organisms offers an alternative to the use of chemicals. Currently control of Ggt by Pseudomonads involves the production of the anti-fungal agent phenazine-1-carboxylic acid or 2,4 diacetylphloroglucinol. The Australian non-fluorescent bacterial isolate *Pseudomonas* strain AN5 (*Ps*. str. AN5) has been shown to have new mechanisms in inhibiting Ggt. *Ps.* str. AN5 produces the sugar gluconic acid as an anti-fungal agent, a protease which attacks take-all fungal hyphae, and also has specific bacterial invasion genes responsible for intracellular colonization of wheat roots. *Ps.* str. AN5 is able to protect *in vitro* against take-all. *Ps.* str. AN5 biocontrol of take-all in field trials at dryland sites can induce a significant increase in wheat yield of up to 20% by suppression of the take-all disease. *Ps.* str. AN5 is an excellent colonizer of wheat roots in different soil types and is able to survive under very low soil moisture (10%) in Australian soils, paving the way for it to be developed as a biocontrol agent. We have developed TaqMan assays for the bacterial genes involved in root colonization, gluconic acid and protease production. Using Affymetrix genechip microarray technology we have identified

wheat genes up- and down-regulated by *Ps.* str. AN5 symbiosis with wheat roots. From this we have also developed TaqMan assays for wheat genes involved in this symbiosis. The expression of bacterial and plant genes involved in this biocontrol interaction will be outlined.

38.73 SOILBORNE DISEASES OF CHICKPEA AND THEIR MANAGEMENT THROUGH HOST-PLANT RESISTANCE. S. Pande, M. Sharma, P.M. Gaur, C.L.L. Gowda, J.N. Rao, Om. Gupta, L. Kaur, M.S. Sagwan, R.N. Chaudhary, B.M. Jamadagni, D.R. Saxena and H.K. Ramappa. *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502 324, Andhra Pradesh, India. Email: s.pande@cgiar.org*

Chickpea (*Cicer arietinum* L.) is an important cool-season food legume. Because of biotic and abiotic stresses its average yields are < 0.8 tons ha^{-1} globally, while its potential yield is > 3.0 tons ha^{-1}. Among the biotic stresses, soilborne diseases such as fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*s), collar rot (*Sclerotium rolfsii*), dry root rot (*Rhizoctonia bataticola*) and black root rot (*Fusarium solani)* together can cause up to 100% losses annually under favourable environmental conditions. Host plant resistance offers the most sustainable and effective disease management option to combat these soilborne diseases in chickpea, as fungicide application is expensive and impractical against soilborne pathogens. Different resistance screening techniques (field, greenhouse, and laboratory) to identify resistance against these diseases have been developed at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Chickpea lines found resistant to *Fusarium* wilt at ICRISAT were further evaluated through International chickpea wilt and root rot nurseries (ICWRRN) at various locations in Asia from 2004-2007. Eight lines (ICCs 12467, 14344, 14391, 14432, 14433, 14436, ICC X 950106-F4-66P-BP and ICC X 950110-F4-26P-BP-BP) showed stable and broad-based resistance to *Fusarium* wilt. Additionally, four chickpea lines (ICCs 1710, 2242, 2277 and 15441) showed combined resistance to wilt and dry root rot. These resistant lines have been shared with National Agriculture Research Systems (NARS) globally and are being used in resistance breeding at ICRISAT.

38.74 CHARACTERIZATION, PATHOGENICITY AND HOST PLANT SPECIFICITY OF *RHIZOCTONIA SOLANI* ISOLATES ASSOCIATED WITH CAULIFLOWER IN BELGIUM. J. Pannecoucque and M. Höfte. *Laboratory of Phytopathology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium. Email: Joke.Pannecoucque@UGent.be*

Worldwide, the soilborne fungal pathogen *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) is frequently reported in several vegetable crops. In Belgian cauliflower fields, *R. solani* causes girdling of the stem and stunting of the plant, resulting in severe yield reduction. Subdivision of *R. solani* into 13 different anastomosis groups (AGs) and subgroups complicates research on this species. Because AGs differ in host plant spectrum, pathogenicity and susceptibility to control strategies, characterization of the AGs is necessary. In Belgium, AGs from cauliflower isolates of *R. solani* have not previously been determined. In this study, 60 *R. solani* isolates collected from cauliflower fields in Belgium were characterized using different techniques: pectic zymogram, PCR-RFLP and sequencing of the ITS region. Based on the results, we could ascribe two of the collected isolates to AG 1-1B, five to AG 1-1C, 41 to AG 2-1, three to AG 3, four to AG 4 HGII and five to AG 5. Isolate pathogenicity was determined using *in vitro* pathogenicity tests and greenhouse experiments. The results identified isolates of AG 1-1C, AG 2-1 and AG 4 HGII as the most aggressive towards cauliflower, while isolates of AG 3 were the least aggressive. This study is the first report of pathogenic isolates of AG 1-1C and AG 4 HGII associated with cauliflower. Currently, research is focusing on factors involved in host-plant specificity of *R. solani.*

38.75 BACTERIAL WILT: THE PAST AND CURRENT SITUATION IN THE SOUTHEASTERN USA. P.D. Peterson and B.A. Fortnum. *Entomology, Soils, and Plant Sciences Department, Pee Dee Research and Education Center, Clemson University, Florence, S C, 29506, USA. Email: ppeters@clemson.edu*

When bacterial wilt, caused by *Ralstonia solanacearum*, first appeared in 1881 in Granville County, North Carolina, little did people realize the devastating consequences of the disease on tobacco production and people's lives in the increasingly important bright leaf tobacco belt. By the early 20th century, disease losses were commonly in the range of 25 to 100%, leading to a concerted effort by scientists to find successful disease control measures. The best management systems approach developed by North Carolina, agricultural experiment station, and USDA scientists provided effective bacterial wilt control for nearly a half-century. However, by the 1980's, sudden disease outbreaks in previously uninfested areas and a general increase in disease intensity were reported across the region. We investigate the initial scientific response to bacterial wilt, focusing specifically on the work that led to practices of crop rotation, chemical control and the development of resistant tobacco cultivars which combined to produce the best management system. We also review how changes in mechanization, expanded production, and movement of equipment have compromised the management system, resulting in a subsequent rapid increase in disease losses. The potential for disease control in the future and the ramifications for long term tobacco production are also examined.

38.76 *FUSARIUM* WILT AND CROWN ROT DISEASES CAUSED BY *FUSARIUM OXYSPORUM* IN VIETNAM. H.T. Phan, T.M. Luong and L.W Burgess. *National Institute of Medicinal Materials, 3B Quang Trung Str., Hanoi, Vietnam. Email: phanthuyhien@yahoo.com*

Diseases caused by soilborne fungal pathogens are responsible for significant losses in Vietnam in a wide range of crops. These diseases commonly cause non-specific symptoms such as wilting, stunting and reduced yield. The casual pathogens can be difficult to isolate and identify. Many root rot problems were falsely attributed to *Fusarium oxysporum* because this species includes saprophytic strains which colonise diseased roots and are readily isolated on a range of media. Over the past decade many of these root rots have been shown to be caused by *Phytophthora* or *Pythium* species. Furthermore, some bacterial wilt diseases have been mistakenly attributed to *formae speciales* of *F. oxysporum* and vice versa. *Fusarium* wilt of banana is a significant problem in many areas of Vietnam. The cause of a range of vascular wilt diseases in several important cash crops is being re-assessed based on systematic isolation and pathogenicity testing. For example, *formae speciales* of *F. oxysporum* have been shown to be responsible for vascular wilt diseases in carnation, lisianthus, asters and ginger as well as crown rot of gerbera. Integrated disease management strategies are being developed for these diseases. Further re-

search is planned to evaluate the role of non-host crops on the survival of the pathogens.

38.77 ENHANCED BIOCONTROL BY BACTERIAL ANTAGONIST MIXTURES AGAINST BACTERIAL PUSTULE DISEASE OF SOYBEAN. **S. Prathuangwong, P. Chan-On and S. Kasem.** *Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, 50 Paholyothin Rd., Chatuchuck, Bangkok 10900, Thailand. Email: agrsdp@ku.ac.th*

Our previous work showed that four bacterial antagonist strains including *Bacillus amyloliquefaciens* KPS46 and *Paenibacillus* sp. SW01/4, *Serratia marcescens* Spt360, and *Pseudomonas fluorescens* SP007s individually showed biocontrol activity against root and leaf diseases of various plants. In this study, single strains and all possible combinations of them were tested whether they would improve the control of soybean bacterial pustule caused by *Xanthomonas axonopodis* pv. *glycines*. KPS46 was most effective to inhibit pathogen growth in laboratory experiments, followed by Spt360, SP007s and SW01/4 respectively. No in vitro antibiosis was observed among the four antagonist strains, but co-culture demonstrated that SP007s decreased cell numbers of other strains. Ten combinations and four individual strains were tested on 14-day-old soybean cv. SJ4 with pathogen-coinoculation in greenhouse trials. Foliar spray with individual strains had similar effects in disease reduction correlated to pathogen inhibition in the laboratory tests. However, there was a general trend across all treatments toward greater disease suppression using two-strain mixtures. Mixtures of two but not for three or four strains were better than individual strains. The pair of strains KPS46+Spt360 at total 1×10^{11} cfu/ml most significantly reduced disease by 64%, followed by SW01/4+ Spt360, and KPS46+SW01/4 with 55% and 51% respectively. The effect of antagonist spread on soybean leaves at final severity evaluation revealed greater population density of the KPS46 and Spt360 mixture than other combinations or individual strains. The data obtained are essential for optimizing methods to formulate bacterial inoculants contributing to crop improvement.

38.78 IMPROVING COMPOST SUPPRESSIVENESS TOWARDS *PYTHIUM ULTIMUM* BY INOCULATION WITH *TRICHODERMA* SPP. **M. Pugliese, M.L. Gullino and A. Garibaldi.** *AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: massimo.pugliese@unito.it*

Pythium ultimum is a widespread soil-borne pathogen causing seed decay, pre- and post-emergence damping-off and root rot on many crops. Several investigations have shown a suppressive effect of composts on *Pythium ultimum* and its use for biological control has also been proposed. The presence of antagonists to *Pythium* spp. in compost may enhance the disease suppression. Four *Trichoderma* strains, registered in Italy as biological control means, were added to compost and tested under greenhouse conditions to evaluate the ability of the enriched composts to suppress disease. Half of the compost was steam-sterilized before inoculation with antagonists. In all assays the compost was inoculated with *Pythium ultimum* and seeded 0, 30 and 60 days after inoculation with the antagonists. Disease incidence and biomass were measured 2 weeks after seeding. A peat substrate was used as control. *T. harzianum* T-22 (1g/l) (Rootshield, Intrachem), *T. viride* TV1 (1g/l) (T. viride TV1, Agribiotec) and *T. harzianum* ICC012 + *T. viride* ICC080 (5g/l) (Remedier, Isagro) increased the disease suppressiveness of compost not steam disinfested. For compost inoculated with *T. harzianum* T-22 (1g/l), the average disease suppressiveness was 68.9% compared to the peat control. The compost steamed and not inoculated with antagonist showed no suppressiveness. In all other cases the disease suppressiveness attained at least 25.1%. A period of time of 30 days between inoculation with antagonists and use of compost showed best results.

38.79 MONITORING AND INTEGRATED MANAGEMENT OF MELON COLLAPSE CAUSED BY *MONOSPORASCUS CANNONBALLUS* IN CENTRAL ITALY. **R. Reda, M.P. Aleandri, M. Antonelli, L. Varvaro, P. Magro and G. Chilosi.** *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Viterbo, Italy. Email: r.reda@unitus.it*

Recently melon worldwide has suffered significant losses in yield and quality due to collapse caused by the soil-borne fungus *Monosporascus cannonballus*. The objective of a 3-year field study in the greenhouse melon-producing areas in central Italy (Province of Viterbo) was to assess the occurrence of collapse and evaluate different management strategies for controlling it. A severe outbreak of collapse occurred in greenhouses in 2003, and it was was also frequent in 2004. In 2005 and 2006 the disease was found in 13% and 12%, respectively, of the farms surveyed. Among sanitation methods, destruction of crop residues including roots effectively reduced disease incidence. Soil fumigation and solarisation did not provide acceptable results. Steam treatment of soil contributed to control in the short-term. Grafting is effective only when more tolerant varieties are grafted on squash, whereas grafting on melon rootstocks is consistently ineffective. Among chemical resistance-inducers tested, methyl jasmonate was found to decrease disease incidence. Antagonistic fungi and bacteria contribute to soil fertility and crop yield by limiting the development of pathogens and inducing resistance in plants. Beneficial bacteria able to inhibit growth of *M. cannonballus* and induce resistance in melon seedlings were isolated from non-cultivated soils of the same area. The use of local biocontrol agents may provide an additional way to control the pathogen.

38.80 DISPERSION OF DEFOLIATING AND NON-DEFOLIATING PATHOTYPES OF *VERTICILLIUM DAHLIAE* IN HOST CROPS BY IRRIGATION WATER IN SOUTHERN SPAIN. **D. Rodríguez-Jurado, R. Moraño-Moreno and J. Bejarano-Alcázar.** *IFAPA–Centro Alameda del Obispo, Apartado 3092, 14080 Córdoba, Spain. Email: dolores.rodriguez.jurado.ext@juntadeandalucia.es*

The presence and amount of *Verticillium dahliae* propagules of different sizes in irrigation water used in 33 olive orchards and 31 cotton fields affected by *Verticillium* wilt, were monitored periodically from 2004 to 2006 and in 2006, respectively. Fields were distributed in three provinces (13 in Córdoba; 21 in Jaén; 30 in Sevilla) of Andalucía, southern Spain, along the Guadalquivir Valley and were irrigated with water from dams or canalised superficial water from the Guadalquivir river, or with subterranean water from wells. Water samples of 1000 l were sieved for sclerotia (20 µm pore size) or micropropagules (1 µm pore size), and plated on a semiselective medium. Virulence of *V. dahliae* populations in cotton irrigation water was determined on cotton cv. Acala SJ-2. *V. dahliae* was isolated from irrigation water in 64 and 68% of the olive and cotton fields surveyed (61, 71 and 63 % of fields in Córdoba, Jaén and Sevilla, respectively). Both superficial and subterranean waters were infested. Infested water

samples harboured 1–98 sclerotia and 50–108,000 micropropagules per 1000 l, but their presence and amount were highly variable over time. Fifty two of 55 monosporic isolates obtained of irrigation water were pathogenic to cv. Acala SJ-2, and all of them were classified as belonging to the defoliating highly virulent pathotype of *V. dahliae*, except one isolate that induced a moderate disease reaction and was assigned to the non-defoliating pathotype. Research support was provided by grants CAOD.03-3 from CAP, and RTA2006-00012 from INIA, Spain.

38.81 CONTROL OF *MELOIDOGYNE JAVANICA* WITH ANIMAL WASTE MATERIALS AND TOMATO C.V. DIVERSITY REACTION. **F. Saeidi Naeini.** *Plant and Diseases Department, Agricultural and Natural Resources Center of Bushehr, Borazjan, 75615-333, Iran. Email: fsaeidinaeini50@yahoo.com*

Root-knot nematode *Meloidogyne javanica* is one the most important pathogens on vegetables and field crops in southern Iran. In three years research, control of root-knot nematode populations was studied using three tomato cultivars 'Emperial', 'Cal-J' and 'Queen' in response to three additives (chicken, cow and shrimp wastes) in pot culture dig on field condition. Experiments were done as factorial in completely randomized block design with six replications and two levels (sterile and non-sterile soils). Shrimp waste and c.v. 'Queen' were the best treatment combination for control of root-knot nematode populations, considering all edaphic, nematode and yield factors.

38.82 DEVELOPMENT OF BIOMANAGEMENT STRATEGIES AGAINST *FUSARIUM* WILT DISEASE IN BANANA. **T. Saravanan and M. Muthusamy.** *Agricultural Research Station, Kovilpatti 628 501, India. Email: pathsaran75@rediffmail.com*

Fusarium wilt disease, caused by *Fusarium oxysporum* f.sp. *cubense* (Foc), is one of the worst diseases in banana (*Musa* spp.). Greenhouse, *in vitro*, and field experiments were done to find suitable management strategies including biocontrol agents and organic amendments for the control of the disease. Seven biocontrol agents were screened for antifungal activity against Foc *in vitro*. Among the agents, *Pseudomonas fluorescens* strain Pfm (Pf-Pfm) has shown higher inhibition of Foc mycelial growth and conidial germination. Pf-Pfm along with extract of neem cake significantly reduced mycelial growth and conidial germination *in vitro*. When treated with a talc formulation of Pf-Pfm at 10 g/plant along with neem cake, inoculated banana plants had a lower vascular discolouration index and wilt index under greenhouse conditions. In field experiments in Killikulam (Vallanadu) of Thoothukudi district and Cholavandan of Madurai district, we observed that basal application of neem cake at 0.5 kg/plant + soil application of talc formulation of Pf-Pfm at 10 g/plant at 3, 5 and 7 months after planting gave maximum reduction of *Fusarium* wilt disease. Production of 2, 4 diacetylpholoroglucinol (an antibiotic of Pf-Pfm) in the rhizosphere of the Pf-Pfm inoculated plants was also observed. Increased level of disease-resistance enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase were also observed in Pf-Pfm inoculated plants challenged with Foc.

38.83 OCCURRENCE AND CHARACTERIZATION OF A *PHYTOPHTHORA* SP. PATHOGENIC TO *ASPARAGUS OFFICINALIS* IN MICHIGAN. **C. Saude, O.P. Hurtado-Gonzales, K.H. Lamour and M.K. Hausbeck.** *Department of Plant Pathology, Michigan State University, East Lansing, MI, 48824-1311, USA. Email: hausbec1@msu.edu*

Michigan ranks third in the United States for fresh and processing asparagus (*Asparagus officinalis*), following California and Washington. A homothallic *Phytophthora* sp. was recovered from asparagus spears, storage roots, crowns and stems in northwest and central Michigan in 2004 and 2005. In 2004, symptomatic spears were curved, with soft water-soaked lesions and/or shriveling slightly above or below the soil line. In 2005, water-soaked lesions were rarely observed on either spears or storage roots sampled from the field; necrotic lesions on storage roots and curved spears were the most prevalent symptoms. Isolates (N = 131) produced ovoid, nonpapillate, noncaducous sporangia 45 µm long × 26 µm wide and amphigynous oospores of 25 to 30 µm diameter. Mycelial growth was optimum at 25ºC with no growth at 5 and 30ºC. All isolates were sensitive to 100 ppm mefenoxam. Pathogenicity studies confirmed the ability of the isolates to infect asparagus as well as cucurbits. Further studies are warranted to determine the potential host range of this *Phytophthora* sp. in Michigan. Amplified fragment length polymorphism analysis of 99 isolates revealed identical fingerprints, with 12 clearly resolved fragments present and no clearly resolved polymorphic fragments suggesting a single clonal lineage. The internal transcribed spacer (ITS) regions of representative isolates were homologous with a *Phytophthora* sp. isolated from diseased asparagus in France and a *Phytophthora* sp. from agave in Australia. Phylogenetic analysis supports the conclusion that the *Phytophthora* sp. isolated from asparagus in Michigan is a distinct species that requires formal description.

38.84 A NEW *PHYTOPHTHORA* SPECIES ASSOCIATED WITH *EUCALYPTUS GOMPHOCEPHALA* DECLINE IN WESTERN AUSTRALIA. **P.M. Scott, B.L. Shearer, P.A. Barber, T. Jung, T. Burgess, I.J. Colquhoun and G.E. Hardy.** *Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Murdoch, WA 6150, Australia. Email: P.Scott@murdoch.edu.au*

Eucalyptus gomphocephala is a keystone tree species endemic to a narrow (5-10 km wide) coastal strip approximately 300 km in length in south-west Western Australia. *E. gomphocephala* is undergoing a significant decline that was first identified as a spot decline in 1994 and now occurs throughout large sections of its remnant distribution within Yalgorup National Park in the south-west of Western Australia, in some areas resulting in 100% mortality. Multiple factors, including soil-borne plant pathogens, have been identified as possibly contributing to the decline syndrome. Less fine roots are associated with trees on decline sites compared to those on healthy sites. Foliar analysis indicates that declining trees have lower concentrations of some micronutrients, including zinc, the uptake of which is typically impaired by fine feeder root loss. A range of Pythiaceous microorganisms have been isolated from declining roots, including an isolation of a yet to be described *Phytophthora* species. The *Phytophthora* isolates appear morphologically similar to the *Phytophthora citricola* holotype although they are distinct from this based on molecular analysis of the internal transcribed region. The exact phylogeny of the new *Phytophthora* species is being determined using additional gene regions including the amplification of mitochondrially encoding Cox I and II spacer regions. These isolates appear to be contributing to the loss of fine roots. Glasshouse trials are currently underway to determine whether these isolates are indeed pathogenic.

38.85 DETECTION OF SOILBORNE VIRUSES OF SUGAR BEET IN UKRAINE. **N. Senchugova and O. Postoenko.** *National Taras Shevchenko University of Kyiv, Virology Department, Ukraine. Email: nsenchugova@yahoo.com*

We have recently started monitoring sugar beet fields in Ukraine for the presence of *Beet necrotic yellow vein virus* (BNYVV). We have decided to continue our monitoring work on sugar beet, mainly because of the high incidence of this viral infection in Europe. We have now developed a number of fast and highly sensitive RT PCR assays for the detection of different sugar beet viruses including BNYVV, *Beet soil borne virus* (BSBV) and *Beet virus Q* (BVQ). For RT PCR analysis we first isolated total RNA (using the standard phenol method) from the sugar beet leaves collected in 2006 in Kiev, Cherkasu and Vinnitsa regions. cDNA was synthesised using superscript reverse transcriptase (Qiagen, UK) and random primers, and the cDNAs were tested with virus-specific primers described earlier (Meunier *et al*, 2003). PCRs using Taq polymerase (AmpliSence, Russia) were carried out on a Tercis PCR machine (Russia). We detected BVQ but not BNYV or BSBV. The PCR product for BVQ was 291 bp in size and was later confirmed as BVQ (*Pomovirus*). This is the first report of BVQ infection in the Ukraine.

38.86 CROP ROTATION, NITROGEN FERTILISER AND SOIL MOISTURE AFFECT POWDERY SCAB OF POTATO AND *SPONGOSPORA SUBTERRANEA* DNA IN SOIL. **S.F.A. Shah, R.E. Falloon, S.M. Thomas and R.C. Butler.** *New Zealand Institute for Crop & Food Research Limited, PB 4704, Christchurch, New Zealand. Email: shahf@crop.cri.nz*

Powdery scab of potato (caused by *Spongospora subterranea*) is a severe problem for potato production. Resting spores of the pathogen survive in soil for many years. A field trial (split-split plot design, eight replicates) measured effects of two previous crop rotations [potato/peas/potato (PPP); potato/wheat/potato (PWP)], two irrigation levels (high and low) and three nitrogen applications (0, 200 and 400 kg/ha) on powdery scab incidence and severity, the amount of *S. subterranea* DNA in soil, and total potato tuber yield. All of these parameters varied ($P \leq 0.003$) with applied nitrogen. Total yield, number of tubers and powdery scab incidence and severity increased with increasing nitrogen. PWP rotation increased total yield ($P<0.05$), and high irrigation increased powdery scab severity. There was a change in *Spongospora* DNA in soil between the different rotation ($P=0.003$), nitrogen ($P<0.001$) and irrigation ($P=0.02$) treatments. None of the interactions between these factors was statistically significant ($P>0.15$), indicating that the factors acted independently. The mean amount of DNA in soil was 43% greater after the PWP rotation than after the PPP rotation, and 113% greater with high than with low irrigation. There was a reasonable positive correlation ($r=0.63$) between $\log_{10}$ pg DNA/g soil and powdery scab severity. This trial will continue, exploring crop management effects on powdery scab and pathogen DNA levels in soil. Data from a second growing season will be included in this presentation. This study is determining if *Spongospora* DNA levels in soil can be used to predict powdery scab in potato crops.

38.87 DEVELOPMENT OF SUPERIOR STRAINS OF *TRICHODERMA* FOR INTEGRATED CONTROL OF SOILBORNE PATHOGENS OF PULSE CROPS. **R. Singh.** *Institute of Bioengineering and Biological Sciences, Varuna Bridge, Varanasi 221001, India. Email: dr.rajesh.s@gmail.com*

Fusarium udum, F. oxysporum f.sp. *ciceri* and *F. oxysporum* f. sp. *lentis* cause severe economic loss to pigeonpea, chickpea and lentil crops. Forty three isolates of *Trichoderma* were screened for their antagonistic ability against the test pathogens. Potent strains were identified on the basis of *in vitro* antagonistic ability and fungicide tolerance. They were subjected to mutagenesis using NTG for improvement of their antagonistic potential. Two stable mutants which showed enhanced bioefficacy against the soilborne fungi and tolerated carbendazim and copper sulphate were subjected for protoplast fusion in order to achieve a superior strain. Protoplasts were obtained from the mutants with novozyme 234 and fused in the presence of PEG; hybrids were isolated on selective medium. Selected rapidly growing stable fusants were tested for fungicide tolerance and bioefficacy in dual culture experiments. The fusant TPTF3 exerted a high degree of antagonistic ability (90–94%) in limiting the radial growth of test pathogens, and also tolerated carbendazim and copper sulphate up to 125 µm and 120mM respectively. The fusant strain produced higher level of chitinase and β-1, 3 glucanase as compared to the mutants and wild-type parent strains. Hence, in the present study, a superior strain with broad spectrum distinct antagonistic and fungicide tolerant abilities was developed which could be used in IPM programmes for the control of soilborne diseases of pulse crops.

38.88 MULTI-DISEASE PROTECTION WITH NEMATICIDAL SEED COATING IN COTTON. **J. Smith Becker and J.O. Becker.** *Department of Nematology, University of California, Riverside, CA 92521, USA. Email: beck@ucr.edu*

Rhizoctonia damping-off and *Fusarium* wilt of cotton, caused by *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *vasinfectum* (Fov), respectively are destructive soilborne diseases that occur worldwide in most cotton production areas. Root-knot nematodes *Meloidogyne incognita* race 3 are known to enhance both diseases, even at low population densities. In growth chamber and plot trials, seed coating with the nematicide abamectin was evaluated in the presence of either fungus and the nematodes. Abamectin mitigated early damage by *M. incognita* and increased root growth compared to unprotected seedlings. In *R. solani* and *M. incognita*-infested soil, abamectin seed coating in combination with fungicides and an insecticide (Avicta Complete Pac; 1.66 g azoxystrobin, 0.28 g fludioxonil, 0.83 g mefenoxam per 100 kg seed and 0.32 mg thiamethoxam per seed) resulted in the best seedling stand and largest root systems compared to the untreated check or seed coatings without the nematicide. In the presence of both the root-knot nematodes and Fov race 1, seedlings grown from abamectin-coated seeds showed little of the stunting, chorosis and vascular discoloration that were observed with seedlings grown from seeds coated with only the fungicide/insecticide components. These trials indicated that the target efficacy of a nematicidal seed treatment might contribute to protection against other diseases that are promoted by plant-parasitic nematodes.

38.89 EFFICACY OF BOTANICALS AGAINST *RALSTONIA SOLANACEARUM* CAUSING BACTERIAL WILT. **A.K. Sood and Pankaj.** *Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur 176062, H.P., India. Email: soodak@hillagric.ernet.in*

Bacterial wilt caused by *Ralstonia solanacearum* (E.F. Smith) Yabuuchi *et al.* is a serious disease of solanaceous vegetable crops in the mid hill sub-humid areas of the northwestern Himalayan

region of India. The pathogen has a wide host range, survives in the roots of non-host plants and soil and chemical control is not feasible. Eighteen botanicals, five *Azadiracta indica* formulations and nine essential oils were evaluated *in vitro* and *in vivo* against three isolates of *R. solanacearum*. *In vitro* evaluation was done by paper disc, spectrophotometer and plate count methods. Irrespective of the method of evaluation, the aqueous and organic extracts of *A. indica* in general, were found inhibitory against all the three isolates followed by *Ranunculus muricatus* and *Ageratum houstonianum* at 100 per cent concentration. These plant extracts were more inhibitory than test chemicals viz. streptocycline (100 µg/ml), copper oxychloride (0.25%) and their combination. Among the *A. indica* formulations, Wanis was most effective followed by Achook and Neemazal at 100 and 50 per cent concentrations. The *A. indica* oil at 20 µg/ml was the most inhibitory followed by mentha and eucalyptus oils. *In vivo* evaluation revealed that the aqueous and organic extracts of *A. indica* gave the maximum survivability of tomato seedlings followed by *R. muricatus* at 100 per cent concentration after dipping durations of 12 and 6 h. Wanis showed maximum survivability of seedlings followed by Achook and Neemgold at 100 per cent concentration. The efficacy of all botanicals decreased with the decrease in concentration both *in vitro* and *in vivo*.

38.90 EFFECT OF *ASPERGILLUS NIGER* ON GROWTH AND YIELD OF INOCULATED GROUNDNUT PLANTS. **E.N.K. Sowley and A. Lasisi.** *Department of Agronomy, University for Development Studies, P.O. Box TL1882, Tamale, Ghana. Email: enksus@yahoo.com*

Aspergillus niger van Tieghem is a soilborne fungus reported to cause hypocotyl malformation in young plants of groundnut (*Arachis hypogaea* L.). In this study the effect of *A. niger* on the growth and yield of infected groundnut plants was investigated. Two varieties of groundnut namely Chinese and J.L 45 were inoculated with *A niger* isolated from soil. The fresh and dry weight of inoculated plants was determined at 10 day intervals up to 80 days after planting. Also the number of pods per plant was determined at maturity as a measure of yield. Though growth was adversely affected by *A. niger*, this did not have a significant (p=0.05) effect on yield.

38.91 CHARACTERISATION AND DETECTION OF *PYTHIUM* AND *PHYTOPTHTORA* SPECIES ASSOCIATED WITH GRAPEVINE IN SOUTH AFRICA. **C.F.J. Spies, M. Mazzola and A. Mcleod.** *Department of Plant Pathology, University of Stellenbosch, Privat Bag X1, Matieland, 7602, South Africa. Email: cspies@sun.ac.za*

Grapevine decline is caused by a disease complex that includes several trunk-disease pathogens. These pathogens often act in an opportunistic manner, only causing disease when plants are under stress. Root-pathogenic *Phytophthora* and *Pythium* species could potentially cause, and increase such stress. A survey conducted in the 1980's identified four *Phytophthora* and five *Pythium* species associated with grapevines in South Africa. The aim of the current study was to reinvestigate the species composition of these genera on grapevines. Surveys were conducted in grapevine nurseries (4 climatic regions) and established vineyards (10 climatic regions). Grapevine root and crown material were collected from nurseries during spring and summer, whereas in established vineyards only root samples were taken during summer, winter and spring. The highest incidence of oomycetes was observed in spring and winter. In total, twelve *Pythium* and one *Phytophthora* species were recovered from nursery vines. In established vineyards, 25 *Pythium* and three *Phytophthora* species were identified. The most common species recovered included *Pythium vexans*, *Pythium irregulare*, *Pythium heterothallicum* and *Phytophthora cinnamomi*. In order to further investigate the population dynamics of some of the pathogenic species, real-time polymerase chain reaction (PCR) primers and probes were developed and validated on infected plant material.

38.92 EXPERIENCES WITH CLUBROOT ON CANOLA (OILSEED RAPE) IN ALBERTA, CANADA. **S.E. Strelkov, S.F. Hwang, R.J. Howard and J.P. Tewari.** *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada. Email: stephen.strelkov@ualberta.ca*

Clubroot, caused by *Plasmodiophora brassicae* Woronin, was initially found on *Brassica napus* L. (canola, oilseed rape) in the Edmonton, Alberta region in 2003, in the first report of this disease on canola in the Canadian prairies. Surveys conducted from 2005 to 2007 indicate that clubroot is spreading and more widespread than originally thought, with several hundred clubroot-infested fields identified in eight counties in central Alberta, the centre of the outbreak. The disease has also been recently found in canola fields in a county in southern Alberta. The primary mechanism of spread between fields is the movement of infested soil on farm machinery. Yield losses ranging from 30% to 100% have been reported in severely infested canola fields. The occurrence of clubroot is not restricted to fields with acidic soils, but there is a significant negative correlation between disease severity and soil pH. Evaluation of the virulence of populations and single spore isolates of *P. brassicae* on differential hosts has revealed the presence of at least three, and possibly four, pathotypes in Alberta. Pathotype 3, as classified on the differentials of Williams, is predominant and highly virulent on all canola cultivars currently available in Canada. At this time, there are few management options for farmers except extended crop rotations, although studies are underway to assess the efficacy of various fungicides and soil amendments for clubroot control. The Province of Alberta recently made *P. brassicae* a declared pest under the Agricultural Pests Act, as part of its Clubroot Management Plan.

38.93 ADVANCES IN THE EPIDEMIOLOGY OF CARROT CAVITY SPOT CAUSED BY *PYTHIUM* SP. **F. Suffert, M. Guibert and F. Montfort.** *INRA - Agro Paris Tech, UMR1290 BIOGER-CPP, F-78850 Thiverval-Grignon, France. Email: fsuffert@grignon.inra.fr*

Cavity spot, an important soilborne disease of carrot worldwide, is caused by a *Pythium* complex including *P. violae*. Our objectives were to identify and prioritize the processes involved in the spatiotemporal development of the disease for optimizing protection methods. A classical hypothesis which considers primary infection (from soilborne inoculum) and secondary infection (auto- and alloinfections) was favoured. The occurrence of secondary infections, and then the polycyclic nature of cavity spot, was demonstrated experimentally. The analysis of a pathometric incidence-severity relationship between the incidence (i) and the total disease area (tda) given by i=100(1-exp(a(t).tda) and the fitting of models to disease curves (logistic model, bi-logistic model of Hau & Amorim, and model of Brassett & Gilligan) confirmed the occurrence of both types of infections. The effect of three cropping factors (fungicide application, soil moisture, and planting density)

as key variables affecting the disease tetrahedron (host, pathogen, environment, management) was tested. In microcosm experiments Mefenoxam reduced alloinfections by *P. violae* and then slowed the disease progression. A deficit of soil moisture reduced primary infections, presumably by limiting the accessibility of the host tissues for *Pythium* propagules; it also promoted the healing of lesions, thus limiting the potential of alloinfections. Increase of mean root-to-root distance and decrease of sowing density reduced the rate of alloinfections. IPM scenarios based on this epidemiological knowledge and combining agronomic management practices, such as optimizing fungicide application, management of date of harvest vs. soil moisture content, and optimizing host density vs. planting pattern, are discussed.

38.94 INTERSPECIFIC TRANSMISSION OF *SCLEROTINIA SCLEROTIORUM* DEBILITATION-ASSOCIATED RNA VIRUS TO *S. NIVALIS*. **F. Teng, J. Xie, Y. Yu, D. Jiang, Y. Fu, G. Li and X. Yi.** *The key lab of plant pathology of Hubei Province, Huazhong Agricultural University, Wuhan, 430070, Hubei, P. R. China. Email: daohongjiang@mail.hzau.edu.cn*

Because of their intracellular mode of transmission, mycoviruses can only be transmitted vertically via reproduction or horizontally via hyphal anastomosis. However, the occurrence of hyphal anastomosis is limited to strains belonging to the same vegetative compatible group. Recently, several papers showed that fungi in different classes harbor mycoviruses with high identity, suggesting that there might exist an unknown transmission pathway. Here, we report that a mycovirus (SsDRV) which infects *Sclerotinia sclerotiorum* strain Ep-1PN and causes debilitation of its host, can be transmitted to *S. nivalis*, a relative of *S. sclerotiorum*. Strain Ep-1PN and *S. nivalis* strain Let-19 (isolated from a sclerotium on diseased lettuce) were cultured side by side in the same PDA plate, and when the two colonies made contact, hyphal tips were cut from the colony growing margin of Let-19 and placed in fresh PDA plates. Colonies which showed debilitation phenotype similar to that of Ep-1PN were picked to extract viral dsRNA of SsDRV. The viral RNA samples were run in 1% agarose gel, or used to perform Northern blot with α-32P labeled cDNA segment of SsDRV as probe. The results showed that SsDRV in strain Ep-1PN of *S. sclerotiorum* could be transmitted to *S. nivalis* Let-19, and the frequency of transmission was about 29%. Further experiments showed that when strain Let-19 and strain Ep-1PN were inoculated on the same lettuce leaf, the transmission of SsDRV also occurred. Our results suggest that there may be a different way to transmit mycoviruses apart from hyphal anastomosis.

38.95 VARIABILITY IN AUSTRALIAN *RHIZOCTONIA SOLANI* AG-2-1 ISOLATES FROM POTATO. **C. Todd, K. Ophel-Keller, T. Wicks and E. Scott.** *University of Adelaide, Waite Campus, Adelaide, SA 5064, Australia. Email: cathryn.todd@adelaide.edu.au*

The fungus *Rhizoctonia solani* is pathogenic to potato crops world-wide. Several anastomosis groups (AGs) can cause symptoms on potato plants. The type and severity of these symptoms can vary between and within AGs, as these are based on compatibility in a hyphal fusion reaction, not on pathogenicity-linked genes. Knowledge of *R. solani* groups based on pectic enzyme expression has been instrumental in establishing disease management techniques for cereals. However, because of the technical constraints of this method, no association has yet been drawn between pectic enzyme expression and pathogenicity to potato. AG-2-1 is an example of a group commonly found in Australian potato crops and which varies in pathogenicity and fungicide sensitivity. Molecular tools have been developed to identify and quantify AG-2-1 in field soils. These tools have facilitated the collection of multiple AG-2-1 isolates from a range of Australian regions. PCR amplification of the intergenic spacer 1 (IGS1) region of these AG-2-1 isolates has yielded products of different lengths. In the UK, isolates with different IGS1 amplicon length caused different symptoms of stem necrosis in pathogenicity trials. Hence for Australian isolates the relationships between amplicon length, pathogenicity to potato plants and biochemical characteristics are being examined. This will complement existing data on variation in sensitivity of AG-2-1 isolates to fungicides. The findings will be discussed in the context of improving management of *Rhizoctonia* disease on potato crops.

38.96 MODIFICATIONS OF PARP MEDIUM USING FLUAZINAM, MICONAZOLE, AND NYSTATIN FOR DETECTION OF *PYTHIUM* SPP. IN SOIL. **M. Tojo and Y. Morita.** *Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan. Email: tojo@plant.osakafu-u.ac.jp*

The standard *Pythium* selective medium PARP [pimaricin + ampicillin + rifampicin + pentachloronitrobenzene (PCNB) agar], was modified by replacing PCNB and pimaricin with other antifungal agents. Several antifungal agents such as fluazinam, miconazole, 2,4,5,6-tetrachloroisophthalonitrile (TPN), iminoctadine triacetate, tolclofos-methyl, captan, and nystatin, were initially screened for effects on *Pythium* growth. Based on these results, the following three media were developed: PARF (pimaricin + ampicillin + rifampicin + fluazinam agar), NARF (nystatin + ampicillin + rifampicin + fluazinam agar), and NARM (nystatin + ampicillin + rifampicin + miconazole agar). The new media were comparable with PARP on yield of naturally occurring *Pythium* species from two different types of soil using the soil-dilution plating technique. PARF and NARF were significantly better than PARP on inhibition of non-pythiaceous microbes on the soil-dilution plates, but were significantly lower than PARP on the rate of mycelial growth of six of eight isolates belonging to seven species of *Pythium*. NARM was equivalent to PARP on inhibition of non-pythiaceous microbes except for *Fusarium oxysporum*, and was significantly better than PARP on rate of mycelial growth of five of eight *Pythium* isolates.

38.97 MONITORING, FORECAST AND CONTROL OF *HELMINTHOSPORIUM* ROOT ROT IN SIBERIA CONSIDERING THE ECOLOGICAL STRATEGY OF THE PATHOGEN. **E.U. Toropova and V.A. Chulkina.** *Chair of Plant Protection Systems and Plant Epidemiology, Novosibirsk State Agricultural University, P.O. Box 253, Krasnoobsk, Novosibirsk reg., Russia. Email: helento@ngs.ru*

Life cycle strategy of *Helminthosporium sativum* – Kr-strategist. A strategy of protective measures should include bringing the basic number of pathogen propagules in arable soils below the threshold of harmfulness (for different Siberian soils – from 10 to 50 conidia per gram of air-dried soil); bringing the basic number of propagules on seeds below the threshold of harmfulness (10–15%). A forecast of seasonal and long-term dynamics of the plant epidemic process of *Helminthosporium* root rot is based on pathogen transmission factors, the main being transmission through soil with additional transmission through seed.

It is advisable to monitor the number of pathogens in soil and on (in) seeds at large intervals: in spring after sowing or at the end of vegetation after cereal crop harvesting once in 3–5 years (for soil) and yearly (for seeds). Disease control systems include regular treatment with organic fertilizers, the introduction of preceding phytosanitary crops and crop rotation, enrichment of the plant rhizosphere with antagonistic microorganisms by treatment of seed with biologicals, replacement of nitrate forms of nitrogen fertilizers by ammonia forms and the enhancement of norms of potassium fertilizer application, seed heating and disinfection etc.

38.98 MANAGEMENT OF WHITE MOULD (*SCLEROTINIA SCLEROTIORUM*) ON IRRIGATED COMMON BEANS IN BRAZIL. P.J. Trazilbo, R.F. Vieira and J.E.S. Carneiro. *EPAMIG, Vila Giannetti 47, 36570-000 Viçosa (MG), Brazil. Email: trazilbo@epamig.ufv.br*

The purpose of this study was to evaluate the efficiency of integrated managements on white mould control in common bean. Initially, tests in vitro were made to assess the antagonism of 11 *Trichoderma* isolates against *Sclerotinia sclerotiorum* and to investigate inhibitory effects of the fungicides fluazinam and procymidone on those fungi. The following combinations were tested in two field experiments: irrigation frequencies (seven or 14 days), plant densities (six or 12 plants per meter), and three disease controls (untreated control, fungicide or *Trichoderma* sp.). In a third experiment plant densities were replaced by grass mulching treatments (with or without mulching). Fluazinam was applied at 45 and 55 days after emergence (DAE). The antagonists *T. harzianum* (experiments 1 and 3) and *T. stromatica* (experiment 2) were applied through sprinkler irrigation at 10 and 25 DAE, respectively. Most of the *Trichoderma* spp. were effective against the pathogen. Fluazinam was more toxic than procymidone to both the pathogen and the antagonist. Fungicide increased yield between 31.7% and 40.8%. The reduction from 12 to six plants per meter did not decrease yield, and disease severity diminished in one of the two experiments. We conclude that of the strategies for white mould control, just reduction of plant density and especially applications of fungicide were efficient.

38.99 *PHYTOPHTHORA* FOOT ROT OF BLACK PEPPER IN VIETNAM: DISEASE SURVEY, AETIOLOGY AND CONTROL MEASURES. N.V. Truong, L. W. Burgess and E.C.Y. Liew. *Royal Botanic Gardens Sydney, Botanic Gardens Trust, Mrs Macquaries Road, Sydney, NSW 2000, Australia. Email: edward.liew@ rbgsyd.nsw.gov.au*

Black pepper is a high-value export crop in Vietnam; however, its production is reduced remarkably by *Phytophthora* foot rot, which causes lesions and rot at the collar and root system and eventually death. Although the disease was first reported in 1952, the identity of the causal organism was never conclusively determined. A national survey of the disease and collection of disease specimens were conducted in four major black pepper-growing provinces. *Phytophthora capsici* was found to be the main cause of the disease based on morphological characteristics and verified by ITS-RFLP analysis. Phosphite (potassium phosphonate) was evaluated for control. In greenhouse trials 3-month-old vines treated with phosphite by soil drenching (10 to 20 g a.i./l) were significantly less affected by foot rot compared with the untreated control. In field trials black pepper vines were treated with phosphite by soil drench and root infusion. After ten days root, stem and leaf specimens were removed for bioassay by inoculation with *P. capsici* zoospores. Phosphite soil drenching was significantly more inhibitive of disease development on leaf, stem and root, as compared to phosphite root infusion. Our study provides more evidence on the efficacy of phosphite in the management of black pepper foot rot caused by *P. capsici*. In addition, the excised leaf bioassay used in this study is a rapid and reliable technique useful for efficacy testing of systemic fungicides for the control of this disease.

38.100 APPLYING LIGNIN TO SOIL IN ORDER TO REDUCE THE VIABILITY OF SCLEROTIA. S. Van Beneden, J. Audenaert, S. Franca, G. De Backer and M. Höfte. *Laboratory of Phytopathology, Department of Crop Protection, Ghent University, Gent, Belgium. Email: Sarah.VanBeneden@ugent.be*

The sclerotial forming fungi *Rhizoctonia solani* and *Botrytis cinerea* cause a lot of damage to greenhouse lettuce in Belgium. This research focusses on controlling *R. solani* and *B. cinerea* survival structures, as a possible long-term alternative for methyl bromide. One approach we are investigating is adding lignin to soil. The lignin used is Indulin AT, pure unsulphonated lignin, a by-product of the paper industry. Pot-assays were set-up: sclerotia were placed in nylon mesh-bags and buried in soil with 1% of lignin added. After four weeks incubation the viability of *Rhizoctonia* sclerotia was reduced with 65% compared to the control. Remarkably, the sclerotia appeared to be more parasitized by antagonistic fungi like *Trichoderma* and *Fusarium*. When applying lignin in combination with different *Trichoderma* strains, an increase in mycoparasitism of the sclerotia was seen. However, this effect was not reached with all strains tested, and seems to be strain-dependent. Although the number of parasitized sclerotia was higher in soils containing lignin, no increase in the total *Trichoderma* population was observed. With *Botrytis* sclerotia similar results were obtained, but were not consistent, suggesting that the microbial population/activity in soil plays an important role. Currently we are trying to unravel the mechanisms involved. We are studying the changes in microbial population upon lignin application, using phospholipid fatty acid analysis of soil, ergosterol measurements and enzyme-assays.

38.101 THE ROLE OF SILICON IN MANAGEMENT OF BLACKLEG AND SOFT ROT ON POTATOES. J.J. van der Merwe, J.H. van der Waals and J.E. van der Waals. *Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, 0002, South Africa. Email: jacquie.vdwaals@up.ac.za*

Silicon is a beneficial element for plants in that it has been shown to suppress the incidence of diseases and damage by pests in a range of crops. An Si-containing metallurgical slag produced close to Middelburg (Mpumalanga) is already being used as a liming material on the eastern Highveld in maize production as well as to prevent Eldana borer damage on sugarcane in KwaZulu-Natal. This study researched the effect of Si soil additions, especially through the use of the above-mentioned metallurgical slag, on the incidence of the soft rot/blackleg disease complex in potato. No studies have been published on the effect of Si on potato pathogens. This study was initiated after various severe blackleg outbreaks on potatoes in South Africa. Pot trials using three different Si soil amendments were conducted. Tubers were artificially inoculated by dipping for 30 min in a 10^4 cfu/ml bacterial suspension of a mixed population of *Pectobacterium carotovorum* and *P. carotovorum* ssp. *brasiliensis* isolates from South Africa. Total phenolics were extracted from potato stems and tu-

bers and identified using TLC and HPLC as chlorogenic, caffeic and ferulic acids. The phenolic compounds were quantified by means of the Folin-Ciocalteau method. It was found that plants treated with silicon had significantly higher levels of total phenolic compounds than the controls. This preliminary data showed positive results and will open up possibilities in terms of controlling other soil-borne potato pathogens through the application of Si-containing slags.

38.102 SUSCEPTIBILITY OF WINTER WHEAT CULTIVARS TO BUNTS (*TILLETIA CARIES* AND *T. CONTROVERSA*) AND SURVEY OF INCIDENCE IN THE CZECH REPUBLIC. **M. Vanova, M. Kochanova and E. Prokinova.** *Agricultural Research Institute Kromeriz Ltd., Havlickova 2787, 767 01 Kromeriz, Czech Republic. Email: vanova.marie@vukrom.cz*

Bunts [*Tilletia caries* (DC.) Tul. and *T. controversa* Kuhn] cause very important diseases of winter wheat because they contaminate seed, food and feed, affecting the competitiveness of the crop on both domestic and export markets. Microscopic assessments of grain samples collected from all regions of the CR in 2000–2006 documented considerably increased incidence of both bunts, with *T. controversa* dominant. The highest percentage of samples contaminated by *T. controversa* was found in 2006. This was due to long-term accumulation of spores of this bunt in soil and favourable conditions during the 2005 autumn and 2006 winter. A total of 1,058 samples from four years' monitoring in various locations in the CR were analysed for the presence of bunt spores and diagnosed using a microscope. The reaction of Czech and foreign winter wheat cultivars registered in the CR was evaluated in field tests. Seeds of winter wheat were inoculated with teliospores of *T. caries.* Resistance to *T. controversa* was studied under heavy natural infestation from the soil. The heaviest infestation was found in plots with *T. caries* in cvs. Ilias, Drifter and Ebi. Very low incidence was detected in cvs. Bill, Barroko and Carolinum. On average, *T. controversa* incidence was higher than that of *T. caries*. There were also significant differences among the cultivars. 'Bill', 'Barroko', 'Carolinum' and 'Ludwig' showed very low infection by *T. controversa*. This work was supported by Ministry of Agriculture project QH71105.

38.103 EFFECT OF ARBUSCULAR MYCORRHIZA AND BONE CHARCOAL ON *FUSARIUM* DISEASE OF TOMATO. **H. von Alten.** *Institute of Plant Pathology and Plant Diseases, Herrenhaeuser Str. 2, D-30419 Hannover, Germany. Email: von-alten@ipp.uni-hannover.de*

Only a limited percentage of livestock biomass can be consumed by humans directly. In the EU during slaughtering and food processing from cattle alone, about 1 million tons/year bone by-products have to be handled. Bone meal can be turned into animal bone charcoal (ABC) using high temperatures and exclusion of oxygen. This sterile material contains high amounts of calcium and phosphorus and only low amounts of heavy metals; however, the phosphorus is not easily available to plant roots. This makes ABC interesting as a P-fertilizer for organic vegetable production. In greenhouse experiments with mycorrhizal and non-mycorrhizal tomato plants in quartz-sand or a quartz-sand/peat mix, growth and P-uptake was measured, with ABC as the only P-source. *Fusarium oxysporum* f. sp. *radicis lycopersici* was inoculated as a soil-borne pathogen. The severity of the *Fusarium* disease depended on the type of P-source used and the presence of the mycorrhizal symbiont.

38.104 DEVELOPMENT AND VALIDATION OF "IN FIELD" DETECTION KITS FOR THE CLUBROOT PATHOGEN *PLASMODIOPHORA BRASSICAE*. **A. Wakeham, R. Faggian and R. Kennedy.** *Warwick HRI, Wellesbourne, UK. Email: alison.wakeham@warwick.ac.uk*

Clubroot caused by the protist *Plasmodiophora brassicae* Woronin is a major problem of cultivated cruciferous crops world-wide. Symptoms (galls or clubs) form on the roots which reduce plant vigour and yield. Infection often leads to wilting, death and total crop loss. There is a direct relationship between contamination of the soil with clubroot resting spores and visual symptoms on roots. One of the problems in controlling this pathogen has been the inability to detect resting spores in the soil. However lateral-flow tests which detect clubroot resting spores have been successfully developed, and can be used to detect clubroot contamination of soil in the field. The lateral-flow device when used on pure samples of clubroot could detect the positive test line by eye at a concentration of approximately 1×10^4 spores (the threshold for symptom occurence on roots). By using reader devices the test can be made quantitative over a wide range of clubroot concentrations. Preliminary results show that the test can be used directly on soil samples but further work may be required to enable this direct usage on all soil types.

38.105 CONTROLLING BLACK DOT OF POTATOES (*COLLETOTRICHUM COCCODES*) BY UNDERSTANDING ITS EPIDEMIOLOGY. **S.J. Wale, A. Hilton, A. Lees, J. Brierley, N. Bradshaw, P. Gladders and J. Peters.** *SAC, Ferguson Building, Craibstone Estate, Aberdeen, AB21 9YA, UK. Email: stuart.wale@sac.co.uk*

The progress of visual disease development through the growth cycle of the potato crop was studied over three years across the UK in field trials funded by the British Potato Council and the Scottish Executive. Where seed-borne infection was the primary source of inoculum, visual symptoms on stem bases were detected at or before 6 weeks prior to haulm destruction. Thereafter, symptoms developed progressively but slowly on stems stolons, roots and tubers. Where soil-borne contamination was the primary inoculum source, initial visual symptoms were found later but the disease developed faster on below-ground parts than from seed-borne inoculum. There were no differences in disease development between varieties on stems, stolons and roots but tubers exhibited differential resistance. On the whole soil-borne contamination was a more important source of inoculum in terms of causing disease, than seed-borne infection. A RealTime PCR diagnostic soil test has been developed to quantify the level of soil contamination. This test provides guidance in determining the risk of black dot development and whether a more (tuber) resistant variety should be grown and/or soil fungicide treatment is required. The extent of daughter tuber infection increased as harvest was delayed. The longer the duration of the growing season from 50% emergence to harvest the greater the development of black dot on daughter tubers. Harvest date could, therefore, be brought forward to limit development of black dot on daughter tubers.

38.106 STUDIES ON THE CAUSE OF ONION STUNTING IN SOUTH AUSTRALIA. **T. Wicks, S. Pederick, G. Walker and B. Hall.** *South Australian Research and Development Institute, P.O. Box 397, Adelaide, S A 5064, Australia. Email: wicks.trevor@saugov.sa.gov.au*

Over 40% of Australian onion production is grown in South Australia mainly in an area approximately 100–150 km east/north east of Adelaide. Onions are often grown in rotation with cereals. In recent years patches of stunted onions similar to 'bare patch' in cereals have developed in onion plantings in the area. Stunted patches range in size from a few m^2 to >5m^2 and result in yield reduction of up to 15 to 20%. Stunted plants show no obvious root rotting and a similar range of fungi (*Fusarium*, *Pyrenochaeta*, *Pythium* and *Rhizoctonia*) are frequently recovered from the roots of stunted plants as well as non-stunted plants in areas adjacent to the stunted areas. Similarly stubby root, lesion and pin nematodes are found in low numbers in the onion plants and soil in both the stunted areas and other areas not associated with stunting. The cause of the onion stunting is unknown, however recent studies have detected *Rhizoctonia solani* anastomosis groups (AGs) 2.1, 2.2, 3, 8 and 11 in onion soils, with levels of AG 8 highest in soils from stunted areas and low or absent from other areas. Studies are underway to determine if AG 8 or a complex of *Rhizoctonia* AGs is pathogenic to onions and associated with stunting.

38.107 UNDERSTANDING AND MANAGING COMMON SCAB OF POTATO IN AUSTRALIA. T.J. Wiechel, N.S. Crump and R.F. de Boer. *Biosciences Research Division, Department of Primary Industries, Knoxfield Centre, P.B. 15, Ferntree Gully Delivery Centre, VIC 3156, Australia. Email: Tonya.Wiechel@dpi.vic.gov.au*

Common scab of potato, caused by *Streptomyces* spp., is a complex disease that produces a variety of symptoms such as shallow, raised, netted or deep-pitted lesions. Our research is focused on management strategies for this disease, with particular emphasis on: characterising the species of *Streptomyces* associated with common scab on potatoes in Australia and elucidating regions of the genome that may be responsible for pathogenicity; studying the effect of soil inoculum level of *Streptomyces* spp. on the development of common scab symptoms on potato tubers; and reducing the development of common scab symptoms on potato tubers under field conditions. So far numerous strains of *Streptomyces* have been isolated from scab-affected potatoes in Australia. These strains contain pathogenicity factors nec1 and the thaxtomin A gene. This gene was found in more isolates of *Streptomyces* than the nec1 gene, indicating that the txtA region may be a better target for a diagnostic test to identify pathogenic *Streptomyces* spp. in Australia. In glasshouse trials, relatively high levels of soil inoculum are required to cause high levels of disease, and this information is important in determining the target range required for a diagnostic test in soil. In field trials, ammonium lignosulfonate, calcium cyanimide, fish emulsion, meat and bone meal and pH plus ($CaCO_3$) can significantly reduce common scab severity without having an adverse effect on total and marketable yield.

38.108 INFLUENCE OF TEMPERATURE ON ZOOSPORANGIUM GERMINATION AND INFECTION OF *OLPIDIUM VICIAE*. J. Yan, H. Ye, G. Wu and Z. Yuan. *Department of Plant Protection, Sichuan Agricultural University, Yaan, Sichuan, 625014, P.R. China. Email: jimingyan@126.com*

Olpidium viciae Kus. was first reported as a new species by Shunsuke Kusano in Japan in 1912. The fungus parasitizes the leaves and stems of *Vicia unijuga*. In 1982, *O. viciae* was first reported in China as a pathogen attacking broad bean in the northwest. So far, only two reports on the fungus have been found. As a pathogen it only infects epidermal cells of leaves and stems. The epidermal cells at the infected site enlarge and increase number, producing a blister. Due to this symptom, the disease was named as broad bean blister disease. We investigated the pathogen and disease in 2004-2007. The distribution of the fungus and disease are limited to the plateau of north-west Sichuan. The disease is destructive in this area. To understand the relationship between distribution of the fungus and disease, and ecological factors, the effect of temperature on zoosporangium germination and infection of *O. viciae* was studied in growth chambers or incubators. The results showed that the range of temperature for germination was 5-18ºC, and the optimal temperature was 8-13ºC; the temperature range for infection of zoospores was 10-25ºC. The percentage of diseased leave at 10, 15, 20 and 25ºC was 38.3, 64.7, 66.9 and 4.3%, respectively. The incubation period was 6.0, 5.5, 4.5 and 7.0 days, respectively.

38.109 DISEASE SEVERITY AND GRAIN YIELD LOSS CAUSED BY MAIZE SHEATH BLIGHT. H. Ye, J. Yan and Y. Qin. *Department of Plant Protection, Sichuan Agricultural University, Yaan, Sichuan, P.R. China. Email: yhz@sicau.edu.cn*

Maize sheath blight, caused by *Rhizoctonia solani* Kuhn, is one of the major maize diseases in the world. In recent years, the disease has become a big problem in south of China, especially in Sichuan province, causing grain yield losses of 10-15%. The *R. solani* strain AG1-1A predominates as the causal agent. The pathogen attacks the sheath and ear, causing sheath blight and ear rot. To understand the relationship between disease severity and yield loss, artificial inoculation experiments were conducted in the field. Sheath blight severity was scored using a scale of 0–5 based on whole-plant symptoms. Ear rot severity was assessed by determining the percentage of each ear covered by symptoms, using a 4-point scale. Grain dry weight of each severity scale of sheath blight and ear rot was determined. Results showed that the disease causes grain yield losses depending on the disease severity level. Yield loss rose gradually with the disease severity rating. Reduction of grain yield caused by sheath damage on the 1–5 scale was 5.1, 10.8, 13.8, 19.8 and 38.7%, respectively. The regression equation between severity (X) and loss (Y) was Y=5.124+7.554X, r=0.9269**. Grain yield loss caused by maize ear damage on the 1–4 scale was 14.0, 19.3, 23.8 and 71.1%, respectively, with a regression equation Y=11.919+17.59X, r=0.8620**.

38.110 RELATIONSHIP BETWEEN THE *NEC1* GENE IN SOIL AND THE OCCURRENCE OF COMMON SCAB OF POTATO. H. Yoshida and O. Koyama. *Faculty of Bioproduction, Tokyo university of Agriculture, Yasaka 192, Abashiri, Hokkaido, 099-2493, Japan. Email: h-yoshid@bioindustry.nodai.ac.jp*

We monitored the amounts of the *nec1* gene, possessed by the main potato scab pathogen, in soil on a potato production farm, because there are few reports on quantitative changes of potato scab pathogen in soil in relation to occurrence of potato scab disease. At every potato growth stage, there was a positive correlation between *nec1* quantities in soil in the interhill space, and the scab index. We also examined the changes over time in amounts of *nec1* in soils of fields suffering different degrees of the disease. At the beginning of tuber initiation, which is the time of infection, the quantity of *nec1* in soil of the field with low scab index was lower than that in soil of the high scab index field. However, *nec1* quantities after the period of tuber initiation were not greatly different between the low and high scab index fields. This sug-

gests that we can understand the quantitative changes in scab severity by using this quantitative *nec1* gene analysis.

38.111 CHARACTERISATION OF THE SNOW PEA WILT PATHOGEN POPULATION IN AUSTRALIA. **A.L. Yousiph, A. Watson, L.W. Burgess and E.C.Y. Liew.** *Royal Botanic Gardens Sydney, Botanic Gardens Trust, Mrs Macquaries Road, Sydney, NSW 2000, Australia Email: ameera@yousiph.com*

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *pisi*, is the most devastating disease of snow peas in Australia. Severe outbreaks were observed in 2005 and 2006 throughout the main growing regions in Queensland, New South Wales and Victoria. In Victoria, pea crops were also similarly affected. Current disease management strategies include crop rotation, fungicide application and seed dressing. There is, however, a need for the establishment of more sustainable control measures, which requires information on the pathogen population. A total of 110 isolates were collected from the main growing regions and analysed on the basis of their haplotypes, VCG and pathogenic races. RAMS and rep-PCR were chosen for the haplotype analysis. No genetic differentiation was observed between isolates collected from pea and snow pea plants. It was also found that the population contained two large haplotype groups with 62% of isolates in one group and 30% in the other. The remaining isolates were found to be unique haplotypes. There was no correlation between haplotype group and geographic origin. These findings indicate that there is a moderate level of genetic diversity within this pathogen population in Australia, which needs to be taken into consideration in the development of management strategies. As there is a lack of clonality and correlation with geographic location, control measures cannot be targeted to specific haplotypes or regions.

38.112 BIOLOGICAL CONTROL OF *FUSARIUM* CROWN AND ROOT ROT DISEASE IN FOUR VARIETIES OF TOMATO USING *PSEUDOMONAS FLUORESCENS* AND *BACILLUS AMYLOLIQUEFACIENS*. I. GERMINATION AND GROWTH OF SEEDLINGS. **Y. Yusran, M. Weinmann, T. Müller and V. Römheld.** *Institute of Plant Nutrition (330), Hohenheim University, 70593 Stuttgart, Germany. Email: yusran_ysrn@yahoo.ca*

Fusarium crown and root rot of tomato (FCRR) induced by *Fusarium oxysporum* Schlecht f.sp. *radicis-lycopersici* Jarvis and Shoemaker (FORL) is one of the most damaging soilborne diseases of tomato, causing heavy economic losses. Once established in the soil FORL is difficult to control with available pesticides without treating the whole rooting zone. For ecological and economic reasons, alternatives for disease management are required. In this study, the efficacy of commercial fluorescent pseudomonad strains *Pseudomonas* sp. *proradix* (Proradix®, Sourcon-Padena, Tübingen, Germany) and *Bacillus amyloliquefaciens* FZB 42 (RhizoVital®, ABiTEP, Berlin, Germany) to suppress FORL were evaluated in growth chambers. Ten tomato (*Lycopersicon esculentum* Mill.) seeds were planted in each pot containing 0.5 kg substrate (Einheitserde Typ P, Einheitserde- und Humuswerke Gebr. Patzer, Sinntal-Jossa, Germany) with and without FORL-inoculation (50 ml 0.5 kg^{-1} substrate with spore concentration 10^{-7} ml^{-1}) and treated with either (1) Proradix® seed dressing (4.5×10^{10} cfu l^{-1}), (2) FZB 42 seed coating (5-15 g kg^{-1} seed) or (3) neither. After FORL-inoculation, both bacterial inoculants significantly increased seed germination and seedling growth measured as dry and fresh weight production and seedling height compared to the treatments without any of the two bacteria. This effect differed between the four tested tomato varieties depending on their susceptibility to FORL. In generally, this study demonstrated the efficacy of *Pseudomonas* sp. *proradix* and *Bacillus amyloliquefaciens* FZB 42 to control FORL and to enhance germination and plant growth when applied early before pathogen attack, particularly for susceptible varieties.

38.113 BIOLOGICAL CONTROL OF *FUSARIUM* CROWN AND ROOT ROT DISEASE IN FOUR VARIETIES OF TOMATO USING *PSEUDOMONAS FLUORESCENS AND BACILLUS AMYLOLIQUEFACIENS*. II. SEEDLING INFECTION AND DISEASE SEVERITY. **Y. Yusran, M. Weinmann, T. Müller and V. Römheld.** *Plant Nutrition Institute (330), Hohenheim University, 70593 Stuttgart, Germany. Email: yusran_ysrn@yahoo.ca*

Fusarium crown and root rot of tomato (FCRR) induced by *Fusarium oxysporum* Schlecht f.sp. *radicis-lycopersici* Jarvis and Shoemaker (FORL) is one of the most damaging soilborne diseases of tomato causing heavy economic losses. Once established in the soil FORL is difficult to control with available pesticides without treating the whole rooting zone. For ecological and economical reasons alternative measures for disease management are required. In this study, the efficacy of commercial fluorescent pseudomonads strain *Pseudomonas* sp. *proradix* (Proradix®, Sourcon-Padena, Tübingen, Germany) and *Bacillus amyloliquefaciens* FZB 42 (RhizoVital®, ABiTEP, Berlin, Germany) to suppress FORL were evaluated in growth chambers. Each ten tomato (*Lycopersicon esculentum* Mill.) seeds were cultivated in pots containing 0.5 kg substrate (Einheitserde Typ P, Einheitserde- und Humuswerke Gebr. Patzer, Sinntal-Jossa, Germany) with and without FORL-inocculation (50 ml 0.5 kg^{-1} substrate with spore concentration 10^{-7} ml^{-1}) and treated with either (1) Proradix® seed dressing (4.5×10^{10} cfu l^{-1}), (2) FZB 42 seed coating (5-15 g kg^{-1} seed) or (3) none of both. After FORL-inoculation, both bacteria significantly reduced the percentage of seedlings infected by FORL and the disease severity. This effect was most pronounced with the tomato variety Money Maker followed by Marmande, Hellfrucht Hilmar and Cal-J, indicating that the varieties differed in susceptibility to FORL. In generally, this study demonstrated the efficacy of *Pseudomonas* sp. *proradix* and *Bacillus amyloliquefaciens* FZB 42 to control FORL and to enhance plant health when applied early before pathogen attack particularly for susceptible varieties.

38.114 HYPOVIRULENT STRAIN XG36-1 OF *SCLEROTINIA SCLEROTIORUM* FROM DISEASED PLANT OF RAPESEED (*BRASSICA NAPUS*). **L. Zhang, Y. Fu, D. Jiang, G. Li and X. Yi.** *The key lab of plant pathology of Hubei Province, Huazhong Agricultural University, Wuhan, 430070, Hubei, P.R. China. Email: yanpingfu@mail.hzau.edu.cn*

Sclerotinia sclerotiorum is a ubiquitous inhabitant of soils in many parts of the world. In China, this pathogen causes stem rot on rapeseed, and serious losses in the middle and low drainage areas of the Yangtse River. Previously we have reported a mycovirus (SsDRV) associated strain Ep-1PN of *S. sclerotiorum* isolated from a sclerotium on a diseased eggplant; here we report a new hypovirulent strain of *S. sclerotiorum*, named XG36-1, which was isolated from a stem lesion on rapeseed in Xiaogan County, Hubei Province, P R China. Strain XG36-1 grew on PDA plates very slowly with sectoring, and could not induce lesions even on detached leaves of oilseed rape. Sclerotia of XG36-1 rarely produced apothecia, but occasionally a few sclerotia could bear apothecia

successfully. 104 single-ascospore-isolation sexual progeny were obtained, and all of them were not different from the healthy strain of *S. sclerotiorum*. About 26% of XG36-1 protoplast regeneratants showed normal phenotypes of *S. sclerotiorum*. The hypovirulence and its associated traits could be transmitted to sexual progeny of XG36-1 by hyphal anastomosis. DsRNA could not be detected with the cellulose CF-11 extraction method, but virus-like particles (about 40 nm in diameter) in hyphae of XG36-1 could be observed by transmission electron microscopy, but could not be observed in the sexual progeny of XG36-1. These particles could be co-transmitted with the hypovirulence and its associated traits through anastomosis. The characteristics of the new mycovirus in *S. sclerotiorum* will be studied.

38.115 OCCURRENCE AND CONTROL OF *FUSARIUM* YELLOWS OF CABBAGE IN YANQING COUNTY OF BEIJING, CHINA. **Y. Zhang, J. Zheng, J. Li, X. Wu, Y. Shi and Y. Ma.** *Zhibaolou 126, 2 Yuanmingyuan West road, Haidian, Beijing 100094, P.R. China. Email: zhangyang0309@126.com*

Yanqing county is an important production and export base of cabbage for Beijing and other cities in China. In summer 2001, *Fusarium* yellows of cabbage first occurred in this area and caused 30% losses in production, with seriously diseased fields yielding no harvest. The disease became prevalent between 2002 and 2006, with the incidence area enlarging from 1,330 acres to 8,670 acres and production losses increasing from 0.18 million kg to 1.37 million kg. The crop acreage of cabbage was reduced quickly from 218,890 acres in 2003 to 66,750 acres in 2005. This study identified *Fusarium oxysporum* and *F. verticillioides* as causative agents of the disease. Screening of ten fungicides in vitro suggested that prochloraz, SPY-Z048 and carbendazim could significantly inhibit the pathogens, with EC_{50} less than 1 mg/l. The EC_{50} of 0.5% hydrochloric berberine aqua, a fungicide of plant origin, was 30 mg/l. When applied twice during the seedling age, once during field planting and three times during transplanting, hydrochloric berberine was effective in preventing and controlling the yellows. It was superior to water-dispersible powder treatment with carbendazim in field trials. Follow-up investigation indicated that the disease in Yanqing was spreading rapidly and was serious and hard to combat. Irrigation might facilitate its waterborne transmission. The disease does not threaten other crucifers such as radish, Chinese cabbage and cauliflower. Crop rotation with disease-resistant varieties may offer an effective prevention and control measure.

Taxonomy of plant pathogens

44.1 PRECISE IDENTIFICATION OF *GANODERMA BONINENSE* AND ITS PATHOGENIC IMPACT ON OIL PALMS. **F. Abdullah and M. Nelson.** *Dept. of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. Email: fidah@fsas.upm.edu.my*

The bracket fungus *Ganoderma boninense* has been implicated as the cause of basal stem rot of oil palms (*Elaies guineensis*). Initially, species identification of this fungus was somewhat controversial as the macromorphology of the tropical species are prone to gradations of features, making difficult the identification of species on hosts other than oil palm. Studies using interfertility and molecular data have resulted in precise identification of individuals belonging to the species. *In vivo* artificial infection tests conducted on *G. boninense* isolated from other oil palms as well as from coconut (*Cocos nucifera*) in Malaysia showed that they were all equally infective although the latter was only found on stumps and not known to kill living coconut palms, whereas oil palm isolates can kill their hosts. *G. boninense* (on *Livistona* sp.) from Japan, whose taxonomy was verified from the integrative studies above, was found to be non-pathogenic to oil palms. In addition, "*G. boninense*" from Taiwan, verified as a non-*boninense Ganoderma* from our studies, were also found to be non-pathogenic to oil palms. In conclusion, even though the biological identity of the pathogen *G. boninense* can be verified, there seem to be gradations of infectivity within the species, for those isolates not naturally exposed to oil palms seemed to show no inclinations to infect this host.

44.3 SYSTEMATICS OF THE GENUS *PUCCINIA* (UREDINALES) ON CUCURBITACEAE. **G. Bagyanarayana.** *Department of Botany, Osmania University, Hyderabad 600 007, A.P., India. Email: gbagyan@rediffmail.com*

A taxonomic revision of the species of *Puccinia* parasitizing cucurbitaceous hosts is made. Infected herbarium specimens were obtained from both national and international herbaria viz., B, FH, IMI, PREM, PR, S and HCIO. The author also made several personal collections from different parts of India. Type specimens were examined in most cases. Out of 16 taxa described (15 species and 1 variety), 11 species are recognized as valid viz., *Puccinia cephalandrae*, *P. cephalandrae-indicae*, *P. citrulli*, *P. physedrae*, *P. momordicae*, *P. cucumeris*, *P. melothriicola*, *P. trochomerie*, *P. ctenolepidis*, *P. gymnopetali-wightiae*, *P. vanderysti*, *and P. windhokensis* and redescribed. *P. citrulli* var. *vulgaris* is treated as a synonym of *P. citrulli*; *P. citrullina* as a doubtful species; *P. anguriae* and *P. melothriae* as excluded species since the host is not cucurbitaceous. *P. arisanensis* is transferred to *Uropyxis* as *U. arisanensis*. The uredinial stage of *P. trochomeriae* is reported for the first time. A dichotomous key to the recognized species of *Puccinia* on Cucurbitaceae is made.

44.4 MYCELIAL COMPATIBILITY GROUPS AMONG *SCLEROTINIA SCLEROTIORUM* ISOLATES IN IRAN. **H. Barari, S.M. Badalyan and V. Alavi.** *Agriculture and Natural Resources Research Center of Mazandaran, Department of Plant Protection, P.O. Box 48175-556, Sari, Iran. Email: hosseinbarari1385@yahoo.com*

Sclerotinia sclerotiorum (Lib.) de Bary is an important fungal pathogen on more than 400 host-plant species in Iran and worldwide. The pathogen overwinters as sclerotia in the soil; germination in spring causes direct infection on crop roots, or apothecia are produced which release wind-dispersed ascospores infecting leaves and stems. Classification of isolates into mycelial compatibility groups (MCGs) is used routinely as a quick marker for genotyping *S. sclerotiorum* within populations. Mating type genes regulate sexual compatibility and sexual reproduction of fungi. Sixty-five samples were collected from six infected plants (*Lactuca sativa*, *Brassica napus*, *Faba vulgaris*, *Sinapis arvensis*, *Lycopersicum esculentum* and *Cucumis sativus*) from northern Iran. These isolates had different colours (white, red and grey) on PDA culture after 21 days. To determine the extent of differentiation in populations of this pathogen, MCGs were assessed by pairing isolates on PDA amended with Wilton red food coloring (75 µl/l), using the Kohn (2006) procedure. Assessment of incompatibility was based on the failure of two colonies to fuse, which was indicated by the formation of a red line and of a strip of thin mycelium or aerial

mycelium at the intersection. Thirty-nine different MCGs were distinguished, with all self-pairings compatible. Of these, twenty-six, eight, one, three and one groups had one, two, three, four and eight isolates, respectively. Most identified isolates fell within a single MCG. A high rate of out-crossing as well as evolutionary potential was found within the population. Variation within the population of the pathogen is reported for first time from Iran.

44.5 NEW HOSTS OF *SCLEROTINIA SCLEROTIORUM* IN IRAN. **H. Barari, S. Badalyan and V. Alavi.** *Agriculture and Natural Resources Research Center of Mazandaran, Department of Plant Protection. P.O. Box 48175-556 Sari, Iran. Email: hosseinbarari1385@yahoo.com*

Sclerotinia sclerotiorum (Lib.) de Bary is an important fungal pathogen of many plants and is responsible for substantial losses in agricultural and horticultural crops in Iran and world wide. The symptoms of *Sclerotinia* stem rot appear as bleached stem and shredding. Sclerotia are formed on infected tissues. Previously 5 hosts (*Helianthus annus*, *Brassica naous*, *Nicotiana tabacum*, *Gossypium herbaceum* and *Lattuga sativa*) were reported in Iran. The aim of this study was to find new hosts of *S. sclerotiorum* in northern Iran. Three hundreds samples of *S. sclerotiorum* were collected from six infected plants (*L. sativa*, *B. naous*, *Faba vulgaris*, *Sinapis arvensis*, *Lycopersicum esculentum* and *Cucumis sativus*) in Golestan, Mazandaran and Gillan provinces of northern Iran during 2007. Mycelial cultures isolated from sclerotia had white to grey pigmentation on potato-dextrose-agar. Colonies covered Petri dishes (80 mm) during 3 days at 22 °C, and rounded to elongated sclerotia from 2 to 20 mm size were formed. Cupulate and stipitate brown apothecia formed on the surface of the sclerotia. Hyaline, single-cell and elongated ascospores (8 per ascus) were similar in size. Rinds of sclerotia were composed of 1 to 3 layers of dark paranchyma cells and the medulla consisted of compact white hyphae. Based on the key of Kohn (1979) the causal fungus was identified as *Sclerotinia sclerotiorum.* Among 6 plant hosts, *Faba vulgaris*, *Sinapis arvensis* and *Lycopersicum esculentum* were identified for the first time as a new hosts of *S. sclerotiorum* in Iran.

44.6 FIRST REPORT OF *RHIZOCTONIA CIRCINATA* VAR. *CIRCINATA* AND BNR AG-FA IN RICE-PASTURE-SOYBEAN ROTATION IN ARGENTINA. **V.A. Barrera, S. Gutiérre, M. A. Cúndom, R. Rojo and L. Gasoni.** *IMYZA, INTA, CC 25 (1712) Castelar, Buenos Aires, Agentina. Email: vbarrera@cnia.inta.gov.ar*

In Argentina the area cultivated with soybean has increased during the last 5 years, displacing traditional crops such as wheat, maize and sunflower, among others. Flooded soils, in the northeast, give appropriate conditions for rice cropping, and although rice has recently been displaced by soybean, they coexist in rotation with cattle-feeding pastures. There is evidence that some phytopathogenic fungi, particularly the soilborne *Rhizoctonia* complex, might attack rice plants and soybean. The aim of this work was to survey the presence of *Rhizoctonia* species in rice and soybean soils in Argentina. *Rhizoctonia* isolates were collected from soils at Entre Ríos, Santa Fe, Santiago del Estero and Buenos Aires provinces in the Pampa region. Morphological and cytological features were examined. Anastomosis groups were assigned by confrontation among the isolates and the corresponding testers and fusion frequencies were scored. Cytological identification was complemented with ITS-rDNA sequence comparison with GenBank database. Pathogenicity tests were performed on rice and soybean with representative isolates from different species. As a result of this survey *R. circinata* var. *circinata*, *R. circinata* var. *zeae* and BNR *Ceratobasidium* AG-Fa were identified. *R. circinata* var. *circinata* isolates were non-pathogenic while, *R. circinata* var. *zeae* and C*eratobasidium* AG-Fa gave high disease incidence on rice and soybean plants. This is the first report of the presence of *R. c.* var. *circinata* and *Ceratobasidium* AG-Fa in Argentina. Further work is in progress to determine the frequency of this species in the Pampa Region.

44.7 A COMPARATIVE STUDY OF *PHYTOPHTHORA* SPECIES. **H. Brouwer, A.W.A.M. de Cock, C.H.A. Gerritzen and P.J.M. Bonants.** *Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. Email: h.brouwer@cbs.knaw.nl*

Over the last decade, the number of newly described *Phytophthora* species has increased markedly, and among them are several pathogens of quarantine significance. Existing identification keys (Stamps *et al*., 1990) and reviews of the morphological literature on the genus (Erwin and Ribeiro, 1996) are over a decade old and outdated as they do not include important new species like *P. ramorum*, *P. alni*, and *P. kernoviae*. We aim to create a database for identification of *Phytophthora* species, using both molecular and morphological characters. The database will be built on a comparative study of isolates in the CBS collection. Living ex-type strains, or if not available, representative strains are being grown and morphologically compared under controlled conditions. The following DNA regions will be sequenced: the ribosomal DNA internal transcribed spacer (ITS), mitochondrial cytochrome c oxidase subunit (Cox1), β-tubulin, and elongation factor 1-alpha. Phylogenetic relationships and morphological data observed in this ongoing project will be evaluated and discussed.

44.8 BOTRYOSPHAERIACEAE AS POTENTIAL PATHOGENS OF *PRUNUS* SPECIES IN SOUTH AFRICA. **U. Damm, P.H. Fourie and P.W. Crous.** *Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa. Email: ulrike@sun.ac.za*

Botryosphaeriaceae are common dieback and canker pathogens of woody host plants, including stone fruit trees. In the present study, the diversity of members of the Botryosphaeriaceae isolated from symptomatic wood of *Prunus* species (plum, peach, nectarine and apricot) was determined in different stone fruit growing areas in South Africa. Morphological and cultural characteristics as well as DNA sequence data (5.8S rDNA, ITS-1, ITS-2, EF-1α and 28S rDNA) were used to identify known, and describe novel members of Botryosphaeriaceae. From 258 wood samples collected, 69 isolates of Botryosphaeriaceae were obtained, from which nine species were identified. All species were associated with wood symptoms. *Diplodia seriata* (*Botryosphaeria obtusa*) was dominant, and present on all four *Prunus* species sampled, followed by *Neofusicoccum vitifusiforme* and *N. australe*. First reports from *Prunus* spp. include *N. vitifusiforme*, *Dothiorella viticola* and *Diplodia pinea*. This is also the first report of *D. mutila* from South Africa. Three species are newly described, namely *Lasiodiplodia plurivora* sp. nov. from plum, *Diplodia africana* sp. nov. from peach, and *Aplosporella prunicola* sp. nov. from nectarine. Based on phylogeny of the 28S rDNA (LSU) gene, the genus *Aplosporella* was recognised as a previously unknown anamorph lineage within the Botryosphaeriaceae (Botryosphaeriales). All species tested, except *Dothiorella viticola*, caused lesions on green nectarine and/or plum shoots in a detached shoot pathogenicity assay.

44.9* NOVEL *PHAEOACREMONIUM* SPECIES ASSOCIATED WITH NECROTIC WOOD OF *PRUNUS* TREES. U. Damm, L. Mostert, P.W. Crous and P.H. Fourie. *Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa. Email: lmost@sun.ac.za*

The genus *Phaeoacremonium* is associated with opportunistic human infections, as well as stunted growth and die-back of various woody hosts, especially grapevines. In this study, *Phaeoacremonium* (*Pm*) species were isolated from necrotic woody tissue of *Prunus* spp. (plum, peach, nectarine and apricot) from different stone fruit growing areas in South Africa. Morphological and cultural characteristics as well as DNA sequence data (5.8S rDNA, ITS-1, ITS-2, β-tubulin, actin and 18S rDNA) were used to identify known, and describe novel species of this genus. From 257 wood samples collected, 42 *Phaeoacremonium* isolates were obtained, from which 14 species were identified. *Pm. scolyti* was most frequently isolated, and present on all four *Prunus* species sampled, followed by *Togninia minima* (anamorph: *Pm. aleophilum*) and *Pm. australiense*. Almost all taxa isolated represent new records on *Prunus*. Furthermore, *Pm. australiense*, *Pm. iranianum*, *T. fraxinopennsylvanica* and *Pm. griseorubrum* represent new records for South Africa, while *Pm. griseorubrum*, hitherto only known from humans, is newly reported from a plant host. Five species are newly described, two of which produce a *Togninia* sexual state. *T. griseo-olivacea*, *T. africana* and *Pm. pallidum* are newly described from *Prunus armeniaca*, while *Pm. prunicolum* and *Pm. fuscum* are described from *Prunus salicina*.

44.10 MORPHOLOGY VERSUS DNA SEQUENCE COMPARISONS: CONFLICTING GENERIC CONCEPTS IN THE OPHIOSTOMATALES. Z.W. de Beer, R. Linnakoski and M.J. Wingfield. *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Insitute, University of Pretoria, Pretoria, South Africa. Email: wilhelm.debeer@fabi.up.ac.za*

The Ophiostomatales currently accomodates at least 169 teleomorph and 61 anamorph species. Most are wood-inhabiting fungi associated with bark beetle vectors, while several species are serious tree pathogens. Recent DNA-based studies have revealed that *Ophiostoma* species with *Leptographium* anamorphs as well as those with falcate ascospores and *Hyalorhinocladiella* anamorphs forms two discrete monophyletic lineages. The genera *Grosmannia* and *Ceratocystiopsis* have thus been re-instated to represent these groups. An additional 35 *Leptographium* species without known teleomorphs, are associated with *Grosmannia*. *Ophiostoma* remains, having a rather broad definition and including 126 teleomorph species. In addition, anamorph species associated with *Ophiostoma* include 15 *Sporothrix*, 10 *Raffaelea*, seven *Ambrosiella*, two *Pesotum*, and one *Dryadomyces* species. In the present study we explored LSU, SSU and beta tubulin exon data for as many species as possible, to determine whether additional monophyletic lineages exist in the Ophiostomatales. Resulting phylogenies revealed at least four major groups in addition to *Grosmannia* and *Ceratocystiopsis*. One of these, although not always well-supported, includes the type species of *Ophiostoma*, *O. piliferum,* species in the *O. piceae*-complex, and species with pillow-shaped ascospores. A second group accommodates only species with *Sporothrix* anamorphs, including the human pathogen *S. schenckii*. A third group is comprised of *O. pluriannulatum* and three similar species, and a fourth group includes *O. rectangulosporium* and *P. fragrans*. We suggest that these four groups represent distinct genera. Interestingly, all the *Ambrosiella* and *Raffaelea* species grouped within *Grosmannia*, although this is based only on SSU data and deserves further exploration.

44.11 TAXONOMY, PHYLOGENY AND IDENTIFICATION IN *PYTHIUM*. A.W.A.M. de Cock, C.A. Lévesque and H. Brouwer. *Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. Email: a.decock@cbs.knaw.nl*

It is more than 25 years since the monograph of the genus *Pythium* was published by van der Plaats-Niterink (1981). Since then more than 70 new species have been described and critically studied with regard to their phylogenetic position and morphological characteristics. Living ex-type strains, if available, or otherwise representative strains, were grown under controlled conditions and compared with related and morphologically similar species. For phylogeny and species delimitation the following DNA regions were sequenced: the ribosomal DNA internal transcribed spacers (ITS) and 28S gene (partial) and the mitochondrial cytochrome c oxidase subunit 1 (Cox I). If no living culture could be obtained, literature and GenBank data were used as data source. Based on the observed phylogenetic relationships, morphological characters were evaluated in order to find key characters for morphological identification.

44.12 INVASION OF OAK POWDERY MILDEW IN EUROPE: EVIDENCE OF SEVERAL CRYPTIC TAXA. M.-L. Desprez-Loustau, A. Mougou and C. Dutech. *INRA, UMR1202 BIOGECO, Equipe de Pathologie forestière, 71 Avenue Edouard Bourleaux, B.P. 81, F33883 Villenave d'Ornon Cedex, France. Email: loustau@bordeaux.inra.fr*

Oak powdery mildew was first recorded in 1907 and rapidly spread in Europe, causing severe damage. The taxonomy and origin of the causal agent remained controversial in the early 20th century and, surprinsingly, was never revisited with molecular tools. In this study, we analysed the variability of the ribosomal DNA of 33 European (mostly French) samples of oak powdery mildew. The internal transcribed spacer (ITS) and the intergenic spacer (IGS) regions were amplified and PCR products were subsequently sequenced. Four different haplotypes were obtained for ITS among the various samples (ITS_A, ITS_B, ITS_C and ITS_D). Each ITS sequence corresponded to a different IGS sequence. Comparison of our ITS sequences with sequences accessible in the GenBank database revealed very high homologies with different taxa. Of these, three taxa had already been described on oaks in Europe, i.e *Erysiphe alphitoides* (100% homology with ITS_A), *Erysiphe hypophylla* (99.4% homology with ITS_C) and *Phyllactinia guttata* (97.64% homology with ITS_D). Our data also confirmed the 100% homology between ITS_A and the sequence described for *Oidium mangiferae*, the agent of mango powdery mildew. The fourth haplotype, i.e ITS_B, represented by nearly 25% samples, showed 100% homology with the recently described *E. quercicola* from *Quercus* spp. in Asia, and several tropical and sub-tropical powdery mildew species, including *Oidium heveae*, a major pathogen of rubber trees worlwide. Our results suggest that oak powdery mildew might originate from host shifts of several tropical *Erysiphe* species introduced to Europe through infected exotic host plants.

44.13 PHYLOGENY AND TAXONOMY OF *PHAEOACREMONIUM* AND *PHAEOMONIELLA* SPECIES ON GRAPEVINE. S. Essakhi, L. Mugnai, P.W. Crous, J.Z. Groenewald and G. Surico. *Dipartimento di Biotecnologie Agrarie, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. Email: salwa.essakhi@unifi.it*

Petri disease and esca are serious diseases of young and mature vines in most countries where grapevines (*Vitis* spp.) are grown. *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* are among the principal hyphomycetes, which are believed to be associated with esca disease symptoms, both producing a range of enzymes and phytotoxic metabolites. Although 13 species of *Phaeoacremonium* have been associated with grapevines, only one species of *Phaeomoniella* is known from this host. The present study compared the phylogeny and genetic diversity of a global collection of 250 *Phaeomoniella* and 165 *Phaeoacremonium* isolates from grapevine, in order to gain a better understanding of their geographic distribution and possible modes of dispersal. Phylogenetic analyses of DNA sequence data sets of the actin, β-tubulin and calmodulin genes revealed the presence of additional novel species of *Phaeoacremonium*, which are currently being described based on cultural and morphological characters. Further work is in progress to investigate the genetic diversity among isolates of *Phaeomoniella* by means of Amplified Fragment Length Polymorphism (AFLPs) and Inter Sequence Simple Repeats (ISSRs).

44.14* GENETIC RELATEDNESS IN A WORLDWIDE COLLECTION OF *FUSARIUM OXYSPORUM* F. SP. *CUBENSE*, THE CAUSAL AGENT OF *FUSARIUM* WILT OF BANANA. G. Fourie, E.T. Steenkamp, T.R. Gordon and A. Viljoen. *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa. Email: gerda.fourie@fabi.up.ac.za*

Fusarium oxysporum is a highly diverse soil-borne fungal pathogen responsible for wilt diseases of many agricultural crops. The species is subdivided into *formae speciales*, of which *F. oxysporum* f.sp. *cubense* (Foc), the causal agent of *Fusarium* wilt of banana (*Musa* spp.), is one of the most destructive. Isolates of this pathogen are routinely characterized using vegetative compatibility, and 24 so-called vegetative compatibility groups (VCGs) are known for Foc. To study the genetic relatedness among and within the VCGs of Foc, two nuclear and two mitochondrial regions were sequenced. Phylogenetic analysis separated the isolates into two main clades that were each subdivided into three subgroups. Multi-gene sequence analyses further divided Foc into lineages according to their VCG status. We also determined the mating-types of these isolates using PCR-based assays for detection of conserved regions in the MAT-1 and MAT-2 mating-type idiomorphs. Isolates of opposite mating-type were then crossed in an attempt to induce a sexual stage. All of the isolates examined harboured one of the mating-type idiomorphs, and never both. This is consistent with the idea that the mating system of this fungus is heterothallic, should sexual reproduction occur. Although no sexual structures were observed, some lineages of Foc harboured MAT-1 isolates and MAT-2 isolates, suggesting that these lineages have a sexual origin. Overall our findings therefore show that Foc is a polyphyletic taxon consisting of multiple lineages, most probably representing cryptic species that might have more recent sexual origins than initially anticipated.

44.15 ASSESSMENT OF GENETIC DIVERSITY OF *FUSARIUM OXYSPORUM* F. SP. *CUBENSE* USING MICROSATELLITE MARKERS. G. Fourie, E.T. Steenkamp, T.R. Gordon and A. Viljoen. *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa. Email: gerda.fourie@fabi.up.ac.za*

Fusarium wilt of banana, caused by *Fusarium oxysporum* f.sp. *cubense* (Foc) is one of the most important and destructive wilting diseases within the *Fusarium oxysporum* species complex. Isolates of Foc are routinely grouped according to vegetative compatibility and the specific host they affect. Thus, isolates of the fungus are divided among 24 vegetative compatibility groups (VCGs) and three races. The aim of our study was to assess the genetic diversity of 257 Foc isolates from Africa, Asia, Australia and the Americas by using DNA-based microsatellite markers targeting 9 unlinked loci. Populations were defined according to geographic origin, VCG and phylogenetic lineages determined by multi-gene sequence analysis. Based on the various size differences of the microsatellite markers, measures of gene and genotypic diversity, reproductive mode, population differentiation and gene flow were determined. Southeast Asian isolates showed the highest level of genetic diversity, supporting the hypothesis that Asia is the centre of origin of Foc. Low gene and genotypic diversity were observed within the various Foc phylogenetic lineages. Gene flow among these lineages and the VCG-based populations, as well as some of the geography-based subpopulations was also limited. In all of the subpopulations the hypothesis of sexual recombination was also rejected. Our data therefore show that the Foc VCGs and phylogenetic lineages represent stable clonal groups that reproduce asexually. Furthermore, the results presented here demonstrate that VCGs, in combination with their phylogenetic association, remain a powerful tool for characterizing isolates of the causal agent of *Fusarium* wilt of banana.

44.16 FURTHER GENETIC CHARACTERISATION OF A *XANTHOMONAS TRANSLUCENS* INFECTING PISTACHIO IN AUSTRALIA. D. Giblot Ducray, M.R. Gillings, A. Marefat, K. Ophel-Keller and E.S. Scott. *The University of Adelaide, Waite Campus, PMB 1, Glen Osmond, 5064 SA, Australia. Email: daniele.giblotducray@adelaide.edu.au*

The genus *Xanthomonas* contains a large number of species and pathovars that were originally characterised by their ability to cause disease on specific plants. The groupings were later supported by genetic data. Shortly after onset of commercial production, the Australian pistachio industry was affected by a dieback disease that eventually resulted in the death of affected trees. Strains of *X. translucens*, here designated *X. translucens pistachio* (Xtp), were identified as the causal agent. Previous biochemical and pathogenicity tests as well as genetic analyses (rep-PCR, rRNA sequencing and DNA:DNA hybridization) showed that Xtp is biologically and genetically closely related to, but distinct from, other *X. translucens* pathovars, particularly in being pathogenic to dicotyledonous woody hosts. To further characterise Xtp, we tested a collection of isolates for integrons and plasmids, and assessed the diversity of house keeping (*atp*D) and pathogenicity-related (*rpf*B) genes by PCR-RFLP. The genome of Xtp contains an integron like other *Xanthomonas*, but the difference in the amplification patterns of the gene cassette array associated with the integron confirmed that Xtp differs from all other *Xanthomonas* tested to date. Initial plasmid isolation experiments suggest that Xtp contains a low-copy number, high molecular weight plasmid. Further investigation is needed to confirm the result and to characterise the plasmid. Finally, PCR-RFLP analysis of *rpf*B and *atp*D genes, which have been shown useful in clarifying the phylogenetic relationships among *Xanthomonas* species, is being performed. Results will be used to improve understanding of the position of Xtp within the genus *Xanthomonas* and to elucidate the origin of this intriguing pathogen.

44.17 A NEW SPECIES OF *OPHIOSTOMA* FROM HARDWOODS IN AFRICA. J.W. Grobbelaar, Z.W. de Beer, P. Bloomer, M.J. Wingfield and B.D. Wingfield. *Department of Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa. Email: mike.wingfield@fabi.up.ac.za*

The genus *Ophiostoma* encompasses a suite of wood-inhabiting taxa. Many are insect-vectored sapstaining fungi, but some species are tree pathogens. The majority of known *Ophiostoma* spp. are considered to be native to the northern Hemisphere. However, as surveys on these fungi in the southern hemisphere increase, the number of new species discovered from this part of the world is rising. *Ophiostoma quercus*, for example, was reported in recent studies from Africa, South America and New Zealand. Polymorphic microsatellite data from a recent population study on *O. quercus* showed that some African isolates from hardwoods were genetically distinct from *O. quercus*. In this study we compared African isolates with authentic *O. quercus* isolates using morphological characters, growth in culture, mating compatibility, and ITS and β-tubulin DNA sequences. African isolates were substantially variable in culture and morphologically similar to *O. quercus*. Phylogenetic analyses of the ITS and β-tubulin gene regions confirmed that the African group represents a distinct species in the hardwood clade of the *O. piceae*-complex, more closely related to *O. ulmi* and *O. himalulmi* than to *O. quercus*. Mating studies between *O. quercus* and the African isolates showed that isolates predominantly mated with isolates of their own group, although there were rare cases of crosses between the groups. The new species is presently being described as *O. tsotsi* nom. prov. The biology, pathogenicity, and ecological role of this species is unknown, and needs serious consideration in view of its relationship to the Dutch elm disease fungi.

44.18 PHYLOGENETIC LINEAGES IN THE MYCOSPHAERELLACEAE AND TERATOSPHAERIACEAE. J.Z. Groenewald, U. Braun and P.W. Crous. *CBS Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands. Email: e.groenewald@cbs.knaw.nl*

The teleomorph genus *Mycosphaerella* (Capnodiales, Mycosphaerellaceae), and its sister genus *Teratosphaeria* (Capnodiales, Teratosphaeriaceae), contain almost 3000 species, with thousands of additional species described in more than 30 anamorph genera. *Mycosphaerella* sensu lato was considered as being monophyletic, until it was recently shown to consist of at least two distinct teleomorph genera, namely *Mycosphaerella* sensu stricto and *Teratosphaeria*. These genera and their anamorphs encompass many important plant pathogens, saprobes and endophytes, as well as extremotolerant species. Co-occurrence of multiple species or genotypes within the same lesion has been observed, as has jumping of species between diverse hosts in different plant families. While some species are highly host-specific, others can occur on several hosts. Homo- and heterothallic species are found, with species observed as being strictly sexual, strictly asexual, or being able to readily produce the teleomorph as well as several anamorph states. In an attempt to understand speciation within the Mycosphaerellaceae and Teratosphaeriaceae, we sequenced the translation elongation factor 1-alpha gene, the complete 18S nuclear ribosomal RNA (nrRNA) gene and the complete 28S nrRNA gene. Compared to other genera, for example *Venturia* (Pleosporales, Venturiaceae), we observed well-supported phylogenetic lineages within the families, indicating the presence of several genera. Due to convergent evolution, several anamorph genera are dispersed across these phylogenetic lineages, emphasizing the plasticity of anamorph form-genera. We conclude that in order to understand the force driving speciation in these families, we should first revisit our definition of what constitutes a genus within the Capnodiales.

44.19 RADICAL REVISION OF THE IMPORTANT PATHOGEN GENUS *CRYPHONECTRIA* AND ALLIED FUNGI EMERGES FROM PHYLOGENETIC INSIGHT. M. Gryzenhout, B.D. Wingfield and M.J. Wingfield. *Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa. Email: Marieka.Gryzenhout@fabi.up.ac.za*

Cryphonectria has traditionally included the devastating and well-known forest pathogens *Cryphonectria parasitica* and *C. cubensis*. Until relatively recently, this was a small genus of only ten species. Multi-gene DNA sequence comparisons have, however, revealed that *Cryphonectria* is polyphyletic and this has led to the creation of eight genera encompassing existing *Cryphonectria* spp. as well as many newly discovered species. Most of these new genera include important tree pathogens. DNA sequence comparisons of the more conserved large subunit of the ribosomal operon have also revealed that species of *Cryphonectria*, *Endothia* and the newly recognized genera represent a distinct family. This has been described as the *Cryphonectriaceae* in the order *Diaporthales*. Although this radical revision of *Cryphonectria* might at first sight appear confusing, the genera and species can be distinguished by robust morphological characters that have recently been summarized in a monographic study. The morphological characteristics and DNA sequence data can now be combined to produce interactive polyphasic keys, which will be relatively simple to use and readily available to researchers working with these fungi.

44.20 A NEW GENUS IN THE *CRYPHONECTRIACEAE* PATHOGENIC TO *EUCALYPTUS* IN INDONESIA. M. Gryzenhout, M. Tarrigan, P.A. Clegg and M.J. Wingfield. *Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa. Email: Marieka.Gryzenhout@fabi.up.ac.za*

The *Cryphonectriaceae* (*Diaporthales*) is a recently described family including numerous important tree pathogens. The best known of these are the chestnut blight pathogen *Cryphonectria parasitica* and *Chrysoporthe cubensis* that causes a serious stem canker disease on *Eucalyptus*. The *Cryphonectriaceae* includes ten genera, the majority described recently. A recent investigation of dying *Eucalyptus grandis* clonal hedges in northern Sumatra, Indonesia, revealed the presence of an unknown member of the *Cryphonectriaceae*. Inoculations with this fungus on two clones of *E. grandis* showed that it is highly pathogenic. DNA sequence comparisons with other members of the family showed that the Indonesian fungus represents a new monotypic genus in the family. It can be distinguished from other *Cryphonectriaceae* on *Eucalyptus*, such as *Chrysoporthe*, *Microthia*, *Holocryphia* and *Cryphonectria*, by its orange, limited and predominantly pseudoparenchymatous stromatic tissue, single septate, fusoid to cylindrical ascospores and the absence of paraphyses. The importance of this newly discovered pathogen of *Eucalyptus* is unknown and its potential threat to *Eucalyptus* forestry in Indonesia must be assessed.

44.21 COMPARATIVE D2/D3 LSU rDNA SEQUENCE STUDY OF SOME IRANIAN *PRATYLENCHUS LOOSI* POPULATIONS. **B. Hajieghrari.** *Department of Plant Production, Moghan Junior College of Agriculture, University of Mohaghegh – Ardabili, Pars Abade Moghan, Ardabil, Iran. Email: bhajieghrari@uma.ac.ir*

The nematode *Pratylenchus loosi* Loof attacks roots of tea bushes in Guilan province, northern Iran. For phylogenetic analysis, we amplified and sequenced the D_2/D_3 LSU rDNA expansion segment of 13 isolates from Guilan. Amplification yielded one fragment of 787 bp for all isolates. The DNA sequences were aligned using Clustal X1.81 together and with three *P. loosi* sequences available in GenBank database (isolate T from Srilanka and isolates N1 and N2 from Florida, USA). Also the genetic distances between sequences were calculated using four methods: Uncorrected distance (UC), Jukes-Cantor (JC) Kimura distance (K) and Jin-Neigamma distance (JNG). For generating phylogenetic trees both Neighbor-joining (NJ) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) were used. The results indicated that very short genetic distances (close relationships) exist among the Iranian isolates and between the Iranian isolates and isolate T from Srilanka, whereas this group was genetically distinct from isolates N1 and N2.

44.22 WHEAT LEAF SPOT DISEASES IN THE CZECH REPUBLIC 2001-2006. **A. Hanzalova and J. Palicová.** *Research Institute of Crop Production, Drnovsk 507, 161 06 Prague, Czech Republic. Email: hanzalova@vurv.cz*

Our aim was to summarize results dealing with the incidence of wheat leaf spot pathogens in the last five years in the Czech Republic and to give preliminary information about race spectrum of PTR in our country. *Pyrenophora tritici-repentis, Phaeosphaeria nodorum* and *Mycosphaerella graminicola* were the most important pathogens in the Czech Republic during the last five years. In 2001, 2003, 2004 and 2005 *P. tritici-repentis* was the most often detected pathogen in samples collected from different parts of the country. On the other hand *M. graminicola* was the most often isolated pathogen in 2001 and 2002. In the Czech Republic race 1 seems to be the most frequent (67%). Races 2, 3, 6 and avirulent race 4 were detected only sporadically (less than 4%). The occurrence of race 3 in the Czech Republic is the first report of this race outside of Canada (Ali *et al*. 2004). Other races were not found. *Didymella exitialis* (anam. *Ascochyta* sp.) was found very often in 2001. *D. exitialis* has not been described as a pathogenic fungus in the Czech Republic yet. *D. exitialis* was the most common fungus identified on senescent leaves in Canterbury wheat crops (New Zealand) in 1993-94 season (Cromey *et al*. 1994). *Ascochyta* sp., *Cochliobolus sativus* (anam. *Bipolaris sorokiniana*), *Cladosporium cladosporioides* and *Epicoccum nigrum* were detected in the samples very rarely.

44.23 DIFFERENTIATION OF NEW ZEALAND *FUSARIUM* POPULATIONS USING MORPHOLOGICAL AND MOLECULAR APPROACHES. **S.A. Harrow, A.R. Pitman and M.G. Cromey.** *New Zealand Institute for Crop & Food Research Limited, Private Bag 4704, Christchurch, New Zealand. Email: harrows@crop.cri.nz*

Fusarium is an economically important genus worldwide that includes many pathogens of agricultural crops such as rice, wheat, potatoes, and maize. Within the genus, limited research has been conducted on *F. avenaceum* and *F. acuminatum*, that cause disease of legumes and cereal grains in temperate regions. The research described here demonstrates the sub-specific variation within New Zealand populations of these two fungi using morphological and molecular tools. Samples of soil, grass stem bases, cereal root and stem bases were collected from 28 sites in the South Island of New Zealand, representing agricultural and native ecosystems. A total of 58 single spore isolates, suspected of being either *F. avenaceum* or *F. acuminatum*, were obtained from the samples and identified to species level using classical taxonomic methods. Molecular tools were also used since these species were difficult to distinguish on morphological and physiological characteristics alone. To confirm the identity of the isolates, the DNA sequences of the translation elongation factor (EF1α) and the beta-tubulin (βTUB) nuclear genes were compared to those of reference cultures from international and national collections. Analysis of the molecular data provided greater resolution of *Fusarium* strains than the morphological data, showing the value of a multiphasic approach for discriminating *Fusarium* isolates at the species and sub-species level.

44.24 NEW HYPOTHESES ON THE ORIGIN OF THE INTERSPECIFIC HYBRID OOMYCETE *PHYTOPHTHORA ALNI*. **R. Ioos and P. Frey.** *INRA, Nancy-University, UMR1136 Tree-Microbe Interactions, F-54280 Champenoux, France. Email: frey@nancy.inra.fr*

An emergent disease of alder in Europe is caused by a complex of three taxa belonging to the genus *Phytophthora* (Oomycetes): *P. alni* subsp. *alni* (Paa), *P. alni* subsp. *uniformis* (Pau) and *P. alni* subsp. *multiformis* (Pam). Paa was previously hypothesized to be an allopolyploid hybrid between two genetically close species, *P. cambivora* and *P. fragariae*. Furthermore, Pau and Pam were thought to be genetic breakdowns of Paa. In order to examine these hypotheses, we studied (i) the occurrence and the allelic distribution of four nuclear and two mitochondrial genes, (ii) the expression of elicitin genes, a multigenic family specific to the genus *Phytophthora*, and (iii) the distribution of microsatellite alleles, on a wide collection of *P. alni* and closely related species. Altogether, our results clearly demonstrate that *P. cambivora* and *P. fragariae* are not the progenitors of *P. alni*, which thus is not a superpathogen gaining a new host range. Furthermore, our results show that Paa was generated on several occasions by hybridization between Pam and Pau. This study raises new issues about the geographic origin, host range, and ecology of Pam and Pau.

44.25 A NEW *FUSARIUM* SPECIES IN THE *GIBBERELLA FUJIKUROI* COMPLEX FROM PINEAPPLE IN SOUTH AFRICA. **A. Jacobs, P.S. van Wyk, W.F.O. Marasas, B.D. Wingfield, T.A. Coutinho and M.J. Wingfield.** *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa. Email: riana.jacobs@fabi.up.ac.za*

Pineapple (*Ananas comosus*) is native to South America and widely planted in the tropics and sub-tropics. It is susceptible to a number of fungal diseases of which fusariosis is the most serious. This disease is caused by *Fusarium guttiforme* and it is only known to occur in South and Central America. The appearance of a similar disease on pineapples in South Africa has prompted a re-evaluation of the *Fusarium* spp. associated with pineapple fruit rot. Phylogenetic relationships of isolates from pineapples col-

lected in Brazil and South Africa were assessed based on sequence data for the translation elongation factor-1α and β-tubulin gene regions. Analyses of these sequence data showed that the South African isolates represent a species distinct from Brazilian isolates. Based on an evaluation of morphological characteristics, the South African isolates are characterised by a concentration of aerial mycelium at the centres of the colonies. In contrast, the Brazilian isolates have an even distribution of aerial mycelium. Both phylogenetic and morphological data indicate that the disease on pineapple in South Africa is caused by a *Fusarium* species different to *F. guttiforme*, which will be described as *F. ananarum* prov. nom.

44.26 GENOMIC DIVERSITY AND DIFFERENTIATION AMONG PHYTOPLASMAS IN SOUTH KOREA. **H.Y. Jung, Y.H. Kim, S.S. Han and J.T. Lee.** *Division of Applied Biology & Chemistry, Kyungpook National University, Daegu, Republic of Korea. Email: heeyoung@knu.ac.kr*

Plant pathogenic phytoplasmas cause devastating damage to many plants worldwide, but studies on phytoplasmas in Korea have been surprisingly limited. Only about 30 plant diseases have been identified as caused by phytoplasmas, and their phylogenetic positions are largely unknown. Here we present the results of the comprehensive detection, classification and characterization of phytoplasmas in Korea. Many plant species showing phytoplasma-like symptoms were collected from crop or non-crop fields during the past five years. Transmission electron microscopy, fluorescence microscopy, and PCR were used to detect phytoplasmas from 30 plant species belonging to 15 families. Based on the full-length 16S rRNA gene and elongation factor Tu gene sequences, the 30 phytoplasma isolates were classified into six *Candidatus* phytoplasma species (*Ca*. P. asteris, *Ca*. P. solani, *Ca*. P. pruni, *Ca.* P. castaneae, *Ca*. P. trifolii, *Ca*. P. ziziphi). These phytoplasmas caused characteristic symptoms of little leaf, proliferation, and floral abnormalities. Our findings show that non-crop species have the potential to function as reservoirs of phytoplasmas causing crop diseases. This work also provides evidence for the unexpected diversity of phytoplasmas in Asia.

44.27 MANGO MALFORMATION IN THE SULTANATE OF OMAN. **M. Kvas, E.T. Steenkamp, A.O. Al Adawi, M.L. Deadman, A.A. Al Jahwari, W.F.O. Marasas, B.D. Wingfield, R.C. Ploetz and M.J. Wingfield.** *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa. Email:emma.steenkamp @fabi.up.ac.za*

Mango (*Mangifera indica*) malformation is an economically important disease in most mango-growing countries worldwide. Symptoms typically include abnormally branched and thickened panicles that are sterile and bear no fruit. Various *Fusarium* species have been associated with the disease but only *F. mangiferae*, a member of the *Gibberella fujikuroi* species complex, has been proven to cause the disease. In 2005, mango trees bearing abnormally enlarged inflorescences with branched and heavy panicles were observed in a Sohar orchard, 250 km northwest of the Omani capital, Muscat. To confirm the presence of mango malformation in Oman, isolations were made from symptomatic tissue, followed by morphological examination and phylogenetic analyses. Isolates produced microconidia in false heads from mono- and polyphialides, which is characteristic of, but not unique to *F. mangiferae*. Similarity searches and sequence comparisons revealed that the EF1α and β-tubulin sequences of the Oman isolates were identical to those of *F. mangiferae*. This result was confirmed using phylogenetic analyses. This is the first record of mango malformation and *F. mangiferae* in Oman. At present the disease is not serious and every effort must be made to ensure that its severity does not increase.

44.28 POWDERY MILDEW FUNGI (ERYSIPHALES) ON *CARICA PAPAYA*, WITH A CHECK-LIST OF THE SPECIMENS IN AUSTRALIAN ARCHIVES. **J.R. Liberato.** *Department of Primary Industry, Fisheries and Mines, Diagnostic Service Division, P.O. Box 3000, Darwin, NT 0801, Australia. Email: jose.liberato@ nt.gov.au*

Samples of Australian powdery mildew, collected on papaya (*Carica papaya)* and preserved in the Australian National Collection of Fungi (herbaria BRIP, VPRI and DAR) were examined. Three species were identified, namely *Oidiopsis haplophylli* (anamorph of *Leveillula taurica*), *Oidium caricae* and a new species of *Cystotheca*. Additional specimens were obtained from herbaria worldwide and re-examined. *O. caricae* also occurs in Brazil, New Zealand, India and South Africa. *O. haplophylli* also occurs in India and Portugal. *Streptopodium caricae* occurs in Brazil and *Ovulariopsis papayae* in South Africa. *Podosphaera fusca* s. lat. occurs in the Cook Islands and New Zealand, *Podosphaera xanthii* in India and *Podosphaera caricae-papayae* in Japan. Only *Phyllactinia caricaefolia*, *O. haplophylli*, *O. papayae*, *S. caricae* and *O. caricae* were associated with adult papaya plants. *Oidium indicum* is conspecific with *O. caricae*. The status of *P. caricaefolia* remains unclear.

44.29 OPHIOSTOMATOID FUNGI IN FINLAND AND RUSSIA. **R. Linnakoski, Z.W. de Beer, M. Rousi, P. Niemel, A. Pappinen and M.J. Wingfield.** *Faculty of Forest Sciences, University of Joensuu, P.O. Box 111, FIN-80101 Joensuu, Finland. Email: riikka.linnakoski@joensuu.fi*

Several ophiostomatoid fungi are economically important forest pathogens and many are agents of sap-stain. These fungi are well-adapted for dispersal by bark beetles (Coleoptera: Scolytinae), which include numerous primary pest species, especially on conifers in the Northern Hemisphere. There is global concern that growing international trade is increasing the risks posed by these pathogens and pests. In this regard, the volume of timber originating in the boreal forests of Finland and Russia and moved across international boundaries is also increasing, but little is known regarding the fungi potentially moved with these logs. The aim of this study was to isolate and identify ophiostomatoid fungi associated with different bark beetle species infesting economically and ecologically important boreal tree species such as *Picea abies*, *Pinus sylvestris* and *Betula pendula*. The survey was conducted in the Karelia region, on both the Finnish and Russian sides of the border. Resulting fungal cultures obtained from bark beetles as well as from their galleries were grouped according to morphology. Where these were required, ITS, β-tubulin, LSU and elongation factor 1-α sequences were obtained for isolates representing the groups identified based on morphology. Phylogenetic analysis revealed at least 26 different ophiostomatoid taxa. These included *Ophiostoma ainoae*, *O. bicolor*, *O. canum*, *O. floccosum*, *O. minus*, *O. quercus*, *Grosmannia cucullata*, *G. olivaceae*, several *Leptographium* spp., *Ceratocystis polonica*, and at least 13 undescribed species. The results of this survey greatly expand our current knowledge of the diversity of ophiostomatoid fungi in the boreal forests. The novel species will be characterized and described.

44.30 TAXONOMIC STUDIES ON TROPICAL ISOLATES CLASSIFIED AS *PHYTOPHTHORA CITROPHTHORA*. **E.D.M.N. Luz, M.C.A. Paim and J.T. de Souza.** *Ceplac/Cepec/Sefit, Cx. Postal 07,45600-970, Itabuna, BA, Brazil. Email: ednadora@cepec.gov.br*

Phytophthora citrophthora is pathogenic to *Citrus* and other economically important crops. In Brazil, a species putatively classified as *P. citrophthora* is the most aggressive among the ones causing black pod disease on *Theobroma cacao*. Taxonomic studies were conducted to compare Brazilian isolates from cacao and *Citrus*. Random amplified polymorphic DNA (RAPD) and sequence analyses were performed on 83 isolates (69 from cacao, 12 from *Citrus* spp., one from *Erythrina glauca* and one from *Anthurium andraeanum*). Fragments of three genes (ITS region, translation and elongation factor 1α and β-tubulin) were sequenced and used for phylogenetic analyses. RAPD and sequence analyses of each or the three gene fragments combined showed the existence of two distinct groups: one formed by isolates from cacao, *Erythrina glauca* and *Anthurium andraeanum* and the other composed of isolates from *Citrus* spp. Isolates from cacao were more closely related to *P. capsici* than to isolates obtained from *Citrus*. Sporangia were bigger (63.9 × 30.6 µm in average) in cacao isolates than in *Citrus* isolates (42.6 × 29.0 µm). The shape of the sporangia and the ability to form chlamydospores also differed between the two groups. Cacao isolates were unable to infect *Citrus* fruits while the latter group was capable of infecting cacao pods. The significance of these findings for the taxonomy of the studied isolates is discussed.

44.31 CHARACTERIZATION OF POPULATION STRUCTURE OF *RHIZOCTONIA* RICE SHEATH PATHOGENS BY REP-PCR GENOMIC FINGERPRINTING. **M. Matsumoto, S.S. Aye, T.H. Cuong and K. Ogata.** *Institute of Tropical Agriculture, Kyushu University, Fukuoka 812-8581, Japan. Email: mmatsu@agr.kyushu-u.ac.jp*

In previous research, we investigated the potential of repetitive-sequence-based polymerase chain reaction (rep-PCR) fingerprinting of fungal genomic DNA as a rapid and simple alternative to random amplified polymorphic DNA (RAPD) analysis in the study of population structure, with isolates of rice sheath pathogen *Rhizoctonia solani* AG 1-IA. We confirmed the existence of 4 clonal populations from Japan (Matsumoto, 2006). DNA primers (BOX and ERIC) corresponding to conserved repetitive element motifs were used to generate genomic fingerprints of *R. solani* AG 1 IA, *R. oryzae* and *R. oryzae-sativae*, where isolates were collected from Myanmar, Thailand and Vietnam, and compared with Japanese isolates. Computer-assisted analysis of the database of combined fingerprints clearly distinguished each taxon and indicated phylogenetic relationships. Clonal populations of *R. solani* AG 1 IA isolates were distinguished into 6 types from Myanmar, 3 types from Thailand, 4 types from Vietnam and 4 types from Japan, respectively. The population structure consisted of three main clusters with isolates showing 40 to 60% similarity based on the combination of fingerprints. Clonal populations of *R. oryzae-sativae* isolates were also grouped into 3 types from Myanmar, 2 types from Thailand, 3 types from Vietnam and 2 types from Japan, respectively, and population structure consisted of two main clusters with isolates showing 75 to 90% similarity. Clonal population structures of *R. oryzae* isolates, however, presented similar fingerprints from Myanmar, Thai, Vietnam and Japan, and showed homologous clonality.

44.32 CHARACTERIZATION OF NEW MEMBERS OF THE GENUS *ENDORNAVIRUS*, LARGE DSRNA VIRUSES WITH PLASMID-LIKE PROPERTIES. **H. Moriyama, C. K. Yong, R. Okada, N. Aoki and T. Fukuhara.** *Department of Applied Biological Sciences, Tokyo University of Agriculture and Technology, 3-5-8 Saiwaicho, Fuchu, Tokyo 183-8509, Japan. Email: fuku@cc.tuat.ac.jp*

Endornaviruses are unique double-stranded (ds) RNAs, which the International Committee on Taxonomy of Viruses has recently accepted as forming a distinct virus taxon, the unassigned genus *Endornavirus*. Endornaviruses have a linear dsRNA genome approximately 14-17 kbp in length. They have important properties in common that differ from those of conventional viruses: they have no obvious effect on the phenotype of their host plants, and they are efficiently transmitted to the next generation via seeds. The five endornaviruses sequenced completely share a common structure, though they infect disparate hosts from the plant, protist and fungal kingdoms. Their genomes possess a single unusually long open reading frame encoding a protein of about 4,500 to 5,500 amino acid residues with RNA-dependent RNA polymerase and RNA helicase domains. All five endornavirus genomes contain a site-specific nick in the 5' region of the coding strand. Here we report partial nucleotide sequences from large dsRNAs in kidney bean, bell pepper, barley, melon, bottle gourd, Malabar spinach and seagrass. Phylogenetic comparison of these seven dsRNAs with those of known endornaviruses and single-stranded RNA viruses indicate that these dsRNAs constitute new taxa in the *Endornavirus* genus that are widely distributed among monocotyledonous and dicotyledonous plants.

44.33 ASSOCIATION OF *PHELLINUS* SPECIES WITH JAPANESE PEAR DWARF DISEASE. **H. Nakamura, K. Yoshida, A. Sasaki and T. Shimane.** *National Institute of Fruit Tree Science, 2-1 Fujimoto, Tsukuba 305-8605, Japan. Email: nakamh@affrc.go.jp*

Japanese pear dwarf disease occurs on mature, especially more than 20 year-old trees in Japan, and diseased trees show delay of sprouting and leaf production in spring, and have rugose leaves with necrosis. Wood decay is also observed in trunks or branches of diseased trees. The pathogen has not been determined, but *Phellinus igniarius* or other *Phellinus* species were presumed to cause the disease (Sakuma *et al*., 1993). In this study, to clarify the association between the disease and *Phellinus* species, the frequency of *Phellinus* infections in decayed wood of the diseased trees was investigated. Fungal isolates were obtained from decayed wood in the trunks or branches of the diseased trees, and *Phellinus* isolates were identified on the basis of cultural characteristics. The appearance frequency of *Phellinus* isolates on 35 diseased trees in six Japanese prefectures was ca. 75%. When the frequency of *Phellinus* isolations on 15 diseased trees was compared with that on 47 trees without dwarf symptoms in a prefecture, a significant correlation was observed between the occurrence of Japanese pear dwarf and the isolation of *Phellinus*. A few *Phellinus* isolates obtained in this study were considered to belong to the *P. robustus* group, referred to as the genus *Fomitiporia*, by phylogenetic analyses using ribosomal DNA sequences (Yasuda *et al.* 2005). These results demonstrated the association between Japanese pear dwarf disease and *Phellinus* species in decayed wood of the diseased trees.

44.34 NEW SPECIES OF THE BOTRYOSPHAERIACEAE DISCOVERED ON BAOBABS AND OTHER NATIVE TREES IN

WESTERN AUSTRALIA. D. Pavlic, M.J. Wingfield, B. Slippers, P. Barber, G.E.StJ. Hard and T.I. Burgess. *Forestry and Agricultural Biotechnology Institute, Department of Microbiology and Plant Pathology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, 0002, South Africa. Email: draginja.pavlic@fabi.up.ac.za*

New species of the Botryosphaeriaceae were identified and described in this study. Isolates were collected from baobab (*Adansonia gibbosa*) and surrounding endemic tree species growing in north-western Australia. Cultures resembling those of the Botryosphaeriaceae were predominantly endophytes isolated from apparently healthy wood and bark, but some also came from dying branches. Based on phylogenetic analyses of ITS and EF1-α sequence data, isolates were placed in clades representing the genera *Pseudofusicoccum*, *Botryosphaeria*, *Lasiodiplodia*, *Dothiorella* and *Neoscytalidium*. Sequence comparison with known species of the Botryosphaeriaceae revealed that several isolates obtained in this study represent undescribed species. The new taxa were characterised based on ITS and EF1-α sequence data combined with anamorph morphology. Six new species are recognised including three *Pseudofusicoccum* spp., a *Botryosphaeria* sp., a *Lasiodiplodia* sp. and a *Dothiorella* sp. *Neoscytalidium* isolates from this study formed a separate sub-clade in this genus, but a lack of sequences for previously described *Neoscytalidium* spp. precludes their identification at this stage. Very few studies have been conducted to identify Botryosphaeriaceae on trees in natural ecosystems, and almost nothing is known regarding the fungi on baobabs. Thus, the relatively large number of new species emerging from this study is not surprising. The role of these fungi in the ecology of the trees from which they were collected will be considered in future studies.

44.35 CRYPTIC SPECIES IN THE *NEOFUSICOCCUM PARVUM / N. RIBIS* COMPLEX REVEALED BY MULTIPLE GENE GENEALOGIES. **D. Pavlic, B. Slippers, T.A. Coutinho and M.J. Wingfield.** *Forestry and Agricultural Biotechnology Institute, Department of Microbiology and Plant Pathology, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, 0002, South Africa. Email: draginja.pavlic@fabi.up.ac.za*

Neofusicoccum parvum and *Neofusicoccum ribis* are closely related species within the Botryosphaeriaceae (Ascomycetes). These fungi are widely distributed endophytes and opportunistic pathogens on many woody hosts, world-wide. In a previous study, *N. parvum* and *N. ribis* were identified as the most abundant Botryosphaeriaceae on native *Syzygium cordatum* trees in South Africa. Species boundaries for the *N. parvum/N. ribis* complex are not clearly defined, but a previously developed PCR- restriction fragment length polymorphism (RFLP) fingerprinting technique can distinguish between isolates representing *N. parvum* and *N. ribis* sensu lato. The aim of this study was to consider the identity of isolates in the *N. parvum/N. ribis* complex obtained from native *S. cordatum* in South Africa. This was achieved by combining the RFLP marker data with multiple gene DNA sequence data for five nuclear loci, including the internal transcribed spacer rDNA (ITS1, 5.8S, and ITS2), partial translation elongation factor 1-α (EF-1α), β-tubulin (βt-2a/b), a portion of the RNA polymerase II subunit (RPB2) and locus BotF15 (an unknown locus containing a simple sequence repeat). All analyses used supported the demarcation of three phylogenetic species in the *N. parvum-ribis* species complex of isolates from native *S. cordatum* in South Africa. Our results reflect the critical importance of using multiple gene genealogies to identify cryptic species and to characterise the true diversity within the Botryosphaeriaceae.

44.36 HOST SPECIFICITY AND POPULATION BIOLOGY OF *COLLETOTRICHUM ACUTATUM* FROM FRUIT AND ORNAMENTAL CROPS. **N.A. Peres, S.J. Mackenzie and L.W. Timmer.** *University of Florida, Gulf Coast Research and Education Center, 14625 CR 672, Wimauma, FL, 33598, USA. Email: nperes@ufl.edu*

Colletotrichum acutatum causes foliage blights and fruit rots of numerous fruits and ornamental plants. It caused serious epidemics on citrus and leatherleaf fern recently in the Caribbean area and is endemic on strawberry, blueberry, and tropical fruits in many areas of the world. Isolates of *C. acutatum* were collected from Key lime and leatherleaf fern foliage, strawberry and blueberry fruit, and citrus flowers affected by postbloom fruit drop (PFD). Cross-pathogenicity tests showed that all isolates were highly pathogenic to their host of origin. Isolates were not pathogenic on foliage of heterologous hosts, but some nonhomologous isolates were mildly or moderately pathogenic to detached fruit of strawberry and blueberry or citrus flowers. Sequencing of the ITS1-5.8S rRNA-ITS2 region of the rDNA, the glutaraldehyde-3-phosphate dehydrogenase (GPDH) intron 2, and the glutamine synthase (GS) intron 2, showed that isolates from the same host were identical or very similar to each other and distinct from those isolated from heterologous hosts with the exception of isolates from fern. Among fern isolates, there were two distinct GPDH and GS sequences that occurred in 3 of 4 possible combinations. The results indicate that isolates of *C. acutatum* from these hosts have a high level of specificity and that they are genetically distinct from each other.

44.37 CHARACTERIZATION OF A UNIQUE *MONILINIA* ISOLATE FROM HUNGARY. **M. Petroczy and L. Palkovics.** *Department of Plant Pathology, H-1118 Menesi Road 44, Corvinus University of Budapest, Budapest, Hungary. Email: marietta.petroczy@unicorvinus.hu*

Monilinia species are the most important fungal pathogens of pome and stone fruits. Unusual symptoms were observed on apples at the 10 mm fruit stage in an orchard in north-eastern Hungary. Yellowish exogenous stromata appeared on the shoots and small fruits with shoot blight symptoms. The pathogen was characterised by classical and molecular methods. Iwas isolated (isolate: UFT) on Leonian malt agar. By culture characteristics the pathogen was identified as *Monilinia fructigena* but the colony showed some strange features. Colony growth rate was higher than for *M. fructigena* isolates, and produced zones of black stromatal plates in culture. Conidial chains formed on the exogenous stromata. The conidia were slightly smaller than the *M. fructigena* average. For molecular comparison an unknown genomic sequence of *Monilia* sp. found in the databank was compared with of our *M. fructigena* and UFT isolates. UFT showed higher nucleotide sequence similarity with *M. polystroma* (99.2%) than with *M. fructigena* (97.7%), while the similarity between *M. fructigena* and *M. polystroma* was only 96.1%. The phylogenetic tree indicated that UFT is evolutionarily closer to *M. polystroma* than to *M. fructigena*. A repetitive sequence motif was also identified, five times in *M. fructigena*, seven times in *M. polystroma* and six times in UFT, as an insertion in the genome. *M. polystroma* has been reported only from Japan. The origin and the identity of the UFT isolate will be further characterised.

44.38 *TURNIP RINGSPOT VIRUS* AND *RADISH MOSAIC VIRUS* – CLOSELY RELATED COMOVIRUSES INFECTING *BRASSICACEAE*. **K. Petrzik, I. Koloniuk and J. Špak.** *Department*

of Plant Virology, Institute of Plant Molecular Biology, Biology Centre, Academy of Sciences of the Czech Republic, Čáeské Budějovice, Czech Republic. Email: petrzik@umbr.cas.cz

Two comoviruses can infect cruciferous plants – *Radish mosaic virus* (RaMV) and the recently described *Turnip ringspot virus* (TuRSV). The taxonomic status of TuRSV was not yet resolved, as only fragments of its genome were known. We showed that RaMV and TuRSV differ serologically. In double diffusion test neither the TuRSV isolate from Toledo (OH, USA) nor the isolates from Moscow (Russia) reacted with antisera against the Californian and Czech RaMV isolates, respectively. We sequenced the large (L-) and the small (S-) capsid protein genes of several TuRSV isolates and compared them with those of RaMV isolates from Japan and Europe. The intra-species variability was lower than 3% in both viruses, but was about 25% between the species in the CP-L and CP-S genes. Similarly, the differences in the RNA polymerase gene of RaMV and TuRSV were also 25%. We conclude that TuRSV and RaMV have the closest relationship among comoviruses. They are different in serology, and probably represent distinct species in the genus Comovirus. This research was funded by GACR No. 522/07/0053.

44.39 CHARACTERIZATION OF *DIAPORTHE/PHOMOPSIS* SPECIES ON HERBACEOUS AND WOODY PLANTS IN ITALY. L. Riccioni and A. Haegi. *CRA-PAV, Centro di Ricerca per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Rome, Italy. Email: luca.riccioni@entecra.it*

Recent studies have shown that host specificity, cultural characteristics and morphology can no longer be used to distinguish among species of the fungal pathogen *Diaporthe/Phomopsis* (D/P), due to the wide host ranges of some species and the morphological variability of others. For this reason, beside morphology, D/P isolates obtained from soybean, sunflower, kiwi and some ornamental plant showing symptoms of canker, stem decay and/or wood discoloration have been studied by sequencing genomic regions for ITS rDNA, translation elongation factor (EF) 1α and large subunit (28S) LSU rDNA, followed by comparison with sequences in GenBank and phylogenetic analysis. We identified more then 10 taxonomic groups of D/P distributed among the hosts studied. Few of these groups are associated to well-defined species, while most of them have no established name. Most of them are polyphagous (e.g. *Phomopsis theicola*), while others are host-specific (e.g. *Diaporthe phaseolorum* var. *caulivora* and *P. longicolla*). These studies aim to help clarifying the complex taxonomy of D/P species and to offer new tools for diagnosis, especially when pathogens, like D/P on soybean seed, are under regulation.

44.40 *HENBANE MOSAIC VIRUS* IN HUNGARY: NATURAL HOSTS, PATHOLOGICAL VARIABILITY AND THE FIRST SEQUENCE DATA. P. Salamon, R. Michaletzky and L. Palkovics. *Corvinus University of Budapest, Department of Plant Pathology, H-1118 Budapest Menesi road 44, Hungary. Email: laszlo.palkovics@uni-corvinus.hu*

Henbane mosaic virus (HMV, Genus *Potyvirus,* Family *Potyviridae*) has been found naturally occurring in jimsonweed (*Datura stramonium* L.), belladonna (*Atropa belladonna* L.), chinese lantern (*Physalis alkekengi* L.) and woody nightshade (*Solanum dulcamara* L.) in Hungary. Isolates of HMV were identified on the bases of reactions of diagnostic test plants, virion morphology and serological tests in comparison with the Rothamsted type isolate (HMV-R). Host range studies and serological tests revealed that the potyvirus tentatively named Hungarian Datura innoxia virus (HDMV) should also be considered a strain of HMV. Isolates of HMV varied in virulence, and different strains were distinguished by the severity of symptoms caused in *N. tabacum* cv. Xanthi-nc. *A. belladonna*, *Ph. alkekengi* and *S. dulcamara* act as reservoirs of HMV. *S. dulcamara* is a new perennial host of this virus. For molecular characterization an isolate from *Ph. alkekengi* marked HMV-Phys/H was amplified by PCR using a modified unipoty and polyT primers. The PCR product was cloned and the viral sequence determined. The sequence contained parts of the NIb, CP and the 3' UTR region. Data on Phys/H isolate of HMV (Acc.No.: AM184113) was compared with 47 other potyviruses. The coat protein amino acid sequence identity between HMV and the other potyviruses ranged from 61.4 to 80.5%. Sequence alignments and phylogenetic analyses of the potyviral coat protein sequences reveal that HMV is the closest to *Chili veinal mottle virus* and *Pepper veinal mottle virus.* This appears to be the first sequence data on *Henbane mosaic virus*.

44.41 *ACIDOVORAX VALERIANELLAE* AS A PATHOGEN OF MELON, HYDRANGEA AND *MACLEAYA CORDATA*, AND ITS DIFFERENTIATION INTO PATHOVARS OF DIFFERING HOST SPECIFICITY. Y. Takikawa, H. Tsujimoto, K. Yamamoto, H. Kawada, S. Kusumoto and T. Makino. *Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizoka 422-8529, Japan. Email: abytaki@agr.shizuoka.ac.jp*

Several new bacterial diseases of plants have emerged in Japan during recent decades. They are leaf blight of melon, leaf spot and blight of hydrangea, and leaf spot of *Macleaya cordata* (plume poppy; Fam. Papaveraceae). Pathogenic bacteria were isolated from the diseases and all of them were presumed to belong to the genus *Acidovorax*. Sequence analysis of 16SrDNA showed that they closely resemble *A. valerianellae*. The characteristics of the bacteria were almost identical with each other and with those of *A. valerianellae*. *A. valerianellae* was described as a pathogen of corn salad and has recently been reported as a pathogen of tea plants in Japan. Our cross-inoculation tests including the type strain and tea isolates of *A. valerianellae* revealed that the bacteria isolated from each plant species have host specificity and could be distinguished from each other. These results suggest that *A. valerianellae* could affect a wider range of plants than previously anticipated, and is differentiated into several pathogenic groups that may deserve consideration as pathovars or subspecies.

44.42 MOLECULAR AND BIOLOGICAL STUDIES OF *ASPARAGUS VIRUS 1* (GENUS *POTYVIRUS*). L. Tomassoli, A. Tiberini, A. Zaccaria and H.J. Vetten. *CRA-Plant Pathology Research Centre, Via C.G. Bertero 22, 00156 Rome, Italy. Email: l.tomassoli@entecra.it*

Asparagus virus 1 (AV1) (genus *Potyvirus*, family *Potyviridae*) has been reported only from *Asparagus officinalis* L. (family Liliaceae) and occurs worldwide in all asparagus production areas. AV1 has no effect on spear and fern growth but, when present in mixed infection with *Asparagus virus 2* (AV2), is thought to seriously contribute to asparagus decline and to increase the susceptibility of roots and rhizomes to fungal attack. Nevertheless, AV1 has not been studied in detail, and its biological, serological and molecular properties are poorly understood. Using specific asparagus potyvirus primers in RT-PCR, the 3' portion of the genome of isolate AV1-1770 from northern Italy has been previ-

ously amplified. The sequence (Accession N. EF576991) of the resulting PCR product (500 bp) encompassing the coat protein (CP) shared the highest nucleotide (nt) sequence similarity (75%) with *Turnip mosaic virus* (TuMV). On the basis of CP amino-acid (aa) sequence analysis (Neighbor-Joining analysis) AV1 was shown to form a clade distinct from TuMV within the genus *Potyvirus*. Sequence identities between AV1-1770 and TuMV did not exceed 40.7% at aa level. We also determined the CP gene sequences of several asparagus isolates from various regions in southern Italy and compared them with AV1-1770. Two main AV1 subgroups were found, with identities of 96.8% (nt) and 85.3% (aa). This sharing of AV1 isolates was mainly associated with geographical area and asparagus varietal origin.

44.43 A PREVIOUSLY UNREPORTED *TOSPOVIRUS* SPECIES ISOLATED FROM MELON CROPS IN MEXICO. M. Turina, M. Ciuffo, V. Masenga, E. Vivoda, B.W. Falk and C. Kurowski. *CNR–Istituto di Virologia Vegetale, Strada delle Cacce 73, 10135 Torino, Italy. Email: m.turina@ivv.cnr.it*

During the 2007 melon growing season, samples from the state of Guerrero in Mexico showing mosaic and growth deficiency were collected. Electron microscopic examination of negatively stained leaf dip extracts revealed the abundant presence of virus-like particles with features characteristic of the family *Bunyaviridae*, but ELISAs specific for the most common tospoviruses gave negative results. No other viral particles were observed in these preparations. The virus was mechanically transmitted to a number of test plant species, including *Nicotiana benthamiana*. Purified nucleocapsids analyzed by SDS-PAGE and Coomassie staining showed a protein with different electrophoretic mobility from proteins of *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* purified nucleocapsids. Antibodies raised against purified nucleocapsids reacted specifically with leaf extracts in Western blots and DAS-ELISA. The viral RNA was used as a template for a cDNA library, and several clones were isolated and analyzed. Nucleotide sequence analysis identified cloned cDNAs representing regions of each of the three tospovirus genome segments. BLAST analysis of the nucleotide sequence of a number of cloned cDNAs showed that the new virus had highest similarity to TSWV and *Chrysanthemum stem necrosis virus*. Phylogenetic analysis of various genome regions confirmed that this virus, provisionally named Melon severe mosaic virus, is an undescribed tospovirus species.

44.44 RE-IDENTIFICATION OF *COLLETOTRICHUM* (*GLOEOSPORIUM*) *CARTHAMI* (FUKUI) HORI ET HEMMI AND *C. CHRYSANTHEMI* HORI BASED ON MORPHOLOGICAL AND MOLECULAR CHARACTERISTICS OF HERBARIUM SPECIMENS. S. Uematsu, K. Kageyama, J. Moriwaki and T. Sato. *1762 Yamamoto, Tateyama, Chiba Prefecture, 294-0014, Japan. Email: s.uemts@mb.pref.chiba.lg.jp*

Colletotrichum (*Gloeosporium*) *carthami* (Fukui) Hori et Hemmi was first described as a leaf blight pathogen of *Carthamus tinctorius* in Japan (Fukui 1916; Hemmi 1919). Arx (1957) transferred *Colletotrichum carthami* to *C. gloeosporioides* based on the original description. *C.* (*G.*) *chrysanthemi* Hori was also described as a pathogen of *Chrysanthemum coronarium* var. *spatiosum* in Japan (Takimoto, 1924). *C. chrysanthemi* has not been fully re-examined until now. We therefore re-examined herbarium specimens of *C. carthami* collected by Hemmi in 1915 and of *C. chrysanthemi* collected by Takimoto, in 1919, deposited in the Hokkaido University Museum, based on morphological and molecular characteristics. Appressoria were not observed, conidia on the acervulus were hyaline, fusiform to cylindrical, 7.0-17.8 × 3.1-6.8 µm. These characteristics almost almost agreed with those of the isolates from three species of Compositae, *Calendula officinalis*, *Ch. coronarium* var. *spatiosum* and *Car. tinctorius* (Uematsu *et al.*, 2003) as well as the descriptions of Hemmi (1919) and Takimoto (1924). When the genus-specific primer set for *Colletotrichum*, Cc1F1/Cc2R1 (Cullen et al., 2002), was used for amplification of the internal transcribed spacer (ITS) region of rDNA, amplicons were obtained from these specimens and the isolates from three composite species. The sequences of two herbarium specimens showed not only 100% homology with each other but also 99.8-100% with those of the composite isolates and *C. acutatum*. The results suggest that the two herbarium specimens of *Colletotrichum* would be *C. acutatum* described by Simmonds (1965, 1968), later than *C. carthami* and *C. chrysanthemi*.

44.45 MOLECULAR CHARACTERIZATION AND TAXONOMY OF LOLIUM LATENT VIRUS, A NOVEL MEMBER OF THE *FLEXIVIRIDAE*. A.M. Vaira, C.J. Maroon-Lango, H.S. Lim and J. Hammond. *USDA-ARS, USNA, FNPRU, 10300 Baltimore Avenue B-010A, Beltsville, MD 20705, USA. Email: John.Hammond@ars.usda.gov*

Lolium latent virus (LoLV), originally discovered in Europe, was recently detected in the United States for the first time in ryegrass hybrids (*Lolium perenne* × *multiflorum*). The genome of one U.S. isolate, LoLV-US1, has now been fully sequenced. The positive-strand genomic RNA is 7674 nt long and is organized in five open reading frames (ORFs) encoding the replication-associated protein, the movement-associated triple gene block proteins and the capsid protein. A sixth small ORF partially overlaps the capsid protein ORF; its predicted 45 amino acid product is highly basic (pI 9.56) and has no significant homology to characterized proteins, but may act as a nucleic acid binding protein. With the exception of the putative sixth ORF, this genome organization is similar to that of viruses in the genera *Potexvirus* and *Foveavirus*; however, phylogenetic analysis of the deduced amino acid sequences of the polymerase and the capsid proteins, together with the analysis of the complete LoLV genomic sequence, highlight features peculiar to LoLV. These characteristics indicate that LoLV forms a monotypic group for which we propose the genus name *Lolavirus*, as it is separate from existing genera-assigned and other unassigned species within the family *Flexiviridae*. We have generated an infectious clone of LoLV, and have also expressed individual genes and combinations of genes via Agro-infiltration in order to examine interactions between gene products and infected cells.

44.46* COMPLETE SEQUENCES OF TWO DISTINCT POLEROVIRUSES INFECTING CUCURBITS IN CHINA. H. Xiang, Q. Shang, C. Han, D. Li and J. Yu. *Department of Plant Pathology and State Key Laboratory for Agrobiotechnology, China Agricultural University, No.2 Western Road, Haidian District, Beijing, P.R. China. Email: hanchenggui@cau.edu.cn*

Two poleroviruses co-infecting cucurbits were identified by RT-PCR detection with a pair of universal primers for poleroviruses and partial sequence analysis. Subsequently, the complete genomic sequences of a Chinese *Cucurbit aphid-borne yellows virus* (CABYV) isolate (CABYV-CHN) and the new proposed polerovirus (CABYV-NEW) were determined respectively. Sequence analysis showed that both CABYV-CHN and CABYV-

NEW had genomic organization and characters very similar to the reported French isolate (CABYV-FRA) and other poleroviruses. The entire genome of CABYV-CHN was 5682 nucleotides in length and shared 89.0% sequence identity with CABYV-FRA and 50.8%-68.5% with other reported poleroviruses. The whole genome of CABYV-NEW was 5674 nt long and is predicted to contain six large open reading frames (ORFs), and non-coding sequences of 21 nucleotides at the 5' terminus and 167 nucleotides at the 3' terminus; and an intergenic non-coding region (NCR) of 195 nucleotides between ORF2 and ORF3. Sequence analysis revealed that it shared 74.2% sequence identity with CABYV-CHN, 73.7% with CABYV-FRA, and 50.7%-68.6% with the other poleroviruses, respectively. Further sequence comparison showed that divergence of all the gene products was greater than 10%. Therefore, we propose that CABYV-NEW be considered as a new distinct *Polerovirus* species according to the demarcation criteria in the family Luteoviridae and should be referred to as CABYV II, accordingly renaming the former CABYV as CABYV I. This is the first report on two poleroviruses involved in yellowing disease of cucurbitaceous crops.

44.47 IDENTIFICATION OF THE *ALTERNARIA* BLIGHT PATHOGEN ON AMERICAN GINSENG IN CHINA. X.F. Yuan, J. Chen, Y.Liang and G.Z. Zhang. *Department of Plant Pathology, China Agricultural University, P.R. China. Email: zhanggzh@cau.edu.cn*

Alternaria leaf and stem blight is one of major diseases of American ginseng (*Panax quinquefolius* L.) and causes root yield losses. But so far the only recorded pathogen is *Alternaria panax* Whetzel. Ten isolates were obtained from leaves of American ginseng with *Alternaria* blight symptoms by standard isolation. The morphological characteristics of the colony and conidia indicated that there were other species of *Alternaria* apart from *A. panax*. To test the pathogenicity of the isolates, we inoculated intact three-year-old leaves using 4-mm-diameter plugs from PDA colonies. The inoculated leaves were put on moist paper in 20-cm Petri dishes and incubated at 28°C in the dark for 24 h. Then the leaves were incubated under the light at 28°C. The incidence and severity of disease were recorded after 6 days. ANOVA results showed that all isolates were pathogenic to the ginseng and there were significant differences in spot area among them (p=0.016). Isolates BJ-9-18, YQ-2-2 and BF2-1-1 caused larger spots than others but there was no difference between these three isolates. rDNA ITS regions were amplified using ITS5 and ITS4 and sequenced. The morphological and molecular characteristics indicated that the four isolates belonged to *A. longipes* Mason (YQ-2-2) and *A. tenuissima* Wiltshire (BF2-1-1, XWZ-1-2 and BJ-7-26), respectively, which could cause leaf blight of American ginseng in China, in addition to *A. panax*.

Teaching Plant Pathology

42.1 NORPATH: A NEW INTERNATIONAL MASTER OF SCIENCE PROGRAMME IN PLANT PATHOLOGY. D.B. Collinge, B.D. Jensen, L. Munk, A.M. Tronsmo, L. Sundheim, M. Pirhonen, A.-L. Laine, H. Sverrisson, A. Djurle, H. Friberg and J. Yuen. *University of Copenhagen, Faculty of Life Sciences, Department of Plant Biology, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark. Email: dbc@life.ku.dk*

A consortium of Nordic universities is offering a new international two year MSc Programme in Plant Pathology from 2008 to students with a relevant BSc degree. The study programme has developed from a long-term collaboration between these institutions, and provides a unique platform for students to learn about the fascinating world of plant pathology. All study activities are offered in English. Students will enrol at one of the participating universities and will take a compulsory introductory summer school course in Plant Pathology taught jointly. Here, students will meet teachers and will be able to discuss and identify an MSc thesis subject. Though the student will be enrolled on existing MSc programmes at a single university, the programme entails a compulsory study visit to at least one of the other participating universities, and joint courses, for instance in molecular plant pathology, will be part of the programme. MSc thesis work will be based on research topics ranging from molecular and post-genomic studies of plant-microbe interactions to epidemiology and disease management through biological control and disease resistance. The thesis work will provide an opportunity to obtain laboratory or field-based experience with research groups at the participating universities. In addition, the programme will give students a unique opportunity to meet others who share the same interests and thereby build a plant pathology network to solve problems for the future. URL see http://www.nova-university.org/index.htm This project is supported by the Nordic Council of Ministers and the NOVA Universities network.

42.2 EDUCATION AND SKILLS POSSESSED BY BRITISH PLANT PATHOLOGISTS: A PROFESSIONAL AUDIT. G.R. Dixon and P.R. Mills. *GreenGene International, Hill Rising, Horsecastles Lane, Sherborne, Dorset, DT9 6BH, UK. Email: 113541.1364@compuserve.com*

Analysing quantitatively the education, training and careers of current cohorts of practitioners gives professions like Plant Pathology an understanding of why and how they are evolving. The effects of changing educational and training systems and the impact of new demands from the labour market can be assessed. Professional bodies may then align their activities and services more closely with the needs of new recruits. This knowledge also raises the effectiveness of interactions with those formulating more general scientific and educational policies. In 2006, the British Society for Plant Pathology (BSPP) commissioned GreenGene International to undertake an audit of: the education, training and careers of members, changing demands of prominent employers and a short comparative survey of recruitment advertisements. Each member received a questionnaire, predominantly sent electronically. Employers responded by telephone interviews based on previously circulated electronic questionnaires and recruitment advertisements in 'New Scientist' for 2005 and 1985 were compared. Respondents ranged from 1945 to 2006 cohorts and employers were drawn from a cross section of organisations. The data gives a uniquely comprehensive view of BSPP's membership. Practitioners are highly qualified, first degrees contained options related to plant pathology followed by a doctoral degree. Most respondents completed one cycle of post-doctoral work. Employers now demand more extensive knowledge and skills compared with previously. Shortages of practical skills and abilities to analyse multi-faceted problems are significant deficiencies. A wealth of further information will be discussed.

42.3 PAKISTAN: STATUS AND CONSTRAINTS IN PLANT BIOTECHNOLOGY. T. Ismail and A. Hussain. *Balochistan University of Information Technology and Management Sciences, H.no 7-5/52-392 Toghi Road Near bari Eid Gah, Quetta, Balochistan-87300, Pakistan. Email: tariqali84@hotmail.com*

Pakistan is a developing country in Asia. About 70% of its population is rural and depends on agriculture for its livelihood. The Pakistan Agricultural Research Institute prioritises exploration of modern biotechnology options to improve agriculture. Universities, research foundations, and private laboratories are involved in tissue culture research and commercial production. To date there are no commercialised molecular marker assisted selection (MMAS) products in Pakistan. However research is proceeding by collaborators using MMAS to develop maize lines resistant to *Maize streak virus*, and drought-tolerant maize. The National Biosafety Committee has approved research trials on genetically engineered sweet potato, maize and cotton, and a seedless citrus has been produce by NIAB (National institute of agriculture Biology). Funding for research and development is mostly from donor agencies while state funding caters for recurrent expenditure of institutes. Public awareness on biotechnology issues has increased due to the efforts of NCB – National Commission, on Biotechnology and others. Major constraints include the fact that research is donor-funded, hence Pakistan cannot drive her own agenda. The rate of brain drain is very high. Institutional linkages are weak and there is an absence of explicit policies to govern biosafety. The way forward includes intensified efforts to retain capacity and create linkages. Curriculum development should incorporate biotechnology and allow for early exposure. The enactment of laws should be expedited to spell out national priorities and investment strategies for biotechnology research and development.

42.4 IMPROVED DETECTION OF SEED-BORNE PATHOGENS USING A SINGLE-SPOT ISOLATION TECHNIQUE. **G.N. Ngala and M.O. Adeniji.** *Department of Crop Protection and Environmental Biology, Faculty of Agriculture & Forestry, University of Ibadan, Ibadan, Nigeria. Email: gnngala@yahoo.com*

Rice dirty panicle is a serious disease in tropical Africa and other parts of the world. Different fungi and other factors have been implicated as causes. Detection of these causes as reported from various sources has always been difficult probably because of variation in their prevalence with region, season, and importantly, methodology. Detection methods have only been based on plating the whole discoloured grains either by blotting or directly on agar, which gave inconsistent and conflicting results. Here we report a new significantly improved method, the single spot isolation technique. It favoured the relative importance of the candidate fungi, eliminating most saprophytes, which out-grow the important, slow-growing pathogens, and proved superior to plating whole grains. Using this technique, *Sarocladium attenuatum* was the most important fungus in 21 survey isolations from wet and dry season crops, with the highest frequency from different locations, cultivars and breeding lines. The other fungi were *Fusarium (moniliforme) verticilloides* (17.25%), *Curvularia lunata* (14.64%), *Nigrospora oryzae* (15.33%), *F. semitectum* (14.25%), *Pyricularia oryzae* (4.75%), *Helminthosporium (Drechslera) oryzae* (3.0%), *Alternaria* species (1.67%), and a species of *Helminthosporium*. Field and laboratory pathogenicity tests with 6 most frequently isolated fungi confirmed, except *N. oryzae*, that they were pathogenic, *S. attenuatum* being the most virulent. Also that many fungi are associated with this disease, their prevalence varying with location and season, and that the different causal fungi reproduce similar symptoms on the same rice hosts.

42.5 PHYTOPATHOLOGY TEACHING IN AGRICULTURAL UNIVERSITY SCHOOLS IN ARGENTINA. **M.C. Plencovich and M.V. López.** *Facultad de Agronomía, Universidad de Buenos Aires, Avenida San Martin 4453, CP 1416 Buenos Aires, Argentina. Email: palmucci@agro.uba.ar*

In recent years Argentina has increased its activities as an agricultural exporting country. Many universities grant degrees in Agricultural Studies, and the graduates are mainly Agricultural Engineers. In the curriculums, Phytopathology is considered as fundamental, and a gateway to healthy food. We collected information on the status of Phytopathology teaching in Argentina in 23 National Universities. The aim was a) to gather information about the differences and similarities in the academic curriculum organization and methods. b) To determine how program contents addressed teaching goals. c) To know the teaching methods used. Twenty-three teaching teams from different universities answered a questionnaire by email. The questionnaire was addressed to the Phytopathology Chairs, who are responsible for epistemological and teaching aspects and for human resource development. Responses were analysed and information obtained about the status of Phytopathology in university programs, curriculum characteristics (denomination and character of the discipline, epistemological approaches, objectives and contents, course organization, and teachers' operative definitions of Phytopathology, teaching team characteristics (professor categories, undergraduate and postgraduate qualifications) and teaching methods. The information was analyzed in order to draw some conclusions.

TRANSGENIC PLANTS

47.1 POTENTIAL THREAT OF A NEW PATHOTYPE OF *PAPAYA LEAF-DISTORTION MOSAIC VIRUS* INFECTING TRANSGENIC PAPAYA RESISTANT TO *PAPAYA RINGSPOT VIRUS*. **H.J. Bau, Y.J. Kung, J.A.J. Raja, S.J. Chan, Y.K. Chen, H.W. Wu and S.D. Yeh.** *Department of Plant Pathology, National Chung Hsing University, Taichung, ROC. Email:sdyeh@nchu.edu.tw*

A virus designated TW-WF was isolated from diseased papaya in an isolated test field in central Taiwan where transgenic papaya lines resistant to *Payaya ringspot virus* (PRSV) were being evaluated. Papaya plants infected with this virus displayed severe mosaic, distortion and shoe-stringing on leaves, stunted apex, and water-soaking on petioles and stems. The virus did not react in ELISA with antiserum to the coat protein (CP) of PRSV and infected only papaya, but not 18 other species tested. Electron microscopy revealed the presence of filamentous particles of about 800 nm in length and cytoplasmic inclusions including pinwheels, scrolls, and laminated aggregates in infected cells. RT-PCR with primers specific to potyviruses generated a 1927 nucleotide product representing the 3' non-coding region of a potyvirus. Sequence analyses revealed that TW-WF shares 94.9% amino acid identity in the CP gene and 96.2% nucleotide identity in the 3' non-coding region with those of *Papaya leaf distortion mosaic potyvirus* (PLDMV). Search for similar isolates using an antiserum against bacteria-expressed CP of TW-WF revealed wide occurrence of PLDMV in Taiwan. Phylogenetic analysis of four PLDMV Taiwan isolates and four Japanese isolates indicated that the Taiwan isolates belong to a separate genetic cluster. Since all Taiwan isolates infected only papaya, unlike cucurbit-infecting Japanese isolates, they are considered as a new pathotype of PLDMV. All our PRSV-resistant transgenic papaya lines were susceptible to PLDMV, indicating that the virus is an emerging threat for the application of PRSV-resistant transgenic papaya in Taiwan and elsewhere.

47.2 TRANSGENIC MELONS OVER-EXPRESSING GLYOXYLATE-AMINOTRANSFERASE ARE RESISTANT TO *PSEUDOPERONOSPORA CUBENSIS*. **I. Benjamin, D. Kenigsbuch, M. Galperin and Y. Cohen.** *Bar Ilan University, Ramat Gan, Israel. Email: idobinyamin@yahoo.com*

In a previous study (Taler *et al*. 2004, Plant Cell 16, 172-184) we reported that resistance of the wild melon PI124111F against *P. cubensis* is controlled by enhanced expression of the enzymatic resistance (eR) genes At1 and At2. These constitutively expressed genes encode the photo-respiratory peroxisomal enzyme glyoxylate aminotransferases (AGTs). At1 and At2 of susceptible melons Hemed and AY share 98% homology with PI124111F but both genes are down-regulated at the transcriptional level. At1 and At2 of the resistant and susceptible melons were cloned into *E. coli* (the empty vector served as control). AGT activity in all clones was 10 times higher than the control, suggesting that At1 and At2 from Hemed encode for AGTs and that the minor differences in deduced amino acid sequence is not responsible for the suppressed AGT activity. Indeed, abundant mRNA transcripts of At1 and At2 were detected in PI124111F but not in Hemed or AY. Transgenic melons over-expressing either At1 or At2 from PI124111F displayed enhanced AGT activity, and remarkable resistance against *P. cubensis*. Western blot analyses also confirmed these findings. In the present study, At1 and At2 from Hemed driven by the CaMV35S promoter were each introduced into the susceptible melon BU21/3. The transgenic plants showed enhanced AGT activity (as PI124111F) and high resistance against *P. cubensis*. The data suggest that (i) eR genes do occur in susceptible genotypes but are suppressed at the transcription level, and (ii) eR genes isolated from a susceptible genotype and over-expressed in a susceptible melon can induce resistance against downy mildew.

47.3 EVALUATION OF RESISTANCE TO *CANDIDATUS* LIBERIBACTER SPP. IN TRANSGENIC PLANTS OF SWEET ORANGE. **R.L. Boscariol-Camargo, T.S. Simões, C. Haeck, B.M.J. Mendes, F.A.A. Mourão Filho, E.F. Carlos and M.A. Machado.** *Centro APTA Citros Sylvio Moreira-IAC, Rodovia Anhanguera km 158, 13.490-970, Cordeirópolis, SP, Brazil. Email: raquel@centrodecitricultura.br*

Huanglongbing (HLB) or citrus greening, caused by *Candidatus* Liberibacter spp., is a serious citrus disease with no varietal resistance within the genus *Citrus* and its relatives. The bacteria inhabit the phloem of the host and cause symptoms like yellow shoot and leaf mottle. Considering its importance and the need to find alternative sources of resistance, the main objective of this work was to evaluate transgenic plants of sweet orange carrying the attacin A gene, an antibacterial peptide. The titre of the bacteria in the tissues was evaluated by real time PCR (qPCR) with TaqMan probes after six, nine and twelve months post-inoculation by grafting. The primers and probes used were designed previously. Different lineages of transgenic sweet orange were used. Five replicates of each transformation event and a control (non-transformed plant) were inoculated with infected buds and evaluated for disease symptoms and presence of bacteria. A pool of these five plants was subjected to qPCR analysis in duplicate. HLB symptoms started after six months in the majority of the plants. Only one plant did not show symptoms during the period analyzed. The amount of bacteria detected varied from 1.5×10^3 to 1.9×10^7 molecules of 16S rDNA, and in some cases, these levels were higher than the control. Two transgenic plants of cultivar 'Pera' showed lower levels of bacteria than the non-transgenic same-cultivar control, and this level was maintained during twelve months.

47.4 EXPRESSION OF OPBP1 FROM TOBACCO ENHANCES SALT TOLERANCE IN TRANSGENIC RICE. **X.J. Chen and Z.J. Guo.** *Department of Plant Pathology, China Agricultural University, Beijing 100094, P.R. China. Email: chenxj@cau.edu.cn*

AP2/EREBP transcription factors play important roles in plant development and in the responses of plants to biotic and abiotic stresses. Overexpression of osmotin promoter binding protein 1 (OPBP1), an AP2/EREBP-like transcription factor of tobacco (*Nicotiana tabacum*) have led to increase salt tolerance and disease resistance in tobacco. Here, we generated OPBP1-overexpressing rice lines. The overexpression rice plants had enhanced resistance to infection by *Magnaporthe grisea*. They also had increased tolerance to salt stress as well as oxidative stresses such as paraquat treatment. Compared with wild-type plants, the transgenic plants accumulated relatively high levels of *OsP5CS, OsGSTu1* and *OsIM1*, reported to be involved in a salt-tolerance signalling pathway. The results suggest that OPBP1 may participate in different signalling pathways that mediate responses to disease resistance and abiotic stresses.

47.5 INMUNOMODULATION OF *PLUM POX VIRUS* INFECTION IN TRANSGENIC *NICOTIANA BENTHAMIANA* PLANTS EXPRESSING SPECIFIC SINGLE-CHAIN ANTIBODY FRAGMENTS. **M. Gil, O. Esteban, S. Santiago, J.A. García, L. Peña and M. Cambra.** *Instituto Valenciano de Investigaciones Agrarias, Carretera Moncada-Náquera km 5, 46113 Moncada, Valencia, Spain. Email: maitegil@ivia.es*

The constitutive expression of genes or gene fragments of antibodies (recombinant antibodies or rAbs) specific for viral proteins can interfere with the viral infection. This is an alternative strategy to plant transformation with viral sequences to obtain plants resistant to virus. *Nicotiana benthamiana* plants were transformed with the scFv2A antibody fragment, specific for the NIb RNA replicase protein of *Plum pox virus* (PPV). Different expression versions addressed to different cell compartments were generated by addition of several peptides, and used for plant transformation: A cytosolic version (scFv2A), a nuclear version (NLS-scFv2A), and a cytosol-facing endoplasmic reticulum membrane targeted version (6K2-scFv2A). Transgenic *N. benthamiana* plants expressing each different scFv2A version were obtained, and some lines from each construction were selected for viral challenge. Challenge experiments were performed by mechanical inoculation with a PPV-D/NAT isolate at two different inoculum doses. Also, mechanical inoculations with a R3 PPV-GFP infective clone were performed. After mechanical virus inoculation, the percentage of infected plants was lower in some transgenic lines than in control plants. Most transgenic lines expressing the cytosolic scFv2A version showed reduction in viral infection when inoculated either with PPV-D/NAT or R3 PPV-GFP. One transgenic line expressing the nuclear scFv2A version showed the highest reduction in viral infection when inoculated with PPV-D/NAT. Aiming to obtain more information about how scFv fragments interfere with viral infection, we carried out agroinfiltration experiments. *N. benthamiana* plants were agroinfiltrated with a co-culture of a recombinant *Agrobacterium tumefaciens* carrying a PPV-GFP infective clone and different versions of the scFv2A fragment or the scFv5B fragment (specific for the viral coat protein). Results are discussed.

47.6 EFFICIENT GENERATION OF TRANSGENIC BARLEY (*HORDEUM VULGARE* L.) TO MODULATE ITS INTERAC-

TION WITH FUNGAL PATHOGENS. **G. Hensel, C. Marthe, V. Valkov, J. Middlefell-Williams and J. Kumlehn.** *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Plant Reproductive Biology, Corrensstr. 3, D-06466 Gatersleben, Germany. Email: hensel@ipk-gatersleben.de*

Stable genetic transformation is the gold-standard approach to the functional analysis of plant genes. This is particularly relevant in barley, an important crop as well as an experimental model widely used in applied molecular, genetic and cell biological research and biotechnology. Here, the establishment of a protocol for *Agrobacterium*-mediated gene transfer to immature embryos is presented which enables the highly efficient generation of stable transgenic barley. Advances were achieved through comparing the influence of various explant treatments and co-culture conditions. Analysis of representative numbers of transgenic lines showed that the T-DNA copy numbers obtained are typically low, transmission of the recombinant DNA is Mendelian, and the vast majority of the primary transgenics produce progeny that express integrated transgenes. Moreover, the new protocol turned out to be useful to transform not only the highly amenable 'Golden Promise', but also other spring and winter barley genotypes. In this study, we have developed a very useful tool for functional gene analyses as well as for genetic engineering of barley. Examples are presented that show how the method is helping to reveal the functions of genes associated with plant-fungus interactions.

47.7* TRANSGENIC COTTON EXPRESSING A DEFENSIN GENE FOR FUNGAL CONTROL. **J.M. Hinch, Y.M. Gaspar, F.T. Lay, J.A. McKenna, M.A. Anderson and R.L. Heath.** *Hexima Limited, c/o School of Botany, The University of Melbourne, VIC 3010, Australia. Email: jhinch@unimelb.edu.au*

A defensin gene (*NaD1*) was transferred to cotton plants and tested against fungal pathogens. NaD1 was isolated from the female sexual tissues of *Nicotiana alata*. Transgenic cotton plants were tested in field trials in Queensland, Australia, in the growing seasons 2006-2007 and 2007-2008. The trials involved planting cotton in fields naturally infested with the fungus that causes Fusarium wilt, *Fusarium oxysporum* f.sp. *vasinfectum*. The 2006-2007 trials showed that the defensin technology gave very clear advantages to cotton plants, with almost three times the survival rate compared with plants that lack the defensin gene. The surviving plants had higher numbers of bolls per plant and consequently a higher yield per plant.

47.8 ANALYSIS OF RECOMBINATION BETWEEN RNA3 OF *CUCUMBER MOSAIC VIRUS* AND TRANSGENE MRNAS UNDER CONDITIONS OF HIGH SELECTION PRESSURE. **M. Morroni, J.R. Thompson and M. Tepfer.** *Plant Virology Group, ICGEB Biosafety Outstation, via Piovega 23, 31056 Ca' Tron di Roncade (TV), Italy. Email: morroni@icgeb.org*

Cucumber mosaic virus (CMV) infects over 800 plant species, causing economically important diseases, and isolates are divided in two main subgroups (I and II), distinguished by serological and nucleic acid properties. Mixed infections (with two or more viruses) happen frequently in nature, and when two viruses replicate simultaneously in the same cells, recombination between them can occur. There is evidence that recombination can also occur between cellular RNAs and viral RNAs. Thus, virus-resistant transgenic plants (VRTPs) that express viral sequences could be a source of additional recombinants. From a biosafety point of view, it is important to understand if the recombinants that may be generated in VRTPs could contribute to the emergence of new viral diseases. The best way to evaluate this risk is to compare the recombinants that appear in infected transgenic plants with those that appear in doubly infected non-transgenic ones. Detection of host-messenger/viral RNA recombinants under conditions of low selection pressure is problematic because of the low number of recombinant molecules in relation to parental molecules. It was therefore decided to analyze under conditions of high selection pressure for recombination between I17F-CMV (subgroup I) and the mRNA of transgenic tobacco plants expressing a transgene coding for the subgroup II R-CMV coat protein (CP) and 3' non-coding region (3'NCR), infected with I17F-CMV. The selection pressure may simply restrict the recombinants to a subset of those observed under low selection pressure, but it may also reveal novel recombinants not previously observed.

47.9 DEVELOPMENT OF NUCLEOCAPSID GENE-MEDIATED RESISTANCE IN TOMATO AGAINST *GROUNDNUT BUD NECROSIS VIRUS*. **P. Raja and R.K. Jain.** *Department of Plant Pathology, College of Horticulture and Forestry, Pasighat, Arunachal Pradesh, India. Email: prajachf@gmail.com*

Tomato bud blight, characterized by bronzing and necrosis of leaves and concentric rings on fruits, is an emerging viral problem in India. Bud blight-affected samples collected from Coimbatore (Tamil Nadu, TN-Co), Kanpur (Uttar Pradesh, UP-Ka), Pune (Maharashtra, MH-Pu) and Rahuri (Maharashtra, MH-Ra) were subjected to biological and molecular assays. On the basis of bio- and immuno-assays, it was established that the bud blight is caused by *Groundnut bud necrosis virus* (GBNV). The nucleocapsid protein (N) and movement protein (NSm) genes of the GBNV isolates from three locations were cloned and sequenced. Sequence comparison revealed that the N genes of Tospovirus isolates shared 97–100% predicted amino acid sequence identity with GBNV and were highly conserved, suggesting their common origin. Similar results were obtained with the NSm genes. The N gene from GBNV isolate TN-Co was used as transgene to confer resistance in tomato. The gene was sub-cloned into pBI121 in sense orientation and in pBINAR in antisense orientation. The gene constructs were then separately mobilized into *Agrobacterium tumifaciens.* Regeneration of popular tomato varieties Co3, Pusa Early Dwarf (PED) and Pusa Ruby (PR) was standardised. MS medium supplemented with IAA (0.2ppm)+BAP (2ppm) was best for PED and PR regeneration. Both leaf and cotyledon explants were co-cultivated with *Agrobacterium* containing the constructs. Leaf explant was the best choice for *Agrobacterium*-mediated transformation. Nine transgenic lines (To) which were Southern positive, did not show symptoms upon mechanical inoculation with TN-Co isolate. Further evaluation of transgenic lines is in progress.

47.10* VIRUS GENE-MEDIATED SMUT AND BUNT RESISTANCE IN GM WHEAT. **T. Schlaich, A. Fammartino, B. Urbaniak, W. Gruissem and C. Sautter.** *Institute of Plant Sciences, ETH Zurich, Universitaetsstr. 2, CH-8092 Zurich, Switzerland. Email: fammarta@ethz.ch*

A viral gene (KP4) encoding an anti-fungal protein in genetically modified spring wheat varieties (*Triticum aestivum)* resulted in a 30% reduction in symptoms of *Tilletia caries* (stinking smut). In a dose-response greenhouse-based experiment using isolated fungal strains, in which infection pressure was varied via the spore concentration, the transgene behaved as a quantitative re-

sistance gene and shifted the S-shaped dose-response curve towards greater resistance. A field test confirmed a 10% increase in resistance against *T. caries* under high infection pressure. To the best of our knowledge, this is the first report of improved resistance in wheat to fungus achieved using genetic engineering techniques. The same genetically modified wheat lines also showed up to 60% increase in resistance to *Ustilago tritici* (loose smut) in greenhouse experiments. The transgene was shown to be highly specific for fungi of the order Ustilaginales. Toxicity tests of the transgene using cultures of eukaryotes, including hamster and human cells, showed no significant side effects with respect to biosafety. Endogenous pathogen-related genes were also activated upon fungal infection in the presence of the kp4 transgene as shown by micro-array analysis and confirmed by real-time PCR. Flavonoid content, as an example of a metabolic profile with putative environmental interaction, showed greater difference between different conventional varieties than between KP4-GM wheat and wild-type plants.

47.11 LARGE-SCALE PRODUCTION OF RUTIN FROM *TEPHROSIA PURPUREA* THROUGH CALLUS, SUSPENSION CULTURE AND HAIRY ROOT CULTURE. **M.S. Kavitha, E.G. Wesely and M. Rajasekara Pandian.** *Center for Biotechnology, Muthayammal College of Arts and Science, Rasipuram 637408, Tamil Nadu, India. Email: mskavi272003@gmail.com*

Tephrosia purpurea in the family Fabaceae is a multipurpose medicinal plant. It is known to produce rutin, which is found to have many medicinal properties. Since rutin concentration is too low in naturally occurring plants, we have selected tissue culture and hairy root culture to enhance rutin production. Callus was induced from five different explants (cotyledon, hypocotyls, leaves, stem and root) by utilizing suitable hormones. The conditions (plant growth regulator, pH, temperature, and antioxidant) for the production of maximum amount of callus were optimized. Transformed hairy roots of *T. purpurea* were established by infecting explants with *Agrobacterium rhizogenes* (ATCC 15834). The effect of bacterial concentration, silver nitrate, co-cultivation period and pH on *T. purpurea* transformation was investigated. Finally, formation and accumulation of rutin in callus, suspension culture and hairy root culture was enhanced by precursor feeding (phenyl alanine) and elicitor treatment (yeast extract, biotic elicitor). The content of rutin was found to be higher in hairy root culture than in normal roots, callus and suspension culture.

Tropical plant pathology

2.1 DETECTION AND CHARACTERIZATION OF OOMYCETES ASSOCIATED WITH EXPORT CROPS IN CENTRAL GUATEMALA. **P. Abad-Campos, L.A. Álvarez, A. Pérez-Sierra, J. Armengol, E. Rodríguez-Quezada, A. Sánchez-Pérez, R. López-Pineda and G. Álvarez-Valenzuela.** *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia Spain. Email: pabadcam@eaf.upv.es*

Guatemala is turning to the production of new crops destined for export and for rapidly growing urban markets within the country. Diseases caused by Oomycetes have been detected with increasing incidence in these crops in central Guatemala. Surveys of ornamental, vegetable and fruit crops were carried out in 2007. Isolations were made from soil, roots, and stems of symptomatic plants on medium selective for Oomycetes. Isolates obtained were identified based on colony morphology, mycelial characteristics, cardinal growth temperatures, and production, morphology, and dimensions of sporangia, oogonia, and antheridia. The ITS regions were amplified with the primers ITS4 and ITS6, sequenced and compared with sequences available in the EMBL/GenBank database. Six different *Phytophthora* species were identified associated with different crops: *P. capsici*, *P. cinnamomi*, *P. citrophthora*, *P. palmivora*, *P. parasitica*, and *P. tropicalis*. These species are typical of tropical regions. *Pythium* species were also isolated and identified based on the sequence of the ITS region: *P. cucurbitacearum*, *P. splendens*, *P. sylvaticum* and *P. ultimum.*

2.2* POPULATION DYNAMICS OF SOME PHYTONEMATODES ON OKRA PLANTED BETWEEN ROWS OF *LEUCAENA LEUCOCEPHALA* AND *GLIRICIDIA SEPIUM.* **O.K. Adekunle.** *Dept. of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife, Nigeria. Email: kolaade2002@yahoo.co.uk*

Field trials were conducted for two consecutive years to investigate the effects of *Meloidogyne incognita* on okra planted in alleys of *Leucaena leucocephala* and *Gliricidia sepium*. Other nematodes on the experimental site were *Pratylenchus* spp., *Paratylenchus* spp. and *Hoplolaimus* spp. Okra was planted in 4m alleys of three-year-old *L. leucocephala* and *G. sepium* infested with *M. incognita* and was also planted in a control field. At the end of the study, okra plants in the control field had higher root gall indices than those in the gliricidia-leucaena field in both 2005 and 2006. Similarly, okra plants in the gliricidia -leucaena field gave higher fruit yields than those in the control field. In test and control fields, okra cultivar 47-4 gave the highest fruit yield in 2005 and 2006. Percentage soil population densities of four genera of plant parasitic nematodes isolated from the test and control fields increased in both trial years. A higher increase in percentage soil nematode population density was however recorded in the control field for the three cultivars of okra. In the test field, percentage soil nematode population increase was highest in soil sown to okra cultivar Ewela, followed by the percentage increase in soil sown to cultivars LD-88 and 47-4. The results of this study suggest that *L. leucocephala* and *G. sepium* planted as alley crops have potentials to suppress population densities of phytonematodes and enhance performance of the associated crop.

2.3 SPREAD OF CITRUS HUANGLONGBING (GREENING DISEASE) '*CANDIDATUS* LIBERIBACTER ASIATICUS' IN NORTHERN THAILAND. **A. Akarapisan, C. Santasup and C. Sittigul.** *Department of Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand. Email: aangsana@chiangmai.ac.th*

Huanglongbing (HLB) is already known in many countries as greening disease. It is the most destructive disease of citrus in Thailand. HLB is caused by the bacterium '*Candidatus* Liberibacter asiaticus' that can be transmitted by grafting and vectored by the Asian citrus psyllid *Diaphorina citri.* A survey showed that the disease was widespread in the Chiang Mai, Chiang Rai, Lampang, Nann, Phrae, and Mae Hong Son Provinces of Northern Thailand. Symptoms of the disease include erect, yellow new leaves, mottling and blotchy yellowing of leaves and development of interveinal chlorosis. These symptoms are the same as, and can be confused with, those of nutrient deficiencies or other disorders. Characteristic of HLB, however, is that one branch or part of the tree first shows symptoms, which then spread throughout the canopy. A total of 85 samples from the surveys in five Provinces were tested by

polymerase chain reaction (PCR), with specific primers A2/J5 for amplification of the *rpl*KAJL-*rpo*BC gene cluster, producing specific bands of 703 bp. Thirty-four of the samples were HLB-positive. The vector was present in each of these locations.

2.4 EVALUATION OF MANGO VARIETIES FOR RESISTANCE TO POST-HARVEST DISEASES. **C. Akem, G. MacManus, Z. Baron and P. Boccalatte.** *Horticulture and Forestry Science, Department of Primary Industries and Fisheries, P.O. Box 15, Ayr, QLD 4807, Australia. Email: Chrys.Akem@dpi.qld.gov.au*

Managing post-harvest rots of mangoes caused by anthracnose (*Colletotrichum gloeosporioides*) and stem end rots (*Fusicoccum parvum*) through fungicide sprays or dips and other unsustainable methods continue to present a challenge to the Australian mango industry. There are concerns of residue levels and the efficacy of these treatments as a whole in managing these diseases. Genetic resistance has been a powerful tool in the control of several diseases but has not been fully exploited to manage postharvest disease of mangoes. This study was initiated on a mango gene pool collection at the DPI&F Research Station in North Queensland, to evaluate the collection for reactions to the different postharvest diseases under natural infection conditions. Thirty varieties were selected from each of which 50 fruits were harvested in December and another 50 from a further 30 varieties in January and evaluated for resistance to post-harvest rots, following incubation for 12-14 days at 22°C. The incidence and severity of anthracnose and stem-end rots as well as any other rots was recorded. The varieties reacted very differently to the postharvest rots, suggesting a large variation in genetic resistance to the diseases among the mango varieties tested. Varieties from the December harvest showed higher levels of resistance than those from the late January harvest. This may be attributed to their exposure to less disease pressure. Some of the highly resistant varieties identified have been propagated to initiate a rootstock development program for future mango disease management research.

2.5 HISTOPATHOLOGICAL STUDIES OF WILT SYMPTOMS ASSOCIATED WITH MANGO SUDDEN DECLINE DISEASE IN OMAN. **F. Al-Ouweisi, A.M. Al-Sa'di, A. Al-Hinai, M.L. Deadman and Evylen.** *P.O. Box 34, AlKhoud 123, Oman. Email: aalhinai@squ.edu.om*

Mango sudden decline is the most serious disease of mango (*Mangifera indica*) in Oman. Three fungi have been linked with the disease, including *Ceratocystis fimbriata*, *C. omanensis* and *Lasiodiplodia theobromae.* This study focused on investigating histological changes in mango stems in response to infection by these pathogens. *C. fimbriata* and *L. theobromae* were inoculated on six-month old mango seedlings. Following inoculation and optimization of the sectioning and staining methods, the development of tyloses and gums were investigated. The inoculated fungi caused typical wilt symptoms of mango decline and induced discoloration of the vascular system at varying rates. Tyloses were observed in sections of diseased plants with differences between different pathogen-mango combinations. The severity of discoloration was highly correlated with the number of days to wilt initiation ($r=0.99$, $P<0.0001$). Tyloses, gums and mycelium were detected in the xylem of the infected plants resulting in the blockage of xylem, giving evidence that these factors contribute to the overall wilt symptoms in infected mango trees.

2.6 MANAGEMENT OF ANTHRACNOSE, A DISEASE OF INCREASING IMPORTANCE IN ONION IN THE PHILLIPINES. **R.T. Alberto, M.V. Duca and S.E. Miller.** *Dept. of Crop Protection, College of Agriculture, Central Luzon State University, Munoz, Nueva Ecija 3120, Philippines. Email: ralbrtco@mozcom.com*

A field study was conducted at PhilRice Central Experiment Station in Maligaya, Science City of Muñoz, Nueva Ecija to develop integrated management strategies against anthracnose of onion caused by (Penz.) Penz. & Sacc. Different combinations of disease management practices showed that wider spacing (18 × 20 cm) of seedlings with low nitrogen (60 kg/ha) application and mancozeb application at 7 day intervals significantly reduced the incidence, severity and AUDPC of anthracnose of onion. However, the yield did not differ significantly from that of the other treatments except with the treatment with no mancozeb, standard spacing and low nitrogen.

2.7* GENEALOGY AND GENETIC DISSIMILARITY AMONG COCOA GENOTYPES SELECTED AS RESISTANT TO *CRINIPELLIS PERNICIOSA*. **P.S.B. Albuquerque, A. Figueira, S.F. Pascholati, A. Seebben, L.D.D. Teixeira and J.C. Albino.** *Ceplac-Erjoh, CP 46, CEP 67105-970, Marituba-PA, Brazil. Email: psbalbuq@oi.com.br*

A sample of 87 cacao genotypes, including 78 selected in plantations in the southern Bahia region in Brazil, based on resistance to *C. perniciosa*, were analyzed for genetic dissimilarity by microsatellite markers. The accessions resistant to *C. perniciosa* CAB 0208 and CAB 0214 plus seven samples from the CEPLAC-CEPEC germplasm collection, including five parental candidates of the selections, plus two controls were included in the analyses. The accessions ICS 1, ICS 6, SCA 6, SIC 824 and SIAL 70 were considered putative parents of the selection because they were often used in the hybrid seed production program in southern Bahia, while the hybrids TSH 516 and Theobahia, derived from crosses between ICS 1 and SCA 6 were included as controls. Genealogy and genetic dissimilarity analyses of the genotypes were based on the allelic frequency of 20 microsatellite loci. Degree of relationship estimates were based on a co-ancestry coefficient and the genetic dissimilarity on correspondence factorial analyses. From the 78 selections, 42 presented some significant relationship with some of the parental candidate accessions, mostly related to SCA 6. The dissimilarity analyses allowed accession to be classified into two large groups. Fellowships from CNPq are acknowledged.

2.8 VEGETATIVE COMPATIBILITY AMONG *FUSARIUM FUJIKUROI* ISOLATES FROM BAKANAE-DISEASED RICE PLANTS IN MAZANDARAN PROVINCE, IRAN. **S.A. Alian, M. Javan-Nikkhah, H. Aminian and V. Khosravi.** *Department of Plant Protection, Aboureyhan Campus, University of Tehran, Pakdasht, Iran. Email: aminalian@gmail.com*

Sixty nine isolates of *Fusarium* species belonging to section *Liseola* were established from bakanae-diseased rice plants from different regions in Mazandaran province during 2004. Of these isolates, 21 (30.43%) were identified as *F. fujikuroi* Nirenberg and their pathogenicity were confirmed. *Nit* mutants were used to force heterokaryons to determine distribution of vegetative compatibility groups (VCGs). Of 294 mutants generated, 237 utilized both nitrite and hypoxanthine (*nit1*), 33 utilized nitrite but not hypoxanthine (NitM), and 24 utilized hypoxanthine but not nitrite (*nit3*). *Nit* mutants obtained from three isolates were *nit1*,

only. These isolates were discarded. A *nit1* mutant from each isolate was paired on nitrate medium to either a *nit3* or NitM mutant from all isolates. We placed 18 isolates into 17 vegetative compatibility groups (VCGs). Of the 17 VCGs identified, 16 were represented each by a single isolate and the remaining 2 belonged to one multimember VCG. This VCG was placed in a special limited area where multimember VCGs of *F. proliferatum*, as the major causal agent of rice bakanae disease in Mazandaran province, were also placed. These data confirmed genetic similarity of this area's populations. This diversity should be considered when screening rice germplasm for bakanae disease resistance.

2.9 COMPARISON OF KUMQUAT ISOLATES OF *PHYTOPHTHORA CITROPHTHORA* WITH CITRUS ISOLATES IN TAIWAN. **P.J. Ann, I.T. Wang, J.N. Tsai and L.F. Liou.** *Plant Pathology Division, Taiwan Agricultural Research Institute, 189 Chung-Cheng Rd., Wufeng, Taichung 413, ROC. Email: pjann@wufeng.tari.gov.tw*

In 1995, a severe disease caused by *Phytophthora citrophthora* broke out in Kumquat (*Fortunella margarita*) orchards in northeastern Taiwan. In order to determine if the pathogen is a recent introduction, a survey of *P. citrophthora* on members of the Rutaceae in Taiwan was conducted from 1995 to 2006. Among 200 orchards in 14 counties surveyed, *P. citrophthora* was detected in 23 orchards, and 83 isolates (all A1 mating type) were obtained including 35 isolates from kumquat and 48 isolates from citrus species. Kumquat isolates showed colony patterns on PDA plates different from other citrus isolates and produced more sporangia, and were more pathogenic to kumquat than citrus isolates. The similarity values of rDNA sequences in ITS regions ranged from 97 to 100% among all isolates tested, the length of ITS regions was 779 bp for kumquat isolates and 782-784 bp for citrus isolates. In comparison with citrus isolates, all kumquat isolates have 4 base pair deletions and 2 base substitutions in ITS1 regions, and 6 base substitutions in ITS2 regions. Our results suggest that the pathogen causing severe kumquat decline in Taiwan in recent years is a new invasive strain of *P. citrophthora*.

2.10 MOLECULAR EPIDEMIOLOGY OF *XANTHOMONAS CAMPESTRIS* PV. *MUSACEARUM*, THE CAUSAL AGENT OF *XANTHOMONAS* WILT OF BANANA AND ENSET, FROM 1968 TO 2006. **V. Aritua, N. Parkinson, R. Twaites, D.R. Jones, W. Tushemereirwe and J. Smith.** *National Agricultural Biotechnology Center, Kawanda Agricultural Research Institute, P.O. Box 7065, Kampala, Uganda. Email: arituavalentine@yahoo.com*

Xanthomonas wilt of enset and banana, first described in Ethiopia in 1968, is now a serious bacterial disease that is spreading in East and Central Africa. Earlier studies identified *Xanthomonas campestris* pv. *musacearum* (Xcm) as the causal agent. Phylogenetic analysis of partial nucleotide (nt) sequences of the gyrase B gene and ITS region, genomic amplicon fingerprints using REP-PCR and fatty acid methyl esters of 20 strains originating from Ethiopia, Uganda, Democratic Republic of Congo and Rwanda isolated from 1968 to 2006, belonged to same genotype. The pathogen was unrelated to *X. campestris* but very similar to *X. vasicola*, which formerly comprised pathogens of sorghum (*X. vasicola* pv. *holcicola*) and a group of sugarcane and maize pathogens, that were known to be atypical of *X. axonopodis* pv. *vasculorum* and had a proposed re-classification as *X. vasicola* pv. *vasculorum* (Xvv). Pathogenicity studies also indicated that strains of *X. vasicola* pv. *holcicola* and Xvv induced no symptoms on banana, whereas Xcm produced severe disease. Together, our data supports the reclassification of Xcm as *X. vasicola* pv. *musacearum*. In addition, our data showed that the recent occurrence of the banana disease in Uganda and other East African countries is a consequence of the spread of the previously recognised Xcm pathovar. Possible hypotheses to explain the evolution of the three strains of *Xanthomonas* infecting banana, sorghum, sugarcane and maize are proposed.

2.11 VARIETAL DIVERSIFICATION FOR MANAGEMENT OF RICE BLAST IN THE UPLANDS OF INDONESIA. **N.P. Castilla, S. Santoso, Y. Sulaeman, T.W. Mew and C.M. Vera Cruz.** *Plant Breeding, Genetics, and Biotechnology Division; International Rice Research Institute, DAPO Box 777, Metro Manila, Philippines. Email: ncastilla@cgiar.org*

The effectiveness of varietal interplanting in controlling rice blast under high disease pressure was evaluated in the uplands of Indonesia. In 2003-04 and 2004-05, one row of modern varieties Cirata (highly susceptible to blast) or Way Rarem (moderately susceptible) was interplanted with four rows of a resistant, improved variety. Interplanting did not significantly reduce neck blast on Cirata in 2003-04, but there was 52% (P=0.024) reduction of the disease on the same variety the following year. Interplanting reduced neck blast incidence on Way Rarem by 29% (P=0.022) in 2003-04 and by 77% (P=0.036) in 2004-05. In 2005-06 and 2006-07, two to six rows of Cirata or Way Rarem were interplanted with one row of Sirendah, a moderately resistant, traditional variety. Interplanting did not reduce leaf blast and neck blast incidence on Cirata in either period. In 2005-06, interplanting two rows of Way Rarem with Sirendah reduced neck blast incidence by 81% (P=0.004). Neck blast incidence on Way Rarem decreased as the proportion of this variety in the mixed stand decreased (r=0.995, P=0.0005). However, in 2006-07, interplanting did not significantly reduce leaf blast and neck blast incidence on Way Rarem. These results show that the resistance level of components should be increased to improve the effectiveness of cultivar diversification in highly conducive environments. Interplanting one row of a moderately susceptible variety with at least four rows of a resistant variety has the potential to control blast consistently in such environments.

2.12 CONIDIAL GERMINATION OF *GONATOPHRAGMIUM* SP., THE CAUSAL AGENT OF RED STRIPE DISEASE OF RICE. **T.U. Dalisay, E.G.M. Oreiro, N.P. Castilla and T.W. Mew.** *Crop Protection Cluster, University of the Philippines Los Baños, College, Laguna, Philippines. Email: tess_d17@yahoo.com*

Gonatophragmium sp., the causal agent of red stripe, has not been previously reported on rice. As part of the studies on the etiology of the disease, conidial germination of the pathogen on the adaxial surface of rice leaves was examined from 0.5 to 48 hours after inoculation (hai). Observations on formation of secondary conidia were extended until 15 days after inoculation (dai). Most conidia germinated by 4 hai and all had germinated by 48 hai. Conidial germination over time was described well by the monomolecular model (r^2 = 0.87, P<0.0001). The average size of conidia was 20.73±0.58 µm × 5.11±0.13 µm from 0.5 to 48 hai. Conidia produced germ tubes within 0.5 to 48 hai. Germ tubes elongated continuously and developed into aseptate hyphae with profuse branching, starting from 6 hai. Lengths of germ tubes and hyphae did not significantly differ between 0.5 to 6 hai (7.50±1.32 µm), but were significantly (P<0.0001) shorter than

those at 12 hai (55.57±8.73 µm). Germ tubes formed complex intertwining branches after 12 hai. The number of germ tubes increased linearly with time (r^2 = 0.97, P<0.0001). Germination type was initially unipolar and later became bipolar or multipolar. Germ tubes emerged more often from apical and basal cells, and less frequently, from middle cells. Germ tubes and hyphae entered the leaves through the stomata. Secondary conidia were formed from 9 dai. Studies on the effects of nitrogen rate and relative humidity on conidial germination and penetration are in progress.

2.13 CHARACTERIZATION OF PATHOGEN DIVERSITY AND GEOGRAPHICAL DISTRIBUTION OF *MAGNAPORTHE ORYZAE* IN THE PHILIPPINES. F.A. dela Peña, A.G. Tagle, E.Y. Ardales, M.S. Manalo, I.P. Oña, N. Kobayashi, Y. Fukuta and C.M. Vera Cruz. *Philippine Rice Research Institute (PhilRice), Maligaya, Science City of Muñoz 3119, Nueva Ecija, Philippines. Email: fadelapena@philrice.gov.ph*

A total of 197 rice blast samples were collected in irrigated lowlands (111), rainfed lowlands (54), uplands (31) and cool-elevated areas (1) to determine the population structure of *Pyricularia grisea* and its distribution in the Philippines in 2004–2006. Rice blast has become common in irrigated lowlands particularly those planted with PSBRc14, PSBRc82, NSICRc112, NSICRc122 and IR64 which had intermediate to resistant reaction to the rice blast pathogen when released as varieties but have succumbed to the disease in recent years. Analysis of the 167 selected monoconidial isolates (138 were from PhilRice collection and 29 from IRRI) showed that there were 33 Pot2 and 27 URP5 haplotypes defined. Based on Nei's genetic diversity index, high genetic diversity estimates of 0.94 (Pot2) and 0.89 (URP5) were obtained for the entire population. The phylogenetic relationship among the isolates was determined by cluster analysis by the Unweighted Pair Group Method with Arithmetic Means (UPGMA) using the NTSYS statistical package. Using Pot2, 7 clusters were formed at 75% similarity level while use of URP5 revealed that there were 10 major clusters at 76% similarity level. Most of the isolates having the same haplotype were detected from more than one location and in most cases; isolates exhibiting particular haplotypes that clustered together were derived from different geographical locations. This indicates that there was no correlation between haplotype and geographical location among the isolates. Moreover, the reaction of monogenic lines to the different rice blast isolates revealed significant differences in terms of virulence.

2.14 *ALTERNARIA* SPP. OCCURRING ON ONION FOLIAGE: MORPHOLOGY, PATHOGENICITY AND DNA ANALYSIS. J. Fernandez and L.I. Rivera-Vargas. *Department of Crop Protection, P.O. Box 9030, College of Agricultural Sciences, University of Puerto Rico, Mayagüez, Puerto Rico, 00681-9030, USA. Email: lyrivera@uprm.edu*

Alternaria species occurring on onion foliage from southern Puerto Rico were characterized on the basis of morphology, pathogenicity and DNA analysis. A total of 280 isolates were obtained, from which 34 were selected as morphotypes belonging to *A. destruens*, *A. tenuissima*, *A. palandui* and *A. porri* (= *A. allii*) species group. Sixty two percent of selected isolates were placed within a taxonomically undescribed *Alternaria* sp. group with a "semi-arborescent" sporulation pattern. Pathogenicity tests, conducted under laboratory and field conditions, showed that *A. porri* (= *A. allii*), *A. tenuissima* and *Alternaria* sp. ("semi-arborescent" sporulation pattern) were pathogenic to onions, with *A. porri* (=*A. allii*), isolates being the most virulent. DNAs of the various *Alternaria* spp. isolates were analyzed using RAPD-PCR, and PCR amplification and sequencing of the nuclear internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) gene. RAPD-PCR technique using primer OPA-13 did not differentiate between species. Phylogenetic relationships based on rDNA ITS sequences from *Alternaria* isolates and other Pleosporaceae (*Ulocladium* sp., *Embellisia* sp. and *Stemphylium*) from the GenBank were analyzed by neighbor-joining and by Kimura distance methods. Three clades were distinguished with strong bootstrap values. A first clade with large filiform-beaked spores including *A. porri* (= *A. allii*), *A. solani*, *A. macrospora*, *A. zinniae* and *A. sesamicola* formed a monophyletic group, discrete from other members of the genus. A second clade, including a diverse group of small-spored *Alternaria*, and a third clade that included *Stemphylium* spp., are described.

2.15 PATHOGENIC RACES OF *XANTHOMONAS ORYZAE* PV. *ORYZAE* STRAINS FROM NORTHERN VIETNAM. N. Furuya, S. Taura, B.T. Thuy, P.H. Ton, N.V. Hoan, T. Goto, A. Yoshimura and K. Tsuchiya. *Department of Agronomy, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan. Email: nafuruya@agr.kyushu-u.ac.jp*

Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is becoming very severe in northern Vietnam because of the introduction of hybrid rice from China. Pathogenic variability of Vietnamese Xoo and varietal resistance have not been intensively investigated. We characterized 84 Xoo isolates collected from 11 regions of northern Vietnam using virulence analysis. This analysis, using 11 differential lines (IR 24, IR-BB 1, IR-BB2, IR-BB3, IR-BB4, IR-BB 5, IR-BB 7, IR-BB 10, IR-BB 11, IR-BB 21 and Taichung Native 1), each containing a single resistance gene, identified 4 pathotypes (race; G1-G4). All strains were virulent to resistance genes *Xa1, Xa2, Xa10, Xa11, Xa14, Xa16* and *Xa18,* and were avirulent to resistance genes *xa5* and *Xa21.* Strains from northern Vietnam were composed of two major races (G2 and G3) and two minor races (G1 and race G4), which were widely distributed. Most of Vietnamese hybrid varieties cultivated in northern Vietnam were susceptible to all races, and race G4 was virulent to all hybrid varieties tested. By a combination of DNA and pathotypic analysis we present here the first picture of Xoo recently isolated in northern Vietnam. This will facilitate further understanding of the population structure.

2.16 SPATHE ROT OF ANTHURIUM CAUSED BY *CORYNESPORA CASSIICOLA*. W.C. Ho, S.Z. Wu and C.Y. Hu. *Department of Biotechnology, Tajen University, Yenpu, Pingtung, ROC. Email: pp202wcho@yahoo.com.tw*

Anthurium is an expanding ornamental commodity in Taiwan. Recently, a spathe disease was noticed on cv. 'Nitta Orange' in two commercial anthurium nurseries. The disease initially appeared as a water-soaked spot which turned brown and expanded rapidly covering more than half of the spathe. *Corynespora cassiicola* was consistently isolated from the diseased spathes. Upon inoculation, the fungus caused symptoms on anthurium spathes similar to those observed in nature. *C. cassiicola* was reisolated from diseased spathes, fulfilling Koch's postulates. *C. cassiicola* also caused elongated sunken brown lesions on leaf petioles and spathe stems and seedling damping-off of anthurium. Two isolates of *C. cassiicola* from diseased tomato leaves were also patho-

genic to anthurium but not one from diseased cucumber leaf, indicating diseased tomato leaves as the possible source of inoculum for this new anthurium disease in Taiwan. Among anthurium cultivars tested, spathes of cultivars 10, M01, M22, 'Anuenue', 'Rose', 'Vanilla' and 'Pistache' were immune to *C. cassiicola*, while those of 'Nitta Orange', 'Mini Obake' and LOB1 were highly susceptible. Anthurium seedlings of cultivars M01, M02, M03, M06, M12, M15 and H5-4 were immune to *C. cassiicola*, while those of 'Mini Obake', LOB1 and M23 were highly susceptible.

2.17 CONTROL OF BLACK LEAF SPOT OF SPOON CABBAGE CAUSED BY *ALTERNARIA BRASSICICOLA* WITH EXTRACTS OF THE ORIENTAL MEDICAL PLANT SPEEDWEED (*POLYGONUM PERFOLIATUM*). W.C. Ho, T.Y. Wu, H.J. Su and W.H. Ko. *Department of Plant Pathology, National Chung Hsing University, Taichung, ROC. Email: kowh@dragon.nchu.edu.tw*

Among 65 species of oriental medicinal plants tested, 39 contained substances inhibitory to conidial germination of *Alternaria brassicicola* with the most inhibitory extracts coming from speedweed (*Polygonum perfoliatum*). The inhibitory substances in dried speedweed were insoluble in water but were readily extracted with ethanol or methanol, but not with acetone, ether or chloroform. The ethanol extract was very effective in controlling black leaf spot of spoon cabbage (*Brassica campestris* subsp. *chinensis*) caused by *A. brassicicola*. The inhibitory effect of the extract was not affected by treatment with anion exchange resins, but was partially reduced by cation exchange resins, indicating the presence of two inhibitory substances in the extract, one with a positive charge and the other with no charge on its molecule. The inhibitory substances in the extract were dialyzable with molecular weight minimums of 10,000 or 1000, but not 500 or 100, suggesting that both inhibitors have molecular weights between 500 and 1000.

2.18 FURTHER MORPHOLOGICAL, PATHOGENICITY AND MOLECULAR STUDIES ON *CERCOSPORA* ON YAMS (*DIOSCOREA* SPP.). A.N. Jama, S.R. Gowen and J.C. Peters. *Department of Agriculture, P.O. Box 326, University of Reading, Reading, RG6 6AT, UK. Email: a.n.jama@reading.ac.uk*

Cercospora leaf spot, which causes severe defoliation, is an economically important disease of yam. Although the cause of this disease was initially identified as *Cercospora dioscoreae-pyrifoliae* (teleomorph *Mycosphaerella papuana*), recent studies have acknowledged close morphological similarities between *C. dioscoreae-pyrifoliae* and *Cercospora apii*, deeming the two species synonyms. However, because of the lack of pathogenicity tests and the absence of the teleomorph of *C. apii,* these studies showed the need for further investigation. To elucidate further the relationship between these two organisms, we have conducted comparative studies on the morphology and cultural characteristics of 14 *Cercospora* isolates from yam, three *C. apii* and two *C. apiicola* from celery. Although there were no clear differences in morphological structures and colony characteristics among these isolates, significant differences were observed in their radial growth at different temperatures. Optimum radial growth was measured at 26°C or 30°C for yam isolates and at 26°C for *C. apii* and *C. apiicola* isolates. Moreover, in cross-inoculation tests with representative isolates of the three pathogens, only yam isolates were able to colonize and sporulate on detached leaves of *Dioscorea alata* whereas *C. apii* and *C. apiicola* failed to establish infection. Furthermore, DNA sequence studies on five genomic loci revealed that yam isolates consist of more than one genotype showing strong affinity to either *C. canescens* or to *C. acaciae-mangii.* This indicates that these yam isolates have no close homology either with *C. apii* or *C. apiicola.*

2.19* FAILURE AND SUCCESS IN CHEMICAL MANAGEMENT OF COFFEE BERRY DISEASE IN KENYA. G.M. Kairu. *Coffee Research Foundation, P.O. Box 4, Ruiru 00232, Kenya. Email: crf@kenyaweb.com*

Coffee berry disease (CBD) caused by *Colletotrichum kahawae* Waller & Bridge is an important disease of coffee (*Coffea arabica* L.) grown in Kenya. The pathogen attacks green expanding berries. The disease can cause more than 80% crop loss and is therefore managed with regular sprays of recommended fungicides. In fungicide evaluation trials during 2006 and 2007, sprays of chlorothalonil 75WP (0.4%), cuprous oxide 75WP (0.35%) and copper oxychloride 50WP (0.7%) each applied alone, failed to control CBD. Instead, the sprays stimulated the incidence of CBD by up to 4.6 times compared to the unsprayed treatment. This happened because the spray deposits were eroded and washed down by heavy rains soon after application. Consequently, the microbial balance was upset in favour of *C. kahawae* inoculum which was supplied from CBD lesions on the early crop (off season crop) in an environment already cleared of competitors by the pre-rains spray. In 2007, tank-mixture sprays of chlorothalonil 720 SC and cuprous oxide 75 WP formulations (0.2% + 0.25%) succeeded in controlling CBD effectively at the beginning of the epidemic but failed thereafter. In contrast, sprays of a proprietary formulation (660 SC) made of chlorothalonil and azoxystrobin active ingredients were highly successful in maintaining effective control of CBD throughout the period of crop susceptibility. The success of this proprietary mixture was derived from protective as well as systemic activity of azoxystrobin in stopping inoculum supply from CBD lesions. The implications for crop yield and management of pathogen resistance to fungicides will be discussed.

2.20 FACTORS AFFECTING OCCURRENCE AND MANAGEMENT OF COFFEE BERRY DISEASE IN KENYA. G.M. Kairu. *P.O. Box 4, Ruiru 00232, Kenya. Email: gmkairu@yahoo.com*

Nearly all cultivars of *Coffea arabica* L. grown in Kenya are susceptible to coffee berry disease (CBD) caused by *Colletotrichum kahawae* Waller & Bridge. Coffee berries become susceptible one month after they are formed. The primary source of inoculum is the mature bark on branches. The first CBD lesions on expanding green berries supply more inoculum than the bark and are therefore considered a major driving factor for an epidemic. The conidia of *C. kahawae* require a water film and a temperature range of 17°C to 28°C for a duration of ≥5 h to germinate, form appressoria and penetrate. Against this background, disease incidence and meteorological data were examined in two high-altitude sites, usually more prone to CBD attack, with a view of determining the factors affecting occurrence, and chemical management of the disease. The outcome of the study was that lower incidence of CBD in the higher altitude site (Yara Estate 1970 m above sea level) was surprisingly associated with lower frequencies of wet periods (≥5 h) compared to the lower altitude site (Kamundu Estate, 1830 m above sea level) which experienced a higher frequency of similar wet periods and a higher CBD incidence. The contribution of other factors will be discussed. These are: number of infection targets, the critical period of susceptibility, changing weather patterns, and the history of disease management at each site.

2.21 INOCULUM DYNAMICS OF *PHYTOPHTHORA INFESTANS*: SURVIVAL IN DEBRIS, ALTERNATIVE HOSTS, AND AIRBORNE SPORANGIA. M.A. Lima, L.A. Maffia, R.W. Barreto and E.S.G. Mizubuti. *Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, 36570-000, Brazil. Email: mizubuti@ufv.br*

Better understanding of inoculum dynamics of *Phytophthora infestans* in tropical and subtropical areas of the world can improve late blight management strategies. The objective of this work was to investigate three events associated with initial inoculum of late blight epidemics: survival of *P. infestans* on crop debris (stems, leaflets, and tomato fruits) either buried or not in soil; pathogenicity of *P. infestans* to different hosts, mostly solanaceous species commonly found in Brazil; and temporal dynamics of airborne sporangia. *P. infestans* survived in tomato plant organs less than 100 days and 35 days under greenhouse and field conditions, respectively. In the greenhouse, pathogen structures were more promptly identified on crop debris kept on dry than on wet soil. Isolates of two clonal lineages of *P. infestans*, US-1 and BR-1, obtained from tomato and potato respectively, were inoculated on 43 plant species. Plants of two species, *Petunia hybrida* and *Nicotiana benthamiana*, were susceptible to *P. infestans*. Sporangia were monitored by Rotorod and Burkard spore traps as well as by tomato and potato trap plants throughout 2004 and in January 2005. Sporangia were trapped in most weeks throughout 2004 and in the first two weeks of 2005. Airborne inoculum was detected in 42 out of 46 and in 41 out of 52 weeks with Rotorod and Burkard traps, respectively. Under our conditions, airborne inoculum is thus available most of the time, and is likely to be more important to late blight outbreaks than inoculum from crop debris or alternate hosts.

2.22 OOSPORES OF *PHYTOPHTHORA COLOCASIAE* AS AN IMPORTANT SURVIVAL STRUCTURE IN NATURE. M.J. Lin and W.H. Ko. *Department of Plant Pathology, National Chung Hsing University, Taichung, ROC. Email: jiayi@dragon.nchu.edu.tw*

Taro (*Colocasia esculenta*) is a staple food in many parts of southeast Asia and the Pacific regions. Leaf blight and corm rot caused by heterothallic *Phytophthora colocasiae* is the major limiting factor in the cultivation of taro. Isolates previously collected in different parts of the world consist of A1, A2 and neuter A0 types. Recently, we resurveyed the mating type distribution of *P. colocasiae* in Taiwan. Although most isolates obtained were of the A2 type, five single-hyphal isolates recovered produced oospores in single cultures. These homothallic (A1A2) isolates produced deciduous semipapillate sporangia indistinguishable from those produced by type A2. The 53 single-zoospore isolates obtained from one of the A1A2 isolates consisted of 35 A1A2, 15 A1 and 3 A2. Pathogenicity tests showed that the A1A2 type and the A1 type segregated from the A1A2 type were able to cause leaf blight symptoms similar to those caused by A2 types in the field. The A1A2, A1 and A2 isolates obtained from Taiwan showed 99.3 to 99.9% similarity in ITS nucleotide sequence. When taro corms and petioles were inoculated with zoospores of the A1A2 isolates or mixed zoospores of A1 and A2 segregates, oospores were produced in both kinds of tissues. Our results suggest that in certain areas oospores may serve as an import survival structure and source of variation of *P. colocasiae* in nature.

2.23 TISSUE-SPECIFIC REALTIME QUANTITATIVE ANALYSIS OF TRANSCRIPT LEVELS OF THE ANTIFUNGAL OSMOTIN GENE IN RESISTANT *PIPER* SPECIES. T. Mani. *JRF. PMB Division, Rajiv Gandhi Centre for Biotechnology, Thycaud PO, Trivandrum, Kerala 695014, India. Email: tomsonmani@gmail.com*

Black pepper (*Piper nigrum* L.) is a renowned spice crop with extensive culinary and medicinal uses. It is a native of tropical southern India and is cultivated extensively in various other tropical parts of the world. The growth and yield of the crop is seriously threatened by fungal pathogens especially by the oomycete *Phytophthora capsici* which causes foot rot disease. None of the available cultivars of black pepper are known to be resistant. *Piper colubrinum* Link. is a wild relative of black pepper and it shows higher degrees of resistance against many of the fungal pathogens attacking black pepper. Recently we cloned the full length of the antifungal osmotin gene belonging to the PR-5 family, which was found to be differentially expressed upon treatment with the signaling molecule salicylic acid. Osmotin is potent against many fungal pathogens. Here we present the transcript level of osmotin in different tissues of the resistant wild pepper, measured by real-time quantitative analysis.

2.24 CHARACTERIZATION OF *PHYTOPHTHORA INFESTANS* FROM THE ANDEAN REGION OF COLOMBIA. V. Mesa, H. García, J.M. Cotes, S. Jaramillo, L.E. Lagos and M. Marín. *National University of Colombia - Medellín; University of Nariño, Colombia. Email: mamarinm@unal.edu.co*

Late blight caused by *Phytophthora infestans* is one of the most limiting diseases in solanaceous crops worldwide. This pseudofungus is especially restrictive in the highlands of the Andes, where continuous crops are grown under conditions of high humidity. The aim of this research was to increase the information on the biology of *P. infestans*, specifically concerning to its level of genetic variation in the Andean region of Colombia. We used AFLP markers, mating type analysis, mtDNA haplotypes and sensitivity to fungicides RIDOMIL® GOLD, CURZATE® M-8, PREVICUR® N SL and MILDEX® 711 WG. Results indicated a low level of genetic variation among the isolates evaluated, with 13.4% of AFLP amplicons being polymorphic, a Nei´s genetic diversity index of 0.04 and a Shannon´s information index of 0.06. The UPGMA dendrogram divided the population into two main groups, the first being isolates from potato, while the second included isolates from tree tomato; both groups represented clonal lineages EC-1 and EC-3, respectively. Fungicide tests showed that the population studied was highly sensitive to all the formulations, with sporangium production decreasing from an average of 41,181 sporangia·l^{-1} in the control treatment to less than 500 sporangia·l^{-1} when the maximum doses of the fungicides were used. The results support the hypothesis of a population structure of *P. infestans* base on hosts; however this requires confirmation by testing cross pathogenicity.

2.25 OUTBREAK OF PINK DISEASE IN TEAK (*TECTONA GRANDIS*) PLANTATIONS IN KERALA, INDIA AND ITS MANAGEMENT. C. Mohanan. *Forest Protection Division, Kerala Forest Research Institute, Peechi 680 653, Kerala, India. Email: mohanan@kfri.org*

Teak (*Tectona grandis* L. f.), the prime forestry species in India, is one of the most sought after hardwoods all over the world.

Due to the warm-humid tropical climate prevailing in Kerala State, diseases play a major role in the establishment and productivity of teak stands. Recently, an outbreak of disease caused by *Corticium salmonicolor* occurred in an intensively managed 18-month-old teak plantation affecting > 70% of the plants in 60 ha. The disease epidemiology was studied and management measures adopted. Under high humidity and temperatures (90-95% R.H., 28-32°C), the disease spread very fast and caused multiple cankers on the main shoot. Splitting of the bark was followed by partial to complete girdling of the stem. *C. salmonicolor* produced its cob-web and pustule stages during the initial phase of infection, basidial stage under high humid condition and necator stage during the dry period. Association of an insect belonging to the Pyralidae was noticed in the canker, making galleries in the fleshy callus and causing discoloration of the affected tissues. To manage the disease, we applied Bordeaux mixture (10% paste) and tridemorph (0.1% a.i.) singly or in combination. In severely infested areas, an insecticide (Ekalux @ 0.1%) was mixed with the fungicide. Monitoring of the efficacy of pesticide treatments fortnightly for three months revealed that application of Bordeaux paste (10%) followed by tridemorph (0.1% a.i.) after an interval of two weeks was very effective in healing the cankers and protecting the plants from new infection.

2.26 FUNGI ASSOCIATED WITH STEM CANKER AND SHOOT DIEBACK ON *GREVILLEA ROBUSTA* ACROSS FIVE AGRO-ECOLOGICAL ZONES IN KENYA. **J. Wangu Njuguna, P. Barklund, K. Ihrmark and J. Stenlid.** *Swedish University of Agricultural Sciences (SLU), Department of Forest Mycology and Pathology, Sweden. Email: jane.njuguna@mykopat.slu.se*

The exotic Australian tree species *Grevillea robusta* is widely grown in East and Central Africa mainly on small-scale farms for its multipurpose benefits. Since its introduction about a hundred years ago, it has largely been regarded as a tree without major diseases and pests. Unexpected deaths of young *G. robusta* trees initiated a disease survey in Kenya covering 90 farms spread across five agro-ecological zones, from which, about 2,160 samples of cankered stems, branches and twigs with dieback were collected. More than 45 types of fungi were isolated. *Botryosphaeria* species followed by *Fusarium*, *Pestalotiopsis* and *Phomopsis* were the most commonly isolated fungi. Morphological identification together with phylogenetic analysis of the internal transcribed spacer region (ITS1 and ITS4) of the nuclear rDNA, a partial sequence of the β-tubulin and α elongation factor genes showed that six major species of *Botryosphaeria* (*B. parva*, *B. theobromae*, *B. corticola*, *B. obtusa* and *Botryosphaeria* sp. and or *B. dothidea*), four types of *Fusarium* (*F. solani*, *Nectria* spp., *F. graninearum*, *F. oxysporum*), two types of *Pestalotiopsis* (*P. gracilis* and *P. microspora*) and *Phomopsis* spp. were the major pathogenic fungi isolated from cankered *G. robusta* trees of all ages from the five agro-ecological zones. *Botryosphaeria* spp have increasingly become important pathogens of many woody crops of economic importance including forest and farm trees, fruit trees and many herbaceous woody species. The impact of this disease on mixed farming (tree-crop) systems with *G. robusta* in Kenya is discussed.

2.27 PATHOGENICITY OF *BOTRYOSPHAERIA* SP., *FUSARIUM* SP., *PHOMOPSIS* SP., AND *PESTALOTIOPSIS* SP., ON FIVE TREE SPECIES IN KENYA. **J. Wangu Njuguna, P. Barklund, K. Ihrmark and J. Stenlid.** *Swedish University of Agricultural Sciences (SLU), Department of Forest Mycology and Pathology, Sweden. Email: jane.njuguna@mykopat.slu.se*

Eleven species of fungi belonging to four genera (*Botryosphaeria parva*, *Botryosphaeria rhodina*, *Botryosphaeria obtusa*, *Botryosphaeria corticola*, *Botryosphaeria* sp., *Fusarium solani*, *F. oxysporum*, *Fusarium* sp., *Nectria haematococca*, *Pestalotiopsis microspora* and *Phomopsis* sp.) were tested for pathogenicity on five tree species that are widely grown in Kenya on small-scale mixed farming systems in Kenya. The tree species include *Grevillea robusta*, *Melia volkensii*, *Senna siamea*, *Azadirachta indica*, *Eucalyptus grandis* and *E. camaldulensis*. The experiment was set up in two different sites under glasshouse conditions on 6-month-old seedlings and monitored for 10 months. Site one was characterized by warm, wet weather with fertile soils while site two was characterized by hot dry weather and poor soils. Pathogenicity results indicated that there is both inter- and intra-pathogen variation in virulence on the five tree species. Further there was a significant difference in all the parameters measured between the two sites especially mortality (<10% in site one by the most virulent pathogen compared to about 60% at site two) by the same pathogen. The results further show that pathogens travel much further inside a plant system than can be seen when measuring external lesion size.

2.28* *PYTHIUM* SPECIES ASSOCIATED WITH ROOT ROT DISEASES IN VIETNAM. **H.T. Phan, T.M. Luong, L. Tesoriero, L. Forsyth and L.W. Burgess.** *National Institute of Medicinal Materials, 3B Quang Trung Str., Hanoi, Vietnam. Email: phanthuyhien @yahoo.com*

There has been little research on *Pythium* root rots in many tropical areas of South East Asia. They are insidious diseases which cause non-specific symptoms such as stunting, yellowing and reduced yields. Some species are adapted to hot wet conditions. The identity and role of *Pythium* species in root rot diseases in a range of crops in Vietnam was investigated in 2007, the first systematic study of these pathogens in this country. *Pythium* species were associated with serious diseases including damping-off, root rots and complex root and stalk rot diseases. For example, *P. myriotylum*, a known pathogen of peanuts, was commonly isolated from necrotic roots and hypocotyls of peanut seedlings and mature plants from the central provinces. *P. spinosum* was isolated less frequently from peanuts. *P. aphanidermatum* was commonly isolated as follows: from the roots and lower stem of chrysanthemums affected by severe root rot in Quang Nam and Thua Thien Hue provinces; from tomato seedlings affected by damping-off in a nursery in Lam Dong province; from roots of maize affected by stalk and root rot in association with *Rhizoctonia* sp. in Quang Nam province. Two other *Pythium* species were isolated from other hosts and are the subject of further taxonomic studies using morphological and molecular characteristics. For these diseases, integrated disease management strategies are being developed. They highlight improved drainage using raised beds, the use of pathogen-free transplants, timely application of fungicide and crop rotation. Improved hygiene in commercial nurseries is being promoted.

2.29 PROTEOMICS OF *MONILIOPHTHORA PERNICIOSA*, CAUSAL AGENT OF THE WITCHES'-BROOM DISEASE OF CACAO. **S. Pierre, R.N. Birch, J.V. Hamilton, R. Morphew, I.M. Scott and G.W. Griffith.** *Institute of Biological Sciences, Aberystwyth University, Aberystwyth, Ceredigion, SY23 3DD, Wales, UK. Email: ssp06@aber.ac.uk*

Witches' broom disease (WBD), caused by the basidiomycete *Moniliophthora* (formerly *Crinipellis*) *perniciosa*, is one of the greatest threats to world cacao production, having already deci-

mated the industry in South America. Its biology and genetics have also been studied over 30 years at Aberystwyth, Wales and increasing research interest worldwide has led to a number of recent advances, for instance a genome sequencing programme and extensive transcriptomic studies in Brazil. This unusual hemibiotrophic basidiomycete has two life stages, exhibiting intercellular growth *in planta* after basidiospore germination and infection of meristems, followed by a prolonged saprotrophic (lignocellulose-degrading) phase in necrotic host tissues. The mechanisms involved in host specificity, broom formation and the switch to saprotrophy are still poorly understood. We have taken a proteomic approach (2D gel electrophoresis combined with mass spectrometry) to identify proteins specifically associated with isolates of differing pathogenicity on cacao and also to compare different biotypes of the fungus (infecting non-cacao hosts). A protein specifically expressed in a particularly virulent strain from Bahia – where the disease is particularly destructive – has been identified, by microsequencing, as an aldo-keto reductase. This protein could be related to stress and/or hormonal response in this strain. We have also detected host defence (PR) proteins during infection of a tomato model system.

2.30 *FUSARIUM* SPECIES ASSOCIATED WITH VANILLA STEM ROT IN INDONESIA. **A. Pinaria, L.W. Burgess and E.C.Y. Liew.** *Faculty of Agriculture, Food and Natural Resources, The University of Sydney, NSW 2006, Australia. Email: apin3761@mail.usyd.edu.au*

Indonesia is one of the world's leading producers of vanilla, an important crop offering high economic returns to small-holder farmers. A major constraint in vanilla production in Indonesia is stem rot disease, which has caused significant economic losses over the last decade. Previous reports of vanilla stem rots in the Asia pacific region include those caused by *Fusarium*, *Colletotrichum*, and *Phytophthora* species. In this paper, we report *Fusarium* species associated with the disease. Seven major vanilla-producing provinces were surveyed for disease incidence. Isolates were obtained from diseased stem tissues using selective media. Pure cultures were subcultured onto CLA and PDA for species identification. A total of 542 *Fusarium* isolates were recovered, comprising 7 species, namely *F. decemcellulare, F. oxysporum*, *F. proliferatum*, *F. pseudograminearum, F. semitectum*, *F. solani*, *F. subglutinans*, and 14 isolates of undescribed species. *F. oxysporum* was most commonly isolated from all the areas surveyed, followed by *F. solani and F. semitectum*. Of the species tested in pathogenicity studies, only *F. oxysporum* was shown to be pathogenic on vanilla. Further studies to investigate genetic diversity of the pathogen and host resistance are underway.

2.31 VARIATION IN *PHYTOPHTHORA PALMIVORA* ISOLATES ON COCOA IN PAPUA NEW GUINEA. **J. Saul Maora, E.C.Y. Liew and D.I. Guest.** *Faculty of Agriculture Food and Natural Resources, The University of Sydney, NSW 2006, Australia. Email:d.guest@usyd.edu.au*

In Papua New Guinea (PNG), cocoa is the major source of income for 150,000 smallholder families in the lowlands and island regions, who produce over 80% of the national cocoa crop. The main cocoa-producing areas are East New Britain (ENB), Bougainville, Madang, Karkar and East Sepik. Growers lose about 40% of their production to *Phytophthora* pod rot and canker annually. Currently all cocoa planting material is bred in ENB and distributed throughout the country without any comprehensive knowledge of the pathogen population structure. Furthermore, all current *Phytophthora* control recommendations for the country were developed in ENB. This study tested the hypotheses that 1. *Phytophthora palmivora* is the sole *Phytophthora* species causing pod rot on cocoa in PNG, and 2. that there is variation in the pathogen populations from the 5 major cocoa growing locations. Diseased cocoa pods were sampled hierarchically from each of the 5 locations, including 8 farms/location and 8 diseased pods/farm. Morphological, physiological, biological, molecular and pathological variation of 263 isolates were studied. While *P. palmivora* was found to be the sole *Phytophthora* species causing disease on cocoa in PNG, the variability in colony and sporangial morphology, pathogenicity, and DNA polymorphisms revealed a high level of diversity. In Madang both mating types were found in similar abundance. Hierarchical sampling revealed that the level of diversity within farms is very high, and that there is a similar level of diversity at the local, regional and national scales. Disease management strategies should not be affected by regional differences in the pathogen population.

2.32 PCR DETECTION OF *CANDIDATUS* LIBERIBACTER ASIATICUS FROM *MURRAYA PANICULATA* AND PSYLLID VECTOR IN THAILAND. **R. Sdoodee, P. Tothaum and A. Jumpaduang.** *Dept. of Pest Management, Fac. of Natural Resources, Prince of Songkla University, Hat Yai, 90112, Thailand. Email: ratana.sd@psu.ac.th*

Candidatus Liberibacter asiaticus is a causal pathogen of Huanglongbing (HLB), the most destructive disease in citrus worldwide including Thailand. The pathogen is spread by the Asian citrus psyllid, (*Diaphorina citri* Kuwayana). Orange jasmine (*Murraya paniculata*), a preferred host of *D. citri* has been reported to be a cryptic or symptomless host of *Ca.* L. asiaticus. Since orange jasmine is widely grown as an ornamental in Thailand, it could be harboring *Ca.* L. asiaticus and act as inoculum source of for HLB. During 2002-2006, *M. paniculata* and *D. citri* were collected from Chiang Mai and Songkla provinces and assayed by directional PCR using primers specific to 16s rDNA of *Ca.* L. asiaticus. PCR amplicons were sequenced and analyzed using the GenBank database. Results from the PCR and sequence analysis indicated that 2/6 and 3/10 *M. paniculata* samples collected from Chiang Mai and Songkla, respectively, were infected with *Ca.* L. asiaticus. The sequences from Songkla shared 100% identity to an Okinawa isolate of *Ca.* L. asiaticus. Eighty percent of the *D. citri* collected from naturally infected Shogun mandarin (*Citrus reticulata* Blanco) grown near the infected *M. paniculata* (600 m apart) at Prince of Songkla university were carrying *Ca.* L. asiaticus. *D. citri* adults and nymphs were found on the *M. paniculata* trees. It is speculated that *D. citri* might have transmitted *Ca.* L. asiaticus from the infected Shogun mandarin to *M. paniculata.*

2.33 CACAO WITCHES' BROOM CAUSED BY *MONILIOPHTHORA PERNICIOSA*: CAN NUMBER OF BASIDIOMATA PRODUCED BE USED AS A MEASURE OF RESISTANCE? **S.D.V.M. Silva, E.D.M.N. Luz and L.P. Santos Filho.** *Plant Pathology & Socio-economics Science Division, CEPLAC Cocoa Research Center, P.O. Box 07, 45600-970 Itabuna, Bahia, Brazil. Email: stela@ceplac.gov.br*

Witches' broom disease (WBD) caused by *Moniliophthora perniciosa* is a devasting disease of cacao in Latin America and the Caribbean islands. Vegetative dried brooms are epidemiologically important as the most productive and consistent source of

inoculum in the field. Several cacao genotypes are under evaluation for resistance to WBD in Bahia, Brazil. Ten dry vegetative brooms for each of twenty genotypes evaluated in field trials were collected and hung inside a shade house with ideal conditions for basidiomata production. The number of basidiomata produced per broom was counted daily during a year and evaluated as a tool to discriminate between levels of resistance, and its correlation to field resistance. There were differences among the genotypes evaluated, but this measure of resistance was not correlated to field resistance as measured by number of brooms per plant, or to number of infected pods. Some genotypes under field conditions showed low numbers of brooms and or infected pods but the brooms from these genotypes under optimum humidity conditions produced a high number of basidiomata. The experiment was repeated for a second year and similar results were obtained.

2.34 MANAGEMENT OF BUNCH ROT COMPLEXES OF GRAPES IN HIGH SUMMER RAINFALL AREAS OF AUSTRALIA. **C.C. Steel, S. Savocchia, L.A. Greer and C. Haywood.** *National Wine & Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. Email: csteel@csu.edu.au*

Bunch rot of grapes results in economic losses particularly in regions where rainfall is common during the ripening period. Frequently a range of bunch rotting fungi can be isolated from the one vineyard and from the one bunch. Surveys of a sub-tropical vineyard in the 2004/5 and 2005/6 growing seasons at various stages of fruit development revealed seasonal differences in the fungal profile of the reproductive structures which can be attributed to climatic differences from year to year. Despite this, the predominant bunch rot pathogens were *Colletotrichum acutatum* and *Greeneria uvicola* (responsible for ripe rot and bitter rot of grapes, respectively) in both years. *Botrytis cinerea* (commonly associated with bunch rot of grapes) was largely absent from the vineyard examined. Other bunch rot pathogens recovered were *Alternaria*, *Botryosphaeria*, *Cladosporium* and *Phomopsis*. In field trials fluazinam applied as a spray during the winter dormancy was found to be effective in reducing the overall incidence of bunch rot at harvest, presumably by limiting inoculation load in the vineyard. *Pyraclostrobin* applied at flowering also reduced bunch rot incidence, suggesting that latent flower infections may lead to bunch rot at harvest. Bunch rot incidence at harvest was further reduced by applying strobilurin fungicides post-veraison during the ripening period. However, this option is of limited use to grape-growers in Australia because of withholding periods set for grapes destined for the wine export market. Management of non-Botrytis bunch rot of grapes is currently not sustainable in areas of high disease pressure.

2.35 THE ANTHRACNOSE DISEASE COMPLEX OF CHILLI PEPPER. **P.W.J. Taylor, O. Mongkolporn, P.P. Than, P. Montri, P. Mahasuk, N. Ranathunge, R. Ford, S. Pongsupasamit and K.D. Hyde.** *Center for Plant Health/BioMarka, Faculty of Land and Food Resources, The University of Melbourne, VIC 3010, Australia. Email: paulwjt@unimelb.edu.au*

Anthracnose disease is the most important biological constraint to chilli pepper (*Capsicum annuum* L.) production in south east Asia. Three major *Colletotrichum* species: *C. capsici*, *C. acutatum*, *C. gloeosporioides*, were isolated from chilli fruit in Thailand, Sri Lanka, Korea, Malaysia and Australia that showed typical anthracnose symptoms. These pathogens were isolated separately or together from infected fruit however, *C. acutatum* and *C. capsici* appeared to be the most severe being able to infect a range of *Capsicum* species and resistant genotypes. Each species can be distinguished by distinct spore shape, growth rate in culture on potato dextrose agar and by phylogenetic analyses from DNA sequence data of the ITS rDNA and b-tubulin (*tub2*) gene regions. Three pathotypes, based on qualitative infection on differential genotypes, for each of *C. capsici* and *C. acutatum* isolates have been identified. This will have major impact on chilli breeding programs. The population genetics of *C. capsici* is being studied using species-specific sequence-tagged microsatellite sites (STMS) markers to analyse allele flow within and between populations. 10 polymorphic loci have been identified producing 3-8 alleles among *C. capsici* isolates. Preliminary results identified a high level of genetic diversity both within and between populations of *C. capsici* confirming the usefulness of these markers to study genetic diversity and the adaptability of the pathogen to overcome control measures such as host resistance genes and fungicides.

2.36 *COLLETOTRICHUM* SPECIES CAUSING PEPPER ANTHRACNOSE IN BRAZIL. **H.J. Tozze Jr., M.P.S. Camara, R. Gioria, O. Suzuki, K.R. Brunelli, R.S. Braga and N.S. Massola Jr.** *Setor de Fitopatologia, Universidade de São Paulo, P.O. Box 09, Zip Code 19.418-900, Piracicaba, SP, Brazil. Email: nmassola@esalq.usp.br*

Anthracnose is one of the major diseases of pepper in Brazil. In this country only *Colletotrichum gloeosporioides* is reported as its causal agent. However, there are no thorough studies concerning species identification in Brazil. Our objective was to identify the *Colletotrichum* species associated with pepper anthracnose in Brazil. Fifty six *Colletotrichum* isolates from the major pepper-producing areas were characterized through morphology, cultural characteristics, PCR, and sequencing of the ITS region. Morphology procedures were based on conidial shape and size. Cultural characterization was achieved by aspect and colour of the colony, as well as its growth rates on PDA at 25°C. Genetic characterization was done by PCR with species-specific primers, as well as by sequencing the ITS1-5.8S-ITS2 region of the rDNA. Results showed that *C. acutatum* is the most frequent species in the major producing regions of the country, representing 72% of samples. *C. capsici* and *C. coccodes* were also found, respectively in frequencies of 14% and 2%. The former species was found in São Paulo and Minas Gerais states and the latter was restricted to Rio Grande do Sul state. *C. gloeosporioides*, so far reported as the only causal species, showed a frequency of 5% and was only found in São Paulo and Federal District. Isolates identified as *C. boninense* were found in São Paulo and Rio Grande do Sul states with a frequency of 7%. This is the first report in the world of this latter species affecting solanaceous plants.

2.37 IDENTIFICATION OF *COLLETOTRICHUM* SPECIES ASSOCIATED WITH PASSIFLORA ANTHRACNOSE IN SAO PAULO STATE, BRAZIL. **H.J. Tozze Jr., I.H. Fischer, M.P.S. Camara, M.P. Moreno and N.S. Massola Jr.** *Setor de Fitopatologia, Universidade de Sao Paulo, P.O. Box 09, Zip Code 19.418-900, Piracicaba, SP, Brazil. Email: nmassola@esalq.usp.br*

Anthracnose is the major disease of passiflora in the state of São Paulo, Brazil. In this country, only *Colletotrichum gloeosporioides* has been reported as its causal agent. The goal of this study was to identify the *Colletotrichum* species associated with passiflora an-

thracnose in Sao Paulo state. Thirty six *Colletotrichum* isolates from the major passiflora producing regions were characterized through morphology, cultural characteristics, pathogenicity and, sequencing of the ITS region. The morphological markers were conidial shape and size. Aspect and colour of the colony, as well as its growth rates on PDA at 25ºC were noted. The ITS1-5.8S-ITS2 region of the rDNA was sequenced. All procedures showed that at least three *Colletotrichum* species were associated with passiflora anthracnose in Sao Paulo. *C. boninense* was the most frequent species (n=8) followed by *C. gloeosporioides* (n=4) and *C. capsici* (n=1). Besides these species, two distinct groups of isolates were also identified based exclusively on the sequences. The first one (n=4) grouped with *C. magna*. The second one (n=19) grouped close to *C. boninense* but the ITS sequencing showed these isolates to be quite distinct from this species. All isolates were pathogenic to passiflora fruits and pathogenicity was highly variable. The results clearly showed that passiflora anthracnose in São Paulo state is a complex disease. This fact can directly affect the efficiency of measures adopted for controlling this disease.

2.38 GROUPING BASED ON SEQUENCE LENGTH OF THE ITS REGION AMONG ISOLATES OF *PHELLINUS NOXIUS* FROM TAIWAN. J.N. Tsai, P.J. Ann and W.H. Hsieh. *Plant Pathology Division, Taiwan Agricultural Research Institute, 189 Chung-cheng Road, Wufong, Taichung 41362, ROC. Email: tsaijn@wufeng.tari.gov.tw*

Brown root rot caused by *Phellinus noxius* is widespread in tropical countries in Southeast Asia, Africa, Oceania, Central America, and the Caribbean. In Taiwan, the pathogen attacks more than 120 species of woody fruit and ornamental trees in both tropical and subtropical districts below 1000 m elevation. We examined the internal transcribed spacer region (ITS1, 5.8S, ITS2) of rDNA of 30 isolates of *P. noxius* obtained from various hosts at different locations in Taiwan. Based on the nucleotide sequence length of the ITS region, the Taiwanese isolates of *P. noxius* could be divided into two types, type L with 614 bp and type S with 606 bp. There was a 8 bp deletion between positions 135 and 142. The majority of isolates tested belonged to type L, only about 20% being type S. Isolates from many plant species contained both types L and S. However, isolates from some plant species were either type L or type S. At some locations, isolates of *P. noxius* contained both types L and S, while at other locations, they were either type L or type S.

2.39 CHARACTERIZATION OF *GIBBERELLA FUJIKUROI* SPECIES COMPLEX ASSOCIATED WITH BAKANAE DISEASE OF RICE IN AFRICA AND ASIA. E.G. Wulff, M. Lübeck and J. Torp. *University of Copenhagen, Faculty of Life Sciences, Department of Plant Biology, Thorvaldsensvej 40, entrance 2, 1st floor, DK-1871 Frederiksberg C, Denmark. Email: ewu@life.ku.dk*

Isolates of *Gibberella fujikuroi* species complex associated with bakanae disease of rice (*Oryzae sativa* L.) were collected from rice seed samples originating from Asia (China, Vietnam, India and Nepal) and Africa (Tanzania, Ghana, Burkina Faso and Ivory Coast). Universal Primer PCR (UP-PCR) DNA fingerprinting and translation elongation factor 1-alpha (TEF) DNA sequences were employed to study species diversity and to elucidate the genetic structure of the African and Asian populations. Preliminary results showed that there is a broad genetic variation present in isolates obtained from China and Vietnam compared to other countries. Studies to determine seed and soil transmission ability and pathogenicity on rice are going to be conducted in order to determine the role of the different sources of inoculum, to verify the ability of the isolates to induce bakanae symptoms on rice seedlings and to correlate the findings with the molecular data obtained.

URBAN PLANT PATHOLOGY

12.1* NEW THREAT TO EUROPEAN URBAN TREES: A CASE STUDY OF *EUTYPELLA* CANKER OF MAPLE IN LJUBLJANA AND SOURROUNDINGS. D. Jurc and N. Ogris. *Slovenian Forestry Institute, Department of Forest Protection, Večna pot 2, SI-1000 Ljubljana, Slovenia. Email: dusan.jurc@gozdis.si*

Eutypella canker of maple (*Eutypella parasitica*), a destructive disease of maples (*Acer* spp.) originating in North America, was recently found in Slovenia, Austria and Croatia. The disease develops slowly and usually does not kill the infected tree. Extensive cankers of the trunk develop during the tree's lifetime and wood discoloration extends much further than the visible bark necrosis. The diseased tree is prone to windbreak and its aesthetic value is reduced. In Europe the disease is still spreading and finally it will probably cover the whole continent. Maple species have different susceptibilities to the disease. For the indigenous species and for many exotic maples grown in Europe there is no data on their susceptibility or disease development. This was the reason for performing an extensive inventory of *Eutypella* canker of maple in Ljubljana and in surrounding forests, to test the observations that the disease is more prevalent in urban areas than in managed forests. We report on the disease incidence in urban trees and in managed forests, the affected species and the probable mode of infection.

12.2* SPREAD AND VIRULENCE OF *BOTRYOSPHAERIA DOTHIDEA* ON BROADLEAVED TREES IN URBAN PARKS OF NORTHERN ITALY. S. Moricca, A. Uccello, E. Zini, F. Campana, R. Gini, B. Selleri, R. Tucci, S. Anderloni, P. Pirelli and A. Ragazzi. *Dipartimento di Biotecnologie Agrarie, Sezione di Patologia vegetale, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy. Email: salvatore.moricca@unifi.it*

The canker pathogen *Botryosphaeria dothidea* (Moug.: Fr.) Ces & De Not (anamorph *Fusicoccum aesculi* Sacc.) was isolated from trees growing in some parks in urban and peri-urban areas of northern Italy. A number of species in the genera *Acer*, *Alnus*, *Carpinus* and *Quercus* were found infected. The fungus caused elongated stem cankers that killed branches and whole trees in a couple of years after symptoms first appeared. A number of climatic, biological and silvicultural parameters were taken into account in an attempt to clarify the aetiology of the disease. Successive yearly and seasonally-extended drought episodes were a major factor in inducing tree susceptibility and determining the onset and spread of the disease. Pathogen virulence was tested within a growing season on 240 1-year-old seedlings of *Quercus robur* and *Acer platanoides* (120 seedlings per species). Plants were artificially infected through bark wounding with 14-day-old colonies of the pathogen. Plants were watered bi-weekly to soil capacity and inspected at regular time intervals to determine disease incidence and severity. Infected seedlings presented typical, sunken and elongated stem lesions, with bark discoloration and necrosis around the inoculation site. The pathogen sporulated profusely on cankered tissues and was easily re-isolated. *Quercus robur* was more susceptible than *Acer platanoides*.

12.3* MOLECULAR DETECTION AND EARLY IDENTIFICATION OF WOOD DECAY AGENTS IN STANDING TREES. G. Nicolotti, P. Gonthier, F. Guglielmo and M. Garbelotto. *DI.VA.P.R.A. Dept. for Exploitation and Protection of Forestry Resources, University of Torino, Via L. da Vinci 4, 10095 Grugliasco (TO), Italy. Email: giovanni.nicolotti@unito.it*

Detection and identification of wood-rotting fungi in standing trees is crucial for predicting the severity and development of decay. With very active root and butt-rot fungi, early identification is important to establish the most appropriate failure risk classification. In this work, we have developed a method for the identification directly from wood of some of the most important and widespread decay fungi. We used taxon-specific primers combined in 5 multiplex PCRs. The method proved to be efficient and specific for the diagnosis and early detection of the target fungi, starting from DNA extracted directly from wood of standing trees.

12.4* FURTHER STUDIES ON THE ETIOLOGICAL AGENTS OF *CARPINUS BETULUS* DECLINE. M. Saracchi, F. Rocchi and S. Quaroni. *Istituto di Patologia Vegetale, Via Celoria 2, 20133 Milano, Italy. Email: marco.saracchi@unimi.it*

In Lombardy (northern Italy), there was recently an increase in reports of decline and death of European hornbeam trees (*Carpinus betulus*), especially in urban green areas and parks. Notes in the literature about such symptoms are few and often differ in some characteristic features. Large lesions, characterized by red resin-like clusters gathered in more or less numerous groups and by globose reproductive structures emerging from cortical surfaces and producing orange-yellowish cirri, were often found on the trunks and branches. Morphocultural and biomolecular studies ascertained the presence of two different fungi, forming red and orange masses respectively, in the genera *Naemospora* and *Endothiella*. Experimental infections confirmed both fungi as hornbeam pathogens. Taxonomic identification to species was attempted using micromorphology and different DNA sequences but no satisfactory correspondences with published data were obtained. Comparison of these two fungi on *C. betulus* with strains of other possibly related fungi confirmed their difference from previously described species. Samples collected in several parks, gardens and green areas in the same region were analysed and the isolates described using different morphocultural and physiological characteristics. The *Naemospora* and *Endothiella* isolates were also compared in relation to some mini- and microsatellite patterns and vegetative compatibility. The results stressed the variability of both populations, showing differences among strains isolated from hornbeam grown in several localities of the same region, sometimes not so far from each other.

Vascular plant pathogens

4.1 GRAPEVINE WILT DISEASE IN JORDAN (AJLOON PROVINCE). A. Al-Momany. *Department of Plant Protection, Faculty of Agriculture, University of Jordan, Amman, Jordan. Email: momanyah@ju.edu.jo*

In Jordan, dieback of grapevine branches is becoming increasingly important and is very common in old vineyards in which establishment is slow and uneven. Twenty nine vineyards older than 7 years were chosen in Ajloon province. The number of vines per farm varied from 181–437 in the study area. The disease symptoms most evident during early March when the new shoots are 15–20 cm long are deformation and discoloration of the shoots. Young leaves are smaller than normal, are cupped, and develop tattered margins. Foliar symptoms often appear only on one cordon of an infected vine. Bunches on affected shoots may have a mixture of large and small berries and ripen unevenly. A cross section of an infected cordon shows a wedge of dead, brown tissue. Pathogens cannot be cultured from green shoots of the current season growth but only from cordons more than two years old. The disease incidence varied from 5-75% where disease severity ranged from 9.5-23 % of the vineyards studied. *Eutypa maura*, *Fusarium*, *Phoma*, *Pestalotiopsis* and *Phialophora* were isolated only from cane stump necrosis for the first time in Jordan. Perithecia with ascospores were found on stromatic tissue on the surface of dead wood and were collected from old pruned branches during April. Ascospores were allantoid in shape, orange-yellow in colour, and from 7.6-12.8 µm in length and 1.9-3.8 µm in width.

4.2 DIFFERENTIAL RESPONSE OF GRAPEVINE ROOTSTOCKS INOCULATED WITH THE MAIN CAUSAL AGENTS OF PETRI DISEASE IN CHILE. J. Auger, M. Esterio, G. Díaz and I. Pérez. *Depto. de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, P.O. Box 8820808, Chile. Email: jauger@uchile.cl*

Incidence of Petri disease in Chile has recently increased. The disease affects grapevines between 1 and 5 years old. Chile is the main exporter of table grapes from the southern hemisphere and one of the biggest exporters of wine from the new world, but studies have not been done to assess the effect of Chilean isolates of the endophytic fungi *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* on the quality of grape rootstock propagation cuttings. With this objective, the impact was assessed of these fungi on quality parameters in five main grape rootstocks at present used in the country: Kober 5BB, SO4, 3309 C, 101-14 MG, 1103 P, top-grafted with Carménère. The treatments consisted of inoculations with *P. chlamydospora*, *P. aleophilum*, *P. chlamydospora* plus *P. aleophilum* and distilled water (control). The parameters evaluated in the nursery were: the base end callus, rooting, grafting callus and sprouting; and in the greenhouse: the length of the streak formed inside of each cutting. Our results indicated that the fungi negatively affect the quality of grapevine rootstocks, but to different degrees. The SO4 rootstock had the highest susceptibility, followed by Kober 5BB and 3309 C. Rootstocks 1103 P and 101-14 MG were the least susceptible.

4.3 *PHAEOMONIELLA CHLAMYDOSPORA* CAUSING A SEVERE OUTBREAK OF PINOT NOIR DECLINE AND BLIGHT BUNCH IN CHILE. J. Auger, M. Esterio and I. Pérez. *Depto. de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, P.O. Box 8820808, Chile. Email: jauger@uchile.cl*

Phaeomoniella chlamydospora has been implicated as the causal agent of Petri vine decline in many countries. In Chile, it has only recently been recognized and little is known on the genetic composition and pathogenic variability of the chilean population. A severe outbreak of disease on Pinot Noir was reported recently. Five to seven year-old grapevines showed reduced vigor, shortened internodes, uneven wood maturity, growth decline, stem necrosis, early defoliation and bunch blight. In wood cross sections, necrotic and plugged xylem vessels with black inclu-

sions and tyloses appeared. *P. chlamydospora* was isolated from Pinot Noir plants with these symptoms. The isolates were collected from grapevines with decline symptoms for morphological and molecular characterization. Pathogenicity was tested on greenhouse Pinot Noir plants. To assess phylogenetic divergence, the isolates were analyzed using sequence data from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA and the beta-tubulin gene region. Based on divergence DNA and morphological characteristics, these *P. chlamydospora* isolates differ from *P. chlamydospora* strains already described from other Chilean grapevine cultivars.

4.4 DEVELOPMENT AND APPLICATION OF MICROSATELLITE AND SINGLE NUCLEOTIDE POLYMORPHIC MARKERS IN *VERTICILLIUM DAHLIAE*. **M. Berbegal, J. Armengol and M.M. Jiménez-Gasco.** *Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain. Email: mobermar@etsia.upv.es*

Verticillium dahliae is a soilborne asexual pathogen that causes vascular diseases of many economically important crops worldwide. Earlier studies based on vegetative compatibility and molecular analyses indicated a high level of genetic diversity in *V. dahliae* populations. Isolates within each vegetative compatibility group (VCG) seem to be genetically similar, but there is a need for suitable markers that are highly polymorphic to characterize *V. dahliae* populations. In this study, 5 microsatellite (simple sequences repeats, SSRs) and 7 single-nucleotide polymorphic (SNP) markers were identified from a genomic DNA library highly enriched for SSRs, constructed using the Dynabead biotin-enrichment strategy. Twenty percent of the sequenced clones contained SSRs, and primer pairs were designed from the flanking regions of the repeats. Allelic variation was assessed on 46 haploid *V. dahliae* isolates from diverse geographic origins, hosts and VCGs. Different strategies were used to screen for polymorphisms: polyacrylamide gel electrophoresis, sequence analysis of the amplified regions and fluorescence-based capillary electrophoresis. Analyses of the 5 SSR and 7 SNP markers showed a total of 50 alleles (each locus produced between 2 and 12 alleles). Phylogenetic analyses of individual and combined datasets of SSR and SNP markers showed that isolates of each VCG subgroup are molecularly similar and can be clearly differentiated from others. Markers identified in this study constitute an outstanding tool for the analysis of *V. dahliae* populations.

4.5 *CERCOSPORA INSULANA* ON STATICE IN BAHIA, BRAZIL. **J.L. Bezerra, A. Santos and N. Santos Vitoria.** *CEPLAC/CEPEC, Plant Pathology Section, km 22, Itabuna-Ilheus Road, Cx. Postal 7, Itabuna-BA, Brazil. Email: jlulabezerra@hotmail.com*

Limonium sinuatum (L.) Mill. (Statice) is cultivated in the municipalities of Morro do Chapeu and Maracas, State of Bahia, northeastern Brazil, where the flowers command a good price. The crop is affected by foliar diseases, mainly *Colletotrichum* and *Cercospora* leaf spots. *Cercospora insulana* (Sacc.) Sacc. was identified in the laboratory as the causal agent of *Cercospora* leaf spot of statice in Bahia. This is the first report of the disease in Brazil.

4.6 RESISTANCE TO SYSTEMIC COLONISATION BY THE VASCULAR PATHOGEN *VERTICILLIUM LONGISPORUM* IN *ARABIDOPSIS THALIANA*. **E. Häffner and E. Diederichsen.** *Freie Universität Berlin, Institut für Biologie, Angewandte Genetik, Albrecht-Thaer-Weg 6, D-14195 Berlin, Germany. Email: haeffner@zedat.fu-berlin.de*

Verticillium longisporum is a soil-borne fungus specifically infecting cruciferous hosts, including *Arabidopsis thaliana*. It penetrates host roots, grows towards the xylem and spreads within the xylem vessels, thus colonising the whole plant. We have observed that ecotypes of *A. thaliana* differ in their susceptibility to systemic colonisation; whereas Landsberg erecta (Ler) shows a high degree of systemic colonisation, the ecotype Burren (Bur) is only rarely colonised. Time-course experiments have shown that colonisation in Ler already starts when the first flowers open and reaches much higher levels towards the end of development than in Bur, which is only colonised to a small degree at the onset of fruit maturation. Other *Verticillium* symptoms such as stunting and fresh-weight loss vary depending on environmental factors such as season. The differences in systemic colonisation are less prone to environmental influence and could be reproduced in nine independent greenhouse tests so far. This indicates a high heritability of this trait. A Bur × Ler F2/F3-mapping population was established in order to locate genes/QTL conferring colonisation resistance. The F1-generation was colonisation-resistant, suggesting dominant inheritance. Frequency distribution of colonisation in the F3-families indicated that more than two genes are involved in conferring resistance. Colonisation resistance in the F3-families was positively correlated with the length of the developmental cycle. We expect that the trait is at least partially controlled by genes linked to genes regulating flowering behaviour.

4.7 RESTORATION OF SEXUALITY IN *FUSARIUM OXYSPORUM*. **S. Imai, T. Teraoka and T. Arie.** *Laboratory of Plant Pathology, Tokyo University of Agriculture and Technology (TUAT), Fuchu, Tokyo, Japan. Email: arie@cc.tuat.ac.jp*

In heterothallic ascomycete fungi, isolates of opposite mating type are required to initiate the sexual cycle. Isolate mating-type is determined by the *MAT1* locus carrying one of two highly divergent alleles known as idiomorphs that are designated *MAT1-1* or *MAT1-2*. *Fusarium oxysporum* is considered to be an asexual ascomycete lacking a sexual phase in its life cycle. However, *MAT1* idiomorphs were identified in the *F. oxysporum* genome, homologous to those in *Gibberella fujikuroi*, a sexual relative of *F. oxysporum*, and the *MAT1* genes were expressed. A recent phylogenetic study of *F. oxysporum* f.sp. *lycopersici* (FOL), the tomato wilt fungus, revealed three well-supported clades, each of which contained isolates of only one mating type. Moreover, whole-chromosome PFGE analysis revealed that electrophoretic karyotypes were highly variable among clades. These data suggest that *F. oxysporum* may be unable to complete the sexual cycle due to chromosomal divergence among clades and lack of a compatible mate within each clade. To address this hypothesis, we generated a transformant of FOL in which the *MAT1-2* idiomorph was replaced with the *MAT1-1* idiomorph. The electrophoretic karyotype of the transformant was identical to that of the parental isolate. When the original isolate (*MAT1-2*) and the transformant (*MAT1-1*) were paired under mating conditions, they were able to complete the sexual cycle producing mating-specific organs such as perithecia, asci and ascospores.

4.8* PHYLOGENETIC ANALYSIS OF *FUSARIUM OXYSPORUM* ISOLATED FROM THE TISSUES AND RHIZOSPHERE OF *LYCOPERSICON* SPP. K. Inami, M. Kawabe, A. Okabe, N. Ishikawa, T.L. Peever, M. Kodama, T. Teraoka and T. Arie. *Laboratory of Plant Pathology, Tokyo University of Agriculture and Technology (TUAT), Japan. Email: keyloveit@gmail.com*

Fusarium oxysporum f. sp. *lycopersici* (FOL) is a soilborne pathogen causing vascular wilt disease of tomato. *F. oxysporum* is also a ubiquitous soil resident and non-pathogenic strains (NPF) are frequently isolated from tomato tissues and rhizosphere. Our working hypothesis is that NPFs have acquired pathogenicity factors during their non-pathogenic association with tomato, allowing FOL strains to emerge. The origin of edible tomato (*Lycopersicon esculentum*) is considered to be in the Andean Plateau and several wild *Lycopersicon* spp. are found there. Some of these were transported to Mexico where cultivated *L. esculentum* is thought to have been selected. After the 16th century, *L. esculentum* was spread worldwide. To address our hypothesis, we sampled fungi from wild, symptomless *Lycopersicon* spp. and the soil in which these plants were grown in Chile, Ecuador, and Mexico. From tissues and rhizosphere soil we obtained approximately 200 *F. oxysporum* isolates. Pathogenicity of these isolates was tested, and not all produced wilt symptoms on tomato. Approximately 600 base pairs of the nuclear ribosomal intergenic spacer was sequenced from each isolate and used to estimate a phylogeny which included FOL and other *formae speciales* of *F. oxysporum*. Isolates sampled from the *Lycopersicon* spp. and rhizosphere soils were distributed randomly throughout the phylogeny. Some of the isolates had sequences identical with FOL isolates. These results support our hypothesis.

4.9 GENETIC DIVERSITY IN POPULATIONS OF *VERTICILLIUM DAHLIAE* INFECTING OLIVE IN ANDALUSIA, SOUTHERN SPAIN. R.M. Jiménez-Díaz, C. Olivares, J.A. Navas-Cortés, B.B. Landa and M.M. Jiménez-Gasco. *ETSIAM, Universidad de Córdoba, Edificio C4 "Celestino Mutis", Campus Rabanales, Carretera de Madrid km 396,14071 Córdoba, and and Instituto de Agricultura Sostenible, CSIC, Apdo. 4084, 14080 Córdoba, Spain. Email: ag1jidir@uco.es*

The genetic diversity among *Verticillium dahliae* infecting olive was studied in 637 monoconidial isolates derived from stratified sampling of 72 olive orchards in the five most important olive-growing provinces in Andalusia. *Verticillium* wilt prevailed in young, irrigated olive orchards. *V. dahliae* isolates were typed to vegetative compatibility groups (VCG) using *nit* mutants from both international OARDC reference strains and local strains, as well as by PCR assays using primer sets that differentiate the olive-defoliating and -nondefoliating *V. dahliae* pahotypes, and DNA sequence analysis of a 539/523-bp *V. dahliae*-specific PCR amplicon. VCG1A, VCG2A and VCG4B were identified among 636 isolates and one isolate was heterokaryon self-incompatible. VCG1A comprised 78% of isolates and was the predominant VCG in Córdoba, Jaén and Seville provinces. A single VCG occurred among isolates within an orchard, but two or three VCGs were found occasionally. Three of the seven currently known sequences (*seq1* through *seq7*) of the *V. dahliae*-specific amplicon were identified among the isolates in the study: *seq1*, *seq2*, and *seq4*. However, there were new correlations among *seq* sequences and VCGs as compared to those previously reported. VCGs and *seq1*, *seq2*, and *seq4* distribution among isolates from different provinces suggests that genetic diversity within *V. dahliae* populations is higher in provinces were VCG1A is not prevalent. Results indicate that the defoliating pathotype comprised in VCG1A has spread extensively in the olive-growing area of southern Spain. This poses a serious threat to olive production in Andalusia because no resistance is available against this pathotype in currently grown olive cultivars.

4.10 GENETICS OF RESISTANCE TO *VERTICILLIUM LONGISPORUM* IN *BRASSICA ALBOGLABRA*. S. Konietzki and E. Diederichsen. *Freie Universität Berlin, Institut für Biologie, Angewandte Genetik, Albrecht-Thaer-Weg 6, D-14195 Berlin, Germany. Email: elked@zedat.fu-berlin.de*

Verticillium longisporum is a plant pathogenic fungus affecting cruciferous plants such as *Brassica napus*, *Brassica oleracea* or *Arabidopsis*. Its relevance in the middle and northern European growing areas of oilseed rape has strongly increased in the last decades. Stunting and growth abnormalities are the most common symptoms in greenhouse assays, whereas in the field the fungus affects yield and seed size by precocious maturation. Whereas *B. napus* shows only little variation for resistance, resistance sources have been identified in its two ancestors, *B. oleracea* and *B. rapa*. Our aim is to identify the genetic basis of these resistance sources and to support resistance breeding in *Brassica* by developing molecular markers. We identified two accessions of strongly contrasting disease reactions in *B. alboglabra*, a close relative of B. oleracea, and used these to generate an F2/F3- mapping population. This population was studied in greenhouse assays for resistance and using PCR-based markers. Different disease parameters were analysed: AUDPC, % colonisation, and fresh weight. Results of the genetic analysis of *Verticillium* resistance in the segregating population will be presented.

4.11 EVALUATION OF ADVANCED TOMATO BREEDING LINES FOR RESISTANCE TO BACTERIAL WILT (*RALSTONIA SOLANACEARUM*). S.N. Kuria, J.M. Kibaki, A.M. Ndegwa, M.M. Waiganjo and R.G. Munene. *National Horticultural Research Center P.O. Box 220, Thika, Kenya. Email: mamamwas@yahoo.com*

Nine advanced tomato breeding lines TKA-193-42, TKA -193-28, TKA-140-33, TKA-155-18, Rodade, TKA-193-2, TKA-155-82, TKA-81-1, and TKA-193-31 were evaluated for tolerance to bacterial wilt *Ralstonia solanacearum* in the screen house. *R. solanacearum* was isolated from diseased plants on a wilt-infested farm. Stems were washed; surface sterilized then dipped in jars containing sterile distilled water for extraction of bacterial cells. The resultant suspension was then standardized to approximately 10^8 cells per ml by visual comparison of turbidity to a Marcfalard standard. 300 ml of the standardized inoculum was added to 600ml sterile distilled water. One-month-old tomato seedlings planted in polythene pots of steam-sterilized soil were inoculated with 10 milliliters of the inoculum suspension by pouring into two holes made with a peg (5 ml per hole). All pots were then gently irrigated. Each treatment (breeding line) had 10 plants in the treated plots and, 5 plants in the control (untreated) plots. The results showed that inoculated plants were generally shorter than uninoculated control plants, and no wilt was recorded on uninoculated plants. The highest wilt index was recorded on line TKA-193-28.

4.12 OBSERVING THE DEVELOPMENT OF ESCA SYMPTOMS IN THE VINEYARD. P. Lecomte, G. Darrieutort, J.-M.

Liminana, G. Louvet, A. Muruamendiaraz, F.J. Legorburu, E. Choueiri, F. Jreijiri, R. El Amil, N. David, C. Vidal and M. Fermaud. *INRA, UMR Santé Végétale n° 1065, Ave E. Bourleaux, BP 81, 3388, Villenave d'Ornon Cedex, France. Email: llecomte@bordeaux.inra.fr*

Esca is a worldwide grapevine trunk disease associated with the development of endophytic fungi into the wood leading to different types of lesion. As a consequence of the development of necroses and a number of interacting factors, esca can manifest a large range of foliar symptoms. In order to clarify its symptomatology, a survey based on regular observations in summer was made in Bordeaux vineyards from 2004 to 2007. Additional data were recorded in other French regions, in Northern Spain (Rioja Alavesa) and in Lebanon (Bekaa Valley). Leaf symptoms were variable in severity and development. Almost all damaged leaves showed, at an early stage of disease, interveinal drying and/or red wine or yellowish pigmentation on the lamina, according to the cultivar. These symptoms have also been attributed to black dead arm (BDA). Most badly damaged leaves dropped rapidly and most severely diseased vines showed apoplectic forms. After few days or weeks, leaves less severely altered went through the BDA foliar symptom before expressing typical esca symptoms. Further discolorations occurred in summer on other leaves or on leaves previously diseased. Except some vines showing slight esca-like leaf discoloration, nearly all vines examined showed an orange or brownish longitudinal stripe of altered wood in the neo-xylem, a symptom previously described as characteristic of either esca or BDA. Longitudinal wood sections of trunks revealed large decayed zones and necroses characteristic of bot canker. Results suggest that a dominant symptom pattern can be identified.

4.13* TEMPERATURE SENSITIVITY AND GRAFT TRANSMISSION EFFICIENCIES OF *CANDIDATUS* LIBERIBACTER ASIATICUS AND *CA.* L. AMERICANUS. **S.A. Lopes, G.F. Frare and N.G. Fernandes.** *Fundecitrus, Av. Adhemar de Barros, 201, Araraquara, SP, CEP 14807-040, SP, Brazil. Email: slopes@fundecitrus.com.br*

In Brazil *Ca.* Liberibacter asiaticus (Las) and *Ca.* L. americanus (Lam) cause huanglongbing (HLB), a destructive citrus disease. First reported in March 2004 in two municipalities, HLB has now been detected in 147 municipalities in São Paulo (SP), 1 in Minas Gerais and 2 in Paraná States. In SP outbreaks are mainly in the central and southern regions. In the upper north and west regions, the cumulative number of hours above 32°C is about five times higher, suggesting some influence of temperature on HLB occurrence. In growth-chamber experiments involving naturally or graft-inoculated sweet orange plants, Las was tolerant and Lam sensitive to high temperatures. All plants affected by Las (26) or Lam (25) had developed characteristic HLB leaf symptoms on the new shoots and tested positive by PCR after 60 days at a 22°C-24°C daily regimen. However, at 27°C-32°C only plants affected by Las (24) developed symptoms and were PCR-positive. All the 25 plants previously affected by Lam were symptomless and PCR-negative. Temporal analysis of Liberibacter occurrence in this 3.5 year period also showed a decrease in proportion of Lam over time. In graft-inoculation experiments, lower transmission was observed for Las (61/235 or 25.6%) than Lam (248/357 or 69.5%), a probable consequence of differences in bacterial titer in the affected plants used as source of inoculum. Insect-transmission and growth-chamber experiments underway should bring information essential for the understanding of the so far irregular progress of HLB in SP, and some perspective on HLB progress in regions still free of the disease.

4.14 MOLECULAR DETECTION OF *VERTICILLIUM DAHLIAE* IN LEAF PETIOLES OF OLIVE CULTIVARS SHOWING DIFFERENT LEVELS OF RESISTANCE. **F.J. López-Escudero, P. Estrada, J. Mercado-Blanco, A. Valverde-Corredor and M.A. Blanco-López.** *Departamento de Agronomía, Universidad de Córdoba, Campus Universitario de Rabanales, Edificio "Celestino Mutis", 14071 Córdoba, Spain. Email: ag2loesf@uco.es*

The objective of this work was to correlate the amount of *V. dahliae* in petioles of inoculated olive plants with different levels of resistance to *Verticillium* wilt. Olive plants were inoculated by stem puncture with a *V. dahliae* isolate representative of the defoliating (D) pathotype, and then incubated in a growth chamber at 22±2ºC. Disease severity was scored weekly over 10 weeks according to a symptom scale (0=healthy to 4=dead) based on the percentage of the plant affected by leaf chlorosis, shoot necrosis and/or defoliation. At the time disease progress was recorded, both fallen and still-attached leaves were separately collected and stored at –80ºC. Freeze-dried petioles of both leaf groups were ground to a fine powder and total genomic DNA was extracted according to a previously described procedure. Nested-PCR assays were carried out to qualitatively assess the presence of pathogen DNA. In addition, real-time quantitative PCR was performed to investigate differences in pathogen DNA content in petioles in relation to the resistance level of the olive cultivars assayed. Preliminary experiments using fallen and attached leaves collected just 10 weeks after stem inoculation showed differences in terms of petiole colonization between 'Cañivano Negro' and 'Cordobés Arroyo Luz' (susceptible) and 'Pajarero' and 'Frantoio' (moderately resistant and resistant, respectively). Ongoing experiments with 'Picual' and 'Arbequina' (susceptible) and 'Frantoio' and 'Empeltre' (resistant) olive plants, with detailed time-course sampling, will provide more information.

4.15 BIOLOGICAL EFFECTS OF POLYPEPTIDES SECRETED BY PATHOGENIC FUNGI ASSOCIATED WITH ESCA, A GRAPEVINE TRUNK DISEASE. **E. Luini, P. Fleurat-Lessard, J.-M. Berjeaud and G. Roblin.** *Université de Poitiers, UMR CNRS 6161, Transport des Assimilats, 86000 Poitiers, France. Email: estelle.luini@wanadoo.fr*

Esca is a grapevine disease caused by a fungal complex characterized by typical foliar symptoms and wood necrosis in the trunk and limbs. Death of the grapevines may occur several years after infection. *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* colonize the wood and allow its subsequent degradation by saprophytic fungi into a spongy and friable substance. *P. chlamydospora* and *P. aleophilum* secreted polypeptides in their culture medium. Although the electrophoretic patterns were different, the molecules secreted by both fungi shared common properties as shown by their similar biological actions. Applied on isolated leaves, they induced the formation of anthocyanins and applied on grapevine cells in culture, they modified proton fluxes, depolarized the cell membrane, inhibited the transport of sucrose and glutamine and, finally, induced cell death. The open question is now to define the part taken by the polypeptides in development of the disease.

4.16 *FUSARIUM* SP., A CAUSAL AGENT OF CROTALARIA WILT DISEASE IN JAPAN, IS A MEMBER OF THE *GIBBERELLA FUJIKUROI* SPECIES COMPLEX. **H. Mizuno and N. Kondo.** *Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan. Email: norikon@res.agr.hokudai.ac.jp*

Crotalaria juncea and *Crotalaria spectabilis* are plants that have been introduced and grown in temperate regions of Japan for green manure, mainly to control nematodes. Eight *Fusarium* sp. strains obtained from crotalaria plants with symptoms of severe wilt were characterized based on morphology and the partial DNA sequence of translation elongation factor 1-α (tef). Aerial mycelia on potato dextrose agar (PDA) were white, and the underside pigmentation was whitish yellow to orange depending on the isolates. On spezieller Nährstoffarmer Agar (SNA), 3-5 septate straight to falcate macroconidia and 0-1 septate oval microconidia formed on conidiophores. Abundant chained chlamydospores and sporodochia were observed on carnation leaf-piece agar (CLA). No strains produced any fertile perithecia on the media. BLAST searches using DNA sequence data for tef showed that these strains appeared to belong to the *Gibberella fujikuroi* species complex. The strains were pathogenic to both *C. juncea* and *C. spectabilis*, which showed wilt or leaf yellowing with vascular discoloration 3 weeks after inoculation. In addition, a vegetative compatibility group (VCG) analysis with non-nitrate-utilizing mutants indicated that the strains belonged to the same group and were incompatible with phylogenetically related species, such as *F. udum*, which is pathogenic to *C. spectabilis* producing wilt or yellowing, but is not pathogenic to *C. juncea*. No *Fusarium* spp. strains were isolated from crotalaria seeds distributed commercially in Japan, suggesting a very low probability of seed transmission. However, further etiological and epidemiological investigation of this disease is needed.

4.17 CULTURAL, PATHOGENIC AND MOLECULAR VARIABILITY OF *FUSARIUM* ISOLATES CAUSING WILT OF CHILLI (*CAPSICUM ANNUUM*). **M.K .Naik, G.S. Devika Rani, R.D. Prasad and M.B. Patil.** *Department of Plant Pathology, University of Agricultural Sciences, College of Agriculture, Raichur, Karnataka 584101, India. Email: manunaik2000@yahoo.co.in*

We studied the diversity of 149 isolates of *Fusarium* spp. obtained from wilted chilli crops from different states of India, using morphological, cultural, pathogenic and molecular methods. Significant variation existed regarding rate and type of growth, mycelial width, sporulation, number and pattern of chlamydospore formation etc. On the basis of radial growth 52 isolates were fast growers and 15 were slow growers. Thirty six isolates had large macro-conidia and 43 had smaller macro-conidia. Abundant sporulation was observed in 52 isolates, 41 showed moderate sporulation and the remaining produced few spores. Chlamydospores were intercalary in 26 isolates, while 55 isolates produced both terminal and intercalary spores. Amongst the 149 Fusarium isolates, 12 isolates representing different Indian states were inoculated into *Capsicum annuum* differentials to study pathogenic variability. Based on the virulence, the isolates could be divided into three types. Isolates F11 (Karnataka), F66 (Andhra Pradesh), F73 (Maharastra), F78 (Tripura), F81 (Orissa), F90 (Delhi), F91 (Rajasthan) and F97 (Gujarat) showed high virulence. Isolates F134 (Karnataka), F63 (Tamil Nadu) and F80 (Meghalaya) shared a moderate level of virulence. The third group, comprising the single isolate F29, possessed less virulence which was a local isolate. Jaccard's similarity coefficient and a dendrogram of the RAPD-PCR analysis using 5 primers of 45 representative *Fusarium* isolates indicated 3 clusters. Most isolates belonged to the second cluster sharing 35% similarity, first cluster with 43% and the last cluster sharing 30%.

4.18 RESISTANCE TO *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* IN *ARABIDOPSIS THALIANA*. **L. Perchepied, I. Kars, R. Louette, T. Kroj, M. Tronchet and D. Roby.** *Laboratory of Plant-Microorganisms Interactions, BP 52627, CNRS/INRA 2594, 31326 Castanet-Tolosan, France. Email: Laure.Perchepied@toulouse.inra.fr*

The cruciferous weed *Arabidopsis thaliana* and the causal agent of black rot disease of Crucifers, *Xanthomonas campestris* pv. *campestris* (Xcc) are both model organisms in plant pathology. However, the genetic bases for resistance to Xcc in *Arabidopsis thaliana* are not yet understood. Using a wound inoculation test, adapted to the invasion strategy (vascular) of the bacterial pathogen, several approaches were developed. First, *Arabidopsis* signalling mutants impaired in resistance to different pathogens were tested. While mutants impaired in specific resistance to other pathogens do not seem to affect resistance to Xcc, different *pad* and *eds* mutants which possess reduced basal resistance, such as *pad1,* were found to be susceptible to Xcc. Then, the genetic bases of resistance to Xcc were investigated by analysis of natural variation of resistance to Xcc in various *Arabidopsis* accessions. The accession Columbia 5 (Col-5) is for example resistant to Xcc whereas Kashmir (Kas) is susceptible. Then a recombinant inbred (RILs) population derived from a cross between Col-5 and Kas was analyzed, allowing the detection of one major (about 60% of the variability) and 3 minor QTLs. Heterogeneous inbred families (HIF) from the RIL population allowed us to validate the effect of the major QTL. Fine mapping of this locus has been done, leading to the definition of a confidence interval of 47 kb. Data will be presented concerning the sequencing of the locus in both parents, phenotyping of the corresponding KO mutants and complementation tests, and hopefully, the cloning of this resistance QTL.

4.19 IDENTIFICATION AND DISTRIBUTION OF *BOTRYOSPHAERIA* SPP. ASSOCIATED WITH GRAPEVINE CANKERS IN THE GRAPE-GROWING AREAS OF NEW SOUTH WALES AND SOUTH AUSTRALIA. **W.M. Pitt, S. Savocchia and C.C. Steel.** *National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. Email: csteel@csu.edu.au*

Botryosphaeria spp. are recognised as important pathogens of grapevines, both in Australia and overseas. Entry of these species via pruning wounds and the initiation of canker formation in the vascular tissue results in a slow decline and dieback often described as 'dead arm'. Vascular damage to the xylem results in the loss of spur positions along the cordon, a feature accentuated by progression of the disease basipetally towards, and eventually down the trunk of the vine. Information on the role of these fungi in grapevines in Australia is currently limited, and a field survey comprising vineyards throughout the major grape-growing regions of New South Wales and South Australia was conducted in order to determine the occurrence of *Botryosphaeria* sp. in these regions. *Botryosphaeria* spp. were the most commonly isolated fungi, and morphological and molecular identification revealed the presence of at least five species on grapevines: *B. obtusa*, *B. rhodina*, *B. stevensii*, *B. dothidea*, and *B. parva*. Other pathogens isolated from field samples included *Eutypa lata*, *Phomopsis viticola*, *Phaeomoniella* sp., *Phaeoacremonium* sp. and the bunch rot pathogens *Greeneria uvicola* and *Colletotrichum* spp.

4.20 *FUSARIUM* SP. AND *CERATOCYSTIS* SP. ARE VASCULAR PATHOGENS OF PEDUNCULATE OAK (*QUERCUS ROBUR* L.). **R. Pleskatsevich and U. Kapitsa.** *Ecology department, BNTU, Nezlezhnosti ave., 65, 220027, Minsk, Belarus. Email: v_stack@rambler.ru*

The cause of pedunculate oak desiccation in Belarus was investigated. The complex of pathogens was found to be dominated by two fungi, *Fusarium* sp. and *Ceratocystis* sp. Protective measures were investigated and proposed in practice for adult trees, seedlings and seed material.

4.21 PHYSIOLOGICAL RACE DETERMINATION OF *FUSARIUM OXYSPORUM* F.SP. *MELONIS* IN NORTHEASTERN IRAN. **N. Shafagh, M. Falahati Rastegar and B. Jafarpour.** *Iran-Hamedan, Pardisan-Pardis 3, No. 30, Iran. Email: noushinshafagh@yahoo.com*

Fusarium wilt of melon caused by *Fusarium oxysporum* f.sp. *melonis* is one of the most important disease of this crop which annually causes tremendous losses in northern and Razavi Khorasan provinces, the two major melon growing area of Iran. Due to the persistent nature of this pathogen, the disease is best managed with wilt-resistant cultivars. For this reason, race determination is important In *F. oxysporum* f.sp. *melonis*, four common races exist worldwide that have been identified on the nomenclature proposed by Risser *et a*l. The races are designated as 0, 1, 2, and 1.2. Forty five isolates of *F. oxysporum* f.sp. *melonis* were tested on the specific differential hosts Charentaise T, Charentaise Fom 1, Charentaise Fom 2 and Isabelle, for physiological race determination. Races 0 and 1 were determined by their reaction on differential melon cultivars. Because *Fusarium* strains vary widely in pathogenicity, the resistance of different melon cultivars to strains of *F. oxysporum* f.sp. *melonis* should be evaluated every year in an attempt to develop resistant cultivars carrying genes *Fom*1 and *Fom*2.

4.22 GENES INVOLVED IN DEVELOPMENT OF *BRASSICA ALBOGLABRA* AND THEIR IMPACT ON DISEASE RESISTANCE TO *VERTICILLIUM LONGISPORUM*. **D. Socquet-Juglard, S. Konietzki and E. Diederichsen.** *Freie Universität Berlin, Institut für Biologie, Angewandte Genetik, Albrecht-Thaer-Weg 6, D-14195 Berlin, Germany. Email: elked@zedat.fu-berlin.de*

Verticillium longisporum is a pathogenic fungus affecting cruciferous plants such as *Brassica napus* or *Brassica oleracea*. Stunting and growth abnormalities are the most common symptoms in greenhouse assays whereas in the field the fungus affects yield and seed size by a precocious maturation. Developmental stages, particularly flower initiation, seem to have an impact on pathogenesis and resistance. Identifying resistance sources that are independent of certain developmental parameters would greatly help to maintain developmental variation in a *Verticillium*-resistance breeding programme. A segregating population was generated in *Brassica alboglabra*, and the developmental parameters of 93 F3 families, the two parents and the F1 were assessed, using the Area Under the Developmental Progress Curve (AUDevPC), fresh weight, stem length, and also disease development (AUDPC). A molecular marker analysis based on microsatellites was applied to the F2 population for genotyping. Results on the number and localization of genes involved in flowering behaviour and their interaction with disease responses will be presented.

4.23 POSSIBILITY OF SPREADING OF STEM CANKER AND BLACK STEM DISEASES BY SUNFLOWER ACHENES. **M. Stajic, J. Vukojevi and S. Duleti-Lauševi.** *Institute of Botany, Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia. Email: stajicm@bfbot.bg.ac.yu*

The sunflower pathogens, *Diaporthe/Phomopsis helianthi* and *Leptosphaeria linquisti/Phoma macdonaldi*, causal agents of stem canker and black stem diseases, respectively, seriously influence the quality and yield. The aim of the study was to monitor the presence of mycelia and reproductive structures presence of these pathogens on sunflower achenes. Twenty seven lines and 2 sunflower hybrids, naturally or artificially infected, were analyzed at the histological level. Pycnidia of both pathogens were formed on the pericarp of all sunflower lines and hybrids analyzed. In the both cases the lines were more sensitive then hybrids and percentage of achenes infected with *P. helianthi* was significantly higher (9.75%) compared to *P. macdonaldii* (3.25%). Besides pycnidia of *P. helianthi* containing conidia, the protoperithecia, were rarely found in deeper layers of the pericarp. Large *P. macdonaldii* pycnidia containing conidia can cause infection. Cell necrosis, protoplasmic coagulation and the formation of pycnidia were unique structural changes observed on seeds of all lines and hybrids tested, when infected by *P. helianthi*. Only seeds of the line L6.Ph.B.98. were completely disintegrated. Seeds infected with. *P. macdonaldii* were considerably damaged, and pycnidia were arranged in rows in the outer parenchyma layers of cotyledons. The results show that spread of infection caused by *P. macdonaldii* by achenes is possible, while in the case of *P. helianthi* it is not clear because of the known pathway of pathogenesis.

4.24* IMPACT OF ESCA AGENTS ON *VITIS VINIFERA* CV. UGNI BLANC: ULTRASTRUCTURAL AND PHYSIOLOGICAL ASPECTS. **C. Valtaud, E. Luini, P. Fleurat-Lessard and A. Bourbouloux.** *Université de Poitiers UMR CNRS 6161, Transport des Assimilats, 86000 Poitiers, France. Email: pfleurat@univ-poitiers.fr*

In grapevine, esca is a devastating wood disease caused by a complex of pathogenic fungi located in the trunk, in particular *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum*. Their mechanisms of action upon the plant physiology were still not clarified. Our data show the presence of the fungi in the xylem tissues, and the structural cell modifications induced at a distance in leaves. Briefly, in leaves showing esca symptoms, cytoplasm was damaged in palisade parenchyma, tannin content was enhanced, starch grains were less developed than in controls, and large plastoglobules appeared in the vacuole and cytoplasm. The number of these plastoglobules, that might contain soluble carbohydrates, was increased compared to controls. It is likely that these vascular pathogens produce effectors that may be transported at a distance and induce modifications in grapevine leaf metabolism. Total glutathione measured in leaves with esca symptoms was drastically (68%) lowered compared to control healthy leaves. Furthermore, on a given stock, GSH level decreased, as much in leaf samples from a cane without foliar symptoms as in those showing esca symptoms. Enzymes of GSH metabolism were also altered. Therefore, glutathione appears as an early marker in esca disease development.

4.25 BAYOUDH DISEASE: MONITORING OF MICROFLORA PRESENT IN THE SOIL OF AN ALGERIAN OASIS. **A.M. Vettraino, A. Prodi, S. Franceschini, B. Ceccarelli, F. Abed, F.**

Bessedik, H. Khelafi, P. Nipoti and A. Vannini. *Dipartimento di Protezione delle Piante,Università degli Studi della Tuscia, Via S. Camillo de Lellis snc, 01100 Viterbo, Italy. Email: vettrain@unitus.it*

Fusarium oxysporum (Schlecht.) Snyd. & Hans., the most common species of the genus, is widely distributed in soil and on organic substrates. The species includes non-pathogenic, plant and human pathogenic strains. *F. oxysporum* f. sp. *albedinis* is the agent of bayoudh disease, causing the death of millions of mature and young date palm trees in North Africa. The aim of this work was to examine any correlation between the total microbial community and the incidence of the disease in an Algerian oasis. A total of 38 soil samples were collected in 12 areas randomly chosen within the oasis of Azoua and screened for the presence of fungi, bacteria and actinomycetes. Microbes were isolated on semi-selective substrates (PDS, TSA and Chitin-Agar media). Microbial entities were identified according to their morphological traits. For each soil sample, a statistical index of richness, dominance and evenness was determined. The microbial load ranged from 1.24×10^3 to 9.07×10^4 CFU/g for fungi, 4.00×10^5 to 3.22×10^7 CFU/g for bacteria and 8.60×10^4 to 9.92×10^6 CFU/g for actinomycetes. The correlation between the incidence of bayoudh disease, evaluated on a visual scale, in each area and the microbial community is reported.

4.26 VARIABILITY IN *FUSARIUM UDUM*, THE PIGEONPEA WILT PATHOGEN, IN INDIA.

Vishwa Dhar, R.G. Chaudhary, S. Datta, S. Mishra, R.K.Prajapati and Md. Shamim. *Indian Institute of Pulses Research, Kanpur 208024, India. Email: dr_vishwadhar@yahoo.com*

Vascular wilt caused by *Fusarium udum* is a serious disease of pigeonpea, an important grain legume in India. Like other *Fusarium* species, the pathogen is reported to exhibit a wide range of variability indicating prevalence of at least 3 variants in different crop-growing regions of the country. We studied cultural, morphological and pathogenic variability in 145 isolates of *F. udum* from 48 districts of north and central India at the Indian Institute of Pulses Research Kanpur during 2004-06. Mycelium colour, growth rate, growth pattern and size, and septation of macroconidia were the important cultural and morphological characters that varied between different isolates. Degree of pathogenicity also differed from weakly pathogenic (1-30%), moderately (31-50%) to highly pathogenic (51-100%) among the isolates. Based on cultural, morphological and relative pathogenicity characters, the isolates could be assigned to 31 distinct groups. The variable reaction of isolates representing 14 categories on a set of differential pigeonpea genotypes revealed the existence of at least five variants of *F. udum* (Vr1 to Vr 5). Molecular characterization of 31 representative isolates from different districts of Uttar Pradesh and 14 isolates from other states (Madhya Pradesh, Bihar, Karnataka, Andhra Pradesh, Haryana, Rajasthan, Punjab and Delhi) using 25 RAPD, 25 SSR and two ITS primers showing good polymorphism, revealed as many as 5 clusters. The isolates from Uttar Pradesh alone clustered into 4 distinct groups indicating prevalence of at least 4 variants in the state. SSR primers were designed from gene-rich regions of the *Fusarium* genome, and amplicons were obtained which were specific to particular variants. These can be easily converted to putative race-specific diagnostic markers.

WALL-LESS, PHLOEM-LIMITED BACTERIAL PLANT PATHOGENS

30.1 OVERVIEW OF RECENT STUDIES ON PHYTOPLASMA DISEASES IN CUBA AND ETHIOPIA.

Y. Arocha, B. Piñol, R. Almeida, K. Acosta, M. Wilson, J. Hanson, J. Proud, T. Zerfy, G. Abebe, P. Jones and J. Lucas. *National Centre for Animal and Plant health, CENSA, Apdo 10, San José de las Lajas, Havana, Cuba. Email: yaimaarocha@yahoo.es*

Phytoplasmas, are mollicutes, naturally transmitted by leafhopper and planthoppers of the *Hemiptera* order, affecting over 700 plant species from temperate to tropical countries. They are not culturable *in vitro* so molecular methods are the best approach for their detection, identification and characterization. As a result, knowledge on their physiology, pathogenicity, plant host-insect interactions, virus and prokaryote-like pathogen relationships and epidemiology is very limited. New insights have been revealed from recent studies on phytoplasma diseases in Cuba, related to 'bunchy top symptom' of papaya, BTS, associated with group 16SrII, *Candidatus* Phytoplasma aurantifolia, and in Ethiopia, related to Napier Grass Stunt (NGS), associated with group 16SrIII, X-disease. Polymerase chain reaction (PCR)-based assays, using phytoplasma universal primers, in combination with restriction fragment length polymorphism and sequencing analysis of the 16S ribosomal RNA gene were used to analyze NGS and BTS affected samples. Samples of other plant species and leafhopper specimens from the Hemiptera order were also collected from NGS and BTS affected fields and similarly analyzed. Sequences of the 16S rRNA were compared to those of phytoplasmas of reference in GenBank through bioinformatics for the final identification of phytoplasma isolates, and phylogenetic relationships were established. New alternative hosts for NGS and BTS phytoplasmas were identified, as well as potential leafhopper vectors, that may have epidemiological implications for the spread of NGS in Ethiopia, and BTS in Cuba. Results suggest that both NGS and BTS epidemics are a result of the phytoplasma-vector-plant host three way interaction.

30.2 TRANSMISSION OF *"CANDIDATUS* PHYTOPLASMA AUSTRALIENSE*"* TO *CORDYLINE AUSTRALIS* AND *COPROSMA ROBUSTA.*

R.E. Beever, M.T. Andersen and C.J. Winks. *Landcare Research, Private Bag 92170, Auckland 1142, New Zealand. Email: beeverr@landcareresearch.co.nz*

"*Candidatus* Phytoplasma australiense", found in New Zealand and Australia, is associated with diseases of New Zealand flax (*Phormium tenax*), New Zealand cabbage tree (*Cordyline australis*), strawberry (*Fragaria*) and karamu (*Coprosma robusta*). Previous work in New Zealand has established that "*Ca.* Phytoplasma australiense" is transmitted from *Phormium* to *Phormium* by the native flax planthopper *Oliarus atkinsoni* (family Cixiidae). We have now demonstrated transmission from *Coprosma* to *Coprosma* and *Coprosma* to *Cordyline* by the native polyphagous planthopper *Oliarus oppositus*. Adult insects were collected from infected *Coprosma* in the field, caged onto seedlings of *Coprosma* and *Cordyline* for a maximum of 14 days, and the plants observed for symptoms for up to 6 months. The presence of phytoplasma was monitored in both insects and recipient plants using nested PCR.

30.3 EFFECT OF DIFFERENT STRAINS OF "*CANDIDATUS* PHYTOPLASMA MALI*"* ON SUSCEPTIBLE AND RESISTANT *MALUS* GENOTYPES INOCULATED BY *IN VITRO*

GRAFTING. C. Bisognin, A.M. Ciccotti, A. Salvadori, M. Moser, M.S. Grando and W. Jarausch. *AlPlanta – Institute for Plant Research, RLP AgroScience, Breitenweg 71, D-67435 Neustadt/W., Germany. Email: wolfgang.jarausch@agroscience.rlp.de*

In vitro culture techniques were employed to study the effect of two different strains of "*Candidatus* Phytoplasma mali" on susceptible cultivars of *Malus domestica* and on apomictic, resistant genotypes deriving from *M. sieboldii*. Inoculation was done by in vitro grafting. The effect of the two "*Ca.* P. mali" strains on the different genotypes was analysed by recording the quality of the grafts, the transmission rates, and the survival rates of infected plants 6 and 12 months p.i.. Significant differences of all parameters were observed among the two strains. Additionally, symptoms on the inoculated plants were recorded and the phytoplasma concentration was determined by quantitative PCR at different times after inoculation. All infected apomictic genotypes had lower phytoplasma concentrations than the susceptible controls for both strains. A highly significant difference between the strains was found for phytoplasma concentration, especially in the susceptible genotypes. In contrast to the susceptible genotypes, the resistant ones did not show apple proliferation-specific symptoms or growth reduction compared to the healthy control. After transferring infected and healthy *in vitro* plants *ex vitro* these results were confirmed. Thus, the *in vitro* system is an interesting new tool to study differences in virulence of phytoplasma strains as well as susceptibility or resistance of the host plant.

30.4 RESEARCH ON GRAPEVINE YELLOWS DISEASES IN PIEMONTE: VECTOR CONTROL AND HOT WATER TREATMENT OF GRAPEVINE PROPAGATION MATERIAL. D. Bosco, F. Mannini, C. Marzachì, A. Alma and I. Gribaudo. *Di.Va.P.R.A. - Entomologia e Zoologia applicate all'Ambiente, Università di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. Email: domenico.bosco@unito.it*

Control of grapevine yellows diseases, especially Flavescence dorée (FD), relies mainly on the control of insect vectors and on planting healthy propagation material. For these reasons research on the activity of insecticides in preventing phytoplasma transmission and on hot water treatment (HWT) of propagation material have been sponsored by the Piemonte Region, northern Italy. Organophosphate and neonicotinoid insecticides have been tested on leafhopper vectors of phytoplasmas in order to evaluate their ability to prevent phytoplasma transmission. We first tested *Macrosteles quadripunctulatus* Kirschbaum, vector of Chrysanthemum yellows phytoplasma to herbaceous plants, as an experimental model, and then *Scaphoideus titanus* vectoring FD. The results suggest that neonicotinoid insecticides have a much higher and longer lasting effect in protecting plant from phytoplasma infection compared to organophosphates. HWT is important in reducing the spread of phytoplasmas via grapevine propagation; HWT, however, may interfere with the vitality of wood. To optimize HWT, we tested, over three years, different combinations (temperature and time) of hot water on different propagation materials (scion, cutting, grafted vine, etc.). For survival of propagation material the best treatment was 50 °C for 45 min, which had no or only slight negative effects compared to the control. For phytoplasma elimination it was best to use 52 °C for 45 min, although this temperature was more risky from a nursery point of view. HWT was found to be a reliable technique and therefore advisable in areas with high rates of phytoplasma infection.

30.5 EPIDEMILOGY, TRANSMISSION AND POSSIBLE ORIGIN OF A PHLOEM-RESTRICTED γ-3 PROTEOBACTERIUM ASSOCIATED WITH SYNDROME "BASSES RICHESSES" DISEASE OF SUGAR BEET IN EASTERN FRANCE. A. Bressan, O. Sémétey, J. Arneodo and E. Boudon-Padieu. *Biologie et écologie des phytoplasmes, UMR PME INRA–CNRS–Université de Bourgogne, BP 86510, 21065 Dijon Cedex, France. Email: bressan@hawaii.edu*

The syndrome "basses richesses" (SBR) is an emerging and highly epidemic disease of sugar beet in eastern France associated primarily with a phloem-restricted bacterium, here called SBR bacterium. According to a phylogenetic study on 16Sr DNA sequences, SBR bacterium resulted to be a member of the γ-3 proteobacteria and it shares high 16S rRNA gene sequence homology with bacteria in the genus *Arsenophonus.* The latter clade affiliates mostly insect-associated bacteria. Also SBR bacterium is closely related to a second plant pathogen: "*Ca.* Phlomobacter fragariae" which is the main agent causing Marginal chlorosis disease of strawberry in western France. SBR bacterium and "*Ca.* Phlomobacter fragariae" are spread to plants by means of two planthopper cixiids (Insecta: Hemiptera): *Pentastiridius leporinus* (Linnaeus) and *Cixius wagneri* (China) respectively. Because very few information is available on the biology and transmission of SBR bacterium to host plants, we carried out a series of laboratory and sugar beet field studies in the years 2005-2007. Our main results revealed that SBR bacterium is transmitted persistently and transovarialy by *P. leporinus*. By using PCR, Real Time PCR, and TEM assays we could detect and quantify SBR bacterium in ovaries, salivary glands, and midgut from dissected planthoppers. In field trials we could identify a positive trend between insect population size, rate of SBR bacterium infected insects, and incidence of diseased sugar beets. In addition, specimens of *C. wagneri* found in sugar beet fields were shown to transmit SBR bacterium. Our overall research suggest that this plant pathogen originated from an insect association.

30.6 ENDOPHYTIC BACTERIAL COMMUNITY IN YELLOWS-INFECTED AND RECOVERED GRAPEVINE PLANTS. P. Casati, D. Bulgari, L. Brusetti, F. Quaglino, D. Daffonchio, G. Belli and P.A. Bianco. *Università degli Studi, Istituto di Patologia vegetale, Via Celoria 2, 20133 Milano, Italy. Email: piero.bianco@unimi.it*

Flavescence dorée (FD) and Bois Noir (BN) are grapevine yellows (GY) diseases that cause severe crop losses. FD is caused by "*Candidatus* Phytoplasma vitis", transmitted by the leafhopper *Scaphoideus titanus* and BN is caused by "*Candidatus* Phytoplasma solani" and transmitted by *Hyalesthes obsoletus*. Up to now, FD control is based on chemical treatments against the insect vector and eradication of diseased plants. Recently, great importance has been placed on recovery, the spontaneous remission of symptoms in GY-diseased grapevines. Its causes are still little known although cytochemical and biochemical studies suggest that systemic acquired resistance (SAR) might be involved. Also, it is known that some species of bacteria are able to protect plants against pathogens. This work has aimed to identify the endophytic bacterial community present in *Vitis vinifera* L. and to examine the possible correlation between recovery and endophytic microrganisms. Endophytic bacteria were identified and characterized by screening the 16S rDNA library. The sequence data showed the presence of bacterial species belonging to the Gamma-proteobacteria, family Enterobacteriaceae. Among them, *Pantoea agglomerans*, *Pantoea ananatis*, *Ewingella americana* and *Erwinia persicina* were frequently identified. In the same samples,

Bacillus, *Enterococcus* and *Curtobacterium* were obtained using cultivation-based methods. Further studies done to examine their possible involvement in phytoplasma recovery in grapevine.

30.7 *"CANDIDATUS* PHYTOPLASMA PYRI", THE CAUSATIVE AGENT OF PEAR DECLINE IN *PYRUS* AND PEAR. M.J.D. de Kock, K.T.K. Pham, J. Hiemstra, F. Maas, H. Helsen, B. van der Sluis and J. van Doorn. *Wageningen UR, Crop Protection, Applied Plant Research, P.O. Box 85, 2160 AB Lisse, The Netherlands. Email: maarten.dekock@wur.nl*

The incidence of pear decline in orchards has increased over the last years in the Netherlands. Pear decline in *Pyrus* and pear trees correlates with the presence of the phytoplasma "*Candidatus* Phytoplasma pyri" (Cpp) in sieve vessels as assessed by molecular identification techniques using the 16 S rDNA sequence variation and other target genes. Both tree production in the nursery as well as fruit production in the orchard are affected by Cpp. In general, the phytoplasma occurs in growing parts (leaves, twigs, rootlets) during the summer while it survives in the roots during winter. Molecular diagnostic tools were used to survey the location of the phytoplasma in diseased pear trees during the summer and winter season. Systemic pear decline disease seems to be cultivar-dependent and might be avoided by combination with a resistant rootstock. However, analysis of diseased trees indicated that Cpp can survive in above-ground parts during mild winters. We have also studied the transmission of the phytoplasma by its vector pear psylla (*Psylla pyri*). First results showed the association of phytoplasma-infected pear psylla on diseased trees. Additionally, there are indications for vertical transmission of the phytoplasma to eggs and larvae. The current study aims to generate a better understanding of the epidemiology of pear decline, its relation with pear psylla and to formulate agricultural practices to avoid pear decline in the future.

30.8 GRAFT TRANSMISSION OF PHYTOPLASMAS ASSOCIATED WITH JAPANESE APRICOT YELLOWS. T. Furukawa, Y. Kawabe, K. Tsujimoto, H. Mizoguchi and K. Kishi. *Div. Biological Sciences, Graduate School of Science and Engineering, Tokyo Metropolitan University, Hachioji, Tokyo, 192-0397 Japan. Email: furukawa-toshiko@tmu.ac.jp*

Japanese apricots are an important agricultural product, widely cultivated in Japan. A new disease of Japanese apricot began spreading in Wakayama prefecture about 20 years ago, decreasing productivity and fruit quality. The number of newly diseased trees a year in 1997 and 1999 reached to about 18,000 with the maximum in Tanabe city, Wakayama prefecture. Japanese apricot yellows occurred suddenly and diseased trees declined within a few years. The early symptoms are as follows: leaves appear yellowed and rolled, new shoots do not grow to normal length, and fruits fall before ripening. Many experiments over the past decade failed to discover the cause. We found that spraying of a pesticide whose main component was oxytetracycline hydrochloride was strongly correlated with decrease of newly diseased trees, suggesting that Japanese apricot yellows was caused by a phytoplasma. We grafted buds and twigs of diseased Japanese apricot with on healthy seedlings of Japanese apricot to confirm phytoplasma transmission. After two years, phytoplasmas were detected in the branch that grew from healthy rootstock as well as in the grafted seedlings, using nested-PCR and universal primers amplifying phytoplasma 16S rRNA. The phytoplasmas detected were the same as those of the mother trees, which were "*Ca.* Phytoplasma asteris" and a phytoplasma associated with elaeocarpus yellows.

30.9 AMP-MEDIATED INTERACTIONS BETWEEN "*CANDIDATUS* PHYTOPLASMA ASTERIS" CHRYSANTHEMUM YELLOWS (CY) AND LEAFHOPPER VECTORS. L. Galetto, D. Bosco and C. Marzachì. *CNR–Istituto di Virologia vegetale, Strada delle Cacce, 73, 10135 Torino, Italy. Email: c.marzachi @ivv.cnr.it*

The chrysanthemum yellows strain of "*Candidatus* Phytoplasma asteris" (CY) is vectored with different efficiencies by the leafhoppers *Macrosteles quadripunctulatus* and *Euscelidius variegatus*. The molecular basis of the interactions between the phytoplasma and the vector are mainly unknown, although phytoplasma membrane proteins must be involved. The recent sequencing of a few phytoplasma genomes has shown that several proteins may be predicted to have a membrane location and, in some cases, hydrophilic portions protruding from the membrane towards the extracellular environment. Among these, the CY antigenic membrane protein (Amp) and the periplasmic binding protein of the ABC-type arginine transporter system (ArtI) were chosen for further studies. In the absence of a cell wall, these proteins represent good targets to address in search of the molecular basis of interaction between the phytoplasma and its host. Preliminary results have shown that several vector-proteins interact specifically with CY-Amp in far-Western experiments. Under non-denaturing *in vitro* conditions, CY Amp specifically interacted with 3 major vector proteins and, to a minor extent, with actin. The interacting vector peptides were identifiied through de novo sequencing and Blast analysis. Molecular characterization of the vector genes is in progress. The pattern of vector membrane-peptides interacting with Amp and ArtI are currently under investigation. Under the same experimental conditions, the interaction of ArtI with vector proteins was aspecific. Identification of the vector proteins interacting with phytoplasma membrane proteins will provide clues to the molecular mechanism regulating phytoplasma colonization of the vector body and, possibly, vector specificity.

30.10 TRANSMISION OF PHYTOPLASMAS BY *BACTERICERA COCKERELLI* TO SOLANACEOUS PLANTS IN SINALOA, MEXICO. C.B. García-Negroe, J.A. Chávez-Medina, M.E. Santos Cervantes, J. Méndez-Lozano and N.E. Leyva-López. *CIIDIR-IPN, Unidad Sinaloa, Juan de Dios Bátiz Paredes 250, Guasave, Sinaloa, CP 81101, Mexico. Email: neleyval@ipn.mx*

Solanaceous plants are important vegetable crops in Mexico, grown for local consumption, industry and export. However, their production is limited by many insect pest and disease problems. Recently, symptoms associated with phytoplama diseases were observed in fields of tomato (*Lycopersicum esculentum*), potato (*Solanum tuberosum*) and pepper (*Capsicum annum*) from Sinaloa, Mexico. *Bactericera cockerelli* has been associated with phytoplasmas diseases in North America and Mexico. However, it has not yet been reported as a vector of phytoplasma diseases. Yellow plastic traps confirmed the presence of *B. cockerelli* in these crops during the 2004-2006 seasons in Sinaloa. Population dynamics studies revealed that during the 2005-2006 season, the number of insects was 30 times greater than during the 2004-2005 season. Individuals of *B. cockerelli* collected by aspirator were used in transmission experiments to determine their vector status. Infected insects reared on healthy plants were tested by nested PCR to determine whether the phytoplasma was transmitted to the plants.

Multiplex PCR using specific primers, RFLP analysis and sequencing of the amplified fragments confirmed that phytoplasmas belonging to groups 16SrI, 16SrII and 16SrXIII were transmitted to tomato, potato and pepper plants. To our knowledge, this is the first report of vector transmission of phytoplasmas by *B. cockerelli* to tomato, potato and pepper crops in Mexico.

30.11 THE IMPACT OF PHYTOPLASMA DISEASES ON AGRICULTURAL, HORTICULTURAL CROPS AND TREES IN ALBERTA. **C. Hiruki and K.-R. Wang.** *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G2P5, Canada. Email: chujihiruki@aol.com*

Since the first report of a yellow disease of alsike clover in Alberta (Chiycowski, 1965), the number of phytoplasma diseases has increased in a variety of plant species in agricultural and horticultural crops as well as forest and urban trees and shrubs. In phytoplasma identification, DNA extraction, selection of primers, DNA amplification, PCR and RFLP analyses were one by the established methods. DNA heteroduplex mobility assay was performed for characterization of phytoplasmas at the subspecies level. A total of 62 phytoplasma were differentiated and classified into five major groups such as Aster Yellows (AY), Clover Proliferation (CP), Western X-disease, Elm Yellows and Fababean Yellows. For the first time, 23 plant species were found to be susceptible to certain phytoplasmas. Plant diseases caused by the AY group phytoplasmas were widespread and caused severe economic losses. In particular, the disease incidence of canola yellows has been rapidly increasing in recent years. CP phytoplasma was prevalent on the central and northern Alberta where seed production of leguminous hay crops such as alfalfa, alsike clover, sweetclover is specialized. CP phytoplasma in alfalfa and alsike clover constituted important overwintering hosts, and served as infection sources to other agricultural crops such as potato, causing severe witches' broom. The fact that certain weeds and trees are infected by AY or CP phytoplasmas and suffer from diseases such as poplar yellows and willow witches'-broom, suggests that they may serve as natural reservoirs of phytoplasmas for incidence of yellows diseases in agricultural and horticultural crops in Alberta.

30.12 SEQUENCE DIVERSITY AND POSITIVE SELECTION ON A SURFACE MEMBRANE PROTEIN OF PHYTOPLASMA. **S. Kakizawa, Y. Ishii, A. Hoshi, H.-Y. Jun, K. Oshima and S. Namba.** *Laboratory of Plant Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan Email: kakizawa@mail.ecc.u-tokyo.ac.jp*

Phytoplasmas are an economically important group of plant-pathogenic bacteria with small genomes in the class *Mollicutes.* They inhabit the phloem sieve elements of plants and cause numerous plant diseases. They are also intracellular parasites of phloem-feeding insects and are transmitted between plants by insects. Since phytoplasmas are endocellular and lack cell walls, their membrane proteins are in direct contact with the cytoplasm of plant or insect host cells and may be important in host–bacterium interactions. However, little is known about phytoplasma membrane proteins because of the difficulty in culturing these organisms *in vitro.* Previous studies have shown that immunodominant membrane proteins (IDP) constitute a major portion of the total cellular membrane proteins in most phytoplasmas. These IDPs are classified into three distinct non-homologous types (Imp, IdpA and Amp). In this study, we cloned and sequenced genomic fragments encoding IDP genes from several phytoplasmas, and tried to detect positive selection on these IDP sequences. The sequence variability of IDP genes between phytoplasmas was lower than that of genes upstream or downstream, or non-coding regions, suggesting that IDPs had quite high sequence diversity, and a strong positive selection was observed in some IDPs. These results implied an important biological role for IDP in host-bacterium interactions.

30.13 YELLOWS OF *ELAEOCARPUS SYLVESTRIS* VAR. *ELLIPTICUS* CAUSED BY A NEW '*CANDIDATUS* PHYTOPLASMA' SPECIES. **Y. Kawabe, M. Kusunoki and T. Yokoi.** *Department of Forest Microbiology, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan. Email: ykawa@affrc.go.jp*

Elaeocarpus sylvestris var. *ellipticus* plants have naturally grown over 1m in diameter at breast height and 20 m in tree height in shrine or temple forests and are now planted as roadsides or as garden trees in the warm-temperate to sub-tropical areas of western Japan. However, for the past 20 years or so, the tree has been plagued by Elaeocarpus yellows (EY), whose symptoms include shortened internodes, abnormal fallen leaves, yellowing leaves and die-back of twigs, often resulting in death. Today, EY occurs in nearly all natural and man-made habitats of *E. sylvestris* var. ellipticus, and its association with phytoplasma has been detected by nested-PCR of the 16S rRNA. EY phytoplasmas were observed in phloem tissues of leaves and transmitted by twig grafting. Recovery from of EY symptoms was achieved by trunk injections of oxytetracycline. The 16S rRNA and ITS sequences of EY phytoplasma samples, collected from different locations in Japan, were completely homologous. EY phytoplasma is a new '*Candidatus* Phytoplasma' species because its nearly complete 16S rRNA gene sequence has less than 97.5% similarity to that of all known reference strains of phytoplasma and its branch represents a distinct lineage in the phylogenetic tree constructed using nearly complete 16S rRNA gene sequences from reference strains of phytoplasma, and using *Acholeplasma palmae* as outgroup.

30.14* THE ACTIVITY OF ELICITORS OF PLANT RESISTANCE ON PHYTOPLASMA INFECTION. **C. Marzachì, R. D'Amelio, G. Berta, G. D'Agostino, F. Faoro, E. Gamalero, M. Iriti, N. Massa, S. Sampo and D. Bosco.** *CNR–Istituto di Virologia vegetale, Strada delle Cacce, 73, 10135 Torino, Italy. Email: c.marzachi@ivv.cnr.it*

Vescicular-arbuscular mycorrhizal fungi are naturally present in the roots of most fruit trees, and establish symbiosis with the plant, giving improved resistance to abiotic and biotic stresses. The presence of non-pathogenic rhizosphere bacteria may induce systemic resistance in plants. Several natural or synthetic compounds, among them chitosan, are widely present in nature and not very toxic to the plant, and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), may also activate the plant defence machinery against several pathogens. Delay of symptom development and of plant death has been used to evaluate the activity of 1) the vescicular-arbuscular fungi *Glomus mosseae* and *G. intraradices*; 2) the rhizobacteria *Pseudomonas putida* S1PF1, and 3084 and *Streptomyces* sp. Sb20; 3) chitosan; and 4) BTH as inducers of resistance against chrysanthemum yellows (CY) phytoplasma infection of daisy. While *G. mosseae* and *P. putida* S1PF1 slightly reduced the number of CY-infected plants and extended the life span of the affected plants, only 2.4 mM BTH provided some protection from

the disease and a delay of symptoms. Two concentrations of chitosan dissolved in acetic or hydrochloric acids were not effective, although some protection was present in plants sprayed only with acetic acid. Both acids were phytotoxic. The most promising biotic and abiotic treatments identified in this screening will be further studied following their application under controlled conditions. The possible interactions between elicitors and insect vectors will also be investigated.

30.15 TRANSMISSION OF *SPIROPLASMA CITRI* TO CARROTS BY *CIRCULIFER TENELLUS*. **A.F.S. Mello, R.K. Yokomi, J. Chen, A.C. Wayadande and J. Fletcher.** *Oklahoma State University, Department of Entomology & Plant Pathology, Stillwater, OK, 74078, USA. Email: afsmello@gmail.com*

Carrot purple leaf disease was reported in Washington State, USA and attributed to *Spiroplasma citri*. The objectives of this work were to confirm *S. citri* as the causal agent of carrot purple leaf by fulfilling Koch's postulates and to evaluate its transmission by the leafhopper *Circulifer tenellus*. *C. tenellus* adults were exposed for 24 h to feeding sachets containing *S. citri* isolated from infected carrots. After a 30 day latent period, 5 to 10 insects were transferred to 20 day old carrot seedlings for one or two days. Plants exposed to insects fed only on buffer served as negative controls while periwinkle plants exposed to infected insects were used as positive controls. Confirmation of plant infection was based on the development of purple leaf symptoms, spiroplasma reisolation and PCR confirmation of bacterial identity. Purple leaves in carrots and small, chlorotic leaves in periwinkle became evident 10 to 15 days after plant exposure to infected insects. No symptoms were present in plants exposed to buffer-fed insects, and *S. citri* was not detected by PCR or culturing. Only symptomatic plants of either species yielded cultures of spiroplasma and amplicons of expected size by PCR. *S. citri* was transmitted to 15% of the carrots exposed to infected leafhoppers, a rate almost 5 times lower than that to periwinkle (around 70%), showing that the transmission of the pathogen to carrot is possible though relatively inefficient. The work completes Koch's postulates and confirms *S. citri* as the causal agent of carrot purple leaf disease.

30.16 GENETIC DIVERSITY AMONG *CANDIDATUS* LIBERIBACTER ASIATICUS STRAINS HAVING DIFFERENT PATHOGENICITY ON PUMMELO. **S. Miyata, K. Tomimura, N. Furuya, M. Okuda, S. Subandiyah, C.H. Tsai, T.H. Hung, H.J. Su and T. Iwanami.** *National Institute of Fruit Tree Science, NARO, Fujimoto 2-1, Tsukuba, Ibaraki 305-8605, Japan. Email: smiyata@affrc.go.jp*

"*Candidatus* Liberibacter asiaticus" is a causal agent of citrus greening disease in Asia. It is quite difficult to investigate its biological features because it is phloem-limited and non-culturable. In Taiwan, citrus greening was already a serious disease fifty years ago, but until the 1970s, pummelo was tolerant. These days, however, pummelo is commonly affected by the disease, suggesting the appearance of a new pathogenic strain of "*Ca*. L. asiaticus". Strains isolated from the island were graft-transmitted to seedlings of mandarin, sweet orange, Eureka lemon and pummelo, and categorized according to symptoms and propagation on indexing plants. Type-I isolates produced symptoms on mandarin and sweet orange, but not on pummelo and Eureka lemon. Type-II and III isolates could propagate and show symptoms on pummelo. Fragments of the 16S/23S rDNA and intergenic region, *tufB-secE-nusG-rplKAJL-rpoB* gene cluster, *omp* gene region, and phage-type DNA polymerase gene region were sequenced and compared among Taiwanese isolates of Type-I, II and III. In the 16S/23S rDNA and intergenic region, nucleotide sequences were identical among all isolates. In the region of *tufB-secE-nusG-rplKAJL-rpoB* gene cluster and *omp* gene, several point mutations were found, however, they were irrelevant to the host plant specificity. In the upstream region of phage-type DNA polymerase gene, there were many point mutations between Type-I and Type-II/III isolates. Further investigation is now in progress on the relationship between these changes and the symptoms produced.

30.17 GENE EXPRESSION STUDIED IN DIFFERENT *CA.* PHYTOPLASMA MALI-INFECTED MICROPROPAGATED *MALUS* GENOTYPES. **M. Moser, C. Sprenger, C. Bisognin, R. Velasco and W. Jarausch.** *AlPlanta-Institute for Plant Research, RLP AgroScience, Breitenweg 71, D-67435, Neustadt an der Weinstrasse, Germany. Email: wolfgang.jarausch@agroscience.rlp.de*

A gene expression study was carried out on five different *Malus* genotypes, susceptible or resistant to apple proliferation disease. *In vitro* cultures were used to produce standardised *Candidatus* Phytoplasma mali-infected plants through *in vitro* grafting. The expression patterns obtained from healthy and infected plants were compared between susceptible and resistant genotypes after performing a cDNA-AFLP analysis. The corresponding cDNA fragments from differentially expressed bands were excised from the gel, cloned and sequenced. Apart from genes for which no putative function could be attributed, the identified genes could be grouped into three classes, associated with stress response, electron transport, or protein degradation and modification. Real-time analysis was chosen as the technique to test their differential expression after phytoplasma infection. A method based on RT-qPCR using SYBR green II® dye was developed and carried out on a first set of sequences.

30.18* ASPECTS OF GENETIC CONTROL OF RESISTANCE IN MAIZE TO CORN STUNT SPIROPLASMA. **E. Oliveira, E.E.G. Gama and A.C. Oliveira.** *Embrapa Milho e Sorgo, C.P.151 35701970, Sete Lagoas, MG, Brazil. Email: beth@cnpms.embrapa.br*

The corn stunt spiroplasma (*Spiroplasma kukelii*) transmitted by the leafhopper *Dalbulus maidis* causes severe losses in maize crops in Brazil. There is little information about the genetic control of resistance to this important disease. In this experiment, one complete diallele (Griffing 1956 – model 1) obtained from two resistant and two susceptible corn stunt maize lines was evaluated using *S. kunkeli* inoculation in a screenhouse. The experiment was carried out with 32 treatments: 16 corresponding to maize plants - lines, F1 and reciprocally crosses, and two inoculation treatments (with and without spiroplasma). Each treatment was replicated five times (total of 160 pots, each with one plant). For inoculation, two infective or two healthy leafhoppers were confined on each plant, eight days after sowing, for four days. At 56 days after sowing, plants were cut and individually weighed (dry weight). Dry weight reduction caused by inoculation compared to healthy plants was calculated for each treatment to express the resistance. The results suggested the presence of dominant genes at least for one maize line, based on the small reduction in dry weight in this line as well in the related crosses. The results also suggested a resistance effect inherited from the female side.

30.19 GENOMIC HETEROGENEITY IN TWO PATHOGENICALLY DIFFERENT LINES OF PHYTOPLASMA. **K. Oshima, A. Hoshi, Y. Ishii, S. Kakizawa and S. Namba.** *Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan. Email: kenro@ims.u-tokyo.ac.jp*

Phytoplasmas are plant pathogenic bacteria that cause devastating damage to over 700 plant species worldwide. We have previously reported the complete genome sequence of "*Candidatus* Phytoplasma asteris", OY strain, OY-M line (Oshima *et al*. 2004, Nature Genet.). The phytoplasma genome lacks several important metabolic genes, implying that the consumption of metabolites by phytoplasmas in plants may cause disease symptoms. To compare the genomic structure between different pathogenic phytoplasmas, we cloned and sequenced the genomic DNA from OY-W phytoplasma, which causes severe symptoms. We found that an approximately 30-kb region including the glycolytic genes was tandemly duplicated in the genome of OY-W. Duplicated genes often became pseudogenes by frameshift and stop-codon mutations, probably because of their functional redundancy. However, five genes, including two glycolytic genes, remained as full-length ORFs, suggesting that it is advantageous for the phytoplasma to retain these genes. In particular, 6-phosphofructokinase is known as a rate-limiting enzyme of glycolysis, implying that the different number of glycolytic genes between OY-W and OY-M may influence their respective glycolytic activities. We previously reported that the population of OY-W was higher than that of OY-M in infected plants. Our present results suggest that the higher consumption of the carbon source may affect phytoplasma growth rate and also may directly or indirectly cause more severe symptoms.

30.20 RESEARCH ON GRAPEVINE YELLOWS DISEASES IN PIEDMONT, NORTHERN ITALY: MONITORING OF DISEASES AND VECTORS, TYPING OF PATHOGENS. **S. Palmano, A. Alma, F. Lessio, P. Margaria, C. Morone, D. Pacifico, L. Picciau, R. Tedeschi, M. Turina and C. Marzachì.** *CNR–Istituto di Virologia vegetale, Strada delle Cacce, 73, 10135 Torino, Italy. Email: s.palmano@ivv.cnr.it*

In 1998 a serious outbreak of Flavescence dorée (FD) was reported in Piedmont (north-western Italy). Despite insecticide treatments against the vector and roguing of infected plants since 1999, the average number of healthy grapevines has decreased and numbers of recovered plants and those with symptoms has increased. Molecular diagnosis has indicated the presence of FD and Bois Noir (BN) phytoplasmas in affected plants. A novel and quick diagnostic procedure based on RT-PCR was developed. The abundance of leafhopper *Scaphoideus titanus* Ball, vector of FD, in woodlands containing American grapevine, the occurrence of the planthopper *Hyalesthes obsoletus* Signoret, vector of BN on different host plants, and the presence in vineyards of other homopterans, suspected vectors of Grapevine yellows (GY) phytoplasmas, have been investigated to study the relationships between suitability of the ecosystem for development of insect vectors and the occurrence of GY. Field samplings included direct counting of nymphs of *S. titanus*, and inspection of roots of different plants for nymphs of *H. obsoletus* and other Cixiidae. Insects were analysed to detect GY phytoplasmas. A protocol for identification of molecular variability among BN isolates from grapevine, weeds and vectors has been developed.

30.21 CHARACTERIZATION OF PHYTOPLASMAS INFECTING WEEDS IN INDIA. **G.P. Rao, M. Singh, S. Mall, Y. Chaturvedi, P.P. Upadhyaya, S.K. Raj and C. Marcone.** *Sugarcane Research Station, Kunraghat, Gorakhpur 273 008, UP, India. Email: gprao_gor@rediffmail.com*

During surveys of weeds in agricultural crops in Eastern UP, India in 2006 & 2007 unspecific yellowing and chlorosis were recorded in many weeds in and around agricultural fields. These suspected infected and healthy plant tissues of weeds e.g. *Cynodon dactylon, Dicanthium annulatum, Imperata gerardiana* and *Parthenium americana* were examined by nested PCR using phytoplasma-specific rRNA operon primers. For amplification of phytoplasmal ribosomal DNA (rDNA) by PCR, the universal phytoplasma primers P1/P7 and P4/P7 were used. All the weeds with unspecific yellowing and stunting yielded phytoplasma-exclusive DNA bands when nested PCR was performed. Negative results were obtained when symptomless plant host samples devoid of phytoplasma DNA templates were used.

30.22 FIRST REPORT OF A 16SRI GROUP '*CANDIDATUS* PHYTOPLASMA ASTERIS' PHYTOPLASMA ASSOCIATED WITH POTATO DISEASE IN IRAN. **M. Rashidi, M. Bahar, A. Ahoonmanesh and Y. Ghosta.** *Department of plant protection, College of agriculture, Isfahan University of Technology, Isfahan, Iran. Email: Rashidi_m642@yahoo.com*

A survey for phytoplasma diseases in potato fields in Iran was conducted during 2002 and 2003. In affected plants, the bases of young leaflets turned purple, red or yellow, petioles stood erect, the internodes shortened, and the whole plant grew straight up. Leaves curled upward, aerial tubers formed in the axillary buds and the tubers were purple. Wilting of purple tops, witches-broom and dwarfing were other symptoms. Affected plants looked bushy and some of them did not survive until harvest or were at such a competitive disadvantage that they produced few harvestable tubers. Forty samples were chosen and were tested by PCR to detect phytoplasma infection. Universal phytoplasma-specific primer pairs fP1/rP7, SN910601/SN910502, were tested, but only a few of samples amplified in the first round of PCR. Nested–PCR with primer pairs, fu5/ru3 and R16F2/R16R2, however, proved more effective. In this study most potatoes showing phytoplasma symptoms did not yield a PCR product after one pair of primers, but it was nessecery to use nested PCR. Results showed that about 60% of affected plants were phytoplasma-positive. A 1250 bp fragment was amplified in nested-PCR with R16F2/R16R2. This was ligated into the pGEM-T vector and was sequenced in both directions. Phylogenetic analysis showed that the potato phytoplasma has high similarity to the aster yellows group "*Candidatus* phytoplasma asteris". To our knowledge, this is the first identification of an aster yellows group phytoplasma infection of potato in Iran.

30.23 DIVERSITY AND GEOGRAPHICAL DISTRIBUTION OF PHYTOPLASMAS ASSOCIATED WITH POTATO DISEASES IN MEXICO. **M.E. Santos-Cervantes, J.A. Chávez-Medina, J. Méndez-Lozano and N.E. Leyva-López.** *CIIDIR-IPN, Unidad Sinaloa, Juan de Dios Bátiz Paredes 250, Guasave, Sinaloa, CP 81101, Mexico. Email: neleyval@ipn.mx*

Potato purple top (PPT) has caused severe losses in Mexico. At least four distinct phytoplasma strains belonging to four different phytoplasmas groups (16SrI, 16SrII, 16SrVI and 16SrXVI-II) have been associated with PPT in different regions of North America and Mexico. However, there has been no previous large-scale survey in the main potato-production states of Mexico to

analyze diversity and geographical distribution of phytoplasmas. We collected 3761 samples (tubers and foliage) during 2003 to 2007 and examined them by nested PCR and sequence analysis. The aster yellows (16SrI) group was distributed in all potato-growing areas in Mexico, whereas that Peanut witches'-broom group (16Sr II) was only found in the Guanajuato potato-growing area and the X-disease (16SrIII) group was found in Guanajuato and Coahuila potato-growing areas. Diversity was determined on the basis of the conserved 16S rDNA gene. All PPT phytoplasma strains varied to different degrees. Phytoplasmas from X-disease (16SrIII) group were found for the first time in Guanajuato and Coahuila potato-growing areas.

30.24 MOLECULAR CHARACTERIZATION OF PHYTOPLASMAS ASSOCIATED WITH DIFFERENT PLANT DISEASES IN VARIOUS REGIONS OF CHINA. **G.-Z. Tian, Y. Li, F. Lai, Q.-C. Xu, C.-G. Piao, L.-F. Wang and M.-W. Guo.** *Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing 100091, P.R. China. Email: tiangz@caf.ac.cn*

There are about 100 plant diseases associated with phytoplasmas reported in China. Some of them cause severe problems in arable and pome-fruit crops, forests and horticulture. Phytoplasmas associated with Chinaberry (*Melia azedarach*) witches'-broom in Zhejiang, Jiangxi, Guangdong and Hainan provinces, Bishopwood (*Bischofia polycarpa*) witches'-broom in Zhejiang, Jiangxi and Anhui provinces, periwinkle (*Catharanthus roseus*) virescence, *Cleome viscosa* witches'-broom, *Crotalaria mucronata* witches'-broom and several other leguminous plant diseases in tropical Hainan province were identified by molecular analyses of the phytoplasma 16Sr DNA, 3S rDNA, 16-23S spacer region, 2 elongation factor (EF-Tu) gene (*tuf*) and ribosomal protein gene (*rp*). The results showed that Chinaberry witches'-broom and periwinkle virescence phytoplasmas belonged to the aster yellows group (16SrI), whereas Cleome viscose witches'-broom, *Crotalaria mucronata* witches'-broom, *Tephrosia purpurea* witches'-broom on wild plants as well as peanut witches'-broom phytoplasmas were classified into the peanut witches'-broom group (16Sr II), and Bishopwood witches'-broom into the elm yellows group (16SrV). On the basis of highly conserved DNA sequence similarity and closest phylogenetic relationship with each other and the existing niche of these 16Sr II phytoplasmas from different plants in Sanya, Hainan, it is assumed that these wild plants would be alternative hosts of peanut witches'-broom phytoplasma and contribute to the prevalence of peanut witches'-broom disease in Hainan island.

30.25 CLONING AND EXPRESSION OF PAULOWNIA WITCHES'-BROOM PHYTOPLASMA (EF-TU) *TUF* GENE IN *ESCHERICHIA COLI* AND ANTISERUM PREPARATION. **J. Wang, X.-P. Zhu, X.-D. Li, C.-L. Lin, R. Gao, Y. Li, Q. Xu, C.g. Piao, H.-F. Li and G.-Z. Tian.** *Research Institute of Forest Ecology, Environment and Protection, Forest Ecology and Protection, Chinese Academy of Forestry, Beijing 100091, P.R. China. Email: tiangz@caf.ac.cn*

The 1185bp-complete *tuf* gene which encodes the tuf protein of 394 amino acids (elongation factor EF-Tu) sequence was amplified, cloned and sequenced from paulownia witches'-broom phytoplasma Nanyang strain (PaWB-NY) of Henan province, China. According to the analysis results of TMHMM2.0 and PROST, we presume that tuf might be a cytoplasmic protein combined to the outside of the membrane protein. The PCR product was ligated to expression vector pGEX-4T-3 and transferred into *E. coli* BL21 (DE3). The PaWB-NY tuf gene was expressed as a 62 kD fusion protein when induced with IPTG. Highly specific antiserum against PaWB tuf recombinant protein was produced in a rabbit. The antiserum titer was 1:5000 in ACP-ELISA and dot blotting. Western blot and indirect immunofluorescence analysis revealed that a disease-specific polypeptide was detected in PaWB, periwinkle virescence and Chinaberry witches'-broom phytoplasma-infected plants, which belong to 16SrI group, but not in healthy plants as well as jujube witches'-broom and Bishopwood witches'-broom phytoplasma-infected plants that belong to 16SrV group. Thus the antibody could be useful for the detection, identification and classification of phytoplasmas as well as for the study of phytoplasma metabolism and plant-pathogen interactions.

30.26 CITRUS STUBBORN DISEASE INCIDENCE DETERMINED BY QUANTITATIVE REAL-TIME PCR. **R.K. Yokomi, A.F.S. Mello and J. Fletcher.** *USDA, Agricultural Research Service, SJVASC, 9611 S.Riverbend Ave., Parlier, CA, 93648, USA. Email: ray.yokomi@ars.usda.gov*

Quantitative real-time (q) PCR was developed for detection of *Spiroplasma citri*, the causal agent of citrus stubborn disease (CSD), using the DNA binding fluorophore SYBR Green I. The primer pair, P58-3f/4r, based on sequences from the P58 putative adhesin multigene of the pathogen, resulted in an amplicon of an estimated size of 119 bp from *S. citri* cultures and from DNA extracts of various tissues taken from field- and greenhouse-grown citrus trees. Using qPCR, estimates of CSD incidence in two 12 ha navel orange groves in Kern County, CA, determined by sampling individual trees in six different groups of 8x8 trees per grove were 60% and 4%. All qPCR positives were confirmed by cultivation of *S. citri* in LD8 liquid medium. A second primer pair, P58-1f/2r, yielded an amplicon of approximately 86 bp and reacted with a smaller group of *S. citri* strains from lab and greenhouse cultures as well as from infected field trees, providing support for the presence of two populations of *S. citri* in California. The qPCR technology is being applied to assess within-tree pathogen distribution as well as in a study of CSD incidence and diversity in central California.

30.27 IDENTIFICATION OF PHYTOPLASMA INFECTING TREE PEONY IN BEIJING. **W.J. Zhao, J. Gao, M.Q. Shang, C.Z. Ding and S.F. Zhu.** *Institute of Animal and plant quarantine, Chinese Academy of Inspection and Quarantine, 100029, Beijing, P.R. China. Email: zhusf@netchina.com.cn*

In the summer of 2007, symptoms resembling phytoplasma infection were observed in tree peony in a park in Beijng. The tree showed symptoms including rolling and discoloration of leaves and uneven lignification of shoots. Total nucleic acids were extracted from fresh leaf midribs and phloem tissue from young branches and indexed by a nested PCR with phytoplasma generic primers P1/P7-R16F2n/R16R2. Specific fragments were amplified from some of the symptomatic leaf samples as well as from positive controls. The symptomless sample gave negative results. PCR products were characterized by direct sequencing, and the 16S rDNA sequences were compared with those of other reference phytoplasmas. A phytoplasma of the 16SrI ("*Candidatus* Phytoplasma asteris" group) were identified. Further studies will be required to analysis more gene sequences and know the vectors involved in the epidemiology of this disease.

9th International Congress of Plant Pathology

Index of Authors

ACKNOWLEDGEMENTS

Patronage

Presidency of the Italian Republic
Italian Ministry for Environment, Land and Sea
European Parliament
European Food Safety Authority
Region Piedmont
Province of Torino
City of Torino
University of Torino
City of Grugliasco

Sponsors

European Commission
Torino Chamber of Commerce
Syngenta Crop Protection
Bayer CropScience, Italy
American Phytopathological Society
Canadian Phytopathological Society
Isagro Italia
Lufthansa
Rijk Zwaan, The Netherlands

Main sponsors

Italian Ministry for Environment, Land and Sea
Region Piedmont
Province of Torino
University of Torino
Foundation Cassa di Risparmio di Torino
Compagnia di San Paolo
Agroinnova
8th International Congress of Plant Pathology
North Atlantic Treaty Organization
International Society for Plant Pathology
Dow AgroSciences

Technical Sponsors

Basket of typical products of the Province of Torino
Coldiretti Piedmont
City of Torino
Intesa Sanpaolo
C.U.S. (Centro Universitario Sportivo) Torino
Menabrea bier
Lavazza coffee
Sant'Anna natural mineral water
Ventura dried fruits
Raineri olive oil
Fantolino eggs
Sparkling elisir
Bosca spumante
F.lli Rabino farmhouse
La Posta restaurant and catering, Cavour

Finito di stampare nel mese di agosto 2008
in Pisa dalle
EDIZIONI ETS
Piazza Carrara, 16-19, I-56126 Pisa
info@edizioniets.com
www.edizioniets.com