## The Chinese Society for Plant Pathology

The Chinese Society for Plant Pathology (CSPP) is an academic organization devoting itself to the research and extension of the field of plant pathology in China. The society was established to promote the development of plant pathology in 1929. Over the years, the organization has grown into a national first-class society, with 14 professional committees, five working committees, 26 local committees and more than 6,500 members from China and abroad. The CSPP became a member of the International Society for Plant Pathology in 1983, and is one of the fundamental members of the Asian Association of Societies for Plant Pathology. It's headquarter is located in the campus of China Agricultural University.

The CSPP annual meeting and other national symposiums are regularly sponsored by CSPP or its professional committees. About 1,000 participants including oversea members attend these meetings, present their recent research achievements, learn about the latest advances in related research areas, and meet with colleagues to promote national and international communication. The CSPP journal "Acta Phytopathologica Sinica" was initiated in 1955. The Journal publishes bimonthly in Chinese or English, covering fundamental and application aspects of plant pathology. As an indicator of the academic level of CSPP, "Acta Phytopathologica Sinica" is one of the most highly rated academic journals in China.

CSPP has carried out a great deal of application research related to the prevention of plant diseases. CSPP maintains a close tie with agricultural producers by offering national and international trainings in the application of new techniques and providing services through agricultural extension. All these measures taken have popularized the knowledge of plant pathology, tightened the connection between theory and practice, and accelerated the development of agriculture in China.

The scientific development of plant pathology is the main concern of CSPP. After successfully sponsored the First Asian Plant Pathology Conference in Beijing in 2000 and co-organized the 15th International Plant Protection Congress in 2004, CSPP has now totally prepared itself for the ICPP in 2013.

Volume 43 (supplement) August 2013

Acta Phytopathologica Sinica

# ICPP 2013
# 10th International Congress of Plant Pathology

# ABSTRACTS

25-30 August 2013, Beijing, China

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## FOREWORD

Meeting the demands of growing global population, reducing loss of crop productivity is essential for long term food security. Plant diseases reduce the production and quality of food, fibre and biofuel crops. Plant pathology contributes tremendously to plant disease control, at pre- and post-harvest stages in agricultural production systems.

The Organizing Committee of the 10th International Congress of Plant Pathology (ICPP2013) strengthened the main theme "Bio-security, Food Safety and plant pathology in a Globalized Economy" by developing its scientific programme into 2 plenary sessions, 5 keynote sessions and 66 concurrent sessions. ICPP2013 also emphasized on the ISPP's initiative on Global Food Security with an evening session on "1 Billion Hungry People: What Can We Do?" in addition to the plenary session on "Can We Improve Global Food Security?" This proceeding contains 1591 abstracts of offered papers that are to be presented at ICPP2013.

The very considerable efforts of people involved in developing the ICPP 2013 programme and the ICPP 2013 abstract publication are commended and acknowledged. The ICPP2013 Scientific Programme Committee planned the programme, and numerous concurrent session organizers, noted in the ICPP2013 programme, helped identify paper presenters for the plenary, keynote and concurrent sessions. Many of my colleagues contributed to editing all the abstracts with incredible patience and compiling the Abstract publications. A group of teachers and students at the department of plant pathology, China Agricultural University, have provided substantial assistance for the preparation for ICPP2013.

ICPP2013 is highly expected to be a successful and worthy successor to previous International Congresses of Plant Pathology. All contributors of offer papers have played an important part in this success. I extend my best wishes to all ICPP2013 delegates for a successful and worthwhile time in China.

**You-Liang Peng**

Chairperson, ICPP2013 Organising Committee
Vice-President, International Society for Plant Pathology
Professor of Plant Pathology, China Agricultural University

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# PROFILES OF PLENARY AND KEY-NOTE SPEAKERS

## Opening Lecture

### Qifa Zhang

Qifa Zhang earned his B. S. in agronomy, in 1976 in Huazhong Agricultural College, China, and Ph.D. in genetics in 1985 in the University of California at Davis, USA. He is currently a professor of Huazhong Agricultural University, Dean of the College of Life Science and Technology, and Director of the National Key Laboratory of Crop Genetic Improvement. He has focused his research and provided leadership in genomics and biotechnology of rice in China. His research achievements include: (1) genetic analysis, identification, mapping and functional characterization of genes for agronomically important traits including yield, grain quality, male sterility and hybrid fertility, nutrition efficiency, drought resistance, disease and insect resistances; (2) elucidating biological mechanisms of heterosis in hybrid rice; (3) establishment of technological platforms for functional genomics studies including a large T-DNA insertional mutant library, global and whole life cycle expression profile, full-length cDNA libraries; (4) rice varietal improvement using molecular marker and transgenic technologies. He has published more than 190 original research and review papers in international journals. He was elected to the Chinese Academy of Sciences in 1999, the Third World Academy of Sciences in 2000, and foreign associate of the National Academy of Sciences, USA in 2007.

## Plenary Session1-The Role of Plant Pathology in a Globalised Economy

### Corrado Clini

Corrado Clini, born in 1947, is General Director of the Italian Ministry for the Environment, Land and Sea. He received a Bachelor of Science in medicine from the University of Parma in 1972. He holds a Ph.D. occupational health at Padova University in 1975 and another Ph.D. in hygiene and public health at Ancona University in 1986.

Clini was the director of the Department of Environmental Protection and Industrial Medicine in Venice-Porto Marghera in the 1980s. He became the director general of the Italian Environment Ministry in the 1990s.

He chaired the Italian expert-level delegation at the Rio Conference on Environment and Development in 1992, and the Kyoto Conference on Climate Change in 1997. From 2000 to 2001 he served as co-chairman of the G8 Task Force on Renewable Energy. He was also co-chairman of the European Environmental and Health Committee of the United Nations Economic Commission for Europe. In 2006, Clini was named as chairman of the Global Bioenergy Partnership. From 16 November 2011 until 28 April 2013, Clini was appointed Italian Minister of Environment, Land and Sea in the Monti Cabinet.

Clini also worked as a visiting professor at the Department for Environmental Sciences and Engineering of Tshingua University (Beijing) and at Harvard's Kennedy School of Government.

### Jan E. Leach

Jan E. Leach is a University Distinguished Professor at Colorado State University and an Adjunct Scientist at the International Rice Research Institute (Philippines). Her research focuses on understanding the molecular basis of durable disease resistance, particularly in rice-pathogen interactions. Leach is a Fellow and a past President of the American Phytopathological Society (APS) and she currently chairs the APS Public Policy Board. She is a Fellow of the American Association for the Advancement of Science (AAAS) and served as Chair of the AAAS Section O (Agriculture, Food, and Renewable Resources) in 2007. Leach is a Fellow of the American Academy of Microbiology. She was recently appointed to the National Science Advisory Board for Biosecurity. Leach is Associate Editor of the Annual Reviews of Phytopathology, and has served on numerous other editorial boards.

### Xiaobing Yang

Dr. XiaoBing Yang is a professor at Iowa State University. He received his B.S. and M.S. degrees from China Agricultural University and Ph.D. from Louisiana State University. He has published over 100 journal articles and 250 non-technical articles. In 1998, he and his colleagues studied the impact of climate change on US Agriculture and twice were invited to present their results at the US Congress. He has been invited to give lecture in 15 countries and elected as an APS Fellow in 2005. In 2006, he and his colleagues received Award for Excellence by USDA Secretary for managing the risk of soybean rust. He also received Golden Global Award from Guangxi Government for international collaboration.

## Plenary Session 2-Can We Improve Global Food Security (ISPP Task Force)

### Richard Strange

Richard Strange is Editor-in-Chief of Food Security. He was attracted to Plant Pathology, a subject in which he has published over 100 papers and two books, by its relevance to food security. Currently he holds an Honorary Chair at University College London and an Honorary Fellowship at Birkbeck College, University of London. He has been involved in numerous overseas projects, several of which were located in different

African countries and has supervised Ph.D. students from these and other countries of the Developing World in topics directly concerned with plant disease problems affecting their food security.

**Zhaohu Li**

Dr. Zhaohu Li is the vice president for research of China Agricultural University and professor of agronomy in College of Agriculture and Biotechnology. He received his B.S. and M.S. degree from China Agriculture University, Beijing, China. He received his Ph. D. in weed science and crop physiology from Auburn University, Alabama, USA. His research program focuses on plant growth regulation by plant growth regulators. Dr. Li received the national award for developing and extension of chemical regulation technology for cotton. He is nominated professor of ChangJiang Scholar Program. Dr. Li was appointed as the vice president for R&D since 2011.

**Fen Beed**

Dr. Fen Beed is an experienced plant pathologist based at the International Institute of Tropical Agriculture (IITA), Dar es Salaam, Tanzania (www.iita.org). He leads research for development activities to mitigate the impact of diseases of maize, soybean, cowpea, cassava, banana and vegetables and promotes plant diseases on problematic weeds. Before joining IITA in 2000, Fen worked as a research scientist at the University of Nottingham, UK and simultaneously served as a consultant for ADAS (executive arm of UK government's Ministry of Agriculture) studying field interactions between crop physiology and pathogen epidemiology and the influence of environmental conditions. Prior to Nottingham he worked and studied at the Universities of Sheffield, Strathclyde and University College London, UK where he focused on biochemical interactions between plant hosts and their fungal pathogens. His first position in IITA was in Benin where he developed a weed biological control programme using fungal pathogens with targets such as water hyacinth, speargrass and Striga and, due to demand from across the region then developed a plant pathogen diagnostic clinic supported by a microbial reference collection. He moved in 2007 to IITA Kamapla, Uganda and created systems for the surveillance, diagnostics and management of banana disease across the Great Lakes Region. In 2010 he moved to the IITA regional hub in Tanzania where he has continued his research profile and added food safety issues including mycotoxins prevalence studies, biological control of aflatoxins and vegetable IPM to increase grower and consumer safety.

**Ulrike Grote**

Ulrike Grote is professor at the School of Economics and Management of the Leibniz University Hannover since 2006, where she heads the Institute for Environmental Economics and World Trade. Her research focuses on international trade, environmental and development economics. She has widely published on social and environmental standards, certification and value chain analysis in the field of agricultural and trade economics, and she is member of the Editorial Board of Food Security. Her regional focus is Southeast Asia and Sub Saharan Africa. She received her postgraduate degrees in Agricultural Economics from Kiel University in 1994 and Bonn University in 2004, and worked from 1998-2006 at the Centre for Development Research (ZEF) in Bonn. Prior to that, she worked for several years at international organizations including the Asian Development Bank in Manila and the OECD in Paris.

**Lise Korsten**

Prof. Korsten is a professor in the Department of Microbiology and Plant Pathology in the Faculty of Natural and Agricultural Sciences at the University of Pretoria. She is the Head of the thematic focus area of Food Safety, Biosecurity, Public Health and Regulatory Control within the Institute for Food, Nutrition and Well-being. Prof. Korsten has trained more than 50 Masters' and Ph.D. students and has published extensively. Prof. Korsten developed South Africa's first biological control agent for fruit and has established a research group in sanitary and phytosanitary aspects of international trade. She has expanded her research portfolio to include aspects related to post-harvest pathology and food safety in the fresh produce supply chain. Prof. Korsten serves on the boards of the National Laboratory Association and the Post-harvest Innovation programme from the Department of Science and Technology and the Fresh Produce Exporters Forum. She is also a member of the Specialist Technical Committee of the South African National Accreditation System (SANAS). Prof. Korsten has received a number of special awards such as the University of Pretoria Exceptional Achiever award.

**Gebisa Ejeta**

Gebisa Ejeta is Distinguished Professor of Plant Breeding and Genetics and International Agriculture at Purdue University. He currently serves as Executive Director of the Purdue Center for Global Food Security. His research is focused on the genetic improvement of sorghum for resistance to pests, diseases, and environmental stress with enhanced productivity and nutritional quality. He has contributed to the welfare of people in developing countries through his research as well as through his human and institutional capacity development efforts. Professor Ejeta has served the United States Government and other agencies in advisory capacity. He was designated Science Envoy for Africa for the US State Department, Special Advisor to the Administrator of USAID, before being appointed by President Obama to the Board for International Food and Agricultural Development (BIFAD) in 2011. Previously, Dr. Ejeta has consulted with the Rockefeller Foundation, the Gates Foundation, the USAID, the Food

and Agricultural Organization (FAO) of the United Nations, the CGIAR, as well as a number of national and regional organizations in Africa. Currently, Dr. Ejeta is serving on the advisory board of the Sasakawa Africa Association, the Chicago Council for Global Affairs, and the Consortium Board of the CGIAR. Dr. Ejeta is Fellow of the American Association for the Advancement of Sciences, Fellow of the American Society of Agronomy, Fellow of Crop Science Society of America, and the 2009 World Food Prize Laureate.

## Keynote Session 1-The Role of Plant Pathology in Bio-security and Food Safety

**Robert Zeigler**

Dr. Robert Zeigler is an internationally respected plant pathologist with more than 30 years of experience in agricultural research in the developing world. Dr. Zeigler assumed his position as IRRI's director general in 2005. His professional life spanned Africa, Latin America, US, and Asia. He has published over 100 scientific works in these areas and often serves as an expert resource on rice security in the regional and global media.

Dr. Zeigler is the founding chairman of the board of the IRRI Fund (Singapore, Hong Kong and India). He is currently the Chairman of the Board of Directors of the Association of International Agricultural Research Centers (AIARC).

Dr. Zeigler earned his Ph.D. in plant pathology from Cornell University, M.Sc. in botany from Oregon State University, and his B.Sc. in biological sciences from the University of Illinois. He has completed corporate governance programs from Harvard Business School and Kellogg School of Management.

**Jacqueline Fletcher**

Dr. Jacqueline Fletcher, Regents Professor, Department of Entomology & Plant Pathology, Oklahoma State University, serves as Director of the National Institute for Microbial Forensics & Food and Agricultural Biosecurity, a multidisciplinary OSU initiative that addresses high priority national and global issues in plant pathogen forensics, crop biosecurity and food safety related to human pathogens on plants. Dr. Fletcher served on the American Phytopathological Society's Council and as APS President in 2003-4. Following September 11, 2001, she led APS responses and input to U.S. biosecurity initiatives, and now directs APS's Working Groups on Microbial Forensics and Food Safety. She is a member of the Institute of Medicine's Forum on Microbial Threats at the National Academy of Sciences, and serves on several federal biosecurity committees. She was named a Fellow of APS in 2005 and a Fellow of the American Association for the Advancement of Science (AAAS) in 2007.

**Sophien Kamoun**

Sophien Kamoun is a Senior Scientist and Head of The Sainsbury Laboratory, Norwich Research Park, United Kingdom. He received his B.S. degree from Pierre and Marie Curie University, Paris, France, and Ph.D. in Genetics from the University of California at Davis. He held a faculty position at the Ohio State University, Department of Plant Pathology, Wooster campus, before joining The Sainsbury Laboratory in 2007. His recent research focuses on plant pathogenomics, filamentous pathogen effector biology, and devising new approaches to breeding disease resistant crops. Dr. Kamoun received the APS Syngenta Award, the OSU Pomerene Teaching Award, the WE. Krauss Award for Excellence in Graduate Research Mentorship, and the Daiwa Adrian Prize. In 2011, he was elected to the Academia Europaea and received a European Research Council (ERC) Advanced Investigator Award. Visit his website at http://www.KamounLab.net

## Keynote Session 2-Genomics, Proteomics and Plant Pathology

**Shouwei Ding**

Shouwei Ding is a Professor in the Department of Plant Pathology & Microbiology and the Institute for Integrative Genome Biology, University of California - Riverside, USA. He received his B.S. degree from Anhui Agricultural College (Hefei, China), M.S. degree from Fudan University (Shanghai, China), and Ph.D. from Australian National University (Canberra, Australia). He held a faculty position in the Institute of Molecular Agrobiology, National University of Singapore before joining UC-Riverside. His recent research focuses on RNAi-mediated antiviral immunity mechanisms in plants and animals and on the discovery of viruses and viroids by next-generation sequencing approaches. Dr. Ding is an elected fellow of the American Academy of Microbiology and of the American Association for the Advancement of Science. He currently is a senior editor of PLoS Pathogens and serves on the editorial boards of Virology and Journal of Virology.

**Lijun Ma**

Dr. Ma studies genome evolution combining theoretical, computational and experimental approaches. Her lab focuses on a model system *Fusarium oxysporum*, a highly adaptive species complex causing destructive and intractable wilt diseases across a broad spectrum of plant hosts. The Fusarium comparative genomics demonstrates that horizontal transfer of entire chromosomes conveys pathogenicity and contributes directly to the niche adaptation. This discovery establishes *F. oxysporum* as an effective model to investigate horizontal transfer in eukaryotes, and the pathogenicity chromosomes provide a focal point to investigate the genetic mechanisms that underlie pathogenesis against different plant hosts.

**Yong-Hwan Lee**

Dr. Yong-Hwan Lee obtained his B.S. and M.S. degrees from Seoul National University, Korea, and Ph.D. degree in plant pathology at Louisiana State University in 1991. Currently he is a professor and director in the Department of Agricultural Biotechnology and Center for Fungal Genetic Resources at Seoul National University, Korea. He is also a Finland Distinguished Professor at University of Helsinki, Finland. His group has taken comprehensive and integrative approaches of forward and reverse genetics to understand molecular mechanisms of pathogenesis in the rice blast fungus, *Magnaporthe oryzae*. His group also developed a bioinformatics portal system, Comparative Fungal Genomics Platform (http://cfgp.snu.ac.kr), and several databases and associated informatics tools for fungal functional genomics and evolutionary biology.

## Keynote Session 3-Host-Pathogen Interactions and Molecular Plant Pathology

**Paul Schulze-Lefert**

Paul Schulze-Lefert was trained in biochemistry and genetics at Marburg, Freiburg, and Cologne Universities, Germany. After a Ph.D. 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. 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 Max-Planck-Institut für Pfanzenzüchtungsforschung (MPIPZ), Cologne, and a Honorary Professor at the University of Cologne since 2003. He is an elected EMBO member since April 2006. In 2010 he was elected as member to the National Academy of Sciences, USA, and to the "Deutsche Akademie der Naturforscher Leopoldina", Germany. He was elected Fellow to the American Academy of Microbiology in 2011. He serves as member of the science advisory board of the Two Blades Foundation.

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. A major recent activity involves the development of methods to identify molecular mechanisms enabling plants to live in intimate association with bacterial microbiota in the rhizosphere.

**Sheng Yang He**

Dr. Sheng Yang He obtained B.S. and M.S. degrees from Zhejiang Agricultural University and a Ph.D. degree from Cornell University. He is a Professor at Michigan State University and an Investigator at Howard Hughes Medical Institute. In the past two decades, Dr. He and his associates have studied how a model bacterial pathogen, *Pseudomonas syringae*, causes disease in Arabidopsis. Their research shed light on some of the most fundamental processes underlying bacterial pathogenesis in plants and illustrates the utility of pathogen virulence factors as useful probes in understanding plant biology, including jasmonate signaling, stomatal function, and immunity.

**Pierre J.G.M. de Wit**

Pierre de Wit did his MSc in biochemistry, plant physiology and plant pathology at Wageningen University. For his Ph.D. he studied physiological and biochemical aspects of the pathosystem *Cladosporium fulvum*-tomato. After his Ph.D. he spent a sabbatical year in the USA at the Department of Phytopathology of KSU, Lexington Kentucky. In 1982 he became assistant, professor at the Laboratory of Phytopathology of Wageningen University, associate professor in 1986 and full professor and head of the Laboratory in 1990. His research is focused on fungal effectors from *C. fulvum* and related Dothideomycete fungi like *Mycospaerella fijiensis* and *Dothistroma septosporum*, and effector-triggered immunity responses in tomato and tobacco. He discovered the biological functions of several fungal effectors. In 2008 he became Royal Netherlands Academy Professor.

## Keynote Session 4-Recent Developments in Disease Management

**Erich-Christian Oerke**

Erich-Christian Oerke received his Ph.D. in Biology from the University of Hannover, Germany, and has joined the Department of Plant Pathology, University of Bonn, in 1993. He has prepared the basic chapters of a study on worldwide crop losses published by Elsevier in 1994. Since 2000, crop loss data have been updated and are implemented into the Economic Impact Module of the Crop Protection Compendium published by CAB International. Experimental research focuses on the infection process of leaf pathogens and on sensing of plant diseases using technical sensors on various scales. He has been editor of the book 'Precision Crop Protection' published by Springer in 2010. He received the Julius Kuehn Award of the German Phytopathological Society in 1998.

**Abraham Gamliel**

Head of the Laboratory for Pest, Management Research, Agricultural Research Organization, ARO), Volcani Center, Bet Dagan, Israel, and Professor at the Hebrew

University, Jerusalem.
Received Ph.D. in 1990, from the Hebrew University of Jerusalem, Israel. Research activities involve the development of various effective crop management strategies and technologies, while minimizing negative environmental attributes. Co-edited books on Crop Biosecurity, and Soil solarization. Authored several book chapters and invited reviews on soil disinfestation and pest management. Chairman of the Soilborne Pathogen Group, the International Society for Horticultural Sciences (ISHS), and the convener of the 2009 7th International Symposium on Soil Disinfestation. Member of the United Nations, Environmental Protection Program, (UNEP), Methyl bromide Technical Option Committee (MBTOC).

**Andy Leadbeater**
Andy graduated from Birmingham University (United Kingdom) in 1979 with a degree in Biological Sciences, specializing in Plant Pathology. Andy has more than 30 years experience in the global Crop Protection Industry, joining Ciba-Geigy in 1980 and working through major reorganisations, culminating in Syngenta. He started his career in the Company as a field scientist and since has worked in several technical roles in Syngenta which has included the successful development and launch of several key fungicides important to the Company's success. He currently heads Syngenta's fungicide product development activities in biology.
Outside the company he is chairman of the International FRAC Steering Committee as well as leading the QoI Fungicides Working Group. He is also an active member of the European Crop Protection Association (European Crop Life International) Efficacy Expert Group. He is also very active in EPPO (European Plant Protection Organisation) and is the ECPA representative to their Working Party. Andy has spoken to several conferences and workshops on issues relating to product development and resistance management.
Andy has a wide range of experience in product Research and Development both from inside industry and from the point of view of government and non-government organisations.

## Keynote Session 5-Plant Pathology in Asia

**Ryohei Terauchi**
Ryotei Terauchi is a plant geneticist interested in evolution in general, and that of plants and interacting pathogens in particular. He obtained Ph.D. from Kyoto University, Japan with a study on yam (Dioscorea) molecular taxonomy. He experienced post-doc positions in IITA, Nigeria and University of Frankfurt, Germany. His current research focus is on the molecular interactions between rice and blast fungus *Magnaporthe oryzae*.

**Xueping Zhou**
Xueping Zhou is currently a professor in the College of Agriculture, Zhejiang University. He received his B.S. degree, M.S. degree and Ph.D. from Nanjing Agricultural University (Nanjing, China). His research interests include plant virus aetiology, molecular aspects of virus pathogenesis, and genetic engineering for virus resistance. Dr. Zhou serves as vice president of Chinese Society of Plant Pathology. He currently is an Associate Editor of Molecular Plant-Microbe Interactions and serves on the editorial boards of Journal of General Virology and Virology Journal.

**M P Srivastava**
Born on 5th November 1942, Professor. M P Srivastava received his Ph.D. in 1967 from the University of Allahabad and subsequently joined Haryana Agricultural University. Besides teaching and conducting research on rice, cotton, moongbean and potato, he championed the cause of Transfer of Technology and Plant Clinic and created a niche for himself as a leading Extension Plant Pathologist of India. His real dalliance for Transfer of Technology and Plant Clinic started when he joined as Extension Pathologist in 1976. He received 'Fellowship' of the National Academy of Sciences in 1988 and 'Outstanding Extension Scientist National Award' in 1996 for excellence in Extension. In recognition of his outstanding contribution in Transfer of Technology, he was invited to deliver a Keynote Address in Transfer of Plant Pathology Knowledge Session at ICPP 2003 at Christchurch, New Zealand. His passion for Plant Clinic continued even after taking over as Professor in 1986, Chairperson of Plant Pathology Department in 1996 and Director in 2000. To popularise plant clinic worldwide, he delivered special lectures in India, China, Singapore, Italy and Germany. He organised and chaired a special session on 'Plant Health Clinic' during 9th ICPP at Turin, Italy in 2008 and at International Conference in Globalized Era at New Delhi in 2010. Presently he offers free service to growers and scientists on diagnosis and control of pests, and establishment of plant health clinic through his web portal www.xsgrowth.com. He is a prolific writer and eloquent speaker. He has guided over dozen students for Ph.D. and has published around 200 research papers, 250 popular articles, 6 review articles, 6 hand books and released Plant Disease Warning and Plant Pathology Courier for over one and a half decade. He has been President of Indian Society of Plant pathologists, twice Member, House of Delegate of Asian Society of Plant Pathology and is Chief Editor, Indian Journal of Plant Pathology, Technical Advisor of Pestology and Member Pesticide Project of Department of Science & Technology of Government of India. His current areas of interest are: Plant Clinic, Transfer of Technology, Pesticides and Integrated Pest Management.

# ABSTRACTS OF PLENARY AND KEY-NOTE

## Opening Lecture

### Crop genomics and biotechnology: feeding the billions

*Q.F. Zhang*
*National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, China*
*Email: qifazh@mail.hzau.edu.cn*

In the past half century, production of major food crops has kept pace with the population increase in the world. The yields of major cereals such as maize, rice and wheat have been more than doubled in most parts of the world and even more than tripled in certain countries. However, food production is facing even greater challenges in the next half a century because of high demands in both quantity and quality, and ever increasing pressures on resources and environments. At the same time, advances in genomics, biotechnology and genetic studies have brought about unprecedented opportunities for crop genetic improvement. Rice is a major food crop feeding approximately half of the world's population, and has been a model system for cereal research. In my presentation, I will describe the demands for increased production for future needs, address the main issues that we are facing as challenges, present current progress in rice functional genomics research, and provide prospect on how the advance in research can be translated into technologies and activities for crop genetic improvement.

## Plenary Session 1-The Role of Plant Pathology in a Globalised Economy

### Climate change, bioenergy and global trade: which role for plant pathology?

*Corrado Clini*
*Director General, Italian Ministry for Environment, Land and Sea, Rome, Italy*
*Email: clini.corrado@minambiente.it*

The present agricultural scenario is strongly affected by the effects of climate change on crops, by the increasing importance of bioenergy crops as well as by the influence of global communication and international trade of goods on the movement of plant pathogens among countries. Plant pathology is probably one of the discipline playing a major role in contributing to mitigate the negative effects incited by such factors, in the mean time providing tools and solutions. An overview of international activities promoted by the Italian Ministry for Environment, Land and Sea in the field of plant pathology and crop protection will show the major role played by this discipline in the current agricultural situation, with special emphasis on its role played in mitigating the negative effects of climate change and securing a safe food and energy supply for a growing global population.

### Plant pathology in a globalized economy

*Jan E. Leach*
*Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1177, USA*
*Email: Jan.Leach@colostate.edu*

Plant pathologists must consider the potential of their research to offer solutions in a changing world where global warming, environmental degradation, reduced land available to food production, and diminishing fossil reserves challenge the agricultural status quo. Added to these issues is the expected increase in the world's population, which is predicted to double the demand for agricultural products by 2050. The UN Millennium Development Goals include a challenge to eradicate extreme poverty and hunger, a condition affecting over 1.1 billion people, by the year 2015. Because most of these people rely on agriculture for their livelihood, the opportunity for scientific advances that improve crop productivity to improve their well-being are substantial. Though the challenges for agriculture are serious and imminent, advances in plant pathology and collaborations across all science disciplines can lead to their resolution and are now available or emerging at a rapid rate. This discussion will provide a snapshot of opportunities for plant pathologists that have the potential to improve crops to meet the demands of tomorrow's global economy.

### Impact of a globalized economy on future roles of plant pathologists in a country/region

*X.B. Yang*
*Department of Plant Pathology and Microbiology, Iowa State University, Ames, Iowa 50014, USA*
*Email: xbyang@iastate.edu*

Economic globalization has been with us for centuries which can be exemplified by 18$^{th}$ century trade between China and Europe or earlier agricultural trade by East Indian Company. Now economic globalization occurs at a faster pace, greatly impacting regional agricultural production. The following trends in a globalized economy will be addressed in relation to plant pathology. First, crop production typically fluctuates 25 percent from year to year in a growing region while less than 3% globally. The changing climate has increased the frequency of extreme weather events, leading to more disease epidemics and higher uncertainty in production for a region.

Because modern transportation can move food to other side of globe in 10% of its value, the essence of food security would be balanced with environmental quality in a region. Plant pathologists, who typically work in a regional level, are challenged to reduce regional uncertainty while protecting environment. These in countries extensively participating in a globalized economy should have a greater role in protecting sustainability and environment quality than in food security. Secondly, a globalized economy means that local farmers have to compete globally and globalized agricultural companies are to influence on local farms. Small farms will be replaced by large farms because of profitability. Large farms prefer simplified measures/products and are clients of giant multinational corporations, which are a major force in a globalized economy. Products or technologies from multinational corporations are increasingly vital to our disease management at all levels. This points us to more collaborative research between plant pathologists in public and private sectors.

## Plenary Session 2-Can We Improve Global Food Security (ISPP Task Force)

### Adequate nutrition for all by 2050

*R. N. Strange*
*Department of Biological Sciences, Birkbeck College, London University, Malet Street, London WC1E 7HX, United Kingdom*
*Email: r.strange@sbc.bbk.ac.uk*

"One cannot build peace on empty stomachs": "It is time that the tide of the battle against hunger was changed for the better – but ebb tide could soon set in if we become complacent." The authors of these statements are John Boyd Orr and Norman Borlaug, respectively, both Nobel Peace Prize Laureates. Complacency did indeed set in during the last 3 - 4 decades of the 20th century. There were stirrings in the early part of this century but it was the price spikes in food commodities in 2008 and 2011 which really set the alarm bells ringing. These were largely socio-economic and political phenomena and, no doubt, will be addressed by the last speaker in this session. As plant pathologists, our attention may be more directly concerned with the next two speakers. There is no doubt that the physical environment, whether it be soil, water or climate has profound effects on plant disease. Moreover, we should be prepared for changes in incidence and severity of plant diseases which may be wrought by climate change. Plant pathogens cause tremendous losses of food crops and have been major reasons for famine. But how much loss do they cause? The ISPP has formed a Subject Matter Committee to find answers to this question. Another difficult question is, "What is food insecurity?" Estimates of the food required to feed the world's population of over 9 billion in 2050 are around 70% greater than today's supply. How are we to achieve this with relatively little arable land left for expansion? These are some of the questions which will be examined in this session and on Wednesday evening.

### Physical limitations and challenges for food security: A story of China

*Z.H. Li*
*China Agricultural University, No. 2 Yunimingyuan West Road, Haidian District, Beijing, 100193, P. R. China*
*Email: lizhaohu@cau.edu.cn*

China's grain output was 590 million tonnes in 2012, with the ninth consecutive year of growth. It has achieved the goal in maintaining a high level of self-sufficiency in grains so far. China has 20 percent of the global population, with only 8.0 percent of the world's arable land and 6.5 percent of water reserves. However, China is going to consume more grains in the coming years, which means the country has to increase its annual grain output continuously as China tries to maintain as much self-sufficiency in grains as possible. To achieve the goal, the world's most populous nation and second-largest economy has to find ways to overcome the physical limitations, such as limited potential for qualified cropland expansion, heavily dependent on chemical fertilizer application, water scarcity and uneven distribution, climate change and environmental stresses. Sustainable agricultural techniques are essential for achieving the goal of food security.

### Managing the biological environment to promote and sustain crop productivity and quality

*F.D. Beed*
*International Institute of Tropical Agriculture (IITA, www.iita.org), East Africa Hub, Plot 25, Mikocheni Light Industrial Area, Mwenge Coca-Cola Road, Mikocheni B, PO Box 34441, Dar es Salaam, Tanzania. International Mailing Address: IITA, Carolyn House, 26 Dingwall Road, Croydon, CR9 3EE, UK*
*Email: f.beed@cgiar.org*

Global crop production needs to double by 2050 to meet projected demands from rising populations, diet shifts and biofuel consumption. However, predicted crop yields are not increasing sufficiently (2.4% p.a.) to meet this demand. Therefore, production must increase through more efficient use of current arable land, to prevent further land from being cleared that otherwise provides vital services to the earth and its people (including biodiversity and carbon emissions). Significant improvements in crop productivity and crop quality can be achieved through improved management of critical diseases of crops that are pivotal to food security and income generation. The biological environment is a complex domain characterised by dynamic interactions

between crops and beneficial and antagonistic organisms. These interactions are influenced by the physical environment and human interventions influence emerging diseases and their movement, and this must be respected if diseases are to be sustainably managed. A crop healthcare system for developing countries will be presented that encompasses national responsibility, regional cooperation and harnesses global excellence; in terms of what is known and which methods are available. The aim is control crop diseases in a pre-emptive and cost efficient manner compared to the current scenario of belatedly combating fully blown epidemics. Components of the crop healthcare system are risk assessment; including predicted impacts on food and feed value chains, targeted surveillance, fit-for-purpose diagnostics, control intervention packages, extension mechanisms; that are endowed with refinement from practical feedback, enabling policy environments and application of research to address knowledge gaps. Specific examples will be presented for viruses of cassava, viral and bacterial diseases of banana, stem rust of wheat, viral disease complex of maize and potato blight. Finally, the link between disease control and improved crop quality, consumer health and safe trade will be demonstrated through biological control interventions for aflatoxin in Africa.

**Can we improve global food security? The economic, sociological and political environment**

*U. Grote*
*Institute for Environmental Economics and World Trade, Leibniz University Hannover, Königsworther Platz 1, 30167 Hannover, Germany*
*Email: grote@iuw.uni-hannover.de*

To ensure global food security for a growing population remains a major challenge. This is especially true against the background of increasing food prices partly due to growing income levels and consequently changing demand patterns in the developing world. At the same time, climate change combined with the occurrence of more frequent and extreme natural disasters, and reduced water availability increase the vulnerability of rural farm households, thus negatively affecting agricultural production. Given the multifaceted dimensions of poverty and food insecurity, no simple solution can be found. Promoting productivity of farming and increasing the efficiency of the food system are effective measures contributing to rural development in developing countries. The latter enhances the opportunities of farmers to participate in domestic staple food and high-value agricultural export markets. Information and communication technologies and innovations as well as an improved access to finance and other inputs are known to promote this development by reducing transaction costs and leading to increased investments in the farming sector. Policy reforms in agriculture and beyond help setting price incentives to farmers, promoting good governance, and changing consumers' awareness with respect to food waste and resource use inefficiencies related to human diets. The reduction of agricultural taxation which is traditionally given in many developing countries, and the reduction of distorting subsidies in many OECD countries remain important. What is new, in this context, is the increasing links with other sectors. Thus, on the one hand the linkages between energy and agricultural policies increase due to the trend towards green growth and bioenergy. On the other hand, the monetization of natural resources and speculation link the agricultural market with financial markets. This calls for further research since additional pressure is put on the global food system.

**Achieving global food security**

*L. Korsten*
*Department of Microbiology and Plant Pathology, University of Pretoria, Private Bag X20, Hatfield, Pretoria, South Africa, 0028*
*Email: lise.korsten@up.ac.za*

Hunger and malnutrition are not only caused by food shortage and - scarcity but also by a lack of access to adequate, safe, nutritious food supplies. Food security is complex and its drivers are interdependent. Global drivers in food security link into availability, affordability, quality and safety of food. Addressing chronic food shortages through improved agricultural technologies shift the emphasis from food security to nutritional security. However, an adequate food supply does not guarantee nutritional security. Further, food security diminishes when food systems are stressed. While food systems reflect the continuum from production to consumption, it also involves a much broader consideration than productivity and production. The right to food is also a human right that is recognized under international law. The right of all human beings to feed themselves in dignity includes two aspects related to the production and purchasing of food. To produce own food, resources are required that include land, seeds, water etc. To "buy food" money and market access is required. The "*right to food"* therefore requires governments to provide an enabling environment in which people can use their full potential to produce or procure adequate food for themselves and their families. Food sovereignty on the other hand confronts head-on the notion that food, unlike other fundamental human rights, should be left purely to market forces. It brings to the fore issues around the environment, technology, economies, governance and human rights. It highlights some of the most controversial and challenging issues of our time: climate change and biodiversity, biotechnology, biofuel, water supplies, human migration, population growth and hunger. This presentation contextualises some of these challenges and provides some potential solutions.

### One billion hungry people: what can we do?

*G. Ejeta*
*Center for Global Food Security, Purdue University, 915 West State Street, West Lafayette, IN 47907, USA*
*Email: gejeta@purdue.edu*

The population of the world has been growing at an alarming rate, outpacing gains in global food production. Of the current 7 billion inhabitants of the world, nearly 1 billion people are hungry. Ironically, many parts of our world produce huge surpluses regularly, making food insecurity more a function of poverty and inefficiencies in distribution. Nevertheless, global food security has emerged as humanity's foremost challenge of the 21st century. Yet, there is a lot that we can do, as people, to reduce poverty and eradicate hunger from this planet. Above all, we must develop a collective revulsion against hunger in our planet. We must advance science, technology, and innovation in parts of the world that have not benefitted from these interventions. With increasing global population, there is a growing demand to produce more food, to increase efficiency in food production, to reduce harvest and post-harvest losses, and to devise better strategies to make food accessible to those that need it. We must strengthen the nascent public and private institutions of developing nations to support the adoption and exploitation of modern technologies to change poor rural communities from subsistence to more productive businesses that create jobs and improve livelihoods. Science and technology will increasingly be needed to produce more on less land, with less water, and with judicious use of inputs. With growing concerns about climate change, increasing energy demands, a looming global water crisis, complexities around global trade, and the overall resource inequities around the world, smarter governance and policy instruments for global resource use and investments focused on elevating human welfare broadly are badly needed.

## Jakob Eriksson Prize Award Address

*Jeffrey B. Jones*
*University of Florida, Gainesville, FL, USA*
*Email: jbjones@ufl.edu*

Dr. Jones is being recognized for his numerous seminal research contributions in plant bacteriology that have substantially increased our understanding of the interactions between bacterial pathogens and their host plants. He conducted in-depth analyses of a worldwide collection of xanthomonads associated with tomato and pepper and identified considerable genetic and phenotypic diversity, resulting in the description of three new *Xanthomonas* species. He sequenced their genomes and compared them to identify host specificity factors, resulting in the characterization of several avirulence genes. Dr. Jones and colleagues worked extensively on host plant resistance and identified the first source of resistance in tomato to the bacterial spot pathogen, *X. euvesicatoria*. He and colleagues also developed several tomato and pepper genotypes with resistance to bacterial wilt caused by *Ralstonia solanacearum*. In addition, Dr. Jones and coworkers demonstrated that bacteriophages could be effectively used to control bacterial spot disease on tomato. Dr. Jones is considered a leader in phage therapy for control of plant diseases. His research was instrumental in the first-ever *EPA Registration for Bacteriophage* usage on agricultural crops in the United States. Another important contribution of Dr. Jones and colleagues was the development of a novel method to genetically modify citrus trees so that a hypersensitive reaction (HR)-inducing gene in citrus is activated by native Transcription Activator Like (TAL) effectors in *Xanthomonas citri*, the causal agent of Asiatic Citrus Canker. He and colleagues demonstrated that multiple effector binding elements upstream of a bacterial avirulence gene can be engineered to recognize multiple *X. citri* TAL effector proteins introduced by the bacterial pathogen. and in turn activates a reporter avirulence gene that results in a resistant reaction. The constructs recognize multiple TAL effectors, thus conferring broad and durable resistance. Because the modified genes are locally activated only in the presence of the pathogen, consumers would likely not be exposed to the trans-gene products. Thus, Dr. Jones' work on host-pathogen interactions with bacterial pathogens has had wide-reaching impact, not only in the scientific community but for plant disease control as well. Professor Jones' research has been extremely productive, with over 190 peer-reviewed journal articles, three edited books on bacterial plant diseases, and numerous reviews, book chapters and articles for growers and the general public. Dr. Jones obtained the prestigious University of Florida Research Foundation Professorship twice, for the periods 2002-2004 and 2008-2010. He was recently honored as Outstanding Alumnus by Virginia Tech University in 2010-2011. Dr. Jones was elected to become APS Fellow in 2001 for his outstanding fundamental and applied research on bacterial plant pathogens and their interactions with their hosts.

## Keynote Session 1-The Role of Plant Pathology in Bio-security and Food Safety

### Assuring food security in an uncertain world

*R.S. Zeigler*
*International Rice Research Institute, College, Los Baños, Laguna 4031, Philippines*
*Email: r.zeigler@irri.org*

After an absence of several decades, food security has returned to center stage in any serious discussion on global affairs. The Green Revolutions in Asia help transform countries glibly dismissed as "basket cases"

by strategic thinkers in the 1960s. These countries are now engines of global economic growth. However, falling food prices from the late 1970s through early in this century led to a sense of complacency towards food supplies and agriculture investment declined. The precarious global food supply system is exacerbated by the stresses that climate change is expected to place on major food producing areas, especially rice in Asia. The challenges facing the world to increase food supplies by more than 50% over the coming decades in the face of climate change are arguably much greater than faced us 50 years ago. There are also powerful social and economic drivers that will transform the nature of agriculture in developing countries. This is particularly the case for rice, the staple cereal for half the world's population and the primary food for over 70% of the world's poor. The agricultural science community is in the midst of a nested set of scientific and technical revolutions that will help fundamentally transform agriculture especially in developing countries. Scientific advances in plant genetics, biology, crop management and the social sciences will directly impact the future of global rice supplies, the sustainability of intensive rice production systems so characteristic of Asian landscapes, and the lives of hundreds of millions of farmers and consumers.

**Human pathogens on plants: role of plant pathologists in a multidisciplinary mitigation strategy**

*J. Fletcher*
*National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK 74078, U.S.A.*
*Email: jacqueline.fletcher@okstate.edu*

Increasing concerns about microbial contamination of food plants and resulting foodborne illness have prompted new collaboration and interactions between the scientific communities of plant pathology and food safety. Around the world, repeated outbreaks of human illness attributed to the contamination of fresh produce, nuts and seeds, and other plant-derived foods by human enteric pathogens such as Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. have led some plant pathologists to broaden the application of their science, to address problems of human pathogens on plants (HPOPs). Recent research has shown that human enteric pathogens can have complex interactions with plants and their associated microbial communities. New collaborations between members of the plant pathology and food safety communities facilitate enhanced research capacity and greater understanding of the issues for which research is needed. High priority research topics include HPOP dissemination, plant colonization and entry into plant interiors, plant responses to HPOP presence, HPOP-plant pathogen interactions, and the development of effective mitigation strategies for field and post-harvest applications. Traditional plant pathology concepts, such as the disease triangle and the disease cycle, as well as the application of new technologies to understand HPOP-plant pathogen-plant-environ-ment interactions, can help to inform cross-over issues that pertain to both HPOPs and plant pathogens, and will facilitate the identification of effective strategies for minimizing the risk of microbial contamination. Continued interactions and communication among interdisciplinary and multi-national communities is essential.

**Plant pathology in the post-genomics era**

*S. Kamoun*
*The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom*
*Email: sophien.kamoun@tsl.ac.uk*

Infectious plant diseases cause havoc to world agriculture and threaten to slow laudable efforts to launch a second green revolution to meet the food security needs of a booming world population. Pathogens such as the rice blast fungus, wheat stripe and stem rust, the Irish potato famine pathogen, and many others continue to trigger recurrent epidemics with far reaching consequences. When faced with opponents like these, we need to know our adversary. The genome sequence of a plant pathogen is a deep look into its soul. From important and often unexpected insights into the biology of the pathogen to the tools needed to develop surveillance and diagnostic DNA markers, the genome is an invaluable resource that accelerates research and output delivery. With the cost of genome sequencing decreasing even faster than Moore's law, the cost-benefit calculation is evident. For instance, countless time and money are spent in developing DNA markers, investigating population structures, debating the pathogen origin, etc. – activities that can be greatly hastened by the genome sequence. In this talk, I will discuss some of ways in which genome biology impacts plant pathology. In particular, I will address how pathogen genomics can drive basic and applied plant pathology, and how state of the art findings on pathogen biology can be exploited to drive the development of new approaches to breeding disease resistant crops. Detailed knowledge of the pathogen genome coupled with novel methods of targeted plant genome engineering is ushering the era of next-generation disease resistance breeding in plants.

## Keynote Session 2-Genomics, Proteomics and Plant Pathology

**Induction and suppression of RNAi-mediated antiviral defense by *Cucumber mosaic virus***

*S.W. Ding*
*Department of Plant Pathology and Microbiology, University of California, Riverside CA 92521, USA*
*Email: shou-wei.ding@ucr.edu*

*Cucumber mosaic virus* (CMV) is an important crop pathogen with perhaps the widest host range of any known plant virus. We have been using CMV as a model to understand the mechanism of induction and suppression of RNAi-mediated antiviral defense in plants. In antiviral RNA interference (RNAi), a host RNase III enzyme Dicer processes viral replicative intermediates double-stranded RNA into small interfering RNAs (siRNAs) of 21 to 24 nucleotides in length. Upon incorporation into Argonaute (AGO) proteins, these viral siRNAs then guide specific degradation of complementary viral RNAs. Our studies have led to the discovery of the CMV 2b protein as a potent RNAi suppressor, which explains in part why CMV has a wide host range. We show that the antiviral RNAi of *Arabidopsis thaliana* against CMV requires production and antiviral activities of viral secondary siRNAs processed from a different pool of viral dsRNA synthesized by host RNA-dependent RNA polymerase (RDR). We note that the two members of each of the Dicer-like (DCL), RDR and AGO families exhibit distinct antiviral activities, indicating that gene duplication is followed by functional diversification in *A. thaliana*. In addition to amplifying viral siRNAs, we found that RDR1 also mediates production of a novel class of endogenous siRNAs targeting more than a thousand of *A. thaliana* genes, suggesting a new mechanism for RDR1-dependent antiviral activity. Strong genetic redundancy in antiviral RNAi may explain why forward genetic screens have not been as successful as in the characterization of the bacteria-induced resistance, for example. I shall report the identification of new components in antiviral RNAi by a forward genetic screen developed in *A. thaliana* using a recently characterized 2b-deletion mutant of CMV, which is silenced only by RDR6-dependent antiviral RNAi pathway.

### *Fusarium* pathogenomics: understanding fungal pathogenicity through genomics

*L.J. Ma*
*Department of Biochemistry and Molecular Biology University of Massachusetts Amherst, Amherst MA 01003, USA*
*Email: lijun@biochem.umass.edu*

Since the publication of the first fungal genome, *Saccharomyces cerevisiae*, in 1996, more than a thousand fungal genomes have been sequenced, which makes the kingdom of fungi the most sequenced eukaryotic taxon. Correlating genotypes based on sequence information to interesting phenotypes, such as pathogenesis, is one of many challenges associated with all genome projects. This presentation will use *Fusarium* comparative genomics as an example to study genetic mechanisms that contribute to pathogenicity among a group of important phytopathogens in *Fusarium oxysporum* species complex. Members of this species complex are responsible for destructive and intractable wilt diseases in over 100 diverse plant hosts, including the recent outbreak of Panama disease of banana that destroyed more than 70% crop in disease manifested areas. In contrast to the broad host range, a single pathogenic form within this species complex usually infects a single plant host, exhibiting strong host specificity. The *Fusarium* comparative genomic study demonstrated that horizontally acquired pathogenicity chromosomes convey host-specific pathogenicity. The genome of the pathogen can be divided into core genomic regions: responsible for essential biological processes, and adaptive genomic regions: contributing to pathogen virulence and host specialization. The application of the genome structure compartmentalization enabled the identification of candidate effectors among the newly sequenced genomes of pathogenic isolates. These candidate effectors hold the potential for monitoring the spread of a particular disease and for the development of management plans.

### The rice blast fungus: genomics and beyond

*Y.H. Lee*
*Department of Agricultural Biotechnology, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea*
*Email: yonglee@snu.ac.kr*

The research goal of my laboratory is to elucidate the molecular mechanisms of fungal pathogenesis and interactions between rice blast pathogen, *Magnaporthe oryzae* and its host plant, rice at the genomic level. Rice blast is a compelling model system for studying host–parasite interactions due to its socioeconomic impact and the availability of both the rice and fungal genomic sequences. In an attempt to understand the molecular mechanisms of rice blast, we have been taking both forward and reverse genetics approaches. Our researches using reverse genetics approach focus on identifying and characterizing the genes involved in signal transduction pathways leading to appressorium formation, genes encoding transcription factors, and genes that are required for post-penetration stages. For forward genetics studies, we carried out a large-scale insertional mutagenesis of the *M. oryzae* strain KJ201 via *Agrobacterium tumefaciens*-mediated transformation, generating over 25,000 mutants. We also developed high throughput phenotype screening system that enables rapid and robust assay of mutant phenotypes. Those mutants are stored and maintained in the Center for Fungal Genetic Resources. In addition to our endeavor to functional and comparative genomics, we built a cyber-infrastructure for storage of heterogeneous data and analysis of such data in multiple contexts. The whole genome sequence information of *M. oryzae* as well as most of the results from experimental biology is housed in our customized database. Our comprehensive and integrative approaches coupled with a web-based Laboratory Information

Management System would provide a novel platform for systems biology initiatives for fungal pathogenesis.

## Keynote Session 3-Host-Pathogen Interactions and Molecular Plant Pathology

### Structure, functions, and evolution of the bacterial root microbiota

*D. Bulgarelli, K. Schlaeppi, P.E. ver Loren van Themaat, N. Dombrowski, M. Rott, Y. Bai and P. Schulze-Lefert*
*Max Planck Institute for Plant Breeding Research, D-50829 Cologne, Germany*
*Email: schlef@mpipz.mpg.de*

Plants host distinct bacterial communities on and inside various plant organs. We show that roots of *Arabidopsis thaliana*, grown in different natural soils under controlled environmental conditions, are preferentially colonized by Proteobacteria, Bacteroidetes and Actinobacteria, and each bacterial phylum is represented by a dominating class or family. Soil type defines the composition of root-inhabiting bacterial communities and host genotype determines their ribotype profiles to a limited extent. Plant cell wall features provide a sufficient cue for the assembly of ~40% of the Arabidopsis bacterial root-inhabiting microbiota, with a bias for Betaproteobacteria. This root sub-community may not be Arabidopsis-specific but saprophytic bacteria that would naturally be found on any plant root or plant debris in the tested soils. A comparison of the bacterial root microbiota of *A. thaliana* with the microbiota from selected *A. thaliana* relatives grown under controlled environmental conditions or collected from natural habitats suggests the existence of an evolutionarily conserved core microbiota with species-specific footprints. These findings also imply that the bacterial root microbiota is surprisingly resilient to environmental changes. We have isolated > 40% of the root microbiota members from *A. thaliana* as pure bacterial cultures. This has allowed us to develop *in planta* test systems for single or combinations of microbiota members under laboratory conditions and to explore their functions in plant growth promotion and for plant health. We combine these *in planta* assays with whole-genome sequencing of microbiota members and assess their metabolic capacity using metabolic arrays to obtain first insights into the molecular basis of rhizobacteria interactions with Arabidopsis roots.

### Bacterial pathogenesis in microbe-colonized and microbe-free plants

*S.Y. He*
*Department of Energy Plant Research Laboratory, Howard Hughes Medical Institute, Gordon-Betty Moore Foundation, Michigan State University, East Lansing, MI, USA*
*Email: hes@msu.edu*

We have been using the *Arabidopsis thaliana-Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 pathosystem to study general principles that underlie plant susceptibility to bacterial pathogens in microbe-colonized plants. *Pst* DC3000 is representative of a large number of bacterial pathogens that infect the above-ground parts (phyllosphere) of plants. During infection, this pathogen produces a battery of virulence factors to engage multiple host cell types (e.g., stomatal and mesophyll cells) and diverse host physical and chemical barriers. Its type III secretion system (T3SS) delivers about 30 "effector" proteins into the plant cell, whereas the phytotoxin coronatine mimics the active form of plant hormone jasmonate. Study of the molecular action of T3SS effectors and coronatine demonstrates the great utility of *Pst* DC3000 pathogenesis as a probe in the discovery of components of the plant immune system and the elucidation of the jasmonate signaling mechanism in plants. Recently, we have initiated studies to understand the effects of phyllosphere microbiota on bacterial pathogenesis and plant immune responses by establishing an experimental set-up for growing microbe-free plants in soil. This set-up begins to reveal a potentially fundamental role of microbiota in training the plant immune system and plant abiotic stress responses.

### Genomics of fungal plant pathogens and adaptation to their host plants

*R.E. Bradshaw[1] and P.J.G.M. de Wit[2]*
*[1]Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand; [2]Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands*
*Email: pierre.dewit@wur.nl*

We compared the genomes of the fungal plant pathogens *Cladosporium fulvum* and *Dothistroma septosporum* belong to the class of Dothideomycetes, are phylogenetically closely related, but have different lifestyles and infect different hosts. *C. fulvum* is a biotroph that infects tomato, while *D. septosporum* is a hemibiotroph infecting pine. The genomes of these fungi have a similar set of genes but differ significantly in size, which is mainly due to the difference in repeat content. Repeat-rich areas in *C. fulvum*, which primarily consist of retrotransposons, were enriched for species-specific genes including those encoding secreted effector proteins. Several previously cloned effector genes from *C. fulvum* are present in *D. septosporum* and some of them (*Ecp2* and *Avr4*) are recognized by tomato Cf resistance proteins and cause a Cf-mediated hypersensitive response. Some gene clusters encoding the dothistromin toxin, well studied in *D. septosporum*, are conserved in *C. fulvum*, although in this fungus some of the genes are pseudogenized or not expressed *in planta*. *C. fulvum* produces the species-specific enzyme α-tomatinase, absent in *D. septosporum*, that detoxifies α-tomatine present

in high concentrations in tomato enabling it to colonize tomato. In the two fungi and other Dothideomycetes introner-like elements were identified; these are highly structured near-identical introns present in different genes that are multiplied by a yet unknown mechanism. Overall, comparison of the two genomes shows that closely related plant pathogens have adapted to different hosts and lifestyles by different mechanisms including gene innovations, pseudogenization and gene regulation.

## Keynote Session 4-Recent Developments in Disease Management

### Contribution of precision crop protection technology to modern disease management – challenges and prospects

*E.C. Oerke*
*University of Bonn, INRES – Phytomedicine, Nussallee 9, D-53115 Bonn, Germany*
*Email: ec-oerke@uni-bonn.de*

Spatial distribution of plant diseases within crops often is heterogeneous – at least in some periods of the growth period. Within-field heterogeneity of disease implies that plants at some sites may need disease control while plants at other sites do not. Automatable detection, identification and quantification of diseases on a small scale are the prerequisites for a site-specific application of fungicides, adequate to disease incidence and precise in space and time. Innovative sensor technologies in combination with informatics and modern application technologies may enable disease control where and when actually needed. Remote sensing covers large areas and provides an overview on the health status of crops and its development in time, but spatial resolution and sensitivity to low disease levels are not adequate to detect early symptoms on individual plants at levels below the action threshold. Proximal sensor systems are currently tested for their suitability for precision crop protection applications. Though optical sensors for imaging spectroscopy, thermography and chlorophyll fluorescence seem to be very promising, other sensor types, ranging from mechanical to biochemical techniques may be also suitable. Information from various sensors may be also used for the definition of management zones, especially suitable for the management of soil-borne pathogens. Because of the investment costs this technology is likely to penetrate high productivity cropping systems and high-valued crops first. Systems for disease sensing may be also used in post-harvest applications, fungicide screening platforms and for plant phenotyping in breeding for disease resistance.

### A system approach: integrating crop production, on-farm practices and pest management measures for a healthy and sustainable crop

*A. Gamliel*
*Institute of Agricultural Engineering, Agricultural Research Organization (ARO) Volcani Center, Bet Dagan, 50250 Israel*
*Email:agamliel@agri.gov.il*

The current trend in modern intensive agriculture is characterized by three major directions: pest control, environmentally safe measures and consumer demand for, healthy and pesticide-free products. A system approach in pest management incorporates and assembles all the agricultural practices which are relevant to the crop production and which are also of impact with regard to pest onset or suppression. A system approach includes and considers: (i) Manipulation of agricultural practices aiming at reducing optimal conditions for pest eruption and providing better conditions for plant development and defense. These also include the use of resistant cultivars and grafted plants and the appropriate manipulation of nutrition, water quality and environmental conditions, to further provide plant tolerance to the relevant pest. (ii) Integrated pesticide application at all crop stages and interfering with all stages of pathogen life cycle. These involve, use of pesticide and biopesticides and other chemically and biologically based compounds. Special attention is given to application methods and technology to assure maximum effect on the targeted past (iii) Minimizing negative attributes to the crop, environment and the consumer, by the reduced use of toxic pesticides, the enhanced dissipation of pesticides from the environment and the development and use of soft pesticides and their optimal application. (iv) Measures to assure food safety at harvest and following storage. Pest management is a complicated set of procedure due to the heterogeneity of the production system, and pest infestation. Thus, assembly of all components within a production system may result in effective management. Examples are shown with specific crops, pests and management strategies.

### New developments in chemical disease management

*A.J. Leadbeater*
*Syngenta Crop Protection AG., Schwarzwaldallee 215, CH-4058 Basel, Switzerland*
*Email: andy.leadbeater@syngenta.com*

The use of chemical fungicides to control plant diseases is an integral component of crop management. Although fungicides have been used to good effect in agriculture since the 1940s, the introduction of new fungicides is an essential element to provide sustained control of major crop diseases. The need for new and innovative fungicides is driven by resistance management, regulatory hurdles and increasing customer expectations amongst others. New fungicides can be discovered either within established mode of action groups, ideally with low resistance risk (robust modes of action), or in areas with

completely novel modes of action. Compounds having a novel mode of action are of course of special interest, since they play a key role in resistance management strategies, but equally important are new fungicides with enhanced characteristics such as systemicity, curativity and longevity of disease control. With the background of increasing registration hurdles, increasing costs and increasing market needs, a review is presented on the current market position of major crop protection fungicides, current and future market needs and new fungicidal compounds in late development or recently introduced to the market. Some key features of the new fungicides will be discussed including biological target segments, business potential and future impact. New MoAs are quite rare in some segments (major new fungicides are all SDHIs), but seem to be more frequently discovered for the control of Oomycetes. Potential reasons for this will be discussed.

## Keynote Session 5-Plant Pathology in Asia

### Whole genome analysis of rice - *Magnaporthe* interactions

*R. Terauchi*
*Iwate Biotechnology Research Center, Kitakami, Iwate, 024-0003, Japan*
*Email: terauchi@ibrc.or.jp*

Rice blast caused by the ascomycete fungus *Magnaporthe oryzae* is the most devastating disease of rice worldwide, therefore understanding of the molecular mechanisms of *Magnaporthe*-rice interactions is important to devise efficient control of the disease. Using *M. oryzae* whole genome sequence information and association genetics approach, we isolated genes for three *AVR*s, *AVR-Pia*, *AVR-Pii* and *AVR-Pik*. Genomics approaches allowed us to isolate rice *Pia* and *Pii R*-genes. Rice *Pik R*-gene has been previously reported by other groups. In this paper, I would like to introduce the whole genome sequence (WGS)-based gene isolation methodologies and report our latest findings on the interactions between AVR-Pik and Pik as well as AVR-Pii and Pii.

### Advances in Understanding Begomovirus Satellites

*X.P. Zhou*
*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Biotechnology, Zhejiang University, Hangzhou, 310058, P. R. China*
*Email: zzhou@zju.edu.cn*

Begomoviruses are numerous and geographically widespread viruses that cause devastating diseases in many crops. Monopartite begomoviruses are frequently associated with betasatellites or alphasatellites. Both betasatellite and alphasatellite DNA genomes are approximately half the size of begomovirus DNA genomes. Betasatellites are essential for induction of typical disease symptoms. The *βC1* genes encoded by the betasatellites have important roles in symptom induction, in suppression of transcriptional, and in posttranscriptional gene silencing, and they can affect jasmonic acid responsive genes. Host plants of begomoviruses have evolved diverse innate defense mechanisms against the βC1 protein to counter these challenges. Alphasatellites have been identified mainly in monopartite begomoviruses that associate with betasatellites and have no known contributions to pathogenesis of begomovirus-betasatellite disease complexes. Applications of current molecular tools are facilitating viral diagnosis and the discovery of novel species of geminiviruses and satellite DNAs and are also advancing our understanding of the global diversity and evolution of satellite DNAs.

### Plant health clinics hold key to food security in Asia

*M.P. Srivastava*
*Haryana Agricultural University, India*
*Email: mpsrivastava28@gmail.com*

Ever-rising population, climate change and huge losses due to pests and diseases pose serious threat to food security. Plant health clinics hold key to food security by monitoring pest-scenario, providing early diagnosis and management strategy. Plantwise-initiated community-based-clinics operate in public places in few Asian countries, whereas in India, clinics operate physically under various organizations. To address growing complexity in pest-scenario, more so due to changing climate, creating more well-organized multispecialty clinics with independent physical identity, better infrastructure and resources, redefined role aimed at total plant-health, is imperative to boost food security. Precisely, clinic should be farmer-centric, with distinct identity, welcome-counter with overhead electronic-display of scrolling-text showing relevant message on plant-health, waiting-cum-exhibition-hall with exhibits and colored signage/ blow-ups of diseases/pests, café/toilet, well-equipped library, agro-pharmacy, laboratories with traditional/ new-age diagnostic tools (microscope with monitor-attachment, digital-camera, laminar-flow, ELISA, PCR, LFD etc.), well-experienced pathologist, entomologist, agronomist/edaphologist providing diagnosis and prescription for various pests/ disorders. Beyond diagnostics, clinics owe responsibility of strengthening plant-health-care by educating farmers on pest diagnosis and management, training/teaching students, producing plant doctors, keeping vigil on bio- terrorism, promoting integrated management to minimize pesticide use and saving biodiversity, monitoring pest/diseases distribution/outbreak, issuing pest alerts/ warning, organizing plant health camps in clinics/ farm-fairs, and strengthening mobile clinic during pest-outbreak. Other major roles involve invigorating farmers' access to 7×12 information

through toll-free- telephony, mobile/internet, providing online-advice, enhancing farmers' knowledge-bank through innovative training/print/electronic devices, maintaining database and networking, conducting impact-assessment, revitalizing farmer-extension-research-government interface for optimizing techno-resources. Creating such clinics with difference, modeled on human clinics, providing wide range of plant health services at zero cost, shall symbolize quintessential 'plant clinic/ hospital', commanding same status/recognition as human clinic, empowering farmers/stakeholders to improve food security. Impact of such clinic has been phenomenal in ushering productivity. Let's collaborate and embrace thoughts and technologies to create more such clinics in Asia, and revolutionize plant healthcare, laying foundations of national plant health services.

# ABSTRACTS OF CONCURRENT SESSIONS

## Concurrent Session 1-Airborne Plant Diseases and Their Control

### O01.001 Sexual stage of *Pucccinia striiformis* f. sp. *tritici* in China

Z.S. Kang[1], J. Zhao[1], Z.Y. Wang[2], W. Chen[2] and L.L. Huang[1]
[1]State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yanling, Shaanxi 712100, People's Republic of China; [2]College of Plant Protection, Northwest A&F University, Yanling, Shaanxi 712100, P. R. China.
Email: kangzs@nwsuaf.edu.cn

Wheat stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) has been considered as one of most serious wheat diseases in China. Although barberry (*Berberris* spp.) serving as alternate host of the rust pathogen, whether sexual reproduction occurs in China is unknown. We surveyed barberry plants for infection by rust fungi in the stripe rust "hotspot" regions in Gansu, Sichuan, and Shaanxi provinces; and tested *Pst* isolates obtained from aecia on naturally infected barberry plants on the wheat genotypes used to differentiate Chinese *Pst* races to determine virulence variations. Different *Berberis* species were widely distributed and most surveyed plants had pycnia and aecia of rust fungi throughout the surveyed regions. A total of 28 *Berberis* species were identified during our study. Of 20 *Berberis* species tested with teliospores of *Pst* from wheat plants, 18 species were susceptible under greenhouse conditions. Among 3,703 aecia sampled from barberry plants of three species, *B. shensiana*, *B. brachypoda*, and *B. soulieana*, under natural infections in Gansu and Shaanxi provinces, four produced *Pst* uredinia on susceptible wheat cultivar Mingxian 169. Sequence of the ITS regions of the four isolates from barberry shared 99% identity with the *Pst* sequences in the NCBI database. The four isolates had virulence patterns different from all previously reported races collected from wheat plants. Furthermore, 82 single-uredinium isolates obtained from the four barberry isolates had high virulence diversity rates, ranging from 9.0% to 28.1%, indicating that the diverse isolates were produced through sexual reproduction on barberry plants under natural conditions. Our results indicated that *Pst* can infect some *Berberis* spp. under natural conditions and the sexual cycle of the fungus may contribute to the diversity of *Pst* in China.

### O01.002 New developments in identification and quantification of airborne inoculum

J.S. West, S. Heard, G.G.M. Canning, B.A. Fraaije and K. Hammond-Kosack
Rothamsted Research, Harpenden, AL5 2JQ, UK
Email: jon.west@rothamsted.ac.uk

Novel diagnostic methods integrated with air sampling and wireless communications now offer the prospect of automated inoculum-based crop disease forecasting. This area of precision agriculture has potential to provide information on both the location and optimal timing of crop protection measures. Traditionally spore trapping has been used to understand the seasonal timing of spore releases of plant pathogens, their responses to weather and to identify likely sources of spores. Application of DNA-based diagnostic methods such as qPCR over the past 10-15 years in particular, has provided new information about species that previously could not be identified accurately by visual microscopy or culturing. These lab-based methods can indicate the emergence of new pathogens and also provide valuable information on changes in genetic traits such as pathotype or race structure or presence of fungicide resistance in pathogen populations. Disease forecasting is not needed for common diseases that occur at well-known times but for damaging sporadic diseases, especially for high-value crops. Air sampling can be used for disease warning if samples can be analysed and results disseminated quickly – ideally within hours. Hence, field-based tests are increasingly attractive and new rapid isothermal DNA-based methods, lateral flow immunological kits and automated biosensors are now being used. In some systems, field test results can be sent as text messages along with local weather data and applied to models to trigger a disease alert. Complexities of spore dispersal processes in the atmosphere necessitate further work to optimise deployment of samplers and interpretation of their results.

### O01.003 Epidemiology of soybean rust in North America

X. Li, A.P. Dias, Z. Pan and X.B. Yang
Department of Plant Pathology & Microbiology, Iowa State University, 351 Bessey Hall, Ames, Iowa, 50011, U.S.A.
Email: linuslee@iastate.edu

Since 2004, occurrences of soybean rust (causal agent *Phakopsora pachyrhizi*) have been closely monitored in North America. The disease had strong annual variations, especially in its geographic distribution, overwintering, and early season movement. During 2005-2012, the disease caused minor damage in the Soybean Belt and occasionally had late season epidemics in the southern U.S. The northern most reports of this disease were in Iowa in the U.S. and Ontario in Canada in 2007. These observations agree with our previous pre-entry assessment that the dispersal risk of this disease was less than southern corn rust with low risks to the Soybean

Belt though in the coastal regions it may lead to limited economic losses. To predict soybean rust progress, various models have been built based on weather variables, such as rainfall, temperature, leaf wetness, cloudiness, and solar radiation. Some of these variables, e.g., rainfall and solar radiation, have showed good abilities in soybean rust forecast at a regional scale. Using coupled disease models and regional weather models that processes spore dispersal of *P. pachyrhizi*, the risks of disease establishment can be assessed using operational weather forecast data. This forecasting system has been used since 2006 and successfully predicted the northward movement of soybean rust to north central region in 2007. Our latest research showed that the regional progress of soybean rust in the U.S. had significant spatial and temporal autocorrelation and was also correlated with seasonal changes of solar radiation. An improved soybean forecasting system is being developed based on the new results.

**O01.004 Novel understanding of the water cycle as a link between unsuspected habitats of airborne pathogens -what consequences for plant disease management?**

*C.E. Morris, C.L. Monteil and O. Berge*
*INRA, Plant Pathology Research Unit 407, Montfavet, France*
*Email: cindy.morris@avignon.inra.fr*

Recent research on the ecology of the phytopathogenic bacterium *Pseudomonas syringae* has revealed that its life history is intimately linked to the water cycle. As a consequence, the current vision of its ecology is moving away from that of a ubiquitous epiphytic plant pathogen to one of a multifaceted bacterium *sans frontières* in fresh water and other ecosystems linked to the water cycle. Its life history involves adaptation to ubiquity that is facilitated by dissemination via the water cycle and includes an important aspect of atmospheric transport as well as infiltration through soil. This new vision of its life history also integrates spatial and temporal scales spanning billions of years and traversing catchment basins, continents, and the planet and confronts the implication of roles that are potentially conflicting for agriculture (as a plant pathogen and as an actor in processes leading to rain and snowfall). Overall, it sets the stage for the integration of more comprehensive contexts of ecology and evolutionary history into comparative genomic analyses to elucidate how *P. syringae* subverts attack and defense responses of the cohabitants of the diverse environments it occupies. In practical terms this new ecological perspective has also yielded insight into epidemiological phenomena linked to disease emergence. This latter point will be explored in particular in this presentation and its pertinence for other plant pathogens will be discussed.

**O01.005 Developing forecasting systems of aerial pathogens for practical use**

*X.M. Xu*
*East Malling Research, East Malling, West Malling, ME19 6BJ, UK*
*Email: xiangming.xu@emr.ac.uk*

One major issue in sustainable agriculture is how to reduce pesticide input while maintaining satisfactory control of pests and diseases. For a given crop, particularly high value vegetable and fruit crops, multiple applications of fungicides are often necessary to manage aerial pathogens. Reliable forecast of disease development may enable better timing of fungicide application to ensure maximum efficacy and to avoid unnecessary applications. Developing disease forecasting models and implementing them in commercial agriculture have been an important area of research activities in plant disease epidemiology over the last two decades. Use of forecasting systems in practice is often not constrained by the accuracy of disease forecasts, but rather by practical issues related to the delivery of forecasts, control options available, and other crop husbandry activities. Several examples of contrasting pathosystems (apple scab, apple powdery mildew, and strawberry grey mould in open field and under protection) will be used to illustrate these practical issues. Potential solutions overcoming these issues and hence increasing the use of disease forecasting systems will be discussed.

**O01.006 Monitoring viable airborne inoculum of *Botrytis cinerea* in the South-East of France over 3 years: relation with climatic parameters and the origin of air masses**

*C. Leyronas and P.C. Nicot*
*INRA, UR407 Pathologie végétale, Domaine Saint Maurice, CS 60094, F-84143 Montfavet cedex, France*
*Email: philippe.nicot@avignon.inra.fr*

Viable airborne inoculum of *Botrytis cinerea* was monitored bimonthly during 3 years (September 2007–December 2010) on a site in the South-East of France located approximately 5 km away from susceptible crops. Viable inoculum was observed on 96 % of the sampling days, including during cold winter periods and hot and dry summer conditions. The concentration of airborne inoculum was significantly higher during daytime than at night. Peaks of concentration were recorded at different periods each year (September–October in 2008, May in 2010). The abundance of viable inoculum was positively correlated with average daily relative humidity and negatively correlated with air temperature and solar radiation. The analysis of backward trajectories suggested that air masses originating from the North or the South brought more viable inoculum than those from the West. This study showed that susceptible crops may be at danger from viable inoculum of *B. cinerea*

during all seasons of the year, but that risk prediction models could be developed on the basis of climatic conditions and the origin of air masses.

**O01.007 Strategies and technologies of integrated management on wheat stripe rust caused by *Puccinia striiformis* Westend**

W.Q. Chen[1], Z.S. Kang[2], Z.H. Ma[3], S.C. Xu[1], S.L. Jin[4] and Y.Y. Jiang[5]
[1]State Key Laboratory for the Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, CAAS, Beijing 100193, P. R. China; [2]Northwest A & F University, Yangling 712100, P. R. China; [3]China Agricultural University, Beijing 100193, P. R. China; [4]Gansu Academy of Agricultural Sciences, Lanzhou 7300700, P. R. China; [5]National Agro-Tech Extension and Services Center, Ministry of Agriculture, Beijing 10081, P. R. China
Email: wqchen@ippcaas.cn

Wheat stripe (yellow) rust caused by *Puccinia striiformis* Westend (*Ps*) is an important disaster on wheat production. It has been fully revealed that the over-summering and over-wintering areas of *Ps*, inter-regional dispersal of inoculum, approach of virulence variation, and breakdown causation of wheat cultivar resistance. Based on the geographical conditions, wheat growth, occurrence and dispersal of disease, wheat stripe rust in China can be divided into three major zones, namely the autumn sources of inoculums, the spring sources of inoculums, and spring epidemic areas. A major strategy of headstream management has been put forward, i.e. "integrated management of wheat stripe rust in the sources of inoculums to protect wheat safety plantation in all over the country". A series of effective measures for the forecast and control of disease has been developed, which include the early molecular diagnosis of stripe rust, wide forecast, improving cultivar resistance, changing cultivation crops, regulating sowing date, seed-dressing with fungicides, and spraying fungicides in the initial stage of disease etc. According to the epidemic rules of wheat stripe rust, the integrated management systems based on the biodiversity have been set up in the areas of inoculum sources of *Ps*, respectively, which have been widely applying in wheat production resulting in the sustainable control of wheat stripe rust epidemics and remarkably economic efficiency. It is also proposed as the priority that variation mechanism of pathogen virulence, development of early forecast system and the ecological control measures of disease in the areas of inoculum sources of wheat stripe rust in the near future in this paper.

**O01.008 Attachment of airborne pathogens to their host: a potential target for disease control**

K. Ikeda, H. Kitagawa, S. Shimoi, K. Inoue, Y. Osaki, H. Nakayashiki, P. Park
*Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501, Japan*
*Email: ikeken@phoenix.kobe-u.ac.jp*

The phytopathogenic fungi have been developed various pathogenic factors, i.e., adhesion molecules, sensor machineries against host plant, and invasion machineries into the host cells. The fungal adhesion to the host cell is a first important step and considers as universal machinery across the plant pathogenic fungi. We are interested in the adhesion molecules during spore differentiation in *Magnaporthe oryzae*. We found that one kind of lectin, concanabalin A (ConA)-reactive glycoprotein(s) was important for the establishment of adhesion. We also found that glycoprotein degrading enzymes such as collagenase and gelatinase were able to detach the germlings and inhibit manifestation of disease symptom. We screened collagenolytic/gelatinolytic bacteria which degraded the fungal extracellular matrix to establish a novel biological control measure inhibiting germling adhesion on the host plant surface against airborne phytopathogenic fungi. The selected bacteria were evaluated for durable disease protection of *Magnaporthe oryzae* on barley leaves. We found that highly gelatinolytic bacteria from the soil were less likely to settle on leaf surfaces. We supplemented gelatin during bacterial treatment to facilitate adhesion and provide nutrition for the bacteria. The gelatin supplementation dramatically improved the settlement of gelatinolytic bacteria *Chryseobacterium* sp. from the soil and the disease protection effect lasted for more than 2 weeks on barley. Moreover, exploitation of gelatinolytic bacteria for disease protection was extended against other airborne pathogens, *Alternaria alternata* Japanese pear pathotype and *Colletotrichum orbiculare*.

**O01.009 Changes in the host range and virulence variation of *Pseudoperonospora cubensis* populations in the Czech Republic**

A. Lebeda, B. Sedláková, J. Pavelková and M. Kitner
*Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71, Olomouc-Holice, Czech Republic*
*Email: ales.lebeda@upol.cz*

*Pseudoperonospora cubensis,* a causal agent of cucurbit downy mildew, is a highly variable pathogen. During the 2009 - 2011 growing seasons, disease prevalence and severity and the host range of *P. cubensis* were evaluated at more than 70 locations in the Czech Republic (CR). Infection by *P. cubensis* was observed primarily on *Cucumis sativus*, medium to high disease severity prevailed. During the years 2010 and 2011, *P. cubensis* infection was also recorded on other cucurbit plants: *Cucumis melo*, *Citrullus lanatus*, *Cucurbita* spp. (*C. moschata*, *C. pepo*, *C. maxima*, *C. ficifolia*) and *Lagenaria siceraria*. *P. cubensis* on *C. melo* and *C. lanatus*

has been formerly reported from the CR, however, infection on *C. moschata* and some other *Cucurbita* spp. was observed for the first time in the CR. Virulence shift (2009 - 2011) was studied in populations of seventy *P. cubensis* isolates. There were found the substantial changes in the pathogen population virulence structure (firstly observed in the year 2009) in comparison with the period 2001 - 2008. These findings have been also supported by our recent studies of pathogen molecular polymorphism. This research was supported by the following grants: MSM 6198959215, QH 71229 and Internal Grant Agency (Prf-2011-3, PřF 2012-001).

**O01.010 Infection of intact canola (*Brassica napus*) roots by the foliar pathogen *Leptosphaeria maculans***

*S.J. Sprague, A. Main, A. Marquardt and M. Watt*
*CSIRO, Sustainable Agriculture Flagship, GPO Box 1600, Acton, ACT 2601, Australia*
*Email: Susan.Sprague@csiro.au*

The fungal pathogen *Leptosphaeria maculans* causes blackleg, a disease that significantly reduces yield in *Brassica napus* (canola, oilseed rape) crops around the world. *Leptosphaeria maculans* commonly enters aboveground tissues directly through stomates or wounds causing spots on leaves and pods, stem lesions and crown cankers. The fungus can also enter intact roots when artificially inoculated but the infection pathway is unknown. Whole and sectioned intact roots of susceptible (cv. Q2) and resistant (cv. Columbus) *B. napus* cultivars inoculated with a GFP-modified strain of *L. maculans* were viewed using confocal and cryogenic scanning electron microscopy. *Leptosphaeria maculans* colonised non-branch regions foremost and penetration was not specific to any root region. Hyphae were often observed in the longitudinal grooves between epidermal cells and most commonly penetrated roots between anticlinal cell walls of epidermal cells. There was no difference in the diameter of penetrating and non-penetrating hyphae and penetration structures were not present. A mucilage-like substance was often seen connecting hyphae to plant cells or other hyphae nearby and was present on hyphae both on the root surface and inside the root. It could not be determined if it was secreted by the fungus as perhaps a cell wall degrading enzyme or by the plant in response to the infection. The resistant cultivar of canola was also found to be susceptible to root infection.

**O01.011 Development of models based on weather and airborne inoculum to describe disease index of wheat powdery mildew under field conditions**

*X.R. Cao[1,2], Y. Luo[3], X.Y. Duan[1] and Y.L. Zhou[1]*
*[1]State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; [2]Key Laboratory of Integrated Pest Management on Tropical Crops, Ministry of Agriculture, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou 571001, P. R. China; [3]Department of Plant Pathology, China Agricultural University, Beijing 100193, P. R. China*
*Email: yilinzhou6@yahoo.com.cn*

Disease severity of wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), was recorded weekly in unsprayed field plots in 3 successive seasons, 2009-2010, 2010-2011 and 2011-2012 in Langfang City, Hebei Province, China. Meteorological data was collected and conidia of *Bgt* in the air were trapped using volumetric spore samplers. Models were derived to relate airborne inoculum and weather conditions to the disease index of wheat powdery mildew using weather variable, airborne inoculum concentration separately, and both two kinds of variables. There were no significant differences between the fitted disease models using the variables mentioned above among the 3 seasons. Therefore, general models were derived based on combined data from the 3 seasons. The model based on airborne inoculum concentration gave more accurate predictions of disease index than did the model using weather variables. The model based on both weather variables and airborne inoculum concentration gave the best predictions, but the improvement over the model based on airborne inoculum concentration was only slightly.

**O01.012 Risk assessment of *Sclerotinia* stem rot in spring oilseed rape using real-time PCR**

*A.C. Wallenhammar[1,2], C. Almquist[1,3], and A. Redner[2]*
*SLU, Dept of Soil and Environment, Precision Agriculture and Pedometrics, PO Box 234 SE-532 23 Skara, Rural Economy and Agricultural Society/ HS Konsult AB, P.O. Box 271, SE-701 45 Örebro, Eurofins Food & Agro Sweden AB, P.O. Box 887,SE-531 18 Lidköping, Sweden*
*Email: ac.wallenhammar@hush.se*

Sclerotinia stem rot, caused by the phytopathogenic fungus *Sclerotinia sclerotiorum*, is a major disease of spring oilseed rape in Sweden. The pathogen survives in the soil for long periods as sclerotia. To improve disease risk assessment, a real-time PCR assay was developed to determine the incidence of *S. sclerotiorum* DNA on spring oilseed rape (SOSR). The presence of *Sclerotinia* DNA on petals and leaves at different leaf levels of the plant of two different cultivars was determined regularly during the flowering period in field experiments 2008-2010. Air samples were collected using a Burkad 7-day continuously recording spore sampler starting in late May 2010 and were analysed using real-time PCR assays. The results indicate that infection levels were highest at the lowest leaf level investigated. Analyses of air samples showed that the spore release occurred prior to early

bloom, thus analysing the petals do not indicate presence of the pathogen. A disease support system will be developed based on predictive tests, field data and local climate.

**O01.013 SCORALERT: An online system for mapping, forecasting and recommendation of sugar cane orange rust control**

*E.A. Giglioti, M.G. Canteri, J.A. de França, C. Fagundes, D.P. Rodero, D.C.P. Rodero, W.C. Soares, M. Cardim, V.A. Belo, C.H. Sumida and K.L. Araújo*
*Londrina State University, Rodovia Celso Garcia Cid. (PR 445), Km 380, CP 6001, Londrina, Paraná, CEP 86051-990, Brasil*
*Email: mcanteri@hotmail.com*

Since the first observation of sugar cane orange rust (*Puccinia kuehnii*) in Brazil, in the Araraquara-SP region, the fungus has spread rapidly throughout the major sugar cane producing regions of the country. Although susceptible cultivars are being replaced by resistant, in regions of high favorability for the occurrence of the disease, there is the need for chemical control in cultivars with intermediate resistance. To guide the integrated disease management, it was developed the SCORALERT – "An online System for mapping, forecasting and recommendation of sugar cane orange rust control" The system uses meteorological microstations to collect data of temperature and relative humidity, which are automatically sent via – mobile and stored in databases. SCORALERT calculates the favorability index (FI) for orange rust occurrence from the microstation data and the optimal temperature and humidity for *P. kuehnii* spore germination. Warning system validation was made correlating the orange rust progress curve with favorability during 18 months. The results allowed to find a mathematical model that predicts the level of severity, with one month in advance, with correlation coefficients above r = 0.80. The SCORALERT (www.scoralert.com. br) allows the construction of spatiotemporal mapping of risk zones and of progress curves and the definition of favorable climate windows in order to predict the occurrence of the disease and to recommend the monitoring and control of sugar cane orange rust, contributing to sustainability of the disease integrated management and precision agriculture.

**O01.014 Staying ahead of blackleg; managing the disease in Australia**

*S.J. Marcroft, A.P. Van de Wouw and B.J. Howlett*
*School of Botany, the University of Melbourne, VIC 3010, Australia*
*Email: steve@grainspathology.com.au*

The blackleg fungus evolves rapidly in response to selection pressure from extensive sowing of canola varieties that have major gene resistance. Accordingly resistance can be 'overcome' within a few years of release of a variety. For example, in 2003 after two seasons of extensive sowing, blackleg resistance of particular varieties broke down in the Eyre Peninsula, South Australia, causing 90% yield losses and withdrawal of these varieties from sale. By 2005, virulence of populations towards these varieties declined appreciably. We are developing management strategies for farmers to control this devastating disease and to avoid breakdown of resistance. Information provided to Australian canola growers and agronomists includes (1) National Blackleg Ratings; (2) Blackleg Management Guide; (3) groupings of varieties based on their complement of resistance genes; (4) data from surveys of regional blackleg severity in a range of varieties. In glasshouse and field trials we have shown that sowing canola cultivars with different complements of resistance genes in subsequent years (rotation of resistance genes) minimises disease pressure. This is because the frequency of virulence alleles of avirulence genes corresponding to particular resistance genes decreases. In 2012 we used this information to avert a breakdown of disease resistance in the Eyre Peninsula by advising farmers to change the canola varieties that they had sown for the previous two years. By switching varieties, not only have local farmers saved $18 million in yield losses, but seed companies have been able to sell these varieties in other canola-growing regions, where resistance breakdown was not predicted. This is a 'win-win' situation for farmers and seed companies.

**O01.015 Effects of host resistance on maturation of pseudothecia of *Leptosphaeria maculans* and *L. biglobosa* (cause of Phoma stem canker) in *Brassica napus* (oilseed rape)**

*S.N.M. Sidique [1,2], Y.J. Huang [1], A.M. Hall[1] and B.D.L. Fitt[1]*
*[1]School of Life and Medical Sciences, University of Hertfordshire, Hatfield AL10 9AB, UK; [2]Department of Agrotechnology, Universiti Malaysia Terengganu, Kuala Terengganu 21030, Malaysia*
*Email: s.n.mohamed-sidique@herts.ac.uk*

The stem canker epidemics are initiated in autumn by air-borne ascospores produced in pseudothecia on infected crop debris from previous crops. This study aimed to investigate the effects of cultivar resistance and environmental conditions (precipitation and temperature) on maturation of pseudothecia on stem debris. In autumn 2011 (2011/2012 growing season), there were no differences in pseudothecia maturation due to dry weather in September 2011 with increases rainfall in December 2011 (average of 0.37mm/h), more pseudothecia matured and more ascospores released was observed. In the third week of January 2012 after the major released of ascospores, the Phoma leaf spots were observed in 6 February

2012. However in 2012 autumn (2012/2013 growing season), when there was more rainfall from September to December (average of 0.28mm/h), there were differences between different cultivars in pseudothecial maturation with the maturation on the most susceptible cv Drakkar faster than on the other cultivars. The first ascospores released in early October 2012 and Phoma leaf spots were observed in 24 October 2012. The early appearance of phoma leaf spotting and high severity of leaf spots in 2012 autumn suggests that there might be severe Phoma stem canker in 2013 summer. Results of this study suggest that weather factors (temperature and rainfall) and host resistance affect the maturation of pseudothecia and the timing of the first major ascospore release. The role of host resistance in pseudothecial maturation requires further investigation.

### O01.016 The importance of asymptomatic infection in sustainable crop protection

*A.C. Newton, B.D.L. Fitt, P. Werner, D. Harrap, J. Southgate, M. Ashworth, S.D. Atkins and M. Looseley*
*The James Hutton Institute, Invergowrie, Dundee DD3 6JL, Scotland, UK*
*Email: Adrian.newton@hutton.ac.uk*

Scald or Rhynchosporium, caused by the fungus *Rhynchosporium commune*, is difficult to control with fungicides and severe epidemics may appear suddenly. Its epidemiology is not well understood as it is based on disease symptoms rather than the presence of the pathogen. Quantitative PCR enables detection and quantification of pathogen DNA in barley plants in both pre-symptomatic phases of infection and where they remain asymptomatic throughout their life cycle. Seed-borne inoculum was identified as a significant source for early infection of barley crops, with substantial amounts of *R. commune* DNA found in crops from infected seed but severity of seed infection correlated poorly with amounts of pathogen DNA (leaves), disease severity (leaves) and yield loss later in the cropping season. *R. commune* can colonise barley crops extensively throughout the cropping season (from seed to seed) in the absence of visual symptoms which has implications for the use of fungicides, breeding programmes and national variety recommended lists. The genetic basis of several different components of resistance to *R. commune* in barley was investigated in a mapping population derived from a winter x spring barley cross. Relative expression of symptoms quantified using the residual values from a linear regression of amount of *R. commune* DNA against visual plot disease score and was generally highly correlated. A QTL on chromosome 7H was identified as having a significant effect on the expression of visual disease symptoms relative to overall amount of *R. commune* colonisation.

### P01.001 Airborne inoculum of the soilborne pathogen *Fusarium oxysporum* f.sp. *cucumerinum* in greenhouse cucumbers

*K. Scarlett, R. Daniel, D. Maffi, F. Faoro, L. Tesoriero and D.I. Guest*
*Faculty of Agriculture and Environment, University of Sydney, NSW, 2006, Australia*
*Email: kelly.scarlett@sydney.edu.au*

*Fusarium oxysporum* f. sp. *cucumerinum* is the fungal pathogen responsible for Fusarium vascular wilt of cucumber. While the pathogen is soilborne there is some evidence that the disease cycle in glasshouses also involves airborne inoculum. Relationships between levels of airborne conidia with changes in temperature and relative humidity were determined by 24 hour sampling within a commercial cucumber greenhouse, using a Burkard mini-cyclone air sampler. The development of a highly specific and sensitive quantitative real-time PCR assay detected as few as 100 *Fusarium oxysporum* f. sp. *cucumerinum* conidia in air samples. High fluctuations in airborne conidia density were observed, however no distinct patterns of conidial release associated with relative humidity or temperature were present. We also investigated the ability of airborne conidia to germinate and infect wound sites on the plant stem. Germination of conidia on the stem wound site was evident, however hyphae were not observed to penetrate into the cortical or vascular tissue. While these results indicate that the pathogen is aerially disseminated, they suggest that spores are deposited on the soil surface, where they infect through the root zone. Germination and hyphal development on wound sites may provide an extra source of inoculum and assist in the infection of healthy plants.

### P01.002 Pathogenicity and identification of *Botryosphaeriacae* spp. causing gummosis and dieback in macadamia

*O.A. Akinsanmi and A. Drenth*
*The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Centre of Plant Science, Ecosciences Precinct, GPO Box 267 QLD 4001, Australia*
*Email: uqoakins@uq.edu.au*

Branch canker and dieback occur sporadically in macadamia trees. The disease is thought to be stress related and only important in young trees, but the severity and distribution of the disease is increasing in mature macadamia orchards in Australia. Very little is known about which pathogen species are responsible for branch canker and dieback in macadamia. Isolations from characteristic dieback symptoms were made from symptomatic and asymtomatic macadamia trees. The symtomatic trees showed dieback, dried or dead branches with dried leaves still attached, extending upward from the point of gummosis at the base of the branch and/or main trunk of

affected trees. The cross-sections through the affected branch showed dark discoloration of the wood. Over 60% of the fungal isolates obtained from symtomatic samples were identified, using morphological identification and molecular analysis, as belonging to the *Botryosphaeriaceae* species complex; *Lasiodiplodia/Diplodia* and *Botryosphaeria*. Pathogenicity tests using wound inoculation technique with mycelial plugs of the isolates, on macadamia trees in a greenhouse, showed that the isolates identified as within the *Lasiodiplodia/Diplodia* clade induced dieback symptoms in the glasshouse three months after inoculation. This provides evidence that Botryosphaeriaceous species are present in symptomatic trees in the field and are able to induce typical disease symptoms in the trunks of macadamia trees in the greenhouse. The increasing development and distribution of severe dieback caused by this pathogen warrants further research into the cause and control of this disease. Further study on detailed understanding of the aetiology of the disease in macadamias will underpin the development of management strategies.

**P01.003 Considerable differences of spore concentration and the composition of *Fusarium* species in air samples from Poland**

*P. Serbiak, W. Irzykowski, J. Kaczmarek and M. Jedryczka*
*Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland*
*Email: mjed@igr.poznan.pl*

Numerous species of the genus *Fusarium* pose considerable threat to agricultural and horticultural crops as well as forest plants. Several species cause Fusarium head blight – a damaging disease of cereals, including wheat. The problem is worldwide and it is constantly increasing, in spite of availability of genetic sources of resistance. Crop damage is not only quantitative but also qualitative, due to persistent mycotoxins, hazardous to animal and human health. The aim of this work was to study spore concentration and composition of *Fusarium* species in air samples at four sites located across different geographical and climatic regions of Poland. Each experiment site was equipped with a Hirst type seven day volumetric spore sampler (Burkard Manufacturing, UK or Lanzoni, Italy) and MicroBio MB1 volumetric spore sampler (Cantium Scientific, UK). The studies lasted a month and covered the period from ear emergence, through flowering to milk development stage. Fungal propagules captured by MicroBio MB1 sampler were cultivated on PDA, SNA and PCNB media. For two study periods (2011 and 2012) over 2000 *Fusarium* isolates were obtained and identified using molecular method based on comparison of subsequent patterns of restriction fragments obtained after the treatment of ITS region with selected endonucleases. The studies revealed considerable differences between the composition of *Fusarium* species in air samples from different experiment sites and periods of detection. In flowering time, the concentration of *Fusarium* spores was high. The most prevailing species was *F. tricinctum*, followed by *F. avenaceum.*

**P01.004 Phytosanitary measures prevented epidemics of sugar cane orange rust in Brazil**

*C.H. Sumida, M.G. Canteri, E.A. Giglioti, C. Fagundes, D.P. Rodero, D.C.P. Rodero, V.A.B. de Oliveira and K.L. Araújo*
*Londrina State University, Rodovia Celso Garcia Cid. (PR 445), Km 380, CP 6001, Londrina, Paraná, CEP 86051-990, Brasil*
*Email: cirosumida@hotmail.com*

In Brazil, first report of orange rust in sugar cane culture occurred in December 2009. The disease has spread and established by the major sugarcane producing regions of the country. Before the arrival of the disease, it has started the planning of strategies to be implemented in order to manage the disease and, consequently, to prevent yield losses in the country. Since then, research projects were developed involving the mapping of risk zones and times of higher favorability for the disease occurrence, identification of susceptible and tolerant cultivars, and tests and selection of efficient fungicides against the pathogen. According to maps were observed favorable conditions for the orange rust in the main sugarcane producing regions of Brazil, concentrated in the states of São Paulo, Minas Gerais, Goiás, Paraná and Mato Grosso do Sul, and the period of higher favorability in September-October to March-April. The cultivars SP84-2025 and CV14 were the most susceptible to the disease and fungicides mixtures of strobilurin and triazole proved to be excellent tools for disease emergency control. In general, strategies development and implementation are assisting the sugar cane orange rust monitoring in Brazil to avoid epidemics.

**P01.005 Population dynamics of aeromycoflora from vegetable growing areas of Rawalpindi by evaluating two sampling methods**

*G. Irshad, F. Naz and C. A. Rauf*
*Department of Plant Pathology, PMAS, Arid Agriculture University Rawalpindi, 46300, Pakistan*
*Email: gulshanirshadpp@gmail.com*

Air-borne mycoflora of three vegetable growing areas (Adyalla, Sohan and Pir Mehr Ali Shah Arid Agriculture University Rawalpindi) of Rawalpindi region were monitored for three consecutive years with main emphasis on its occurrence, diversity. Two air sampling methods namely All Glass Impinger (AGI) and Gravimetric plate culture plate technique on four fungal media. A total of 20,409 fungal spores belonging to fifteen genera namely *Alternaria, Aspergillus, Cladosporium,*

*Curvularia, Drechslera, Epicocum, Fusarium, Geotrichum, Helminthosporium, Mucor, Penicillium, Rhizopus, Stemphylium, Stachybotrys* and *Trichoderma* species were recovered. Out of fifteen genera the highest relative contribution was attributed to genus *Aspergillus* with 16.73% of total fungal spore count followed by *Alternaria, Cladosporium, Mucor* and *Penicillium* with 15.83, 13.79, 10.71 and 9.39 percent share, respectively. *Fuasrium* with 0.33% contribution in the total fungal spore count was established as the lowest in occurrence followed by *Stachybotrys* (0.62%), *Helminthosporium* (0.85%) and *Geotrichum* (0.85%). Gravimetric plate method was outrival and statistically significant than AGI in capturing airborne fungal spores as regards selected area. As regards selected sites, the highest mean count of mycoflora was found at Sohan followed by Adyalla and PMAS-AAUR. This paper reports that a variation in kind of species and their contribution in the ambient air vary significantly and depends mainly upon the extent and type of vegetation and the available decaying substrate.

**P01.006 Identification and biological characteristics of pathogen of cowpea ring spot**

*J.B. Li, X. An, C. Zheng, S.S. Tang and Z.H. Liu*
*College of Plant Protection, Shenyang Agricultural University, Shenyang, 110161, P.R. China*
*Email: lijianbingxingfu@163.com*

The newly occurred cowpea disease, cowpea ring spot, discovered in Liaoning province was studied. The damage and symptoms of this disease were reported. The ITS sequence analysis results show that the homology is up to 99% between the pathogen isolate and *Corynespora cassiicola* (Berk and Curt) in GenBank. Therefore, the pathogen of cowpea ring spot is *Corynespora cassiicola* (Berk and Curt). The biological characteristics of the results showed that best mycelia growth was obtained at 28℃ and pH 9 to 10. The effect of light on the mycelia growth was not evident. The cowpea leaf medium was the most favorable for mycelium growth in the 9 tested media, mannitol and peptone were the most favorable for mycelia growth on all 8 carbon sources and the 8 nitrogen sources. Sporulation temperature ranged from 10℃ to 35℃, the optimum one was 28℃. The optimal pH for spore production was 9. The beneficial medium for the sporulation was cowpea seed medium. In the texted 8 carbon sources and 8 nitrogen sources, spore production is more in sucrose as carbon source and in beef extract as nitrogen source than others. The optimum temperature of conidia germination was 28℃ and the optimal pH was 9. The results showed that the lethal temperature was 60℃ for 10 minutes for the spores, and 60℃ for 10 minutes for the mycelia.

**P01.007 Identification of a tobacco powdery mildew strain highly virulent to multiple families of vegetables**

*L.L. Zhang, X.F. Ma, Y.Y. Huang, J.L. Sun, T.T. Wang and W.M. Wang*
*Rice Research Institute, Sichuan Agricultural University, Chengdu 611130, P.R.China*
*Email: linglizhang2012@yahoo.cn*

Powdery mildews, caused by obligate biotrophic Ascomycetes of the order Erysiphales, are widespread airborne diseases of many dicotyledonous and monocotyledonous plants. Because of the economic and agricultural importance, powdery mildews have gained increasing attention. To better understand the pathogenesis of powdery mildew pathogens on different plants, we isolated a strain from tobacco (*Nicotiana tabacum* L. cv. Wisconsin-38) leaves designated as SICAU1 that was also able to severely infect *Arabidopsis*, raising our interest to investigate its host range. By artificial inoculation in green house, we found that SICAU1could also be highly virulent to varieties of plants from different families of vegetables, including *Brassicaceae* (*Brassica napus* L.), *Cucurbitaceae* (*Cucumis sativus* L.), *Solanaceae* (*Lycopersicon esculentum* Mill.) and *Asteraceae* (*Lactuca sativa* L.). Analysis on DNA sequences for the 5.8S ribosomal RNA and two flanking internal transcribed spacer sequences revealed that SICAU1 belongs to *Golovinomyces cichoracearum* (*G*c). Microscopic examination by environmental scanning electron microscopy was used to identify the morphological features of the asexual state that could be used to differentiate the SICAU1 from the other powdery mildew strains. Compared with *Gc* UCSC1, the strain widely exploited in investigation of the mechanism of plant-powdery mildew interaction, *Gc* SICAU1 had wider range of hosts, including some important vegetables such as cucumber, squash, lettuce and tomato. Thus, the isolation of *Gc* SICAU1 provides us a tool to search for resistant resources and investigate the resistant mechanisms in multiple vegetable species.

**P01.008 Quantification of airborne spores of *Blumeria graminis* f. sp. *triciti* in wheat fields using real-time PCR**

*X.R. Cao[1, 2], Y. Luo[3], X.Y. Duan[1] and Y.L. Zhou[1]*
*[1]State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; [2]Key Laboratory of Integrated Pest Management on Tropical Crops, Ministry of Agriculture, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou 571001, P. R. China; [3]Department of Plant Pathology, China Agricultural University, Beijing 100193, P. R. China*
*Email: yilinzhou6@yahoo.com.cn*

In this study, conidia of *Blumeria graminis* f. sp. *triciti*

in the air were trapped in 2009-2010 and 2010-2011 season using volumetric spore samplers in Langfang city, Hebei province, China. A real-time PCR assay was developed to efficiently quantify the dynamics of spore concentration in the field during the growing season. A significant linear relationship between conidial concentrations counted with a compound microscope and those determined with the real-time PCR assay was obtained, using the same samples of spore traps. Real-time PCR was specific, accurate and more efficiency when compared with compound microscope. The results demonstrated a potential method to quantitatively determine spore inoculum potential in fields by using a real-time PCR assay.

## Concurrent Session 2-Beneficial Plant Pathogens for Biological Control of Weeds

### O02.001 Beneficial plant pathogens for biological control of weeds

*W.L. Bruckart, III*
*USDA, ARS, Foreign Disease-Weed Science Research Unit; Ft. Detrick, MD 21702, USA*
*Email: William.bruckart@ars.usda.gov*

In the late 1970s, a new paradigm emerged in the field of plant pathology, and that is the beneficial use of plant disease for biological control of weeds. Weeds are the most important pests of agriculture and ecosystems internationally, and as a result of modern international trade and commerce, many new, invasive species have emerged around the world that cause major damage to agriculture and ecology. The problems that occur are often intractable and present unique challenges to weed management, including the extreme size of infestations, the physically remote or sensitive locations of plants, and the low return on agricultural investment (economics) or presence in non-profit, public, and natural areas, either of which precludes use of conventional weed control approaches. More recently, resistance to modern herbicides has resulted in additional difficulties in management of weeds. There are two traditional strategies for implementation of weed pathogens, i.e., the inoculative (classical) approach and the inundative (bioherbicidal) approach. Biological control by the deliberate use of plant pathogens has resulted in safe and successful weed management in several countries, culminating in benefit to both agriculture and ecology. Despite progress and successes in the weed biological control arena, this is an underutilized international application of plant pathology and weed science that has importance in the realization of global agricultural and ecological biosecurity.

### O02.002 Successful use of plant pathogens for the classical biological control of weeds

*L. Morin*
*CSIRO Ecosystem Sciences, GPO Box 1700, Canberra, Australian Capital Territory, 2601, Australia*
*Email: louise.morin@csiro.au*

Plant pathogens are playing an increasing role in classical biological control of weeds worldwide. This approach involves the deliberate introduction of host-specific natural enemies of the target weed from its native range into the region where the plant has become a problem. Its aim is to re-establish an ecological balance that will suppress populations of the weed. Foliar pathogens, especially rust fungi, are preferentially selected as biological control agents because they are generally more host-specific, can inflict severe damage on the weed and are more readily dispersed by natural means. Australia has had some major successes with weed biological control using pathogens and most recent examples will be presented (bridal creeper, *Asparagus asparagoides*; mistflower, *Ageratina riparia*). The use of pathogens well adapted to the new environment into which they are introduced, compatible with the genotype(s) of the weed present and capable of causing major, recurrent epidemics was crucial to these successes. Some of the challenges encountered in the development of new pathogens for classical weed biological control in Australia will be illustrated using current programs on crofton weed (*Ageratina adenophora*), boneseed (*Chrysanthemoides monilifera* subsp. *monilifera*) and Cape tulips (*Moraea* spp.). While pathogens may not always be the easiest and most effective solutions to pursue for biological control of weeds, there is no doubt that they offer exciting opportunities that should be more widely explored.

### O02.003 Successful biological control of Canada thistle (*Cirsium arvense*) with the rust fungus *Puccinia punctiformis*

*D. Berner, E. Smallwood, C. Cavin, A. Lagopodi, J. Kashefi, T. Kolomiets, L. Pankratova, Z. Mukhina, M. Cripp[f] and G. Bourdôt*
*AgResearch, Private Bag 4749, Christchurch 8140, New Zealand*
*Email: graeme.bourdot@agresearch.co.nz*

Canada thistle (*Cirsium arvense*, CT) is one of the worst weeds in temperate areas of the world. In the U.S., CT is present in 38 states and noxious in 25. The rust fungus *Puccinia punctiformis* was first proposed as a biological control agent for CT in 1893. The rust causes systemic disease, is specific to CT, and is in all states where CT is found. Systemic infections result in permanent infection of thistle roots, and all of the shoots on infected root systems eventually die. Despite a 120-year lapse, establishment of epiphytotics of the rust, and concomitant biological control of CT, have previously been unsuccessful, due to incomplete understanding of the disease cycle. We now understand the disease cycle, and the objectives of our studies were to use this knowledge to routinely establish epiphytotics of systemic rust disease, in 13 fields in four countries, and demonstrate effective biological control. In each field we inoculated newly emerged rosettes of CT in the fall with leaves bearing telia of the rust that were collected in mid-summer in each country. Rosettes were inoculated 2, 4, 6, or 8 times each with about 1 g of telia-bearing leaves. In the spring following inoculation systemically diseased shoots emerged in all inoculated fields. Proportions of systemically diseased shoots generally increased with increasing number of inoculations. Thistle density declines of 50-100% were observed, demonstrating successful biological control of CT with this rust.

**O02.004 The commercial use of plant pathogens as bioherbicides: Risks and rewards**

*K.L. Bailey[1], R.K. Hynes[1], S. Falk[2], J. Chia[2] and L. Morin[3]*
*[1]Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK, S7N0X2, Canada; [2]The Scotts Company, 14111 Scotslawn Road, Marysville, OH 43041, USA; [3]CSIRO Ecosystem Sciences, GPO Box 1700, Canberra, Australian Capital Territory, 2601, Australia*
*Email: russell.hynes@agr.gc.ca; karen.bailey@agr.gc.ca; stuart.falk@scotts.com; james.chia@scotts.com*

A white and dying Canada thistle (*Cirsium arvense* (L.) Scop.) in a Saskatchewan, Canada agricultural field led to an important discovery and a bioherbicide for broadleaf weed control. *Phoma macrostoma* Montagne was registered in Canada (2011) and the United States (2012) for biological weed control in turf grass. Knowledge of the mode of action, macrocidin toxins that induce photo-bleaching, interaction with the target weeds and non-target plants, benign mammalian and aquatic species interactions and low environmental impact aided its passage through the registration process. The bioherbicide development model that lead to commercialization followed the biopesticide innovation chain which consists of stages/gates that include discovery and screening, proof-of-concept for field efficacy, development of fermentation, formulation, and application platform technologies, and implementation. An open tender process identified The Scotts Company as the industrial partner and through close collaboration with Agriculture & Agri-Food scientists over 10 years, commercialization of this discovery was pursued. A granular formulation has been developed for control and/or suppression of weeds such as dandelion, scentless chamomile, English daisy, white clover, black medic, Canada thistle, chickweed, broadleaf plantain, and ragweed. The bioherbicide may be used safely on a variety of turf types such as Kentucky bluegrass, bent grass, perennial or annual ryegrasses, fescues, bromegrasses, timothy, and Bermuda grass. Optimum product performance occurs above 20 ℃ (15-30 ℃ range) and in soil that is relatively moist. The product will be applied as a broadcast or spot treatment to either established or newly-seeded turf to control weeds as they germinate or ones that are already established.

**O02.005 Use of genomics to enhance biopesticide research**

*G.J. Ash, B.J. Stodart, J.A. Pattemore, B.A. Wilson and A. Wang*
*EH Graham Centre for Agricultural Innovations (an alliance between Charles Sturt University and NSW DPI), Charles Sturt University, Locked Bag 588, Wagga Wagga 2678, NSW, Australia*
*Email: gash@csu.edu.au*

Biopesticides offer an innovative approach to the management of pests in farming systems using formulated microbial agents as the active ingredient. Microbes that have been used in this approach include fungi, bacteria, viruses and nematodes. There are a large number of commercial products now available in most regions of the world, where they are being incorporated into farming systems. The approach is gaining favour in the wider community as the markets for organic produce continue to grow. However, the cost of production, efficacy and selection of the isolates remain a limiting factor for the successful commercialisation of the products. With the availibility of high throughput, low cost DNA sequencing systems the application of genomics (the study of genes and their interactions) to both the target and the biological control agent offer exciting opportunities to improve the efficacy and reliability of biopesticides. Many microbial genomes have been sequenced yielding enormous quantities of data. For example we have sequenced the complete genome of *Bacillus thuringiensis* DAR 81934 (a Bt strain with molluscicidal activity) which revealed six Bt toxin genes in both plasmid and chromosome sequences. This information will give us a better understanding of the genetic basis of its molluscicidal activity as well as being useful in the selection, improvement and environmental tracking of this biopesticidal agent. In this review, the application of genomics to the study of microbial host interactions will be given using specific other examples from biological control of insects, nematodes and weeds.

**O02.006 *Puccinia xanthii* sp. *Ambrosiae-trifidae*, a potential biocontrol agent of giant ragweed (*Ambrosia trifida*) in north of China**

*X.Q. Wang, J.Y. Ding, D.C. Yang, J.D. Chen and B. Qu*
*Beijing Plant Protection Station, No.9 Beisanhuan Zhonglu Road, Xicheng District, Beijing, 100029, P. R. China*
*Email: xiaoqingwang08@163.com*

*Puccinia xanthii* sp. *Ambrosiae-trifidae* was observed in Liaoning that it could cause rust on giant ragweed (*Ambrosia trifida*), one of the invasive weeds in north China. To access its weed control effect, thirty giant ragweeds inoculated with *P. xanthii* sp. *Ambrosiae-trifidae* per acre were transplanted along with the healthy individuals in July, 2010. In the next 2 years, from mid June to mid October, rust can be observed on 95% of giant ragweeds. The death rate from rust was 46.67% (2011) and 27.85% (2012). The average plant weight and seeds yielding per plant of infected individuals were significantly decreased, which was 81.97%, 88.49% in 2011, and 62.75%, 48.13% in 2012. The density of giant weed per square meter was 11.91 in 2012, which was decreased by 19.63% compared to 2011. But for seed germination rate, which collected from infected plants and healthy plants, no significant difference was observed at level of $p < 0.05$. It is thus concluded that *P.*

*xanthii* sp. *Ambrosiae-trifidae* could be used as a potential agent in giant ragweed biocontrol.

### O02.007 Sprout control with a decay fungus *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar) in Finland

*L. Hamberg and J. Hantula*
*Finnish Forest Research Institute, Vantaa Research Unit, P.O. Box 18, FI-01301, Vantaa, Finland*
*Email: leena.hamberg@metla.fi*

Excessive sprouting of broadleaved trees is a problem in regeneration areas, under electric power lines and next to roads and railways. Chemicals are not anymore used widely due to their harmful effects on the environment, and therefore new alternatives are needed to control sprouting as mechanical cutting only is not cost effective. In Finland, we are developing a new biological control agent for sprout control based on a decay fungus *Chondrostereum purpureum* (Pers. Ex Fr.) Pouzar which is able to decay cut stumps and thus prevent sprouting. Several *C. purpureum* strains collected from the field have been tested to find an efficient strain for the commercial biocontrol product. Furthermore, the ability of *C. purpureum* to produce spores and consequently new progenies has been utilized in our project. We have paired *C. purpureum* strains tested for efficient sprout control in the field, and tested the efficacy of the progenies in spout control efficiency experiments. This procedure has resulted in isolates with higher efficacy than that of the parent strains. Two growing seasons after the treatment the mortality of birch (*Betula pendula* Roth. and *B. pubescens* Ehrh.) stumps 1 cm in diameter was 74%, 51% and 3% for the best progeny, the parent strain and the control (cutting and blank inoculum), respectively.

### P02.001 Using biological components combined with reduced doses of herbicide to control broad leaf weeds in wheat fields of Moscow, Russia

*M. Zargar, P. Vadim and E. Pakina*
*Faculty of Agriculture, People's Friendship University of Russia, Moscow, Russia*
*Email: meisam.za_ir84@yahoo.com*

Reducing herbicide application in integration with biological components without compromising yields can lead to less environmental pollution, field trial was laid out to investigate the effect of biological agents in combination with reduced rates of new generation post-emergence herbicide Verdict in four levels including: 0, 0.2, 0.3 and 0.5 kg $ha^{-1}$ to control weeds in winter wheat *(Triticum aestivum* L.), experiment was studied in a randomized, complete block design with four replications in Moscow research institute of agriculture, Nemchinovka, Odintsovskiy region, Russia. Labeled-dose as 0.5 kg $ha^{-1}$ + biological components was desirably effective in controlling of *Viola arvensis* and *stelaria media* population, and also dry weight of mentioned weeds were diminished by the using labeled-dose + biological agents, total weeds biomass was quite reduced by labeled dose of verdict + biological agents. Additionally, weeds population also was reduced by the applying of intermediate Verdict dose as 0.3 kg $ha^{-1}$. Treatments had not significant effect on 1000-grain weight and stem per $m^{-2}$, but grain yield, gluten content, plant height and grain per spike were significantly affected by the using treatments, so that these traits increased when both doses of verdict 0.5 and 0.3 kg $ha^{-1}$ + biological agent were applied.

### P02.002 Changes of ROS and antioxidative enzymes during the infection of *Nimbya alternantherae* in leaf of *Alternanthera philoxeroides*

*Y.F. Nie, Z.Y. Chen, Y.F. Liu and Y.Z. Liu*
*Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, No.50, Zhongling Street, Xuanwu District, Nanjing, 210014, P. R. China*
*Email: nieyafeng@jaas.ac.cn*

*Alternanthera philoxeroides* Griseb is an invasive plant which originated from South America and is currently spreading in many area of China. *Nimbya alternantherae* is considered as a beneficial plant pathogen causing foliar and stem necrosis of *A. philoxeroides*. The equilibrium between the production and the scavenging of reactive oxygen species (ROS) may be perturbed by pathogen attack, and lead to sudden increase in intracellular levels of ROS which can cause significant damage to plant cell. In order to elucidate the effect of *N. alternantherae* strain SF-193 infection on metabolism of ROS in the leaf of *A. philoxeroides*, the dynamic changes of superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide ($H_2O_2$), antioxidative enzymes activity and malondialdehyde (MDA) content were detected in diseased leaves by using nitroblue tetrazolium (NBT), 3,3-diaminobenzidine (DAB) and spectrophotometric method. The accumulation of ROS in infected leaves significantly increased with the time after inoculation. The change in rate of $O_2^{\bullet-}$ production corresponded with that of $H_2O_2$ content rose sharply at 24 hours after inoculation and maximized at 48 hours. The peak values of them were about 2.1 and 2.5 times than that of control respectively. The activity of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) reached the highest level at 8 hours after inoculation, and then declined gradually. MDA content increased gradually all the time in the infected leaves, and the peak appeared at 72 hours after inoculation. Their results suggested that the sudden increase in intracellular levels of ROS caused by *N. alternantherae* attack may significantly damage to leaf cell.

## Concurrent Session 3-Biological Control of Plant Diseases

### O03.001 Induced gene expression by beneficial microbes to enhance plant performance and health

*G. E. Harman*
*Advanced Biological Marketing (retired Cornell University) Geneva, NY 14456 USA*
*Email: geh3@cornell.edu*

Some strains of fungi in the genus *Trichoderma* are now recognized as endophytic plant symbionts. They can be provided to plant roots as seed treatments, in-furrow applications, root dips, granular soil applications, potting soils, or even coated onto fertilizers. Once the fungi come into contact with roots, they colonize the outer layers of the roots and establish chemical communication with the plant. The best strains grow with the root system and provide season-long benefits. This chemical communication induced systemic changes in plant gene, protein and metabolite expression. The fungi only colonize roots, but the changes in gene and protein expression are greater in shoots than in roots. The best of these strains induce a number of beneficial changes to plants including increased resistance to plant diseases, increased resistance to abiotic stresses such as drought, salt, and pollutants, increased nitrogen use efficiency, enhanced photosynthetic efficiency and enhanced root development. These capabilities are the basis of multi-strain mixtures that are being used worldwide to enhance plant agriculture; they result in reliable and consistent increases in plant yields, and return to farmers are in the range of 8- to 12:1. One reason for the high return is that very small amounts of the strains are required to cause the benefits, with only about 1 g of highly active product required for seed treatments per hectare. Since the strains colonize roots and not soil, they are effective in diverse crops and climates. They were highly beneficial in alleviating effects of drought in the American Midwest in 2012.

### O03.002 Polymorphism of *Ralstonia solanacearum* and it's plant vaccin development

*B. Liu, J.M. Che, X.F. Zheng and R.F. Xiao*
*Agricultural Bioresources Research Institute, Fujian Academy of Agricultural Sciences, Wusi Road 247, Fuzhou 350003, P.R. China*
*Email: fzliubo@163.com*

The new idea was put forward to develop plant vaccine with the construction of *R. solanacearum* avirulent strain. The study enclosed the six parts. In the first part, bacterial wilt disease and its pathogen forms were tested with pathogenicity index [PI] in TTC medium. In the second part, polymorphism of *R. solanacearum* was conducted to display the polymorphism in RAMS-PCR, BOX-PCR, PLFAs and RS whole genome. In the third part, the characteristics of RS were to indicate differences of reproductive rate, EPS and protain, HPLC chromatogram between virulent and avirulent RS. In the four part, construction of avirulent *R. solanacearum* was carried out to show insertion of Tn5 transposon to *R. solanacearum* resulted in the attenuation. In the five part, new idea of plant vaccin development with the avirulent was put forward with GFP transformation to show the infect mechanism. In the six part, the titer index of plant vaccine was formed to distingush disease control effeciency. *R. solanacearum* was characterized by high-performance liquid chromatography (HPLC). The chromatography titer index (CTI) was calculated as CTI=S1/(S1+S2+S3)×100%, in which, S1+S2+S3 was the total amount of the strains related to avirulent, interim, and virulent, S1 related to content of an avirulent strain, when S2 and S3 is 0, CTI is 100, when S1 is 0, CTI is 0, then the CTI between 0-100, the greater the CTI value, the better the plant vaccine control the disease. The plant vaccine AVIRULENT got 100% control effeciency against the bacterial wilt disease in 20-30 days.

### O03.003 Functional genomics of the fungal biocontrol agent *Clonostachys rosea*

*M. Karlsson, C. Kosawang, G.S. Tzelepis, H. Väätäz, K. Nygren, M.K. Dubey, M. Brandström-Durling and D. Funck Jensen*
*Uppsala Biocenter, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden*
*Email: magnus.karlsson@slu.se*

The mycoparasitic fungus *Clonostachys rosea* is an efficient biological control agent (BCA) under field conditions for a variety of plant diseases on agricultural crops. *C. rosea* belong to the same order (Hypocreales), but a different family (Bionectriaceae), from the more studied *Trichoderma* spp. BCAs. Comparative studies between *C. rosea* and *Trichoderma* spp. BCAs may thus improve our understanding of critical components of the mycoparasitic lifestyle. We sequenced the genome of *C. rosea* strain IK726 using Illumina/SOLiD technology, and transcriptomes from *C. rosea* interacting with *B. cinerea* and *F. graminearum*. Comparative genomics revealed a significant (P=0.001) increase in the number of ABC-transporters (85 genes) compared with other filamentous ascomycetes, including *T. atroviride* and *T. virens*. Interestingly, the increase of ABC-transporter gene number in *C. rosea* was associated with phylogenetic subgroup G (pleiotropic drug resistance transporters), while ABC-transporter gene number changes in *Trichoderma* spp. involved subgroup C that is putatively involved in secondary metabolite export. Gene expression data indicated that certain *C. rosea* subgroup G ABC-transporter genes were induced by exposure to the

*Fusarium* mycotoxin zearalenone, and deletion of a single ABC-transporter resulted in reduced growth rate (P=0.001) on zearalenone-containing media. In addition, deletion of the zearalenone lactonohydrolase gene *zhd101*, previously shown to encode an enzyme that detoxifies zearalenone, resulted in mutants that failed to protect wheat seedlings against *F. graminearum* Foot Rot disease in growth chamber tests. In summary, our data suggest that mycotoxin tolerance/detoxification is an important component of the biocontrol ability of *C. rosea*.

**O03.004 A biocontrol agent among plant pathogens: the unique case of the "smut fungus" *Pseudozyma flocculosa***

*F. Lefebvre[1], D.L. Joly[2], C. Labbé[1], F. Belzile[1], G. Bakkeren[2] and R.R. Bélanger[1]*
*[1]Département de phytologie, Centre de recherche en horticulture, Université Laval, Québec, Qc, Canada, G1V0A6; [2]Agriculture et Agroalimentaire Canada, Summerville, BC, Canada, V0H 1Z0*
*Email: richard.belanger@fsaa.ulaval.ca*

The anamorph species *Pseudozyma flocculosa* is described as a non-pathogenic Ustilaginale with the rare property of being an effective biocontrol agent of powdery mildews. It is also closely related to *Ustilago maydis*, a maize-infecting model organism in fungal genetics. Recently, their phylogenetic link was supported by the discovery of conserved cellobiose lipid biosynthetic gene clusters in both species. However, in spite of this relatedness, *P. flocculosa* does not appear to share many traits with smut fungi. With the recently available genomic data for *U. maydis, Sporisorium reilianum* and *U. hordei*, we exploited the opportunity to sequence and annotate the genome of *P. flocculosa* in order to unravel the features that could account for such distinct lifestyles among related organisms. Through comparative analyses, genomic features of *P. flocculosa* were found to be very similar to those reported for *U. maydis*, *S. reilianum* and *U. hordei* although the genome of *P. flocculosa* appeared to be mesosyntenic in comparison to the others. In spite of being a non plant-pathogen, *P. flocculosa* contains many genetic traits associated to mating and pathogenicity (mating- type and meiosis loci, cell wall degrading enzymes, secondary metabolite biosynthesis). Striking differences between *P. flocculosa* and phytopathogenic species included the absence of homologs to secreted effector proteins proven to influence virulence in *U. maydis*. In addition, genetic elements unique to *P. flocculosa* including proteins linked to biocontrol properties were identified as putative determinants of its antagonistic activity against powdery mildews.

**O03.005 Insect toxin producing plant-associated pseudomonads: Can they be used for joint biocontrol of plant diseases and insect pests?**

*M. Maurhofer[1], B. Ruffner[1], P. Flury[1], M. Péchy-Tarr[2], P. Kupferschmied [2] and C. Keel[1]*
*[1]Institute of Plant Sciences, Swiss Federal Institute of Technology, 8092 Zürich, Switzerland; [2]Department of Fundamental Microbiology, University of Lausanne, 1015 Lausanne, Switzerland*
*Email: monika.maurhofer@usys.ethz.ch*

Root colonizing fluorescent pseudomonas is known for their ability to suppress fungal plant diseases due to their ability to release antimicrobial compounds and to induce systemic resistance in plants. Some years ago we detected that a specific phylogenetic subgroup of the *Pseudomonas fluorescens* species complex is capable to produce a protein insect toxin (Fit) and to colonize and kill insects when injected into haemocoel of *Galleria mellonella* larvae. We currently explore the potential of Fit producing strains for the biocontrol of insect pests and the mechanisms of insecticidal activity. When applied to leaves Fit producing but not Fit-negative *Pseudomonas* biocontrol strains display potent oral activity against larvae of important lepidopteran pests feeding on the treated leaves. Mutant-based studies with model strains *P. protegens* CHA0 and *P. chlororaphis* PCL1391 excellent biocontrol agents against soilborne diseases showed that the Fit toxin contributes to oral activity. Strain CHA0 can establish high populations in larvae of the root weevil *Otiorhynchus sulcatus* and of the European cockchafer (*Melolontha melolonth)a* and change the composition of the larvae's own bacterial flora. CHA0 further displays oral activity against the aphid *Acyrthosiphon pisum*, but is not toxic to the large earth bumblebee *Bombus terrestris* an important pollinator. Additional experiments showed that CHA0 is compatible with the biocontrol fungus *Metarhizium anisopliae*. Taken together our results suggest that a biocontrol product based on insect toxin producing plant-associated pseudomonads could not only be effective against fungal diseases but also contribute to the protection of crops against insect pests.

**O03.006 The enhancement in plants resistance against biotic and abiotic stresses mediated by PGPRs**

*J.H. Guo*
*Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Nanjing 210095, P.R. China*
*Email: jhguo@njau.edu.cn*

The consortium of three plant growth-promoting rhizobacterium (PGPR) strains (Rhizobacteria *Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21)

termed as BBS for short was a promising biocontrol agent. BBS was shown to be a promising biocontrol agent against a broad spectrum of diseases, such as bacterial wilt caused by *Ralstonia solanacearum*, blight disease caused by *Phytophthora capsici*, and root-knot disease caused by *Meloidogyne incognita* in tomato, cucumber, and some other vegetables. In the study of biocontrol mechanisms, we found that strain AR156 triggered induced systemic resistance (ISR) to *Pseudomonas syringae* pv. *tomato* DC3000 in *Arabidopsis* by simultaneously activating the SA- and JA/ET-signaling pathways in an NPR1-dependent manner. Compared with mock-treated plants, AR156-treated ones showed an increase of biomass and reduction of disease severity and pathogen density in the leaves. We provided evidence suggesting that biofilm production is critical for bacterial colonization on plant root surfaces by using model system for studies of *B. subtilis*–tomato plant interactions. We also prove that the establishment of XY21 and its effects on the bacterial community in the tomato rhizosphere by denaturing gradient gel electrophoresis of 16S rRNA gene fragments PCR-amplified from total community DNA. Besides, we carried some research on stress resistance, and BBS conferred induced systemic tolerance to drought stress in cucumber (*Cucumis sativus*), by protecting plant cells, maintaining photosynthetic efficiency and root vigor and increasing some of antioxidase activities, without involving the action of ACC deaminase to lower plant ethylene levels. After withholding watering for 13 days, BBS-treated cucumber plants had much darker green leaves and substantially lighter wilt symptoms than control plants.

**O03.007 Functional genomic approaches for understanding the mode of action of *Bacillus* sp. biocontrol strains**

*C. A. Dunlap, M.J. Bowman and D.A. Schisler*
*Crop Bioprotection Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604, USA*
*Email: Christopher.dunlap@ars.usda.gov*

Complete genome sequencing of several *Bacillus* sp. strains has shed new light on the mode of action of these antagonists of plant pathogens. The use of genomic data mining tools provided the ability to quickly determine the potential of these strains to produce bioactive secondary metabolites. Our *Bacillus* sp. strains were isolated from wheat anthers and shown to be efficacious in managing Fusarium head blight in field trials. Results from data mining indicate the arsenal of antimicrobial compounds produced by *Bacillus* sp. antagonists goes beyond lipopeptides and includes a variety of antibacterial metabolites and siderophores. The rapidly increasing number of completed genomes of *Bacillus* sp. biocontrol strains offers additional opportunities to increase our understanding of the genetic determinants of biocontrol activity. Comparative genomics was used to identify the core-genome and pan-genome of these *Bacillus* sp. strains. These investigations demonstrate the growing importance of applying genomic-based studies to putative antagonists of plant pathogens to enable the rapid identification of bioactive metabolites produced by the strains. In addition, this work provides a foundation for a mechanistic understanding of the *Bacillus* sp. /Fusarium head blight biocontrol interactions.

**O03.008 Exploitation of microbial resources for biological control of root diseases and crop improvement**

*B. Chakraborty, U. Chakraborty, P. Dey, and K. Sunar*
*Immuno-Phytopathology Laboratory, Department of Botany, University of North Bengal, Siliguri - 734 013, West Bengal, India*
*Email: bncnbu@gmail.com*

Microorganisms isolated from forests, river basins and agricultural field soil were identified by rDNA technology, screened for their beneficial traits and utilized for developing strategies of biological control of root diseases of cereal (*Oryza sativa*), pulses (*Glycine max, Cicer arietinum* and *Vigna radiata*), horticultural plant (*Citrus reticulata*) and plantation crop (*Camellia sinensis*). *In vitro* antagonistic activities of selected fungi (*Talaromyces flavus*, *Trichoderma harzianum*, *T. asperellum, T. erinaceum*) and bacteria (*Bacillus pumilus, B. altitudinis, B. megaterium, Pseudomonas fulva, Streptomyces griseolus* and *S. griseus*) against phytopathogens (*Thanatephorus cucumeris, Sclerotium rolfsii, Rhizoctonia solani, Macrophomina phaseolina, Ustulina zonata, Fusarium oxysporum, F. solani* and *F. graminearum*) were confirmed prior to their application in nursery and field grown plants. Suppression of sclerotial blight of green gram and tea, root rot of chickpea, soybean and mandarin, charcoal rot of soybean, sheath blight of rice and charcoal stump rot of tea were evident either by single or joint inoculation by biological control agents and plant growth promoting rhizobacteria. In all cases, disease suppression was associated with enhanced activity of defense enzymes like chitinase, β-1,3 glucanase, phenylalanine ammonia lyase and peroxidase. Induction of PR-2 and PR-3 and their cellular localization in leaf tissues were further confirmed by indirect immunofluorescence using PAbs of chitinase and β-1,3 glucanase. Enhanced accumulation of phytoalexins following induction of resistance in plants (rice, soybean and tea) were noticed. Disease suppression and health improvement in these plants can lead to development of bioformulations using such potential microbial resources.

**O03.009 Screening of microbial antagonists for the development of commercial biocontrol products**

**against plant pathogens**

*J. Köhl[1], B. Blum[2], P.C. Nicot[3] and M. Ruocco[4]*
*[1]WUR, Plant Research International, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; [2]IBMA, Basel, Switzerland; [3]INRA, Avignon, France; [4]CNR-IPP, Portici, Italy*
*Email: jurgen.kohl@wur.nl*

Screening programs of antagonists for control of plant pathogens often focus on the testing of antagonistic properties *in vitro*, in bioassays and subsequently in crops. However, antagonists must fulfill many more criteria if a commercial application is considered. Within the EU-project ENDURE, various selection criteria have been ranked in a stepwise approach to exclude unwanted candidates in early screening steps using inexpensive tests (Köhl *et al*., 2011. Biological Control 57: 1-12). Consequently, fewer candidates have to be tested in later screening steps when more expensive assessments have to be done. Targeted crops and diseases and the resulting market size are evaluated during the first step. To obtain candidates with the relevant ecological characteristics, the origin of antagonists and isolation techniques are carefully chosen. Candidate antagonists are assessed in rapid tests to exclude those which, for example, produce not sufficient inoculum or show no cold-tolerance. After this first high throughput screening, the remaining isolates are identified and information is collected in relevant data bases. Species with unwanted toxicological or ecological profiles are excluded, but also patent and marketing aspects are reviewed. The antagonistic potential of the pre-selected candidates is subsequently tested on pathogen-inoculated plants. Mass production properties are evaluated in small fermenters in parallel. Tests in crops follow only for a small set of selected antagonists. Feasibility of mass production, formulation and shelf life are tested again in parallel. Consequently, only antagonists which fulfill the major criteria for commercial use will be assessed in field experiments using already suitable pilot-formulation.

**O03.010 Use of plant extracts in plant disease management: the example of Regalia® biopesticide**

*H. Su, T. Johnson, R. Blair, P. Himmel and P.G. Marrone*
*Marrone Bio Innovations, Inc., Davis, CA 95618, USA*
*Email: hsu@marronebio.com*

Regalia® is formulated from the extract of giant knotweed (*Reynoutria sachalinensis*) and was launched in the United States in 2009 by Marrone Bio Innovations, Inc. Regalia® has a broad spectrum of disease control. The mode of action of Regalia® is to stimulate the activity of chalcone synthase and chalcone isomerase in the phenylpropanoid pathway and increase the production of phytoalexins. Accumulation of other simple fungitoxic phenolics was also detected. Regalia® induces papillae formation at pathogen penetration sites and lignification of plant cell walls. It also increases activity of pathogenesis-related proteins such as chitinase, glucanase, and peroxidase. In our extensive tests in the laboratory, greenhouse and field, Regalia as a foliar spray shows efficacy in controlling a wide range of fungal and bacterial diseases, such as powdery mildew of cucurbits, downy mildew of lettuce (*Bremia lactucae*), *Botrytis* of grapes and strawberries, bacterial spot of tomatoes and peppers (*Xanthomonas campestris* pv. *vesicatoria*), and bacterial canker in citrus (*Xanthomonas axonopodis* pv. *citri*), among others. Both laboratory and field tests show that Regalia® is synergistic with commonly used fungicides, such as triazoles, strobilurins, sulfur , copper, mefenoxam, and fertilizers such as calcium. In addition, seed treatment, soil drenches, and dipping seedlings prior to transplanting, can be used to enhance soilborne disease control and increase emergence.

**O03.011 Biological control of *Sclerotinia sclerotiorum***

*H.R. Dillard, K. Waldron and J. Strauss*
*Cornell University New York State Agricultural Experiment Station, Department of Plant Pathology and Plant-Microbe Biology, 630 West North Street Geneva, NY 14456, USA*
*Email: HRD1@cornell.edu*

*Sclerotinia sclerotiorum* is a destructive cosmopolitan pathogen with over 400 species of hosts. The fungus may survive for several years in soil as hardened mycelial structures called sclerotia. When weather conditions are favorable, the sclerotia germinate to produce apothecia, which then produce ascospores that can infect susceptible plant tissues. Biological control offers a potential long term management strategy aimed at reducing populations of the sclerotia in the soil. *Coniothyrium minitans* is a known hyperparasite of the sclerotia of *S. sclerotiorum*. It is now available in the US as a commercial product for use on a wide range of crops. In a preliminary trial conducted in New York State in snap beans, fewer sclerotia of *S. sclerotiorum* were recovered from plots treated with *C. minitans* compared to the untreated control plots. Further studies were conducted in a commercial field of soybeans where over 50% disease incidence was recorded in 2009. Replicate plots were established and treated with the product containing *C. minitans*. Sclerotia populations of *S. sclerotiorum* were determined pre- and post-treatment using a wet sieving technique. A second trial was established in 2011 with a different farmer in a different region. During the growing season, the soybean crops at both locations were scouted for presence of apothecia, disease incidence and severity were recorded, and sclerotia populations were determined pre- and post- treatment. Overall, there was a reduction in the number of sclerotia recovered. Variability in weather had a significant influence on disease incidence and severity.

**O03.012 *Pichia anomala* and *Candida oleophila* in biocontrol of post-harvest diseases of fruits: 20 years of fundamental and practical research's**

*M.H. Jijakli*
*Laboratory of Phytopathology, Gembloux Agro-bio tech, University of Liège, Passage des Déportés 2, Gembloux 5030, Belgium*
*Email: MH.Jijakli@ulg.ac.be*

Fungal pathogens such as *Botrytis cinerea*, *Penicillium expansum* and *Gloeosporides* group are mainly responsible of important economical losses of post-harvest apples and pears. Application of biological control agents (BCA's) is an emerging alternative to synthetic fungicides. However, before becoming an economically feasible alternative to chemical control, BCA's have to satisfy different requirements related to biological, technological and toxicological properties. The different steps for a successful strategy of disease control (selection, production and formulation, study of mechanisms of action, ecological characterization, molecular monitoring, pilot efficacy trials, registration) will be reviewed considering the 2 antagonistic yeasts: (1) *Pichia anomala* strain K as scientific model and (2) *Candida oleophila* strain O. Two decades ago, both strains were selected for their high and reliable antagonistic activity against *B. cinerea* and *P. expansum* on apples and pears. The lack of efficient and reliable BCA's constitutes until now the major drawback to commercialise biopesticides based on these BAC's. In that context, the studies of mode of action and ecological fitness are important because they can lead to a better efficacy of antagonistic yeast strains. Recent advanced molecular techniques have contributed to improve knowledge on the modes of action. Thanks to the identification of genes involved in biocontrol properties, the genetic basis of action mechanisms can be understood. That approach was adopted for *P. anomala* (strain K) and lead to the identification of genes coding for exo-β-1,3-glucanases implicated in the efficacy. Based on that identification, a formulation involving β-1,3-glucans was developed and applied with higher efficacy in controlled conditions. The importance of ecological characterisation is also crucial in the context of pre-harvest application for both antagonistic strains of yeast. UV light, temperature and humidity were identified as major factors influencing strain K and strain O populations. Models taking into consideration temperature and humidity were developed and could be useful in deciding whether pre-harvest treatment is sufficient to allow fast colonization of wounds prior to the arrival of wound pathogens, or whether it is wise to apply further post-harvest treatment to increase the yeast population density.

Furthermore, the scientific background obtained for *Candida oleophila* strain O lead to the development of a formulated biopesticide called Nexy® and registrated in US and some European countries. Practical applications of Nexy® was carried out against postharvest diseases of apples, pears, citrus and bananas. The presentation will concentrate also on these practical applications taking into account the necessity to integrate Nexy® with other methods of post-harvest disease control. Such integration must lead to decrease the global level of chemical residues on fruit surface while keeping a sufficient level of efficacy. Finally, the presentation of 20 years of work will be also the opportunity to highlight the difficulties and challenges to transfer results from scientific laboratory to industrial level, keeping in mind the satisfaction of fruit growers and consumers.

**O03.013 Temperature and incubation period affect *Trichoderma atroviride* conidium production, germination and bioactivity**

*A. Daryaei, R.E. Falloon, K.L. McLean, T.R. Glare and A. Stewart*
*Bio-Protection Research Centre, Lincoln University, P O Box 85084, Lincoln 7647, New Zealand*
*Email: Amir.Daryaei@lincolnuni.ac.nz*

*Trichoderma atroviride* LU132 has been commercialised in New Zealand as Tenet® and Sentinel® for the control, respectively, of *Sclerotium cepivorum* and *Botrytis cinerea*. This study examined the influence of incubation temperature (20, 25, or 30 ℃) on the production of conidia under constant light over a 25 d period. Two measures of quality of the resulting conidia were also assessed-germination and subsequent bioactivity against *Rhizoctonia solani*. Maximum conidium production occurred at 25 ℃ after 20 d. Production of conidia declined at 25 d. Conidia produced at 30 ℃ germinated more rapidly than those produced at 20 or 25 ℃. Incubation at 30 ℃ gave greatest bioactivity against *R. solani* in comparison with incubation at 20 or 25 ℃. An incubation period of 25 d increased bioactivity compared with shorter incubation periods. The experiment was repeated at 25 ℃ for 50 d. Extending the incubation period resulted in a second peak of conidial production at 45-50 d. These conidia had an optimum germination after 20 and 25 d incubation, and the optimum bioactivity for the colonies was achieved with conidia harvested after 15 d. This study suggests that *T. atroviride* formulations based on optimised production of conidia may not result in optimal bioactivity.

**O03.014 Control of *Botrytis cinerea* secondary inoculum within grape bunches by applications of biological control agents and natural products**

*C. Calvo, J. Usall, I. Viñas, P.A.G. Elmer and N. Teixidó*
*IRTA, XaRTA-Postharvest, 191 Rovira Roure, 25198-Lleida, Catalonia, Spain*
*Email: neus.teixido@irta.cat*

Infected necrotic tissues inside the grape bunch represent an important secondary inoculum source for *Botrytis*

*cinerea* in vineyards. Fungicide use in viticulture is becoming restrictive and natural products and biological control agents are promising alternatives to control *Botrytis* bunch rot. However there is a lack of studies evaluating efficacy of alternative treatments to synthetic fungicides controlling *B. cinerea* secondary inoculum in developing bunches. Applications of *Candida sake* and Fungicover®, *Ulocladium oudemansii* and chitosan were carried out in an organic vineyard in 2009 and 2010. At veraison, samples from aborted flowers, aborted fruits and calyptras were collected from wine grape bunches (cv. Macabeu) and incubated in high humidity chambers to evaluate *B. cinerea* incidence. Aborted fruits presented significantly higher incidence of natural infections compared to other tissue types. All evaluated treatments except chitosan significantly reduced incidence in aborted flowers and calyptrae by 46.1% to 84.6% compared to control and sporulation was also reduced by 48.0% to 55.4%. Treatments did not reduce incidence in aborted fruits, which were identified as an important secondary inoculum source of *B. cinerea*. Overall, the tested treatments with biological control agents and natural products demonstrated to be effective controlling pathogen's mycelial growth and sporulation on necrotic tissues within grape bunches.

**O03.015 Cloning and functional analysis of two oxalate decarboxylase genes in the mycoparasite *Coniothyrium minitans***

*L.M. Zeng, J. Zhang, L. Yang, D.H. Jiang and G.Q. Li*
*State Key Laboratory of Agricultural Microbiology and Key Laboratory of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan 430070, P.R. China*
*Email: guoqingli@mail.hzau.edu.cn*

*Coniothyrium minitans* is a mycoparasite and a promising biocontrol agent of *Sclerotinia sclerotiorum*, the causal agent of Sclerotinia stem rot of oilseed rape. Production of oxalic acid (OA) has long been considered to be an important mechanism in pathogenesis of *S. sclerotiorum*. OA is not only toxic to plants, but also toxic to microorganisms including *C. minitans*. Our previous studies showed that *C. minitans* can nullify the toxic effect of OA on *C. minitans* by degradation of OA. However, genes responsible for OA degradation remain unknown. In this study, two genes putatively encoding oxalate decarboxylase, designated as *Cmoxdc1* and *Cmoxdc2*, were cloned from the wild type strain Chy-1 of *C. minitans*. The two genes contain conserved bicupin domains. However, they have different expression patterns, *Cmoxdc1* expressed under pH 3 to 5 and in the presence of OA, whereas *Cmoxdc2* expressed under pH>5 and OA could not trigger expression of this gene. Compared to the strain Chy-1, the Δ*Cmoxdc1* mutants were suppressed to degrade OA and to infect *S. sclerotiorum* mycelia, whereas the Δ*Cmoxdc2* mutants did not differ from the strain Chy-1 in both OA degradation and infection of *S. sclerotiorum*. The function of *Cmoxdc1* in OA degradation and in infection of *S. sclerotiorum* was confirmed through gene complementation. This study suggests that *Cmoxdc1* plays an important role in OA degradation. It also provides direct genetic evidence for importance of OA degradation in mycoparasitism of *C. minitans*.

**O03.016 Iturins play an important role in biological control of apple ring rot by *Bacillus amyloliquefaciens* 9121**

*X.Y. Chen, Y. Li and Q. Wang*
*Department of Plant Pathology, China Agricultural University, No. 2 Yuanmingyuan West Road, Haidian District, Beijing, 100193, P. R. China*
*Email: wangqi@cau.edu.cn*

Apple ring rot disease, caused by *Botryosphaeria dothidea*, was one of the most important diseases on apple fruits in China. Biological control with microbial antagonists has emerged as a promising alternative that can reduce fungicide usage along with safety production of fruit and a lower environmental impact. *Bacillus* strain 9121 has shown dramatically inhibitory effect on the infection of *B. dothidea* YL1 *in vitro* and *in vivo*. *Bacillus* 9121 was identified as *B. amyloliquefaciens* through physiological biochemical characters and nucleotide sequence analysis based on 16S rRNA and partial *gyrA* genea. Cell-free supernatants and lipopeptides crude methanol extract of *B. amyloliquefaciens* 9121 showed comparable inhibitory activities on fungal growth, which indicated that lipopeptides were the crucial compounds responsible for its antifungal activity. PCR detection and nucleotide BLAST analysis showed that the genome of *B. amyloliquefaciens* 9121 contains lipopeptides biosynthesis related genes such as *bamD/bam*, *bacAB/bacD*, *ituD/ituC* and *fenB* which encode iturins and fengycins. Lipopeptides in the crude methanol extract from cell-free supernatants were isolated by thin layer chromatography (TLC) and reversed-phase high performance liquid chromatography (RP-HPLC). Iturins was identified as an active component against *B. dothidea* YL1 by electrospray ionization quadrupole-time of flight mass spectrome-try/mass spectrometry (ESI-Q-TOF MS/MS) along with an in vitro dual culture assay.

**P03.001 Soil solarization for the management of soil borne pathogen in nursery**

*K.D. Thakur and A.K. Meena*
*Department of Plant Pathology, College of Agriculture Nagpur, Dr. Panjabrao Deshmukh Krishi Vidaypeeth, Akola, Maharashtra, India*
*Email: Kdthakur60@gmail.com*

Study of solarization of soil, by covering it with transparent polythene sheets of 100 μm during the October month was examined, for effectiveness in controlling soil borne pathogens. Chilli was selected as nursery crop and the wilt causing pathogen was isolated and identified as *Fusarium oxysporum* f. sp. *capsici*.The experiment was carried out in Randomized Block Design, with 7 treatments and 3 Replication. Each bed was inoculated with *Fusarium oxysporum* f. sp. *capsici* culture at 1g/kg of soil. Weekly data of temperature variation at 5 cm, 10 cm and 15 cm were recorded. Soil was ploughed and then irrigated, polythene sheet wear covered then the recorded maximum rise in temperature at 5 cm varied from 47.6 to 51.0℃, at 10 cm 46.8℃ to 49.1℃, and at 15 cm 45.9℃ to 47.6℃. Soil population of *Fusarium oxysporum* f. sp. *capsici* was reduced up to 90 per cent in irrigated ploughed, 88.3 per cent in irrigated without plough, against 40 per cent in control. *Fusarium oxysporum* f. sp. *capsici* colonies were reduced to non-detectable levels at 5 cm, 10 cm and 15 cm. Among all tested depths maximum reduction was found in irrigated plough 90 per cent, 86.30 per cent and 76.60 per cent followed by irrigated without plough 88.3, 83.6 and 63 per cent against 39.3, 36.6 and 34.6 per cent respectively in control. Seed treatment with Carbendazim (0.1%) has recorded maximum germination percentage 84.33, followed by 82 per cent in irrigated plough solarized treatment, against 43.0 per cent in control. Mortality per cent was found lowest in Irrigated plough 13.66 and 15.6 percent in Carbendazim (0.1%) against 52.3 per cent in control. Plant height after 30 days of germination was found most significant 22.0 cm in Carbendazim (0.1%) which was at par with Irrigated without plough 21.1 cm, against 11 cm in control.

**P03.002 PGPR for the management of major seed-borne diseases of tomato, chilli cabbage and cauliflower and enhancement of production for food security in NE region of India**

*P. Raja[1], P. Nallathambi[2], S.R. Niranjana[3] and A.K. Singh[1]*
*[1]Department of Plant Pathology, College of Horticulture and Forestry, Pasighat, Arunachal Pradesh; [2]Division of Plant pathology, Indian Agricultural Research Institute, Regional Station, Wellington, Tamil Nadu; [3]Department of Studies in Biotechnology, University of Mysore, Mysore-570 006*
*Email: prajachf@gmail.com*

Tomato, chilli, brinjal, cabbage and cauliflower are important vegetable crops grown by tribal people of Arunachal Pradesh, India for their food security. Cumulative analysis of date from recurrent surveys demonstrated that damping off and bacterial wilt of tomato, chili and brinjal were the major disease with disease severity up to 80% and 25% respectively. Similarly, in cabbage and cauliflower damping off is recorded up to 18%. Seed-borne pathogens *viz., Alternaria brassicicola, Alternaria alternata* and *Rhizoctonia solani* were isolated from different samples. Soil samples were collected from more than 40 locations, PGPR bacteria were isolated and identified. Four effective isolates of fluorescent Pseudomonads are identified after a sequence of evaluation both under laboratory and field trials. Bio-priming with effective strains (0.5%) were also evaluated to assess the seed germination and seed borne infection in the nursery. PGPR isolate CHF-2011 32a was promising followed by CHF-2011 43 in tomato, chilli, brinjal cabbage and cauliflower and reduced the diseases incidence ranging from 10-25 per cent. Soil application (5%) also reduced the disease incidence and increased the yield from 6 to 21 percent.

**P03.003 Assessing potentiality of rhizosphere bacteria and fungi from soil in antagonism against sclerotia forming pathogens and growth promotion of cucumber seedlings**

*M.K.A. Bhuiyan and M.A. Khondaker*
*Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur -1706, Bangladesh*
*Email: kabhuiyan55@yahoo.com*

An attempt was taken to control pre and post emergence seedling mortality of cucumber by bacterial and fungal antagonists. A total of 47 bacterial strains and 50 fungal isolates were collected from different rhizosphere soils of Bangladesh and were screened against the selected virulent isolates of *Rhizoctonia solani and Sclerotium rolfsii* in dual culture. Among the screened bacterial strains and fungal isolates in dual plate technique, *Bacillus* sp. AB4 and *Trichoderma* isolate TH-26 were appeared as the most effective antagonist in inhibiting the radial growth of the selected isolates of *S. rolfsii* and *R. solani*. Four other bacterial strains and 12 fungal isolates were also found as effective antagonists. The antagonist bacteria growing on Casein agar glucose medium were identified as gram positive *Bacillus* by observing several morphological and biochemical characters and the fungal isolates were identified as *Trichoderma harzianum.* Thermo stable antifungal components were produced by *Bacillus* strains completely inhibited the radial growth of the test pathogens. *Bacillus* sp. and *Bacillus* sp. strain AB4 and AB1 showed the nitrogenase activity 1.46 and 0.89 $nmolC_2H_4h^{-1}$ $culture^{-1}$, respectively. In the pot culture experiment, *Bacillus* sp. strain AB4 and *Trichoderma* isolate TH-26 showed significantly the highest reduction of pre and post emergence seedling mortality of cucumber over control treatment of *S. rolfsii* and *R. solani* inoculated pots. Growth promotion potentialities of *Bacillus* strains were significantly higher than *T. harzianum* isolates. Increased chlorophyll content was also observed in the *Bacillus* sp. strain AB1 and AB4 treated plants.

**P03.004 Beneficial and deleterious effects of *Trichoderma harzianum* and *T. longibrachiatum* on growth of cotton seedlings and their bioconrol capacity against seedling damping-off**

*A.A. Aly[1], E.M. Hussein[1], A.A. El-Awamri[2], M.M. Habeb[1] and K.A. Abd -Elsalam[1]*
*[1]Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt; [2]Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt*
*Email: abd-elsalam.k.a.@post.com*

*Trichoderma* spp. were isolated from roots of cotton plants showing the typical symptoms of seedling damping-off of root rot of adult plants. Identification of 15 randomly selected isolates of *Trichoderma* to species level revealed that eight isolates (53.3%) were belonging to *T. longibrachiatum*, while seven isolates (46.7%) were belonging to *T. harzianum*. Geographic origins of *T. harzianum* in the sample were restricted to the Nile Delta (Daqahliya and Gharbiya), while isolates of *T. longibrachiatum* were isolated from widely separated geographic origins in the Nile Delta (Minufiya), Middle Egypt (Giza), and Upper Egypt (Assiut). The beneficial and deleterious effects of *Trichoderma* isolates on growth of cotton seedlings were evaluated by planting cotton seeds in autoclaved soil infested with *Trichoderma* isolates. Each of *T. longibrachiatum* and *T. harzianum* included both beneficial and deleterious isolates. Thus, of the nine isolates, which were pathogenic during the postemeregence stage, five were belonging to *T. longibrachiatum* and four were belonging to *T. harzianum*. Similarly, of the 12 isolates that significantly promoted seedling height, seven were belonging to *T. longibrachiatum* and five were belonging to *T. harzianum*. Moreover, the same isolate may exhibit both the beneficial and deleterious effects depending on the variable under consideration. Biocontrol capacity of *Trichoderma* spp. against soilborne fungi involved in cotton seedling damping-off was evaluated by planting cotton seeds in autoclaved soil infested with a mixture of the soil-borne fungi commonly involved in the disease. Before planting, seeds were treated with a fine powder consisted of a mixture of sorghum and *Trichoderma* spp. The tested isolates of *Trichoderma* spp. could be classified into 3 distinct groups based on their biocontrol capacity. The first group included T3, T4, T6, T10, T27, and T42, which were effective biocontrol agents. Isolates of this group significantly increased the percentage of the surviving seedlings. Moreover, isolates T4 and T6 significantly increased dry weight of the surviving seedlings. The second group included T23, T38, and T39, which significantly reduced seedling height. Isolates T23 and T38 also reduced the percentage of the surviving seedlings. The third group included T9, T14, T18, T29, and T31, which were neither biocontrol agents nor pathogens. Each of these groups included isolates of both *T. longibrachiatum* and *T. harzianum*. Isolate T5 showed both pathological effects (increase in preemergence damping-off and reduction in seedling height) and biocontrol activity (reduction in postemergence damping-off).

**P03.005 Pathogenic variation among Fusarium isolates and biological control of Fusarium wilt of Chilli (*Capsicum annumm* L.) by non pathogenic Fusarium solani isolate**

*M.J. Patil, B.T. Raut, S.M. Ghawade, E. Bagde and P.P. Gawande*
*Chilli and Vegetable Research Unit, Dr. Panjabrao Deshmukh Krishi Vidhapeeth, Akola, 444104 Maharashtra, India*
*Email: mohanpatil.1082@rediffmail.com*

Variability among the fifty two isolates collected from different regions of vidarbha of Maharashtra, India was studied in respect of cultural, morphological characteristic and pathogenic ability. Mycelial growth of isolate varied from isolate to isolate. Maximum growth (84.44 mm) was observed in FS-11 followed by FS-9 and FS-13 recording 87.50 and 87.33 mm respectively. The lowest growth was noticed in FS-1and FS-6 (41.66 mm) followed by FS-41 and FS-8 exhibiting 43.33 and 45.66 mm radial growth respectively. The pigmentation also reflects the variation among the isolates. Three milky white, eight dull white to whitish, four reddish brown, seven yellowish, three purple, eleven light red and ten brownish to dark brown. Out of fifty two isolates, 7, 10, 14, 12 and 9 isolates showed non pathogenic, weakly pathogenic, moderately pathogenic, strongly pathogenic and highly pathogenic reactions in pot culture study respectively against X-235 variety of Chilli. The efficiency of non pathogenic Fusarium solani isolates viz. FS-38 and FS-4 for biological control through cross protection of Fusarium wilt of chilli (*Capsicum annumm* L.) was examined in the pot culture. The considerable reduction in wilting was achieved in all inoculum proportion due to pre inoculation of non pathogenic isolates of *Fusarium solani* as compared to control (highly pathogenic isolates). The minimum wilting was recorded in 100 g inoculum by pre inoculation of non pathogenic isolates namelyFS-38 and FS-4 recorded 26.66 per cent where as maximum wilting of 80 per cent was observed in same dose of pathogenic inoculum namely FS-18 and FS-28 per kg of soil.

**P03.006 Investigating antagonistic effect of *Trichoderma viride* on *Rosellinia necatrix* Prill isolation from white root rot apple**

*E. Payghami, S. Nematollahi, A. Zahedifar and M. Jafakesh*
*Department of Plant Pathology, Tabriz Branch, Islamic Azad University, Tbriz, Iran*
*Email: Payghami2011@yahoo.com*

White root rot is an important disease in apple orchards in Iran, particularly in the West and East Azarbaijan provinces. *Rosellinia necatrix*, the causal agent of white root rot and *Trichoderma viride* were isolated for the first time from white root rot of apple trees. The antagonistic effect of *T. viride* on *R. necatrix* in vitro (using dual culture method in the PDA culture) showed that 1) *T. viride* was able to colonise and prevent the growth rate of *R. necatrix*, 2) there was a nutritional competition between the *T. viride* and *R. necatrix* in the PDA culture, 3) plasmolization and vacuolization of the *R. necatrix* hyphae occurred in the presence of *T. viridae*, 4) coiling of the *R. necatrix* hyphae was observed by the effect of *T. viride* hyphae in the PDA culture, 5) *T. viride* hyphae penetrated in to the *R. necatrix* hyphae, and 6) *T. viride* spores stuck to *Dematophora necatrix* spores (asexual form of *R. necatrix*).

**P03.007 *Trichoderma* in valleys of Arica: Salinity and boron tolerance, biocontrol against *Fusarium oxysporum***

*R. Salvatierra-Martínez and G.F. Sepúlveda-Chavera*
*Universidad de Tarapacá. Av. General Velázquez 1775, Casilla 6 – D, Arica, Chile*
*Email: gsepulve@uta.cl*

In the coastal valleys of Arica-Chile (Azapa and Lluta) are developed intensive agricultural systems, closely related to soil salinity (> 3 dS $m^{-1}$) and high levels of boron in water and soil (> 13 ppm). 10 stains native, and an exogenous strain of *Trichoderma* spp. obtained from the comercial product Trichonativa® were used. In "*in vitro*" tests, the strains were submitted to 3 doses of NaCl (8, 15 and 20 g $L^{-1}$) and the same three doses of NaCl + 15 ppm of boron. Native strains were tolerant to NaCl of 20 g $L^{-1}$. The exogenous strain showed less tolerance being inhibited their growth at 20 g $L^{-1}$ of NaCl. The saline tolerance was associated with the capacity to absorb $Na^{2+}$ as a measure of osmotic adjustment, on the other hand saline solutions with boron have an effect marked in growth and sporulation of *Trichoderma*. The antagonism against *F. oxysporum* is not significantly affected by NaCl 8 g $L^{-1}$ in the native strains. *F. oxysporum* improve their competitiveness in saline environments. In vivo tests using plants inoculated with *F. oxysporum* and saline irrigation the protected plants with native *Trichoderma* had higher survival rate, corroborating the "*in vitro*" results and demonstrate the need for salt-tolerant strains in protecting crops in saline and arid areas.

**P03.008 Biological agents of *Erwinia amylovora* which cause a fire blight of fruit crops in Kyrgyzstan**

*T.D. Doolotkeldieva, S.T. Bobusheva and A. Aytbaeva*
*Kyrgyz-Turkish Manas University, Plant Protection Department, Bishkek city, Kyrgyzstan*
*Email: tdoolotkeldieva@gmail.com*

In Kyrgyzstan, *Erwinia amylovora* is presently a quarantine object. Fire blight of fruit crops was found in proximity to borders and there was a threat of its wide spread across the territory of the country. Exudates from diseased apple blossoms were used for isolation of *Erwinia amylovora* pure cultures. Culture-morphological and biochemical properties, immunobiochemical and PCR analyses were conducted for identification of this pathogen. We performed in vitro and in vivo tests using an antibiotic-producing *Streptomyces* and *Bacillus* strains in order to develop environmentally friendly means to control the fire blight. Primary and secondary screening have showed that *Bacillus* sp. *strain P-1002* has a high antibacterial activity in 72 hours and zone of lyses was 8±1.25 mm. *Streptomyces bambergiensis and Streptomyces diastochromogenes* strains have showed a hiperparasitism action to *Erwinia amylovora*. After five days of the experiment, the growth of *Erwinia amylovora culture* was completely inhibited by these antagonists. These biocontrol related strains were screened by polyketide synthetase primers.The diseased branches and flowers of apple trees were sprayed with 1.0% ($3\times10^6$ spore/ml) *Streptomyces diastochromogenes* bio product suspension in the field conditions. The inhibition of growth and development of the disease in the treated branches occurred after 10 days.These results provide promising hope to develop biological agents for control of this dangerous quarantine pathogen in Kyrgyzstan.

**P03.009 Biological control of aggressive races of *Erwinia carotovora* subsp. *carotovora* causing soft rot in potatoes by Streptomyces *fumanus***

*T.D. Doolotkeldieva, S.T. Bobusheva and A. Sulayman kyzy*
*Kyrgyz-Turkish Manas University, Plant Protection Department, Bishkek city, Kyrgyzstan*
*Email: tdoolotkeldieva@gmail.com*

In Kyrgyzstan, potato is a staple product for the population. *Erwinia carotovora* subsp. *carotovora* bacterium is one of the most important factors which cause a soft rot of stem and tubers before and after harvest and greatly reduces a yield. We have isolated *Erwinia carotovora* subsp. *carotovora* races from different regions and analyzed by target primers*: MseF* GACGATGAGTCCT GAG and *MseR* TACTCAGGACTCAT. The 50 isolates were tested for pathogencity in vitro and in vivo tests, as a result of the 10 isolates were pathogenic. We have conducted *in vitro* and *in vivo tests* against *Erwinia carotovora* bacterium using antibiotic-producing *Streptomyces* strains that were isolated from agro-ecosystems in Kyrgyzstan. The 16S rRNA genes were PCR amplified with 27f and 1522r primers, and PKS genes were screened by polyketide synthetase primers. *Streptomyces graminearus, sk-2,* and *Streptomyces fumanus gn-2* have

shown a high antagonistic effect to all races of *Erwinia carotovora bacterium*. Isolates in this category have formed an inhibition zone between 9 and 11 ±0.97 mm. Potato tubers were treated in suspension of *Streptomyces fumanus* gn-2 bioproduct before planting into the soil. It has provided the germination of potato seedlings up to 94.0 ±1.25% and the resistance of crops to pathogen, *Erwinia carotovora in* field conditions. In the storage the potato tubers were treated by powder formulation of *Streptomyces fumanus gn-2* containing $3\times10^9$ spore/g. Percentage of tuber soft rot decreased by 47.8% compared to the control. These results provide promising hope to develop biological agents for control of this pathogen.

**P03.010 Integration of biointensive approaches for management of wilt and rhizome rot of Ginger (*Zingiber officinale*) in Assam**

*L.C. Bora, D. Deuri and G. Goswami*
*Department of Plant Pathology, Assam Agricultural University Jorhat-785 013, India*
*Email: boralohitc@gmail.com*

In Assam, bacterial wilt and rhizome rot caused by *Ralstonia solanacearum* is considered as the major limiting factor for ginger production causing about 92 per cent crop loss. The present study was made to explore the potential of environmentally safe 'biointensive approaches' for management of wilt and rhizome rot of ginger using host resistance and *Pseudomonas fluorescens* and *Trichoderma viride* based bioformulation 'Biofor-Pf' developed in Assam Agricultural University, Jorhat. Twenty seven ginger cultivars and germplasms were collected from various locations of North East Indian states and screened against bacteria wilt and rhizome rot pathogen *R. solanacearum.* The existing variation among these 27 promising cultivars were observed through differential tolerance disease (6.7% to 76.6%) and was found to have a genetic basis using genetic marker, random amplified polymorphic DNA (RAPD). RAPD analysis revealed differential polymorphism of DNA showing a number of polymorphic bands ranging from 26 to 70 among 27 cultivars. Cultivar *Mango ginger* (*Curcuma amada* Roxb.) showed highest degree of tolerance (80.67%) against wilt and rhizome rot followed by cultivars *Kakopathar* (76.13) and *Vairabkunda* (74.45%). UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrograms constructed based on similarity coefficients, and Simple Matching using the RAPD placed the cultivars in four similar clusters in all the three dendrograms revealing the congruence of clustering patterns among the similarity coefficients and a rather less genetic distance among the cultivars. The wilt and rhizome rot incidence in ginger decreased and corresponding yield increased significantly when plants (local cultivar-*Bhola*) were treated with 'Biofor-Pf'. Lowest disease incidence (5.25%) and corresponding highest yield (39 tonnes/ha) was recorded in the treatment comprising of vermicompost + MOC + *P. fluorescens* + *T. viride* along applied as rhizome treatment + soil application 90 days after planting.

**P03.011 Genomic characterisation of microbial antagonists, their interactive effects and utility in management of bacterial wilt of *Bhut Jolokia* (*Capsicum chinense* Jacq.)**

*L.C. Bora, R. Sarkar and L. Kataky*
*Department of Plant Pathology, Assam Agricultural University,Jorhat-785 013*
*Email: lcbora_06@yahoo.co.in*

The *Bhut jolokia* (*Capsicum chinense* Jacq,) is an interspecific hybrid cultivated in Assam and other North East Region of India. Bacterial wilt disease (*Ralstonia solanacearum*), causes complete wilting of the crop and lower its productivity significantly. Different management practices presently available have limitations and do not give results to the desirable level. In the present study two bacterial bioagents, *viz*., *Bacillus cereus*, *Citrobacter freundii*, and two fungal bioagents, *viz*., *Aspergillus flavus*, and *Trichoderma viride* has been tried individually as well as in consortia for management of the crop disease. Genomic analysis of the bioagents was done following standard protocol using RAPD and ITS-PCR and identification was done based on nucleotide homology. Phylogenetic analyses were conducted in Molecular Evolutionary Genetics Analysis (MEGA4) and the bioagents were identified as *Bacillus cereus* CF19 (GenBank Accession Number: JX438690.1), *Citrobacter farmeri*, strain 17.7 KSS (GenBank Accession Number: HE575920.1), *Aspergillus flavus*, strain: IFM 54306 (GenBank Accession Number: AB363745.1) and *Trichoderma viride* strain KSAP113 (GenBank Accession Number: EF639724.1). *In vitro* evaluation of the bioagents and their consortia for suppression of *R. solanacearum*, done by dual culture assay revealed that *B. cereus* alone (22mm) and consortium of *B. cereus* and *T. viride* (28mm) could show highest inhibition of the pathogen. Different bioagents and their consortia evaluated for their ability to suppress bacterial wilt of *bhut jolokia* under green house grown plants revealed that consortium of *B. cereus* and *T. viride* could cause maximum reduction (91.65%) of wilt incidence in *Bhut jolokia.* Our analysis, coupled with the genome sequence data, provides a roadmap for commercial production and application of antagonist consortia based biopesticides.

**P03.012 Antagonism of *Trichoderma harzianum* and *Trichoderma atroviride* on onion pathogens and their effect in the growth promotion and phenolic and flavonoid compounds production of onion bulbs**

*S.G. Ortega García, G. Sepúlveda-Jiménez and R. Montes Belmont*

*Centro de Desarrollo de Productos Bióticos, Instituto Politécnico Nacional, Apartado postal 24, Yautepec, Morelos 62731, Mexico*
*Email: rbelmont@ipn.mx*

*Trichoderma* spp. are common soil fungi used as biocontrol agents due to their capacity to produce antibiotics, induce systemic resistance in plants and parasitize phytopathogenic fungi of major agricultural importance. Some strains of this fungus are able to produce metabolites that enhance plant growth. In the present study, it was evaluated the antagonist effect of *Trichoderma* species on the onion pathogens fungi: *Sclerotium rolfsii* and *Alternaria porri*, and whether colonization of onion seedlings by *Trichoderma harzianum* and *T. atroviride* influenced plant growth and produces changes in phenolic and flavonoid compounds. Six isolates and two commercial products of *Trichoderma* were tested for their antagonism effect and mycelial growth inhibition of *S. rolfsii* and *A. porri* strains. Here it is shown that only *T. harzianum* and *T. atroviride* inhibited from 40 to 62.5% the mycelia growth of the two onion pathogens. *T. atroviride* promotes growth of onion bulbs from Red Satan and Crystal White varieties. Phenolic compounds content was increased in bulbs of Red Satan and Crystal White varieties by the application of *T. harzianum* and *T. atroviride*, respectively; whereas, only the *T. atroviride* inoculation increased the flavonoid content in bulbs of Red Satan cultivar. This is the first report of the *Trichoderma* influence on the biosynthesis of phenolic compounds and flavonoids. Additionally, it is important to evaluate the effect of *T. harzianum* and *T. atroviride* for the biological control of. *S. rolfsii* and *A. porri* under greenhouse conditions.

**P03.013 Enzyme activity of rhizobacterial introduced tomato's seedlings, which the ability to induce the resistance of tomatoes toward bacterial spot (*Xanthomonas axonopodis* pv. *vesicatoria*)**

*T. Habazar, Z. Resti and Y. Yanti*
*Agriculture Faculty, Andalas University, Kampus Limau Manih, Padang 25163 West Sumatera Province, Indonesia*
*Email: trimurtihabazar@yahoo mail.com*

Bacterial spot cause by *Xanthomonas axonopodis* pv. *vesicatoria* is a major constraint in tomatoes cultivation. To control this disease we have found three rhizobacterial isolates, which have the ability to induce systemic resistance (ISR) on tomatoes. One of mechanism of ISR on plant are changes of phenylalaninelyase enzymes activity. The aim of this experiment was to study the activity of phenylalanine ammonia lyase enzyme in rhizobacterial introduced tomatoes which have ISR against *Xanthomonas axonopodis* pv. *vesicatoria*. The experiment has been done in triplicate. Three rhizobacterial isolates were introduced on tomato's seeds and two weeks old seedling. Phenylalaninelyase enzymes activity was assayed on tomato's seedlings (0, 2, 4, and 6 days after rhizobacterial introduction). The results showed that on the rhizobacterial introduced tomato's seedlings (root, stem and leaf) increased the activity of phenylalanine ammonia lyase enzyme compare with control.

**P03.014 Characterization of the *Cryphonectria parasitica* population in Azerbaijan**

*D.N. Aghayeva[1], J. Wall[2], S. Prospero[3] and D. Rigling[3]*
*[1]Institute of Botany, Azerbaijan National Academy of Sciences, Badamdar 40, Baku AZ1073, Azerbaijan; [2]Cornell University Department of Natural Resources, Ithaca, New York; [3]WSL Swiss Federal Research Institute, CH-8903 Birmensdorf, Switzerland*
*Email: adilzara@hotmail.com*

The chestnut blight fungus *Cryphonectria parasitica* originates in eastern Asia and was accidentally introduced into North America and Europe. In both continents, the tree pathogen caused a severe disease epidemic on the endogenous *Castanea* species. Chestnut blight was first recorded in Azerbaijan on the native European chestnut (*C. sativa)* in 2003 (Aghayeva, Harrington 2008). The aim of this study was to assess the diversity of the *C. parasitica* population in Azerbaijan and to determine whether *Cryphonectria hypoviruses* (CHV) occur in this population. CHV are mycoviruses that infect *C. parasitica* and can be used for biological control of the disease. Chestnut blight cankers were sampled in four districts along the Great Caucasus region of Azerbaijan in 2011. In total 157 isolates were obtained of which 149 isolates were *C. parasitica* and seven were putative *C. radicalis*. Among the 149 *C. parasitica* isolates, 142 belonged to the same vegetative compatibility (vc) type. Four additional vc types were identified among the seven other isolates. The dominant vc type did not match with any of the known European vc types. One of the *C. parasitica* isolates revealed specific characteristics (whitish debilitated culture morphology on PDA) for an infection by the hypovirus CHV-1. Sequence analysis indicated that the hypovirus was different from the CHV-1 subtypes that occur in Europe. Our results indicate that the *C. parasitica* population in Azerbaijan is not related to the populations in Europe. Overall, the population has a very low vc type diversity, which will favour biological control of chestnut blight.

**P03.015 Biological recycling**

*M. Abdel-Sattar and H. El-Marzoky*
*Agriculture Botany, FAC Agriculture; Suez Canal Univ., Egypt*
*Email: h_marzoki@yahoo.com*

In spite of the fact that the strawberry Festival and Susana are highly susceptible cultivars to crown rot, root rot,

wilt and anthracnose diseases, it's still required for exportation to Europe countries. Planting fresh strawberry seedling of highly susceptible cultivars on biological decomposed rice straw bags and naturally infested soil carried out in two locations, Abo-Swear at Ismailia and El-Nobaria at El-Behera Governorates revealed that the occurrence of infected strawberry plants grown in decomposed rice straw bags reached 0.7 and 1.6% in El-Behera and Ismailia, respectively. However, the corresponding figures for strawberry plants grown in natural soil under the same conditions were 8.5 and 76.6%, respectively, 120 days after sowing. Strawberry plants grown on rice straw bags under greenhouse or open field conditions showed better growth and an increase in shoot and root systems. Strawberry fruits of good quality and quantity were harvested from plants cultivated on decomposed rice straw bags in comparison with the control plots under natural soil conditions. The pH around the roots in decomposed rice straw bags ranged from 5.5 to 6.5, slightly acidic, while values obtained around the root system in natural soil ranged from 7.5 to 8.5 (alkaline). So, growing strawberry on decomposed rice straw bags avoids and overcome the problem of alkalinity and salinity in the rhizosphere. This is very important, as strawberries are very sensitive to salinity. Cultivating strawberry on rice straw bags keeps the fruits away from contacting the soil and thus limits the possibility to infect by soil borne fungi. Symptoms of crown rots and black root rots yielded several fungi identified as *Phytophthora cactorum*, *Colletotrichum acutatum*, *Pythium ultimum*, *Rhizoctonia solani*, *Sclerotium bataticola*, *Fusarium oxysporum* and *F. solani*. The wilt symptoms observed on a few strawberry plants on rice straw bags might be attributed to transmission of some pathogens through irrigation water or through the highly susceptible strawberry seedling. Based on the above result, it could be recommended that using decomposed rice straw bags as a growing media in replacing natural soil, can improve the production of strawberry under open field conditions in Egypt. Also, avoid the importation of the very expensive grow bags (soil less culture i.e. BVB, Green Isle, Kekkila , Grodan Master and Rock Wool) as compared with the local, cheaper and effective decomposed rice straw bags.

### P03.016 *Paenibacillus* sp. - potential biocontrol agent for black rot of Brassica?

*H. Ghazali-Biglar, J. Hampton, E.van Zijll de Jong, A. Holyoake and A. Stewart*
*Bio-Protection Research Centre, Lincoln University, Lincoln 7647, New Zealand*
*Email: hoda.ghazalibiglar@lincolnuni.ac.nz*

Black rot caused by the seed-borne pathogen *Xanthomonas campestris* pv. *campestris* (Xcc) is a widespread disease of brassicas. The aim of this research was to investigate the use of *Paenibacillus* as a seed treatment for biological control of black rot on cabbage. Twenty-four isolates of *Paenibacillus* were categorized for their interactions with Xcc in a dual culture assay. Nine of these isolates were then screened for their capacity to reduce black rot symptoms on cabbage in pot trial assays. From this work one *Paenibacillus* isolates (P16) was selected as a potential biocontrol agent (BCA). To determine whether this BCA is rhizosphere competent and/or endophytic, cabbage seedlings grown from BCA-treated ($1.5\times10^7$ CFU/seed) seeds were tested for the presence of BCA by real-time PCR using a specific primer pair which targeted the *gyrB* region. Standard curves were generated for soil and plant samples, and the detection limit ($1\times10^3$ CFU/g) determined. In rhizosphere soil, BCA density decreased from $9.9\times10^5$ to $1.1\times10^3$ CFU/g by 11 days after sowing (DAS), and thereafter it was below the limit of detection. BCA population in the bulk soil was only detected up to 6 DAS, and was not recorded in plant samples, indicating either that it is not endophytic or its density in the plant was below the detection limit. This BCA is therefore rhizosphere competent only during early cabbage seedling growth, and is most probably not endophytic.

### P03.017 Microbial biocontrol of Sclerotinia stem rot of canola

*M.M. Kamal, G.J. Ash, K. Lindbeck and S. Savocchia*
*E.H Graham Centre for Agricultural Innovation (an alliance between Charles Sturt University and NSW DPI) School of Agricultural and Wine Sciences, Charles Sturt University, Boorooma Street, Locked Bag 588, Wagga Wagga, NSW- 2678, Australia*
*Email: mkamal@csu.edu.au*

*Sclerotinia sclerotiorum* (Lib.) de Bary is a polyphagous, necrotropic fungal pathogen that infects more than 400 plant species belonging to almost 60 families and causes significant economic losses in various crops including canola in Australia. Five hundred bacterial isolates from sclerotia of *S. Sclerotorium* and the rhizosphere and endosphere of wheat and canola were evaluated. *Burkholderia cepacia* (KM-4), *Bacillus cereus* (W-67) and *Bacillus amyloliquefaciens* (SC-1) demonstrated strong antagonistic activity against the canola stem rot pathogen *Sclerotinia sclerotiorum in vitro*. A phylogenetic study revealed that the *Burkholderia* strain (KM-4) does not belong to the potential clinical or plant pathogenic group of *B. cepacia*. These bacteria apparently produced antifungal metabolites that diffuse through the agar and inhibit fungal growth by causing abnormal hyphal swelling. They also demonstrated antifungal activity through production of inhibitory volatile compounds. In addition, sclerotial germination was restricted upon pre-inoculation with every strain. These results demonstrate that the aforementioned promising strains could pave the way for sustainable management of *S. sclerotiorum* in Australia.

**P03.018 Parasitic capacity of native *Trichoderma* on soil-borne pathogens**

*P. Millas, A. France and C. Santelices*
*INIA-Quilamapu, casilla 426, Chillán, Chile*
*Email: pmillas@inia.cl*

Root and crown rot, wilt, and damping-off are the main symptoms produced by soil-borne pathogens which are causing economic loss in crops. The control is based in chemical fungicides and soil fumigant, however, the use of pesticides is increasingly restricted due to the negative impact on the environment and on human health. Alternative methods to manage soil-borne diseases are therefore needed. The Centre for Biological Control of the Agricultural National Research Institute (INIA) of Chile has been collecting in the last year *Trichoderma* isolates from different ecosystem along the country between parallel 18 º and 54 º South. The variability and adaptation of these isolates allows to select those that are antagonist on several pathogens. This study was made to determinate the parasitic capacity of 50 isolates of *Trichoderma* spp. against *Phytophthora cryptogea*, *Rhizoctonia solani* and *Phoma exigua*. Parasitic capacity was determined by detecting coiling around and/ or hyphae penetration into the pathogen by *Trichoderma*. This was observed under a light microscope from the area of hyphae intermingled in dual cultures on 50% PDA. 56%, 92% and 50% of the evaluated isolates showed parasitism against *P. cryptogea*, *R. solani* and *P. exigua*, respectively. Furthermore, 13 isolates had parasitic capacity against the three pathogens, of which 46% and 23% correspond to *T. harzianum* and *T. asperellum*, respectively. This study shows the potential of several *Trichoderma* spp. isolates of the INIA's collection to simultaneously control of the three soil-borne pathogens.

**P03.019 Iron competition and release of hydrolases are two key tools used by yeast biocontrol agents against fungal pathogens**

*D. Spadaro, D. Zhang, H. Banani, A. Garibaldi and M.L. Gullino*
*AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*
*Email: davide.spadaro@unito.it*

Iron competition and production of cell wall degrading enzymes by the antagonists *Metschnikowia pulcherrima* MACH1 and *Aureobasidium pullulans* PL5 against *Botrytis cinerea*, *Monilinia laxa* and *Penicillium expansum* were studied. In presence of low concentration of $Fe^{3+}$, MACH1 showed higher biocontrol activity against postharvest pathogens on apple. In absence of $Fe^{3+}$, MACH1 exhibited the highest antimicrobial activity, but sufficient $Fe^{3+}$ enabled the disappearance of the activity, suggesting that competition for iron play a key role in the biocontrol activity of MACH1 against the pathogens. In Lilly-Barnett minimal salt medium with the fungal cell walls of the pathogens as sole carbon source, PL5 produced exo-chitinase, endo-chitinase, and β-1,3-glucanase. The extracted crude enzymes produced by the antagonists showed a high activity in inhibiting the growth of the pathogens *in vitro*. An alkaline protease gene was amplified from genomic DNA and cDNA of *A. pullulans* PL5. Expression of ALP5 in *Escherichia coli* and in *Pichia pastoris*, followed by analysis of enzymatic activity, yielded a homogeneous recombinant ALP5 which hydrolysed the substrate casein, inhibited the mycelial growth of the pathogens and the development of postharvest rots on apples. Our results show that competition for iron is the main mode of action of MACH1 and that production of chitinase, glucanase and protease are involved in the biocontrol activities of PL5.

**P03.020 Mechanism of biological control by rhizobacteria against citrus melanose on Unshu mandarin**

*Y.C. Jeun, Y.J. Go, M.H. Oh and K.N. Kim*
*Faculty of Bioscience and Industry, College of Applied Life Sciences, the Research Institute for Subtropical Agriculture and Biotechnology, Jeju National University, Ara-dong, Jeju-si, Jeju 690-756, Republic of Korea*
*Email: ycjeun@jejunu ac.kr*

Melanose in citrus plants caused by *Diaporthe citri* is one of the most important diseases in citrus cultivation. Recently, the interest of environment friendly plant protection is continuously increased. In this study, the possibility of disease control using rhizobacteria against citrus melanose was investigated. Over 100 rhizobacteria were isolated from the rhizosphere of annual plants in Halla Mountain. Some of them such as TRH 415-2, THJ609-3, MRL 408-3 and TRH423-3 showed suppression of disease severity in the leaves of Unshu mandarin after inoculation with the melanose pathogen not only in the green house test but also in the field test. By analysis of internal transcript spaces (ITS) in rDNA sequencing, MRL 408-3 and TRH423-3 were identified as *Burkholderia gladioli*, TRH 415-2 was identified as *Pseudomons fluorescens* and THJ609-3 was identified as *P. pudia*. The microscopical observation using a fluorescence microscope showed that number of conidia was decreased on the leaves of Unshu mandarin pretreated with rhizobacteria. Furthermore, scanning electron microscopical observations revealed some hyphae of melanose pathogen were attached by the rhizobacteria on the leaves of Unshu mandarin. These results suggest that the growth of hyphae was suppressed directly by the rhizobaterial cells.

**P03.021 Isolation and characterization of antifugal metabolite from *Paenibacillus kribbensis* T-9 isolated from soil**

*S.J. Xu and B.S. Kim*
*Department of Plant Science, Gangneung-Wonju National*

*University, 7 Jukhen-gil, Gangneung, Gangwon-do, 210702, Republic of Korea*
*Email: bskim@gwnu.ac.kr*

This study was conducted to select potential soil microorganisms that can be applied to environment friendly control against phytopathogenic fungi. Bacterial strain T-9 was isolated from soil of Samcheok city in Korea and identified as *Paenibacillus kribbensis* on the basis of morphological and biological characteristics and 16S rRNA gene sequence analysis. This bacterium exhibited broad-spectrum antagonistic activity against phytopathogenic fungi *in vitro*. The strain produced cellulase, pectinases, protease, HCN, phosphatase and siderophore. Also this strain had disease control effects against a variety of plant diseases *in vivo*. A bioactive metabolite was isolated from *P. kribbensis* T-9 and also showed potent antagonistic activity towards a range of phytopathogenic fungi. The above-described results indicate that *P. kribbensis* T-9 has the potential ability to be used as an antagonist against various phytopatho- genic fungi.

**P03.022 The effect of *Pseudomonad fluorescens* and AM fungi indigenous isolated from healthy banana rizospheres at endemic Fusarium wilt areas as the potensial biocontrol agents to Fusarium wilt**

*S. Eri, H. Trimurti, F.H. Eti, D. Abdi and N. Nazril*
*Agroekotek, Agriculture Faculty, Andalas University, Indonesia, Padang*
*Email: erisulyanti@yahoo.com*

In this study, to combinate of some biocontrol agents with different mechanisms is alternative to improve the effectiveness of the biological control agents. Single and combined applications of Pseudomonad fluorescens and Arbuscular Mychorrhizae Fungi (AM Fungi) indigenous isolates were tested to induce resistance in susceptible Cavendish banana against *F. oxysporum* f. sp. *cubense* race 4 under greenhouse conditions. These isolates originally isolated from healthy banana rhizosphere at endemic Fusarium wilt areas in the centre of production banana in West Sumatra. These researches were conducted with Block Randomized Design with 16 treatments and 10 replications. The treatments were Three isolates of Pseudomonad fluorescens indigenous (Par1-Cv, Par4-$Rj_1$, Par2-$Jt_1$) and 3 isolates of AM Fungi ($Gl_1BuA_4$, $Gl_2BuA_6$, and $Gl_1KeP_3$). The biocontrol agents were applied as single agents and combination two of them. This study demonstrated that the combined application of biocontrol organisms *Pseudomonas fluorescens* and AM Fungi can provide an effective control option for banana growers dealing with Fusarium wilt where the combination of Par1-Cv + $Gl_1BuA_4$ isolates are the most effective to control Fusarium wilt followed by the combination of Par1-Cv + $Gl_2BuA_6$ and Par2-$Jt_1$ + $Gl_1KeP_3$ isolates, reduced Fusarium wilt incidence by 87.4 and 75.0%, respectively.

**P03.023 The potential of endophytic fungus *Colletotrichum* sp. CKL005 from *Cinnamomum kanehirai* on controlling anthracnose of *Brassica rapa***

*S.Y. Wu[1], W.C. Chung[2] and W.H Chung[1]*
*[1]Department of Plant Pathology, National Chung Hsing University, No. 250, Kuo Kuang Rd., Taichung, 402 Taiwan, China; [2]Taiwan Seed Improvement and Propagation Station, No.46, Xingzhong St., Xinshe Dist., Taichung, 426, Taiwan, China*
*Email: wenchung@nchu.edu.tw*

An endophytic fungus, *Colletotrichum* sp. CKL005 isolate, from *Cinnamomum kanehirai* could inhibit growth of several phytopathogens, including *Alternaria brassicicola, Botrytis cinerea, C. gloeosporioides, C. higginsianum*, *Fusarium oxysporum* f. sp. *lilii*, *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *tracheiphilum*, *Rhizoctornia solani* and *Phytophthora capsici*. Furthermore, the mycelial extract showed higher activity than culture filtrate on growth inhibition of phytopthogens. For examining the efficacy on controlling anthracnose of *Brassica rapa*, the mycelial mass was grinded and extracted by ethyl acetate. Results showed that the disease severity of anthracnose on *B. rapa* could be reduced by 41.7 or 33.4% after the application of 10 or 100 mg/L respectively of mycelial extract. On the contrary, spraying of 10 or 100 mg/L before *C. higginsianum* PA01 inoculation also decreased the disease severity by 27.8% as compared with no treatment. For the identification of the efficacious compounds, the mycelial extract was analyzed by HPLC and GC-MS. Results indicated that the mycelial extract of CKL005 contained [included] β-carotene, glycerol 1-palmitate, prednisolone acetate, ergosterol, gammabufotalin, hydrocortilsoreacetate, digitoxin and gibberellic acid. In addition, the CKL005 is the volatile-producing fungus and can inhibit the mycelial growth of *C. higginsianum*. The GC-MS analysis indicated that several compounds might be associated with the growth inhibition to *C. higginsianum* PA01, including [2,5- Cyclo-hexadiene-1,4-dione,2,6-bis (1,1-dimethylethy)-], [2,6-Bis(1,1-dimethylethyl)-4-methyl-phenol(BHT)] and [(3H)-one, 3,6,7-thimethoxy-isobenzofuran-1]. Based on the molecular phylogenetic analysis, the *Colletotrichum* sp. CKL005 isolate might be a new species which has the potential to act as a bioagent on disease control.

**P03.024 Role of cell wall degrading enzymes and antimicrobial substances in biological control of plant pathogens of sorghum and chickpea**

*S. Gopalakrishnan, V. Srinivas, B. Prakash, A. Shravya, T. Naresh, G. Anusree, R. Vijayabharathi, R.K. Bhimineni and R. Sharma*
*International Crops Research Institute for the Semi-Arid*

*Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India*
*Email: s.gopalakrishnan@cgiar.org*

Biological control of plant pathogens occurs in several ways, the most common mechanisms being parasitism and predation, competition for nutrients (carbon, nitrogen, oxygen, iron and other nutrients) or space, production of antimicrobial substances and induced resistance. A total of eight (CAI-21, CAI-26, MMA-32, CAI-17, CAI-68, CAI-78, KAI-26 and KAI-27) and five (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) strains of *Streptomyces* were earlier reported by us as having potential for the biocontrol of charcoal rot of sorghum, caused by *Macrophomina phaseolina* (Tassi) Goid., and wilt of chickpea, caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC), respectively and plant growth promotion (PGP) of the plant. In the present investigation, all thirteen strains of *Streptomyces* were further evaluated for their production of cell wall degrading enzymes such as B-glucanase, chitinase, cellulase, lipase, protease, siderophore, hydrocyanic acid and indole acetic acid. Further, all the strains were evaluated for their production of secondary metabolite(s) and volatiles. This study confirms that the selected *Streptomyces* strains have broad-spectrum biocontrol and PGP properties.

**P03.025 Biological control of stem blight disease in Asparagus**

*N. Khositchareonkul, A. Somrith, T. Passabuth and T. Tassakorn*
*Plant Protection Research and Development Office, Department of Agriculture, Chatuchak, Bangkok, 10900, Thailand*
*Email: tadsakorn@yahoo.com*

Isolation and efficacy testing of *Bacillus subtilis* and *Trichoderma harzianum* in controlling the stem blight fungal pathogen *Phomopsis asparagi* in asparagus were performed. Ten out of thirty isolates of effective antagonistic bacteria that exhibited mycelia growth inhibition of *P. asparagi* (AS2, AS5, AS8, AS9, AS15, AS18, AS21, AS23 and AS24) on PDA agar were preliminary screened and observed. Spraying method was used to select four *Bacillus subtilis* (AS2, AS5, AS8 and AS9) under greenhouse condition and the results indicated that at 7 days post inoculation, the lesion sizes were reduced to 1.06, 1.58, 1.72 and 1.65 centimeter, respectively. Further study of a hundred of *Trichoderma harzianum* isolated from mushroom materials and mushroom composts in inhibiting the fungal mycelia growth revealed that eighteen species among them exhibiting 90 to 100 percent mycelia growth inhibition. Then, five of *Trichoderma harzianum*, TS15, TS29, TS31, TS33 and TS38 were further selected to test the efficacy controlling of stem blight disease under greenhouse condition by applying *Trichoderma harzianum* on the soil surface before inoculation. The results showed that at 10 days post inoculation, TS29 and TS31 had percent disease incidence at 10.07 and 15.72 percent respectively and revealed significant statistically difference with control treatment at 42.54 percent.

**P03.026 Production and biological evaluation of biopolymers from isolated *Rhodotorula glutinins* against *Botrytis* blight disease**

*W.M. Haggag*
*Department of Plant Pathology, National Research Centre, Egypt*
*Email: wafaa_haggag@yahoo.com*

Yeasts have been receiving great attention in science and industry for over one hundred years because they can produce many kinds of bioactive substances. In recent years, it has been found that *Rhodotorula glutinis* yeast have wide applications as biocontrol and other fields. One of the main characteristics shared among Rhodotorula strains is the ability to produce biopolymers as antifungal compounds active against fungi. *Rhodotorula glutinins* is isolated from garden soil and identified according to morphological and biochemical keys. It has the ability to produce biopolymers as an exopolysaccharide (EPS), siderophore, rhodotorulic acid carotenoids and glucane and showed inhibitory effect against *Botrytis cinerea* causing grey mould disease. In order to standardize the mass and metabolite production some cultural conditions like different incubation time in hours, pH, carbon sources and concentrations and nitrogen source were determined. During fermentation, growth, pH and exopolysaccharide, siderophore, rhodotorulic acid carotenoids and glucane production were monitored. Under artificial and natural condition, *Rhodotorula* formulation was effective in reducing *B. cinerea* disease in strawberry, grape and bean fruits. Pre-harvest treatment protected fruits from *Botrytis* post-harvest disease in comparing of fungicide. In addition, the obtained results showed that *Rhodotorula* treatment significantly increased the growth parameters as well as dry weights and yield. *Rhodotorula glutinis* have proved safe and non-toxic in experimental rat animal.

**P03.027 A high-throughput metabolomics approach for the study of the mycoparasitic interaction between *Stachybotrys elegans and Rhizoctonia solani***

*R. Chamoun, S. Jabaji and K.A. Aliferis*
*McGill University, Department of Plant Science, 21111 Lakeshore Rd., Sainte-Anne-de-Bellevue, QC H9X 3V9, Canada*
*Email: suha.jabaji@mcgill.ca*

The study presents the first proof-of-principle of metabolite profiles of the fungal mycoparasite *Stachybotrys*

*elegans* and the soilborne plant pathogen *Rhizoctonia solani* as the two fungi interact. Both fungi were grown on solid media for 72 hours alone or together in dual confrontation in order to study how this interaction changes their metabolomics profiles. Profiles of direct-infusion Orbitrap mass spectrometry (DIMS) were information rich with a total of 47 identified metabolites. The analysis discriminated between the metabolic profiles of interacting fungal partners from those detected in monocultures of *S. elegans* and *R. solani*. In confrontation, where *S. elegans* hyphae parasitized those of *R. solani*, secondary metabolites belonging to several pathways including, alkaloid biosysnthesis, selenocompound metabolism, fluorobenzoate degrada- tion (pathogen-derived) and furaneol biosysnthesis (myoparasite-derived) were highlighted in the interaction zone compared to monocultures of both fungi. The metabolite, 1-methylxanthine (an inducer of chitinases) known to be implicated in microbial metabolism in diverse environments was highlighted in *S. elegans* indicating higher metabolic activity during mycoparasitic interaction. On the other hand, *R. solani* bioactive secondary compounds, 6-pentyl-2-pyrone, melatonin and rhizoctonic acid were not detected during mycoparasitism and could be connected to the potential suppressive role of the mycoparasite. These results present a global view on the different metabolic pathways involved in mycoparasitic process and can be considered the basis for implementing an efficient biological control strategy against the plant pathogen *R. solani.*

**P03.028 Biological control strategies to reduce Fusarium head blight in Argentina**

*J.M. Palazzini[1], J. Köhl[2], P. Kastelein[2], L. Groenenboom-de Haas[2], A. Torres[1] and S. Chulze[1]*
*[1]National University of Río Cuarto, Rutas 8 Km 601 (5800) Rio Cuarto-Córdoba, Argentina; [2]WUR, Plant Research International, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*
*Email: jurgen.kohl@wur.nl*

*Fusarium* spp. cause Fusarium head blight (FHB) in small grain crops. The devastating disease reduces yield and grain quality and can produce mycotoxins such as deoxynivalenol (DON). Two biological control strategies were evaluated to control FHB under field conditions in Argentina. *Clonostachys rosea* strains 016 and 1457 were applied on wheat stubble after harvest to reduce survival of pathogen inoculum. Bacterial formulations (*Bacillus subtilis* RC218 and *Brevibacillus* sp. RC263) were applied on wheat crops during the anthesis to prevent ear infection. *F. graminearum*, *F. avenaceum* and *F. verticillioides*, quantified by species-specific qPCR, occurred naturally in wheat stubble after harvest. Pathogen concentrations, especially of *F. graminearum*, decreased during the following 6 months. Applications of *C. rosea* further reduced pathogen colonization by up to 98.0%. However, effects were not consistent in the three trials carried out at two locations in two years. The application of the two bacterial formulations during anthesis on ears artificially inoculated with *F. graminearum* significantly reduced disease incidence and severity in two trails below 20% in comparison to control treatments (Fg alone, 22 and 35%, respectively). DON concentrations were >1000 µg/kg in grain harvested from control plots, but below the detection level after biocontrol treatments in both trials.

**P03.029 Leaf microbiota as affected by biological control agents**

*J. Sylla, B.W. Alsanius, E. Krüger, A. Reineke and W. Wohanka*
*Geisenheim University, Institute of Phytomedicine, Von-Lade-Str.1, 65366 Geisenheim, Germany*
*Email: Justine.Sylla@hs-gm.de*

There is an increasing demand for the use of biological control agents (BCAs) against *Botrytis cinerea* in strawberries. However, there is little knowledge of potential effects of foliar applications of BCAs on the indigenous microbiota in the phyllosphere of strawberries. Therefore, the aim of the present study was to investigate this particular issue in a field study performed at Geisenheim University. Strawberry plants were sprayed with three BCAs, namely *Bacillus amyloliquefaciens* FZB42, *Trichoderma harzianum* T22 and *Beauveria bassiana* ATCC 74040 to suppress *B. cinerea* infections. Fungal and bacterial communities of treated leaves were analyzed by means of plate counts and pyrosequencing and compared with those of untreated leave samples. According to plate counts the applied *Bacillus* and *Trichoderma* species survived in the strawberry phyllosphere and no significant effects on the phyllosphere microorganisms could be detected by this technique. However, pyrosequencing of ITS rRNA and 16S rRNA sequences revealed that the fungal composition changed at class level after introducing *Trichoderma harzianum* T22 to the phyllosphere, whereas the bacterial composition was neither affected by *T. harzianum* nor by *B. amyloliquefaciens* and *B. bassiana*. The results from the present study indicate at least temporary effects of *Trichoderma* applications on the indigenous leaf microbiota.

**P03.030 *Trichoderma gamsii* 6085: a new beneficial isolate for the control of FHB of wheat**

*S. Sarrocco and G. Vannacci*
*Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto, 80 - 56124 - Pisa, Italy*
*Email: sarrocco@agr.unipi.it*

Management of Fusarium Head Blight (FHB) through

the use of beneficial fungi represents an environmentally friendly disease control approach alternative to the classical defence strategies. FHB impairs grain yield and quality but the most serious consequence is the contamination of grain and cereal products with Fusarium mycotoxins such as deoxynivalenol (DON) and its acetyl-derivates. In the present work results concerning mechanisms involved in direct interaction between *Trichoderma gamsii* 6085 and *F. graminearum* and *F. culmorum* mycotoxigenic isolates are reported, coupled with promising evidences of the ability of this antagonist to control FHB under field conditions. *T. gamsii* 6085 was able to grow in presence of DON (50 ppm), without degrading or detoxifying the mycotoxins and showed mycoparasitic behaviour against *F. graminearum* and *F. culmorum* mycotoxigenic isolates, with the involvement of chitinase genes. The beneficial isolate showed a strong competitive ability for natural substrates against the two pathogens, whose DON production resulted significantly reduced. All these results made this *T. gamsii* isolate deserving to be tested under field conditions. During two following wheat cropping seasons (2010/2011 and 2011/2012) *T. gamsii* 6085 was used as inoculant of soil before sowing and of spikes at anthesis and reduced naturally occurring FHB in terms of disease severity and disease incidence. The antagonist was able to endophytically colonize spike and spikelet portions with a preference for the basal parts. A third field experiment is in progress (2012/2013) with different spike application procedures.

**P03.031 Beneficial effects of Trichoderma harzianum 6776 on tomato**

*S. Sarrocco[1], L. Moncini[2], G. Pachetti[2], A. Pascale[3] and G. Vannacci[1]*
*[1]Department of Agriculture, Food and Environment, University of Pisa, 56124 - Pisa, Italy; [2]Centro Ricerche Strumenti Biotecnici nel Settore Agricolo-forestale (CRISBA), c/o ISIS "Leopoldo II di Lorena" Cittadella dello Studente, 58100 – Grosseto, Ital; [3]Department of Agriculture, University of Naples, 80055 – Naples, Italy*
*Email: sarrocco@agr.unipi.it*

Several registered *Trichoderma*-based products are available on the market as biopesticides. In addition to biocontrol activity these fungi can stimulate plant growth, an interesting feature that can be commercially exploited to improve plants production. In the present work, the effects of *Trichoderma harzianum* 6776 on tomato are reported, in order to present a new potential beneficial isolate to be employed as active ingredients in new biofertilizers and/or biopesticides. The ability to significantly stimulate tomato plant development was confirmed by several experiments performed under greenhouse conditions according to the standard procedure for production of plantlets to be transplanted. Different tomato cultivars and tomato rootstocks were evaluated. *T. harzianum* 6776 was obtained by fermentation of organic matter derived from food industry and was added as fresh biomass to the peat based tomato growth substrate at the final concentration of 10%. The biostimulating activity resulted in higher stem height and diameter, and increased fresh and dry weight compared with controls on all the tested cultivars. To explain the biostimulating activity of our *Trichoderma*, improvement of nutrient uptake by the plant and secondary metabolites profile of our isolate are under evaluation. Preliminary biocontrol experiments showed that *T. harzianum* 6776 was able to reduce plant mortality due to *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Rhizoctonia solani* and gave promising results against *Fusarium oxysporum* f. sp. *lycopersici.*

**P03.032 Phenotypic characterization of *Trichoderma* isolates from different Chilean ecosystems**

*R.A. France, P. Millas, V. Cisternas and D. Grinbergs*
*Plant Protection, Instituto de Investigaciones Agropecuarias, Chillán, Chile; INIA Quilamapu, Av. Vicente Méndez 515, Chillán, Chile*
*Email: afrance@inia.cl*

*Trichoderma* is a well-known fungus and thoroughly studied due to its properties as biological control agent. On the other hand, Chile is a very long country with vastly differing ecosystems, allowing significant adaptations of the inhabitant species. Thus, the Centre for Biological Control of the Agricultural National Research Institute (INIA) of Chile has been collecting in the last years *Trichoderma* samples along the country, covering from 18 ° to 54 ° South parallels. The main objective of this research was to characterize phenotypicaly 70 isolates of *Trichoderma*, out of the 180 total collections present at INIA. The isolates were cultured on SNA and PDA media at 30, 35 and 40 ℃ and the growth rate was measured over time. Besides, the smell of the colony was characterized as well. Morphology was determined by presence, characteris- tics, and measures of conidiophores, phialides and conidia. These phenotypic traits allow to identify the presence of *T. asperellum* (33%), *T. harzianum* (27%), *T. longibrachiatum* (13%), *Trichoderma virens* (9%), *T. atroviride* (6%), *T. viridecens* (4%), *T. viride* (3%), *T. aureoviridae* (3%), *T. koniingii* (1%), and *T. hamatum* (1%), according with the systematic *Trichoderma* online (Samuels *et al.*, 2013). All species were distributed along the country except *T. aureoviride*, that was only found from parallel 18 to 20 ºSouth. This germplasm is currently used for biological evaluations against plant pathogens.

**P03.033 Purification and characterization of novel antifungal compound, pyrrolnitrin, from the *Burkholderoa pyrrocinia* strain CAB08106-4**

*K.S. Han[1], B.R. Kim[1], S.S. Hahm[1], C.K. Chung[2], Y.G.*

*Nam[1] B.S. Yun[3] and J.E. Choi[4]*
*[1]Chungcheongnam-do Agricultural Research and Extension Services, Yesan 340-861, Republic of Korea; [2]Production Department, Hankook Samgong Co. Ltd., Suwon 443-803, Republic of Korea; [3]College of Environmental & Bioresource Science, Chonbuk National University, Iksan 570-752, Republic of Korea; [4]College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305-764, Republic of Korea*
*Email: hks081@korea.kr*

We were prepared organic solvent fraction for the identification of the antifungal substance producing *Burkholderoa pyrrocinia* strain CAB08106-4 against white rot disease of garlic by adding ethyl acetate and chloroform to the culture medium. With silica gel column chromatography in chloroform layers showing the antimicrobial activity, the antimicrobial activity was detected in chloroform-methanol fraction. This antifungal substance was found to be aromatic compound composed of six proton and ten carbon by $^{1}H$ and $^{13}C$ NMR spectrum. From the $^{1}H$-$^{1}H$ COSY spectrum, HMBC spectrum and HMQC spectrum, this aromatic compound consists of a 1, 2, 3-trisubsti- tuted benzene and pyrrole. As a result of a database and literature search on the basis of results, the compound was found to be in good agreement with pyrrolnitrin which separates as a substance antimicrobial activity from a microorganism of the genus *Burkholderia*, have been reported. In NMR spectroscopy, pyrrolnitrin confirmed the 265 molecular weight, the molecular formula $C_{10}H_6Cl_2N_2O_2$. Among the different culture media and incubation times, the best condition of pyrrolnitrin production was confirmed when cultured for 48 hours in medium potato sucrose broth. These results were carried out for the purpose of manufacturing a high quality biopesticide formulation process by utilizing *B. pyrrocinia* strain CAB08106-4.

**P03.035 Identification of phospho-rhizobacterium from the cassava rhizospere in Thailand**

*J. Nikaji[1], M. Sompong[1], T. Phiwthong[1], C.seangchan[1], K. Vechklang[2], S. Prathuangwong[3], D. Athinuwat[4] and N. Buensanteai[1]*
*[1]School of Crop Production Technology, Institute of Agriculture Technology, Suranaree University of Technology, NakhonRatchasima, 30000 Thailand; [2]Department Applied Biology, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, NakhonRatchasima, 30000 Thailand; [3]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900 Thailand; [4]Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, Pathumthani, 12121 Thailand*
*Email: Saranghayo_jn@hotmail.com*

A phospho-rhizobaterium was screened and isolated from cassava field in northeast of Thailand. The samples were identified as *Bacillus subtilis* based on morphology, physiology, biochemical and genetic characterization. The strain was gram positive, aerobic, oxidase positive, rod shape, endospore forming bacteria and produced extracellular protein as phytase that play the mineral phosphate solubilization role. *Bacillus subtilis* strain CaSUT007 selected as representative strain, its growth was optimal at pH 5.5 and temperature at 55°C. The sequencing analysis of 16S rDNA was closely related to the *Bacillus* species. The identification of CaSUT007 that produced phytase was also determined by PCR analysis of *phyC* gene and phytase activity. The results confirmed that CaSUT007 is a phospho-rhizobacterium, which carried *phyC* gene encoding phytase enzyme.

**P03.036 Identification and bioassay of disease suppressive soils in Western Australia**

*S. Miyan, D. Huberli, M. Connor and W. MacLeod*
*Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth, WA, 6151, Australia*
*Email: daniel.huberli@agric.wa.gov.au*

Previous surveys of cereal root disease incidence and severity in Western Australia conducted in the early 1980's and 2006-2008 showed that there has not been a reduction in the incidence or severity of root diseases during the past 25 years. This study was carried out to identify sites which are suppressive for one or more wheat root diseases. Cereal roots were collected from this region and assessed for *Rhizoctonia solanai* (AG8), *Fusarium pseudograminearum* (fusarium crown rot), *Gaumanomyces graminis* var. *tritici* (take-all) and *Pratylenchus neglectus* (root lesion nematodes; RLN) at anthesis in 2010, 2011 and 2012. We identified 16 paddocks for *Rhizoctonia*, nine for take-all, 22 for crown rot and six for RLN as potentially suppressive in these years. Soil from each potentially suppressive site was amended with different rates of carbohydrate and inoculated with the pathogen being bio assayed (*Rhizoctonia* or take-all) along with a positive control from Esperance, WA and placed in a growth cabinet for two weeks at 10°C with a 12 h light/dark regime. Pots were sown with wheat seeds and harvested after four weeks of growth and roots were assessed for disease incidence and severity. Only the positive control was recorded to be highly suppressive with disease incidence being significantly reduced when 0.5 g of carbohydrate was added in 2010 soils. An additional two paddocks were identified as highly suppressive in the 2012 bioassay. No sites have been identified through bioassays as being suppressive for take-all in 2012.

**P03.037 Traceability and persistence of the postharvest biological control agent *Pantoea agglomerans* CPA-2 applied on apples**

*L. Soto-Muñoz, N. Teixidó, J. Usall, I. Viñas, N. Lamarca*

*and R. Torres*
*IRTA. Postharvest XaRTA, Av. Rovira Roure, 191. 25198 Lleida, Spain*
*Email: neus.teixido@irta.cat*

*Pantoea agglomerans* CPA-2 is an effective biocontrol agent for postharvest diseases of citrus and pome fruit. However, for its implementation as a commercial strategy it is necessary comply with regulatory requirements. For that, the development of an effective monitoring system to detect and quantify the agent in the environment is needed. The main objective of this work was to evaluate the traceability and quantification of a formulated product based on *P. agglomerans* CPA-2, applied on apple under semi-commercial conditions. Traceability of CPA-2 was evaluated sampling surfaces that had contact with CPA-2 and confirmed for conventional PCR using strain-specific molecular markers. The population dynamics on apple surfaces of strain CPA-2 in semi-commercial trials was determined using both, the classical plating technique and quantitative PCR (qPCR). For qPCR development, two sets of primer pairs for use in qPCR and two TaqMan MGB probes were designed from one differential nucleotide sequence of CPA-2. The principal results showed that environmental distribution and persistence of *P. agglomerans* CPA-2 has a low impact since it did not grow and disperse in the environment. Maximum persistence of CPA-2 was three days in the plastic boxes stored at 0 ℃. Concerning population dynamics levels of CPA-2, significant differences were observed between population obtained by qPCR and dilution plating. These differences may indicate the presence of non-degraded DNA from nonviable cells. In conclusion, qPCR represents a novel potential tool to quicky and specifically monitor strain CPA-2 on apple surface in large-scale experiments just after its application.

**P03.038 Hypovirulent isolates of *Rhizoctonia* spp. from orchid mycorrhizae and their potential for control *Rhizoctonia* damping-off in Chinese mustard**

*J.H. Jiang, S.L. Tam and L.C. Chen*
*Department of Plant Pathology, National Chung Hsing University, 250 Kuokuang Road, Taichung, Taiwan, China*
*Email:lcchen@dragon.nchu.edu.tw*

Damping-off of vegetable seedlings caused by *Rhizoctonia solani* will be seriously occurred in plug plants, if the surviving inoculum can get into contact with the emerging plants. Using hypovirulent *Rhizoctonia* isolates to protect host against the damping-off disease is a healthy and eco-friendly strategy. It has been reported that some *Rhizoctonia* spp. form mycorrhizae with orchids and promote plant growth and development. Orchid mycorrhizal fungi were isolated according to existence of fungal pelotons within cortical cells of the root or rhizome in wild orchids. 3 multinucleate *Rhizoctonia* (MNR) isolates (AG 6 GV), 8 binucleate *Rhizoctonia* (BNR) isolates (AG-A, AB-B (O), AG-G, AG-P, AG-R) and 2 BNR isolates (anamorphic *Tulasnella* spp.) were further selected by analysis of anastomosis groups (AGs) and ITS sequences. The results of virulence determination showed that 12 isolates did not cause the death of seedlings with disease severity index ranging from 0.9-1.6 on radish and 0.2-1.4 on cucumber, except for one AG-R isolate produced moderate disease symptoms similar to those caused by *R. solani* AG 4 which caused collapsed hypocotyl with wilted leaves. These 12 isolates hereafter are referred to as hypovirulent BNR and MNR. After inoculating hypovirulent *Rhizoctonia* isolates against soil-challenge inoculation with *R. solani* AG 4, there was a significant reduction in damping-off of seedlings in Chinese mustard compared with those that were challenge inoculated alone. The most effective plant protection occurred with two HBNR isolates from the terrestrial orchid *Cheirostylis hungyehensis* (isolate Cno10-3) and *Calanthe sylvatica* (isolate CalS1-2). This two isolates were AG-P that had lowest disease severity (6.9%-16.7%) and highest plant protection (91.1% -100.0%) in Chinese mustard grown in soil. The present study makes a contribution to orchid mycorrhizal relationships about virulence determination and plant protection of multi- and binucleate *Rhizoctonia* from orchids on other plants based on AGs, and demonstrated that AG-P isolates were effective in controlling damping-off of seedlings in Chinese mustard.

**P03.039 Quorum sensing affects *Pseudomonas* sp. DF41 biocontrol through the regulator RfiA**

*M. Nandi[1], P.C. Loewen[1], W.G. Dilantha Fernando[2] and T.R. de Kievit[1]*
*[1]Department of Microbiology University of Manitoba, Winnipeg, MB R3T 2N2. Canada; [2]Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada*
*Email: Dilantha.fernando@ad.umanitoba.ca*

*Pseudomonas* species DF41 is a biocontrol agent that is able to protect canola from the devastating effects of stem rot caused by the fungal pathogen *Sclerotinia sclerotiorum*. We have discovered that a lipopeptide (LP) called sclerosin is essential for *Pseudomonas* sp. DF41 fungal inhibition both *in vitro* and *in planta*. Production of sclerosin is under control of a complex regulatory cascade that includes a cell-to-cell communication system known as quorum sensing (QS). Quorum sensing enables bacteria to control gene expression in a population density-dependent fashion through production of self-generated signalling molecules. The DF41 QS locus is comprised of two genes, *pdfR* and *pdfI*, encoding a transcriptional activator and an autoinducer synthase, respectively. A third downstream gene, called *rfiA*, is co-transcribed with *pdfI*. In related pseudomonads, RfiA is an activator of an efflux pump and in DF41 genes

encoding an efflux pump lie downstream of *rfiA*. Characterization of a QS-deficient strain no longer synthesizing autoinducer molecules revealed no change in antifungal activity, sclerosin production or protease activity. Conversely an *rfiA* mutant showed a lack of sclerosin production and not surprisingly, was devoid of antifungal activity. Since RfiA is a putative activator of an efflux pump, we hypothesize that this pump is involved in sclerosin export. Interesting, the *rfiA* mutant produced increased levels of alginate and more robust biofilms on an abiotic surface, compared to the DF41 wild type. Collectively, our results indicate that in DF41, QS control of antifungal activity is mediated indirectly through RfiA.

**P03.040 The role of PtrA in *Pseudomonas chlororaphis* strain PA23 biocontrol**

*N. Klaponski[1], K. Duke[1], W.G. Dilantha Fernando[2] and T.R. de Kievit[1]*
*[1]Department of Microbiology University of Manitoba, Winnipeg, MB R3T 2N2; [2]Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2*
*Email: Dilantha.fernando@ad.umanitoba.ca*

*Pseudomonas chlororaphis* strain PA23 inhibits several root pathogens in both the greenhouse and field. We've discovered that a LysR-type transcriptional regulator called PtrA (Pseudomonas transcriptional regulator A) is essential for *Sclerotinia sclerotiorum* antifungal activity. *P. chlororaphis* PA23 produces the antibiotics phenazine 1-carboxylic acid, 2-hydroxyphenazine and pyrrolnitrin, and several additional products that contribute to biocontrol, all of which are markedly reduced in a *ptrA* mutant. In greenhouse studies with *S. sclerotiorum*-challenged canola, the incidence of stem rot and leaf infection was significantly increased in plants inoculated with the *ptrA*-mutant compared to PA23. Thus this LysR-type transcriptional regulator plays an important role in the ability of PA23 to protect canola from the pathogenic effects of *S. sclerotiorum*. Most LTTRs regulate genes that are upstream of and divergently transcribed from the LTTR locus. A short chain dehydrogenase (*scd*) gene lies immediately upstream of *ptrA* in the opposite orientation. We are currently investigating whether *scd* is under PtrA control. In our efforts to understand how *ptrA* itself is regulated, the activity of a *ptrA-lacZ* fusion was measured in a *gacS*$^-$ background. We discovered that expression was markedly reduced compared to the PA23 wild type. Moreover, addition of *gacS* in trans restored the *ptrA* mutant phenotype to that of the wild type; thus this transcriptional activator is tightly linked to the Gac regulatory system. Collectively these findings indicate that PtrA is an essential regulator of PA23 biocontrol and is connected to other regulators involved in fungal antagonism.

**P03.041 Management *Rigidoporus lignosus* on *Hevea brasiliensis* with *Trichoderma* spp. in Indonesia**

*Mulawarman*
*Plant Pest and Diseases Department, Agriculture Faculty, University of Sriwijaya, Jl. Raya Palembang- unsri KM 32 Inderalaya, Ogan Ilir, Sumatera Selatan, Indonesia*
*Email: Mulaunsri@gmail.com*

Management white root fungus diseases (*Rigidoporus lignosus*) on rubber tree (*Hevea brasiliensis* Muell Arg) with local bio control agents (*Trichoderma* spp.) in South Sumatra Indonesia. Diseases loss due to the fungus diseases caused root and stem decay and death of rubber trees. The dry rubber production decreased an average of 2.7kg/tree or 54 kg/tree/20 years and the fungus has infested on rubber area around 80,000 ha and financial fund losses due to plant mortality estimated to US$ 200 million per year with disease severity over 5%. Disease control measures taken by using fungicide especially active ingredient Triadimefon reported cause diseases resistance, pollutes to environment and expensive to farmer. The result showed that the *Trichoderma* spp. could reduce diseases around 24.4% to 33% on the green house and on the field of rubber plantation, they could cure the infected tree from diseases and to stimulate the young leaf on stem. The *Trichoderma* spp. inhibited the hypa of fungal colonization on rubber stem and in the root rezhosphere.

**P03.042 Control of sheath blight caused by *Rhizoctonia solani* on rice leaf by volatile substances from *Streptomyces philanthi* RM-1-138**

*S. Boukaew and P. Prasertsan*
*Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Songkhla 90112, Thailand*
*Email: Topic_47@hotmail.com*

*Streptomyces philanthi* RM-1-138 grown on autoclaved wheat seeds for 7 or 14 days could generate volatile compounds to suppress the growth of four plant pathogenic fungi, (*Rhizoctonia solani* PTRRC-9, *Pyricularia grisea* PTRRC-18, *Bipolaris oryzae* PTRRC-36 and *Fusarium fujikuroi* PTRRC-16) using antifungal assay technique. *R. solani* PTRRC-9 was the most affected strain. The 14 day old culture possessed stronger inhibitory effect on the four fungal strains than the 7 day old culture. Inoculum size and spore concentration of *S. philanthi* RM-1-138 wheat seed inoculum were found to have influence on the suppression of *R. solani* PTRRC-9. Total growth inhibition (100%) was achieved using an inoculum size of $\geq$ 15 g $l^{-1}$ and a spore concentration of $1\times10^7$ spore $ml^{-1}$. The incidence and/or the severity of sheath blight on rice leaf (caused by *R. solani* PTRRC-9) could be reduced by fumigation of volatile substances from *S. philanthi* RM-1-138. Ultrastructure of *R. solani* PTRRC-9 illustrated by SEM and TEM indicated that

the cell-wall structure of *R. solani* PTRRC-9 was damaged and caused the loss of cytoplasm material. This confirmed the effectiveness of the volatile compounds from *S. philanthi* RM-1-138 could effectively control the rice sheath blight disease caused by *R. solani* PTRRC-9.

**P03.043 Elicitor epl1 secreted by several *Trichoderma* species inducing plant defense response to phytopathogen**

*C.T. Lo[1], J.B. Chou[1,2], R.C. Chang[1] and K.C. Peng[2]*
*[1]Department of Biotechnology, National Formosa University, 64 Wenhua Rd., Huwei, Yunlin, 63208, China; [2]Institute of Biotechnology, National Dong Hwa University, Hualian, 97401, Taiwan, China*
*Email: Taiwan. ctlo@nfu.edu.tw*

*Trichoderma* spp. have attracted attention for the biocontrol agents. The accepted biocontrol mechanisms may share in various *Trichoderma* spp. but newly reported host plant-acquired-resistant elicitors were rarely investigated. The ability of *Trichoderma* spp. to induce local and systemic acquired resistance in agricultural crops could be very important character for plant disease management and for understanding the role of *Trichoderma* in natural ecosystems. Small proteins such as Sm1 andEpl1 of *T. virens, and T. atroviride* have been reported to produce elicitors to assist plant acquired resistance against phytopathogens. However, it is still unknown whether Sm 1 and Epl1 commonly present in *Trichoderma* isolates and in acquired resistance of *Trichoderma* treated host plant. In this study, The RAPD technology was used to detect the collective *Trichoderma* strains. The primers were designed by following Seidl, *et al*. for epl1 and by following Djonovic *et al*. for sm1 and then were separately used to detect 30 isolates in 9 species of *Trichoderma*. The results showed that gene of epl1 could be detected and be presented in *T. konigii*, and *T. atroviride* when the epl1 primer was used, however, the gene of sm1was only of *T. virens* as the sm1 primer was used to detect. The small protein of Epl1/Sm1 was also found only in 10 stains of 6 Trichoderma species. The species included *T. virens, T. atroviride, T. harzianum, T. konigii, T. koningiopsis, and T. erinace.* Pretreatment of chinese cabbage with purify Epl1 showed high level of protection to the anthracnose disease caused by *Colletotrichum higginsianum.*

**P03.044 Isolation of Flavobacterium from rhizospheres of resistant or susceptible plants against bacterial wilt**

*E.J. Jung[1], H.J. Lee[2], H.J. Joo[2] and S.W. Lee [1,2]*
*[1]Department of Medical Bioscience, Dong-A University, Busan 604-714, Republic of Korea; [2]Department of Applied Biology, Dong-A University, Busan 604-714, Republic of Korea*
*Email: ampangjoo@naver.com*

Resistant mechanisms of tomato plants against bacterial wilt caused by *Ralstonia solanacearum* have been uncovered, although several resistant tomato lines have been reported. Microbial communities of plants may contribute to specific function of plants such as disease resistance. We investigated the bacterial communities of different rhizospheres soil from crops, such as resistant tomato cultivar Hawaii 7996, susceptible tomato cultivar Moneymaker and a non-host Korean cabbage. Microbial communities were analyzed using RDP Pyrosequencing Pipeline and MOTHUR. The result revealed that phylum *Bacteroidetes* increased in rhizospheres compared to bulk soil using pyrosequencing approach of 16S rRNA gene. Therefore, we conducted bacterial isolation from plant rhizosphere to obtain isolates from phylum *Bacteroidetes.* We randomly selected colonies in the R2A or TSA medium spreaded with suspension of the rhizospheres soil. Large number of bacteria isolates of *Flavobacterium* belonging to the phylum *Bacteroidetes* were identified using the 16S rRNA gene analysis. A total of 28 isolates of *Flavobacterium* was finally selected and further characterized. Some of them were tolerant to kanamycin and formed biofilm on culture plate by producing exopolysaccharides. Several *Flavobacterium* isolates inhibited mycelial growth by colonizing hyphae of *Rhizoctonia solani* and *Phytophthora infestans.* Interaction of the isolates with tomato and arabidopsis plants is being investigated.

**P03.045 Effect of *Chlorella fusca* on the improving of the yield of soybean sprouts**

*M.J. Kim, C.K. Shim, Y.K. Kim, H.J. Jee, J.H. Park, S.J. Hong and E.J. Han*
*Organic agriculture Division, National Academic of Agricultural Science, Rural Development of Administration, Republic of Korea*
*Email: kjs0308@korea.kr*

Fresh water algae, Chlorella species was known as a functional nutrient food for human rather than a biofertilizer for agriculture. This study was carried out to estimate the effect of *Chlorella fusca* as a biofertilizer on the growth of soybean sprout. Five cultivars of domestic breed soybean sprout, Pungsan, Shinhwa, Pungwon, Nokchae, and Wonheuk are treated with two concentrations of *C. fusca* solution, 0.1% and 0.2% for seven days. In the two treatments, the yield of soybean sprout treated with 0.1% of *C. fusca* was higher than the yield of soybean sprout treated with 0.2% of *C. fusca.* Two of five soybeans sprout cultivars, Pungwon and Wonheuk soaking with 0.1% *C. fusca* significantly increased the yield of soybean sprout by 26.2% and 28.2% compared to control treatment, respectively.

**P03.046 The efficacy of biocontrol against *Botrytis cinerea* on tomato depends on the strain of the pathogen**

*M. Bardin, C. Troulet and P.C. Nicot*
*INRA, UR407 Pathologie végétale, Domaine Saint Maurice, BP94, F-84143 Montfavet Cedex, France*
*Email: marc.bardin@avignon.inra.fr*

The objective of the study was to estimate the risk of loss of biocontrol efficacy towards plant pathogens. To this end, the protective efficacy of both biocontrol agents *Microdochium dimerum* and *Bacillus subtilis* (Serenade Max®) was evaluated on tomato against 41 strains of *Botrytis cinerea* differing in their geographic origin, host of isolation and level of aggressiveness. The protective efficacy of *M. dimerum* was evaluated on whole tomato plants after co-inoculation of pruning wounds with *B. cinerea* and *M. dimerum* at the desired dose. Lesion expansion was measured on the stem from the 3rd to the 7th day after inoculation. A very high level of efficacy against all strains was obtained when the biocontrol agent was used at the recommended dose. At a 10-fold reduced application dose, a wide range of sensitivity was observed and a significant correlation was observed between the level of aggressiveness of a strain to tomato and its sensitivity to the biocontrol agent. For *B. subtilis*, tomato leaves were first treated with the biocontrol agent at the desired dose, two days before inoculation with a mycelial agar plug of *B. cinerea*. The resulting lesions were assessed from the 2nd to the 4th day after inoculation. The efficiency of this product was also significantly influenced by the strain of *B. cinerea* but no correlation was observed between the aggressiveness of *B. cinerea* and the protection provided by the biocontrol agent. This study reveals the importance of considering several strains of the pathogen when evaluating the efficacy of biocontrol agents, to obtain a good representation of the pathogen population.

**P03.047 Effect of Chlorella fusca on the enhancing of rot control of soybean sprouts**

*C.K. Shim, M.J. Kim, Y.K. Kim, H.J. Jee, J.H. Park, S.J. Hong and E.J. Han*
*Organic agriculture Division, National Academic of Agricultural Science, Rural Development of Administration, Republic of Korea*
*Email: ckshim@korea.kr*

Chlorella is a one cell type of freshwater green algae. The practicality of utilizing *Chlorella fusca* as a natural material to enhance growth and reduce soybean sprout rot was tested. Five soybean sprout seeds, Pungsan, Pungwon, Nokchae, Wonheuk and Shinhwa were soaked for 6 h in solutions containing two levels, 0.1% and 0.2% of *C. fusca* and cultivated at 20℃ for 7 days. Soaking soybean sprout seeds with two levels of *C. fusca* increased germination percentage and fresh weight of five domestic soybean sprout cultivars. Compared to control treatment, 0.1% of *C. fusca* significantly reduced sprout rot percentage to over 67.4% in five cultivars, and consequently enhanced the marketable sprout yield by over 4.6%. In conclusion, soaking soybean sprout seed with 0.1% *C. fusca* solution seems to be a practical method to enhance the efficiency of soybean sprout production.

**P03.048 Biological control of ginseng diseases using an antagonistic bacterium in Korea**

*H.J. Lee, S.S. Hong, S.H. Won, S.J. Kim, and H.D. Kim*
*Gyeonggi-do Agricultural Research and Extension Service, Hwa-Seong, 445-300, Republic of Korea*
*Email: fungus72@gg.go.kr*

To develop multifunctional microbial inoculant, microorganisms with antagonistic activity and biofertilizing activity were screened. Several antifungal bacteria were selected from cultivated soil in Korea. Among them *Bacillus* spp. YJH-051, *B. amiloliquefaciens* CC178, *Pseudomonas stutzeri* NIST-1 and *Bacillus* sp. GM112 were selected against Anthrocnose (*Colletotrichum* spp.), Gray mold (*Botrytis cinerea*), Sclerotinia rot (*Sclerotinia sclerotiorum*) and Alternaria leaf blight (*Alternaria panax*). Specially, *Bacillus* GM112 had a good antifungal activity against Alternaria leaf blight caused by *A. panax* and Gray mold caused *by B. cinerea* on ginseng. This was identified as *B. subtilis* by morphological, biochemical test of Vitek compact as well as sequence alignment of 16s rRNA. Greenhouse experiment was conducted in a planted ginseng to test effectiveness of *B. subtilis* GM112 to control ginseng fungal pathogens. Spraying application of *Bacillus* GM112 with $1x10^7$ cfu/ml density 4times 7-10 day interval on ginseng, *Bacillus* GM112 suppressed 76.4% disease incidences of Alternaria leaf blight and it showed 60.9% control effect against Gray mold on ginseng. And also, *Bacillus* GM112 showed a good plant growth promoting results in a several vegetable seedling test. So, *B. subtillis* GM112 would be a promising biocontrol agent for the control of several plant fungal pathogens.

**P03.049 Use of *Trichoderma* in the biological control of wood decay fungi in tree wound exposed by pruning**

*J.Y. Wong[1], D.C. Burcham[2], M.I. Mohd Ali[1], M.L. Yap[1], N.V. Abarrientos[2], Y.K. Fong[b], F.W.M.R. Schwarze[3] and F.Y. Tham[4]*
*[1]Animal & Plant Health Centre, Agri-Food and Veterinary Authority, 6 Perahu Road, Singapore 718827, Singapore; [2]Centre for Urban Greenery & Ecology, Singapore Botanic Gardens, House 1, 1 Cluny Road, Singapore 259569, Singapore; [3]Empa, Swiss Federal Laboratories for Materials Science and Technology, Wood Laboratory, Group of Wood Protection and Bio-*

*technology, Lerchenfeldstrasse 5, CH-9014 St. Gallen, Switzerland.; [4]Natural Sciences and Science Education, National Institute of Education, Nanyang Technological University, 1, Nanyang Walk, Singapore 637616, Singapore*
*Email: Wong_Jia_Yih@ava.gov.sg*

As a City in a Garden, tropical Singapore has numerous trees shading our parks, gardens and roads that require a regular maintenance regime. This includes pruning, which improves the canopy structure and the aesthetics of the trees. However, pruning of the various branches exposes the woody tissue to fungal infections, and the subsequent rot may weaken the trees enough for them to become a danger to both property and passers-bys. Our previous work in on the pruned branches of *Khaya senegalensis* and *Samanea saman*, two common roadside trees of Singapore, has revealed the potential of *Phellinus noxius* as an agent of such wood decay. We are thus using these two trees to help develop a *Trichoderma* sp. based sealant with the potential for the biological control of such wood decay fungi through the action of mycoparatism, antagonism and competition. *Trichoderma* isolates, previously recovered from local soils, tree branches and infected *Ganoderma* fruiting bodies, were selected on the basis its *in vitro* growth rate, conidial germination, chlamydospore formation, volatile organic compound production, and antagonism against wood decay fungi when grown on agar culture and on wood blocks. Conidia of the chosen *Trichoderma* isolates were used in the sealant on the freshly cut branches of field grown *Khaya senegalensis* and *Samanea saman* trees, and their persistence, and efficacy in preventing wood decay and improving wound occlusion, are currently being evaluated.

### P03.050 The effect of biochar on the barley pathogen *Rhynchosporium commune*

M.T. Borlinghaus[1,2], *O.G.G. Knox[1], J.M. Fountaine[1] and S.P. Sohi[2]*
*[1]Scotland's Rural College, Crop and Soil Systems, EH9 3JG, Edinburgh, Scotland; [2]University of Edinburgh, UK Biochar Research Center, EH9 3JN, Edinburgh, Scotland*
*Email: Maria.Borlinghaus@sruc.ac.uk*

Barley leaf blotch is caused by the hemi-biotrophic fungal pathogen *Rhynchosporium commune*. It can cause severe crop losses of up to 40% worldwide, especially in cold and wet regions despite the use of fungicides. Biochar is a solid, carbon-rich product obtained by pyrolysis of organic waste material that can sequester $CO_2$-C in soils and also act as a soil conditioner. Improved crop resistance to foliar and soil-borne fungal pathogens following biochar applications to soil or growth media have been previously demonstrated, including recent evidence that biochar can mediate systemic induced resistance. However, the knowledge of specific mechanisms underlying enhanced resistance remains limited, but it is likely to be the result of multiple, direct and indirect mechanisms influencing plant and/or pathogen signalling pathways. The work presented examines the effects of soft wood pellet and *Miscanthus* straw biochars produced under a range of different pyrolysis temperatures on spring barley and pathogen infection from *R. commune*. Comparative *in-vivo* and *in-vitro* studies showed significant reduction in leaf blotch symptoms following the addition of biochar to soil, and restricted mycelial growth of *R. commune* on defined media containing biochar was also observed. Our results suggest that reduced disease levels may be partly attributed to volatile organic compounds derived from individual biochars. Research is now underway to elucidate possible mechanisms of disease reduction by the direct effect of biochar specific volatiles and/or changes in plant defence genes.

### P03.051 Biological control against post-harvest diseases on potato tubers

N. Samils, *M. Karlsson and D. Funck Jensen*
*Department of Forest Mycology and Plant Pathology, Uppsala Biocenter, Swedish University of Agricultural Sciences SE-75007, Uppsala, Sweden*
*Email: nicklas.samils@slu.se*

Post-harvest disease on crops is a serious worldwide problem causing losses of up to one third of the harvested fruits, vegetables and tubers. Potato makes up the fourth biggest crop in the world and is commonly infected with potato dry rot fungi (*Fusarium solani*, *F. roseum* and *F. avenaceum*) during storage. We have developed a standardized infection system where *Fusarium* sp. is allowed to infect potato tubers under a controlled environmental regime. The infection system is developed to mimic the post-harvest environment that tubers undergo (*i.e.* physical damage during harvest, wound-healing, cold storage followed by room temperature). By using this infection system, we tested the critical wound-healing phase where tubers are most sensitive to *Fusarium sp.* infection. This early stage of wound healing is the phase where biological control would be applied and therefore we tested the efficacy of the potential fungal control agent *Clonostachys rosea* against *Fusarium* infections. Furthermore, we also employed another infection system for studies of pathogen: host-interactions in form of transcriptome responses during *Fusarium avenaceum* infections of potato tubers. The acquired results will provide important information on the basis of pathogen aggressiveness vs. host responses and give more insight into control of post-harvest disease on potato tubers. In order to further study pathogenicity factors in *F. solani* and *F. roseum*, we also used Illumina/Solexa technology to perform whole-genome sequencing.

**P03.052 Transcriptome analysis in the mycelium of *Colletotrichum acutatum* exposed to culture extract from a *Streptomyces* sp.**

Y. H. Yun[1], C. W. Choi[2], J. K. Hong[3], Y. S. Kim[4] and S. H. Kim[1]
[1]Dept. of Microbiology, Dankook University, Cheonan 330-714,Republic of Korea; [2]Dept. of Biology and Medicinal Science, Paichai University, Daejeon 302-735, Republic of Korea; [3]Dept. of Horticultural Sciences, Gyeongnam National University of Science and Technology, Gyeongnam 660-758, Republic of Korea; [4]Dept. of Plant Science and Technology, Sangmyung University, Cheonan 330-720, Republic of Korea
Email: qkqhdi0096@naver.com

Tomato (*Solanum lycopersicum*) is one of popular vegetable crops worldwide. Anthracnose fungi have been known to affect the quality and storage of fresh tomato fruits. In this study, we evaluated antifungal activity of the isolate *Streptomyces* sp. DUCC501 against *Colletotrichum spp*. On co-cultivation on PDA, the bacterium inhibited the mycelia growth of *C. acutatum*, *C. coccodes* and *C. gloeosporioides* at the level of 78.4%, 99% and 82.1%, respectively. While ethyl acetate extract from the culture broth of DUCC501 inhibited the mycelia growth of *C. acutatum*, *C. coccodes* and *C. gleosporioides* at the level of 61.5%, 62.5% and 60.4%, respectively. To understand the inhibition mechanism of fungal mycelia by the bacterium at molecular level, we analyzed transcriptomes using Illumina high-throughput sequencing from the mycelia of *C. acutatum* with and without exposure to the ethyl acetate extract. Since there is no information of full genome sequence in *C. acutatum*, we did de novo assembly using Trinity method of 27,249,312 sequence reads from two RNA-Seq libraries from the mycelia of *C. acutatum* with and without exposure to the ethyl acetate extract. About 5600 genes were obtained from both acetate extract treated and non-treated samples. 745 genes were up-regulated and 1368 genes were down-regulated by 2-fold in the acetate extract treated *C. acutatum* mycelium. Up-regulated genes included ABC transporters and genes involved in ion pump. Down-regulated genes involved in glycolysis, TCA cycle and ergosterol synthesis. These results suggest that the bacterial metabolite affects on the cell growth and cell wall synthesis of *C. acutatum*.

**P03.053 Crude extract in filtrate of *Bacillus amyloliquafaciens* KPS46 exhibites a strong biocontrol activity toward *Acidovorax avenae* subsp. *avenae* caused corn bacterial leaf streak**

H. Hirata[2], S. Tsuyumu, S. Prathuangwong[1] and S. Kasem [1]
[1]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand; [2] Plant Pathology Laboratory, Faculty of Agriculture, Shizuoka University, Shizuoka, Japan
Email: agrsupot@ku.ac.th

Previous report revealed that antagonistic *Bacillus amyloliquafaciens* KPS46 was highly effective to suppress bacterial leaf streak of sweet corn caused by *Acidovorax avenae* subsp. *avenae* (Aaa) via antimicrobial compounds in filtrate. The study was undertaken to evaluate the enhanced 2nd metabolites production by KPS46 in different media and conditions. YP-broth + 1% glucose cultured at 30 °C and /or 50-55 °C, and incubated for 72 h was the most effective for cell growth and 2nd metabolites secretion. Crude extract (CE) from cell-free filtrate was prepared using ammonium sulphate precipitation. CE harvested was more effective to increase control efficacy against Aaa than direct KPS46 cell contact. These CE as well as KPS46 cells could induce plant cell death; and HR symptom after cocultured with tobacco cell suspension shown by Evans blue assay; and infiltration onto leaves (*N. tabacum* cv. Bright Yellow), suggesting the possibly plant resistance induction occurred. Seed treatment (corn cv. Insee2) and foliar spray (at 7-day-old seedlings) with 4-CE concentrations (CE:d$H_2O$ from 1:1 to 1:4) and KPS46 cell suspension ($1x10^8$ cfu/ml) followed by a pathogenic Aaa inoculation (at 14-day-old seedlings) were conducted under greenhouse conditions. The results revealed that CE diluted at 1:2 was significantly effective on seed germination, enhanced plant growth, and reduced disease severity ($P \leq 0.05$). The CE at 1:2 was as high effective as 1:1 on disease reduction that correlated with increase of phenol activity level and PAL accumulation in seedlings. The results indicate that *B. amyloliquefaciens* KPS46 is the most effective biological control agent toward antimicrobial activity with also induced plant resistance. The certain biochemical compounds and useful function for biological control activity will be further characterized.

**P03.054 Bacterial mixture increased control efficacy against diseases and insect pests of kale**

A. Sulaiman, C. U-napamnuk, T. Pothiluk, M. Choorin, W. Chuaboon, S. Prathuangwong and L. Kladsuwan
Department of Plant Pathology, Kasetsart University, Bangkok, Thailand
Email: Kladsuwan191@hotmail.com

Recently, a collection of bacterial antagonists including *Bacillus amyloliquefaciens* KPS46, *Paenibacillus pabuli* SW01/4, and *Pseudomonas fluorescens* SP007s have shown individually to suppress diseases and insect pests of kale in that the strain SP007s always provides the best result. To improve their effectiveness, a possible mixture of the two and three strains in ratio of 1:1 formulated in kaolin-based product was tested against various kale pests. In field experiment under natural infection, nine treatments were applied as seed and foliar inoculation with these single and combined-strain products of 6-month-old storage. Two-successful pairs, KPS46+SW01/4 and KPS46+SP007s gave a better control of multiple diseases and insect pests, where three-

strain mixture (KPS46+SP007s+SW01/4) demonstrated less efficacy compared to each of single strain product. Two products, a mixture between 2 strains above exhibited the best protection against 4 diseases (damping off: caused by *Pythium aphanidermatum*, Alternaria leaf spot: *A. brassicicola*, black rot: *Xanthomonas campestris* pv. *campestris* and soft rot : *Erwinia carotovora* subsp. *carotovora*) with an average 74% of disease reduction (P≤0.05) and 3– insect pests (diamondback moth: *Plutella xylostella*, common cutworm: *Spodoptera mauritia*, leaf eating bettle: *Phyllotreta sinuate*) with 81% decrease in population (P≤0.05), resulting highest yield increased with 47% (P≤0.05). In the case of *P. fluorescens* SP007s that consistently showes the best result when applied alone but not for strain combination in this study, the possible action of enzymes or antibiotics involved will be discussed.

**P03.055 Loss of CPSase activity in *Pseudomonas fluorescens* SP007s contributes to control efficacy against soybean bacterial pustule disease**

*D. Athinuwat[3], J. Thowthampitak[2], S. Kasem[1], S. Prathuangwong[1,4] and M. Choorin[1,4]*
*[1] Dept. of Plant Pathology, Kasetsart University (KU); [2]Dept. of Entomology, KU; [3]Major of Organic Farming Management, Thammasat University; [4]Center for Advanced Studies for Agriculture and Food, 50 Ngamwongwan Rd, Bangkok, Thailand*
*Email: jeabmalida@yahoo.com*

*Xanthomonas axonopodis* pv. *glycines* (Xag) is the causal agent of soybean bacterial pustule, a common disease under warm and humid climates in Thailand and many other production areas worldwide. The disease can cause yield loss up to 40% in some susceptible cultivars. Biological control with update strategy focusing on benefit enzymes produced by a control agent is essential as an alternative practice and decreased pressure of producer and consumer. *Pseudomonas fluorescens* SP007s is a PGPR strain that exhibits different mechanisms on disease suppression. We demonstrated that carbamoylphosphate synthetase (CPSase) activity encoded by *carA* and *carB* (control expression by arginine and pyrimidines) of strain SP007s played a crucial role in the inhibition of Xag. Mutants of the *car* genes family resulting lost ability to degrade DSF (diffusible signal factor) produced by Xag and to reduce the disease severity, were observed. All *car*$^-$ mutants produced a lesser extent of EPS, flagelin, and biofilm formation suggesting *car* genes likely intercept other genes during transcription. In the greenhouse experiments found that all mutants including SP007s*carA*$^-$, SP007s*carB*$^-$, and SP007s*carAB*$^-$ coinoculated with pathogenic Xag reduced disease severity by 6, 22, and 25% respectively, where SP007sWT exhibited 31% compared to non-treated control. The *carB* function alone seemed to be sufficient for disease control where *carA* expressed lesser-remarkable change of all phenotype measurement. The results indicate the expression of *carB* potently diminished *carA*-synthesized arginine in SP007s that we also point out a target for fast plant defense response with delayed disease symptoms.

**P03.056 Biological analysis of *Pseudomonas fluorescens*Induced systemic resistance in para rubber seedling against climate change**

*D. Athinuwat[1], W. Chuaboon[2], N. Buensanteai[3], S. Prathuangwong[2] and A. Wongchindakhun[1]*
*[1]Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, 12121, Thailand; [2]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand; [3]School of Crop Production Technology, Institute of Agriculture Technology, Suranaree University of Technology, NakhonRatchasima, 30000, Thailand*
*Email: adusit@tu.ac.th; athinova6@hotmail.com*

The para rubber seed treated and foliar spray with the extrapolysaccharide (EPS) of *Pseudomonas fluorescens* triggered slightly increase expression of defense-related enzymes including peroxidase (POX) and superoxide dismutase (SOD) on 30-day-old under abiotic stress (temperature 50 C) condition. The activity of POX and SOD were increased immediately after para rubber foliar spray with the EPSreaching peak levels at 3 and 4 days at3.14and 16.72 $min^{-1}$ $mg^{-1}$ protein, respectively compared to control treatments (P=0.05). Induction of systemic resistance by EPS of *P. fluorescens* against abiotic stress of para rubber seedling through the biochemical changes brings about accumulation of defense-related enzymes that relates to biosynthesis of phytoalexins (POX) and antioxidance(SOD). These defensive compounds are playing a marked role in stress response and immunity of plants. The application of EPS of *P. fluorescens* as an elicitor against abiotic stress conditions will be compatible and alternative with its use for plant health.

**P03.057 Enhanced biocontrol efficacy by bacterial antagonist mixtures against bacterial soft disease and flea beetle of Chinese kale**

*D. Athinuwat[1], W. Chuaboon[2], N. Buensanteai[3], S. Prathuangwong[2] and J. Sottiwilaiphong[1]*
*[1]Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, 12121, Thailand; [2]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok,10900, Thailand; [3]School of Crop Production Technology, Institute of Agriculture Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand*
*Email: adusit@tu.ac.th; athinova6@hotmail.com*

The mixtures of 2-microbial antagonists including

*Pseudomonas fluorescens* and *Beauveria bassiana* utilization demonstrated in organic Chinese kale production under field conditions. The couple strains most significantly enhanced plant growth and reduced 74% and 65% the incidences of bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora* and flea beetle of Chinese kale, respectively (P=0.05). The community population of *P. fluorescens* in soil was also greatest compared to other treatments. Results suggested that the couple strains had the potential for alternative pests control to replaced synthesis pesticides in organic commercial field application.

**P03.058 Functional analysis of *yutI* gene in nitrogen fixing bacterium, *Bacillus subtilis* TU-Orga1**

*D. Athinuwat[1], W. Chuaboon[2] and N. Doungkaew[1]*
*[1]Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, 12121, Thailand; [2] Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand*
*Email: adusit@tu.ac.th; athinova6@hotmail.com*

A plant growth promoting rhizosphere, *Bacillus subtilis* TU-Orga1 had the potential for nitrogen fixation against *Xanthomonas oryzae* pv. *oryzicola* (Xoc), the causal agent of bacterial leaf streak of rice. A *yutI* targeted mutant was generated with an EZ::TN transposome system. Mutation in *yutI* resulted in lacked nitrogen fixation and antagonism phenotype. Greenhouse experiment using seed treatment ($10^6$ cfu/ml) 1 ml/ 1 kg seeds and foliar sprayed ($10^8$ cfu/ml) 3 ml/ 1 L of water were conducted to test the effectiveness of the *yutI* mutant for nitrogen fixation and protecting rice (cv. Kohdokmali105) against Xoc compared with the wildtype. The result showed the *yutI* mutant altered the efficiency of nitrogen fixation and bacterial leaf streak disease control under greenhouse conditions. Where TU-Orga1 showed the best decrease disease severity by 89.6 percent and related increased the total nitrogen fixation activity 37.1 percent (P=0.05). Results suggested that TU-Orga1 carry the *yutI* gene had the potential for nitrogen fixation against bacterial leaf streak and used as alternative control to replaced synthesis fertilizer and pesticides in organic commercial field application.

**P03.059 Characterization of *Bacillus subtilis* TU-Orga1 to control *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight of rice**

*D. Athinuwat[1], W. Chuaboon[2] and P. Sathitthampana [1]*
*[1]Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, 12121, Thailand; 2Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand*
*Email: adusit@tu.ac.th; athinova6@hotmail.com*

A benefit bacterium strain TU-Orga1 was isolated from rhizosphere soil of rice. Its potentials on suppressed *Xanthomonas oryzae* pv. *oryzae* (Xoo) caused bacterial leaf blight compared with bacteriocide, bacbicure (Canoron)were characterized. TU-Orga1 was significantly greater in Xoo with 11.3 mm inhibition diameter where bacbicureshowed Xoo suppression with 8.2 mm inhibition diameter. Greenhouse and field experiments using seed treatment ($10^6$cfu/ml) and foliar sprayed ($10^8$cfu/ml) were conducted to test the effectiveness of TU-Orga1 for protecting rice (cv. Suphanburi 1) against *X. oryzae* pv. *oryzae* compared with bacbicure. The result showed the best decrease in disease severity by 76.8 and 73.4 percent respectively that better than chemical bacteriocide (P=0.05). The identification of these bacteria using classical diagnosis and 16S rDNA was investigated. The result revealed that strain TU-Orga1 was equivalent to *Bacillus subtilis*. Results suggested that *B. subtilis* TU-Orga1 has the potential for use in organic commercial field application as alternative control or reduced pesticide use.

**P03.060 Impact of LED light on the microbial biogeography of greenhouse grown ornamentals**

*S. Gharaie[1], K.J. Bergstrand[1], S. Windstam[1,2], S. Khalil[1], C.O. Ottosen[3], W. Wohanka[4] and B.W. Alsanius[1]*
*[1]Swedish University of Agricultural Sciences, SLU, Dept of Biosystems and Technology, Microbial Horticulture Laboratory, PO Box 103, SE-23053 Alnarp, Sweden; [2]State University of New York, SUNY, Dept of Biological Sciences, 222A Snygg Hall, Oswego, NY 13126, USA; [3]Department of Food Science, Faculty of Science and Technology, Aarhus University, Kirstinebjergvej 10, DK-5792 Årslev, Denmark; [4]Geisenheim University, Dept of Phytomedicine, von- Lade Str. 1, DE-65322 Geisenheim, Germany*
*Email: Samareh.gharaie@slu.se*

To reduce the use of energy in greenhouse production, alternative methods for heating and artificial lightning have been suggested. In this context, light emitting diodes (LED) have been proposed as alternatives to high pressure sodium lamps which traditionally are used for assimilation lighting in greenhouse production. LED light differs to conventional lighting by high pressure sodium lamps with regard to spectral distribution, light distribution as well as heat emission. Due to the interrelationship between air temperature and humidity, also the water availability on the leaf surface is affected. The use of LED consequently causes changes in the microclimate within the greenhouse and around the crop with a decrease in both air and leaf temperature and larger fluctuations in relative humidity consequently affecting the microbial community structure on the crop and in the cropping system.The objective of the present study was to evaluate the utilization of organic nutrients in the presence of different LED sources (red: 664 nm;

blue/red: 454 nm / 664 nm, white: 444 nm; 556 nm). Bacteria were isolated on King Agar B and 0.1 TSA from the phyllosphere of greenhouse grown *Euphorbia pulcherima* and *Kalanchoë blossfeldiana* and transferred to cryo-culture. All isolates were screened with respect to enzyme activities (protease and chitinase) and production of 2, 4-diacetyl phloroglucinol (phl). Four isolates either overproducing phl or with high enzyme activities as well as the *Botrytis cinerea* and *Trichoderma* sp. were selected for further tests. These test included growth as well as the utilization of different precursor compounds and formation of phl or enzyme activities when exposed to different wavelengths. The results will be presented on a poster. The project was funded by the EU-Interreg project "GreenGrowing" and is facilitated within the framework of the postgraduate school, µHORT, funded by the Swedish research council Formas.

**P03.061 Potential new biopesticides against *Bremia lactucae***

*N. Van Hese[1], I. Van Bogaert[2], J. Deravel[3], F. Coutte[3], G.H. Tran[2], F. Krier[3], P. Jacques[3] and M. Höfte[1]*
*[1] Laboratory of Phytopathology, Departement of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Belgium; [2]Department of Biochemical and Microbial Technology, Faculty of Bioscience Engineering, Ghent University, Belgium; [3]Laboratory ProBioGEM, Polytech-Lille, University Lille 1, Sciences and Technologies, Avenue Paul Langevin, F-59 655 Villeneuve d'Ascq, France*
*Email: Nathalie.VanHese@Ugent.be*

Downy mildew in lettuce (*Lactuca sativa*), caused by the oomycete *Bremia lactucae*, results in high yield losses. The pathogen is usually controlled by growing resistant lettuce varieties combined with the application of fungicides. However, new races of *Bremia* emerge very rapidly, and the pathogen easily develops resistance to chemicals. The aim of this work is to obtain a more sustainable control strategy. Therefore, we study the potential use of biopesticides, such as cellobiose lipids and cyclic lipopeptides. Ustilagic acid is a cellobiose lipid, produced and secreted in relatively large amounts by the basidiomycetous fungus *Ustilago maydis*. This compound is used as biosurfactant, and is also known to have a broad antibacterial and antifungal spectrum. We tested the effect of ustilagic acid and derivatives as biopesticides against *B. lactucae*. Although the application of ustilagic acid seemed to increase the susceptibility for downy mildew, an enzymatic derived cellobiose lipid and a related glucolipid reduced the disease incidence significantly. *Bacillus* spp. is known to produce biosurfactant lipopeptides which are involved in biocontrol activitiy. Iturins have antifungal effects. Surfactins are able to elicit defense mechanisms in some plants, while fengycin is known for fungitoxicity. We tested the effect of mycosubtilin, belonging to the group of iturins, surfactin and fengycin against *B. lactucae*. All three components reduced the disease incidence significantly, depending on the moment of application. With microscopical studies, we try to understand the mode of action of these components.

**P03.062 The effects of three mitoviruses on the virulence of the phytopathogenic fungus *Sclerotinia sclerotiorum***

*M.E. Khalifa and M.N. Pearson*
*Microbiology Department, School of Biological Sciences, The University of Auckland, PO Box 92019, Auckland 1010, New Zealand*
*Email: mkha201@aucklanduni.ac.nz*

Three double-stranded RNAs (dsRNAs) of 2438 nts (A), 2588 nts (B), and 2744 nts (C), from a single isolate of *Sclerotinia sclerotiorum* were sequenced. All three sequences showed similarity to known mitoviruses, consisting of a single open reading frame (ORF) with the characteristic conserved motifs of RNA-dependent RNA polymerase (RdRp). Mitochondrial malformations and reduced virulence and growth were associated with the presence of the dsRNAs. The terminal sequences of the (+) strand of the three dsRNAs could be folded into stem-loop structures and the inverted terminal complimentary sequences of dsRNA-A potentially form a panhandle structure. Sequence A showed 91.6% aa similarity to the previously described *Sclerotinia sclerotiorum* mitovirus 2 and was tentatively assigned the acronym SsMV2/NZ1. Sequences B and C showed only 16.4% aa similarity to each other and 15-48% aa similarity to previously described mitoviruses. Consequently they appear to be new mitoviruses tentatively assigned the names *Sclerotinia sclerotiorum* mitovirus 3 (SsMV3/NZ1) and *Sclerotinia sclerotiorum* mitovirus 4 (SsMV4/NZ1), respectively. Absence of SsMV3/NZ1 in single-ascospore progeny of the parental isolate showed the wildtype phenotype of *S. sclerotiorum*. This finding suggests that SsMV3/NZ1 could be the hypovirulence determinant in *S. sclerotiorum*. However, it is not clear whether there is an interaction between the three mitoviruses to express the reduced virulence.

**P03.063 *Pseudomonas fluorescens* SP007s produces multiple antibiotics to control bacterial pustule disease of soybean**

*L. Kladsuwan[1], D. Athinuwat[3], S. Kasem[1], S. Tsuyumu[2], S. Marach[1] and S. Prathuangwong[1]*
*[1]Department of Plant Pathology, Kasetsart University, Bangkok, Thailand; [2]Faculty of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka, Japan; [3]Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, Bangkok, Thailand*
*Email: agrsdp@ku.ac.th*

Bacterial pustule is a disease caused by *Xanthomonas axonopodis* pv. *glycines* (Xag) that commonly observed in greensoybean field grown in Thailand. Chemical application is not recommended at 15-20 days before harvest (65-day-old plant) due to concerns about exported produces, where plant at a young-pod forming is the susceptible growth stage for infection by the pathogen indicating an alternative control is required. *Pseudomonas fluorescens* SP007s is a biocontrol agent of different pathogens (bacteria and fungi) on various crops of several diseases with exhibiting a strong antimicrobial activity in successful biological control. This was shown by application of SP007s filtrates inhibited disease on soybean plants that the suppression was due to antimicrobial compounds in filtrate. We partially purified antibiotic synthesis using ammonium sulfate precipitation, TLC, and HPLC analyses; and also identified some genes involved. The 5 inhibitory-selected fractions revealed they were 2,4- diacetylphloroglucinol (DAPG), pyoluteorin, phenazine, pyluteorin, and agrocin434. We explored the role of three genes: *Phl*, *Plt*, and *Phz* encoded the 2,4-diacetylphloroglucinol (DAPG), pyoluteorin, and phenazine respctively, that might be a major factor in the biological control of Xag. A site-directed mutation resulted in 5-mutant strains obtained from single-and multiple gene deletion. As expected, plate and greenhouse assays demonstrated that these 5-gene mutants including SP007s*Phz*$^-$, SP007s*Plt* $^-$, SP007s*Phl*$^-$, ΔSP007s*Phz*$^-$& *Plt* $^-$, ΔSP007s *Phz*$^-$ *&Plt* $^-$*&Phl* $^-$ estimably accounted for performance of a reduction to suppress disease by 20, 34, 40, 54, and 96% respectively. The production of antimicrobial compounds and disease suppression was however, correlated with antimicrobial activity against pathogenic Xag. It is interesting to note that among antibiotics produced by SP007s, DAPG plays a key role for disease suppression. We hypothesize that at least, these 3 genes regulate antibiotic biosynthesis in SP007s for essential biocontrol activity in this study.

**P03.064 Disease and insect pest management of kale with mixture of antagonistic microbes and plant extracts**

*V. Pitiyont[1], W. Chongrattanameeteekul[2], J. Thowthampitak[2], B. Pitiyont[3], S. U-raichen[4], D. Athinuwat[5], S. Kasem[6] and S. Prathuangwong[6]*
*[1]Central Laboratory Co., Ltd.; [2]Dept. of Entomology, Kasetsart University (KU); [3]Dept. of Environmental Science, KU; [4]Dept. of Entomology, KPS campus, KU; [5]Major of Organic Farming Management, Thammasat University; [6]Dept. of Plant Pathology, KU ,Thailand*
*Email: agrsdp@ku.ac.th*

Pest control agents, *Bacillus amyloliquefaciens* KPS46, *B. licheniformis* P38, *Paenibacillus pabuli* SW01/4, *Pseudomonas fluorescens* SP007s, *Trichoderma viride* TC42; and *B. thuringensis* KU52; and plant extracts from *Tacca chantrieri* (TE) and *Stemona tuberose* (SE) have been reported individually to control diseases and insect pests of kale under experimental conditions. However, such practices have not been routinely performed in commercial production. This study was then aimed to determine the potential integration of these agents for practical use. The total of 8 formulations including single control agent and combinations of agents were mixed with carriers and tested in this experiment. Seed treatment and foliar applications of these products under field experiment significantly decreased pest problems and increased vegetable yields as compared to those from plots applied with single agent, routine chemicals (fungicides and insecticides), and nontreated control ($P \leq 0.05$). Two- mixture products, (KPS46+ SW01/4+SE) and (KU52+ SP007s) were the most effective for decreasing disease incidence (damping off, *Alternaria* leaf spot, black rot and soft rot) at 37-80%. The landing rate of insect pests onto the treated plants (diamondback moth, common cutworm, and leaf eating beetle) was also reduced at 60-95%, while yield was increased at 20-35%. The population dynamics of natural enemies (lady beetle and assassin bugs) however, was not affected. Other effective products were also recorded. All agents work most effectively in a mixture that the level of bacteria declined from $1x10^{13}$ cfu/ml to $1x10^{9}$ cfu/ml after 12- month-storage at room temperature. This study demonstrates the possibility of utilizing compatible pest control agents for the management of several concurrent pests. The observed effectiveness may be due to the specific mechanisms and effects of these mixed agents against the pests.

**P03.065 Utilizing TU-bioformula to enhance plant growth and soil improvement in organic paddy field**

*D. Athinuwat[1], W. Chuaboon[2] and C. Boonnadakul[1]*
*[1]Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, 12121, Thailand; [2]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand*
*Email: adusit@tu.ac.th; athinova6@hotmail.com*

TU-bioformula evaluated for enhancing growth of rice cultivar Phitsanulok2 compared to bioextract applied by farmers under organic agriculture conditions. The investigation was carried out at Phayao province during January-May, 2012 using RCBD with 3 treatments (soil treated and 3-foliar sprays at 40, 60 and 90-day old plants with TU-bioformula, bioextract and non-treated treatments). The result revealed the treatment of TU-bioformula promoted seedling growth (14-day old) with increased root and shoot length and fresh weight by 8.7 and 5.2 cm and 28.3 g, respectively and increased percentages of rice yield 16.2%. Moreover, the soil property of paddy field as organic matter (3.2%), pH (5.8) and electric conductivity (2.4 µs/cm) tended to be higher than the control treatment. Especially, total

nitrogen and phosphorus in soil significantly increases to 33% and 65.3%, respectively after TU-bioformula was used. It would be the positive impacts of TU-bioformula in producing soil nutrients and organic matter from rice stubble for sustainable enhancing growth and yield of rice (P=0.05).

**P03.067 The activity of plant extracts and biocontrol agents against infection of groundnut by *Aspergillus flavus***

*A. Alamene and S. Rossall*
*Plant and Crop Sciences, School of Biosciences, Nottingham University, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, UK*
*Email: stxaa@exmail.nottingham.ac.uk*

*Aspergillus flavus* infection can result in contamination of groundnut seed with aflatoxin, which poses a potent threat to health of consumers. In preliminary experiments twelve plant oils were screened for inhibition of *A. flavus* on amended PDA plates. Only four extracts (clove, camphor, garlic, and galangal) showed high inhibitory effects *in vitro*. Clove oil and camphor oil also significantly suppressed infection of groundnuts by *A. flavus* in amended compost, when applied as seed treatments, and reduced infection when applied to pods post-harvest. *Trichoderma* spp., three isolates of *Bacillus amyloliquifaciens* and *Pseudomonas chlorophis* was also evaluated. *In vitro* assays on agar plates showed the *Trichoderma* spp. and *Bacillus* isolates 62P and 66P were the most active. These same organisms were also effective in improving emergence when applied as a seed amendment, using *Aspergillus*-inoculated compost. When applied as a preventative treatment to groundnut pods, *Trichoderma* spp., both singly and in combination with plant active oils, were also effective in suppressing infection following inoculation with *A. flavus.* A strategy may be therefore be possible which provides control of *A. flavus* on groundnut, using BCAs and plant extracts, applied alone or in combination. Future work will compare this activity with conventional fungicides. Efficacy as a post-harvest pod treatment will also be evaluated by ELISA determination of aflatoxin accumulation in seed and by quantification of *Aspergillus* infection using qPCR.

**P03.068 Chitosan induced resistance in sugarcane against *Fusarium moniliforme* the causal of sugarcane wilt**

*D.G. Patil[1], B.H. Pawar[2], D.B. Gawade[2], V.C. Vasekar[2] and S.G. Dalvi[3]*
*[1]Kolhapur Institute of Technology, Kolhapur, Maharashtra, India; [2]Plant Pathology Section, [3]Tissue Culture Section, Vasantdada Sugar Institute, Pune, Maharashtra, India*
*Email: bh.pawar@vsisugar.org.in*

World over, sugarcane is being exploited as renewable energy source. High cane, sugar and fiber yielding varieties of sugarcane are needed for meeting the ever increasing demand of sugar and related byproducts. However, such varieties are generally susceptible to many plant parasitic organisms. Many physical, chemical and biological means of diseases management are evolved or identified; however, these methods are sometimes either uneconomical or non-persistent. Therefore, developing the disease resistant varieties is the only way out. Again, sugarcane varietal improvement with reference to disease resistance through present screening methods is a tedious and time consuming process. And hence, inducing disease resistance in present commercial varieties with alternatives is a need of hour. Now days, Chitosan-oligosaccharide is upcoming as potential elicitor for induction of systemic acquired resistance to abiotic and biotic stresses in many crops. Present *in vitro* study was carried out for antagonistic effect of chitosan on the *Fusarium moniliforme and Trichoderma* isolates. Results revealed that chitosan @ 1.5 mg/ml of water showed complete inhibition of *Fusarium* mycelium, whereas, *Trichoderm*a spp. showed rapid colony growth with chitosan. The defense enzyme study with chitosan treated sugarcane leaf discs showed the enhanced level of enzymes like peroxidase and phenylalanine ammonia lyase. Thus, this study highlights intriguing insight that chitosan can be used along with *Trichoderma* as a promising bio-control agent and an elicitor to induce biotic and abiotic tolerance in sugarcane.

**P03.069 Biological control of Orobanche cumana in tobacco by *Fusarium* spp.**

*Y.H. Wu, B. Xia, F.H. Ning and J.C. Tang*
*Shenyang Agricultural University, Shenyang, 110866, P.R. China*
*Email:wuyh09@vip.sina.com; xiabo0522@163.com*

Tobacco in northwest Liaoning province was damaged severely by broomrape (*Orobanche cumana*) in recent years. *Fusarium* spp. was isolated from diseased broomrape and cultured in our lab, which can control *O. cumana* infection on tobacco. The suspension of *Fusarium* spp. were sprayed with five methods, (1) sprayed on soil hole during planting (treatment 1); (2) sprayed on soil surface during the first stage of *O. cumana* for three times every seven days (treatment 2); (3) combined the first and the second method (treatment 3); (4) mixed the soil with sulfur, and then treated as the third method (treatment 4); (5) the field infected by *O. cumana* without treatment as control (CK). The results showed that the treatment 4 was the best method for controlling broomrape. In field treated by method 4, emergence time of *O. cumana* was 3 days later than the control, and there were 31.20% of *O. cumana* infected successfully by *Fusarium* spp., and the death rate of *O. cumana* was 62.44%. The yield of flue-cured tobacco

leaf in treatment 4 was 4895 kg/hm$^2$, and the proportion of super and medium flue-cured tobacco leaf was 89.89%, which was 33.59% higher than the control.

**P03.070 The effects of *Orobanche cumana* infection on tobacco morphology and physiology**

*Y.H. Wu, B. Xia, X.H. Liu and J.C. Tang*
*Shenyang Agricultural University, Shenyang, 110866, P.R. China*
*Email:wuyh09@vip.sina.com; xiabo0522@163.com*

Broomrape (*Orobanche cumana*) is a holoparasitic phanerogam which causes severe damage to tobacco in Liaoning province. Broomrape germinates at the end of June, flowers and seeds in mid-August, and withers after mid-September. In our study, the effects of *O. cumana* infection on tobacco were evaluated by examining leaf and stem morphology, physiological and biochemical properties of tobacco. Broomrape infection had negative effects on the height, length and width of lumbar leaf, and width of stem of tobacco, while little influence on the number of tobacco leaves. The statistic results showed that broomrape parasitism had remarkable effect on tobacco yield and quality. The output of tobacco was 1471.14 kg/hm$^2$ in infected field and 2170.11 kg/hm$^2$ in uninfected field. Broomrape infection increased content of tobacco soluble protein and nicotinamide, decreased the activity of POD, PPO and the content of nitrogen, kalium, soluble sugar, ash and iron, with no obvious effect on the content of phosphorus, organic acid and chlorine.

**P03.071 Isolation and identification of antifungal bacterial strain QD-10 against plant wilt disease**

*C.G. Lu, W.C. Liu, D.P. Zhang, H.L. Wu, D. Dong, T.T. Zhang and T. Liu*
*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100097, P. R. China*
*Email: lcgf88@sina.com*

The antifungal bacterial of plant wilt disease was screened and identified to provide foundation for the study on bio-control preparation of plant wilt disease. Confrontation culture method was used to primarily screen the bio-control bacteria with good antifungal effect against plant wilt disease. 26 bacterial strains were isolated from the seawater collected from Qingdao sea area. Among those strains, 8 isolates showed antifungal activity against *Fusarium oxysporum* f. sp. *conglutinans* (on cabbage), *F. oxysporum* f. sp. *niveum* (on watermelon) and *F. moniliforme* (on asparagus), and antifungal activities of strain QD-10 was the most prominent The inhibition rate of QD-10 strain against three target strains of pathogen reached 80.92%, 72.03% and 79.98% respectively. QD-10 strain was identified of *Bacillus amyloliquefaciens* by the morphological, physiological, biochemical characteristics and the partial sequence of 16S rDNA sequence analysis method. Trials carried out in green house by root-irrigation method, the results demonstrated that the inhibition efficacy of the strain *Bacillus amyloliquefaciens* QD-10 against *F. oxysporum* f. sp. *conglutinans* on cabbages was 80% which was significantly higher than that (61%) of Carbendazol. Our study suggested that strain *Bacillus amyloliquefaciens* QD-10 has a good potential to be the bio-control strain with research and development.

**P03.072 Application of *Bacillus amyloliquefaciens* CGMCC 6978 to control wilt diseases of cabbages**

*C.G. Lu, D.P. Zhang, H.L. Wu, D. Dong, T.T. Zhang and W.C. Liu*
*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100097, P. R. China*
*Email: lcgf88@sina.com*

A bacterium, designated strain *Bacillus amyloliquefaciens* CGMCC 6978, was isolated from the permafrost soil collected from Mo-he River in Heilongjiang province of China, was identified by the morphological, physiological, biochemical characteristics and the partial sequence of 16S rDNA (GenBank KC417346). Tests *in vitro* showed that the strain had strong growth-inhibiting activities against a large number of plant pathogens such as *Fusarium* sp., *Botrytis cinerea, Monilinia fructicola*, *Colletotrichum capsici*, *Pseudomonas syringae* pv. *lachrymans* and *Xanthomonas. campestris* pv. *campsetris*, *etc*.. This strain secreted a great deal of siderophore, protease and cellulose on the detection agar plate. The lipopeptide-like antifungal substance synthesis genes (*itur, mycB, fenB* and *sfp*) and the β-1,3-1,4-glucanase gene (*glu*)were cloned from the genome of strain CGM A bacterium, designated strain *Bacillus amyloliquefaciens* CGMCC 6978, was isolated from the permafrost soil collected from Mo-he River in Heilongjiang province of China, was identified by the morphological, physiological, biochemical characteristics and the partial sequence of 16S rDNA (GenBank KC417346). Tests *in vitro* showed that the strain had strong growth-inhibiting activities against a large number of plant pathogens such as *Fusarium* sp., *Botrytis cinerea, Monilinia fructicola*, *Colletotrichum capsici*, *Pseudomonas syringae* pv. *lachrymans* and *Xanthomonas. campestris* pv. *campsetris*, *etc*.. This strain secreted a great deal of siderophore, protease and cellulose on the detection agar plate. The lipopeptide-like antifungal substance synthesis genes CC 6978. Trials carried out in green house demonstrated that the inhibition efficacy of the strain CGMCC 6978 against *F. oxysporum* f. sp. *conglutinans* on cabbages was 85% which was significantly higher than that (61%) of Carbendazol. To further evaluate the efficacy of strain CGMCC 6978 to control the cabbage

wilt disease, field experiments were consecutively conducted for 2 years. The results showed that the cabbages treated with strain CGMCC 6978 by root-irrigation presented better growth than those of control treatments and the efficacy of strain CGMCC 6978 in controlling the disease was 70%, being higher than that (50%) of Carbendazol. Our study suggested that strain CGMCC 6978 has a good potential to be an alternative for Carbendazol in controlling *F. oxysporum* f. sp. *conglutinans* on cabbages.

**P03.073 *In vitro* effect of plant extract on mycelial growth of leaf spot pathogen( *F. culmorum)* of sweet potato (*Ipomoea batatas*( L. ) Lam)**

*C.A. Amienyo[1] and A.E. Ataga[2]*
*[1]Department of Plant Science and Technology, University of Jos, Plateau State, Nigeria; [2]Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State. Nigeria*
*Email: amienyoc@unijos.edu.ng*

Studies on the efficacy of plant extract on the mycelial growth of the leaf spot pathogen were carried out. Five concentrations of aqueous extract of ten plants were incorporated into potato dextrose broth as growth medium. Discs of 7- day old culture of *F. culmorum* were removed, inoculated into each flask and incubated at 28±2℃. Fungal mycelium was obtained, dried and weighed. The mycelial weight of the fungus grown in the broth containing extract of text plants were significantly (p=0.05) different from the mycelial weight of the control. The broth containing extract of *Alchornea cordifolia, Azardiracha indica, Annona muricata and Carica papaya* indicated no significant (p=0.05) difference in the five concentrations of 5, 10, 15, 20 and 25% in their effect on the mycelial weight. The inhibitory effect of growth medium supplemented with *Rhizophora racemosa, Zingiber officinale, Garcinia cola and Allium sativum* increased with increasing concentration of extract. The mycelial weight of *F.culmorum* in extract supplemented medium showed no significant (p=0.05) difference between 25% aqueous extract of *Vernonia amygalina, Garcinia cola, Ocimum gratissimum* and a fungicide.

**P03.074 Effects of plant extract on leaf spot disease of Sweet potato (*Ipomoea batatas* (L.) Lam.) in a green house**

*C.A. Amienyo[1] and A.E. Ataga[2]*
*[1]Department of Plant Science & Technology, Faculty of Natural Sciences,University of Jos, P.M.B 2084, Jos, Plateau State, Nigeria; [2]Department of Plant Science & Biotechnology, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria*
*Email:amienyoc@unijos.edu.ng*

A Green house evaluation of the potency of plant extract on leaf spot disease of sweet potato (*Ipomoea batatas* (L.). Lam.) was carried out. At the 2-5 leaf stage, spores of suspension of Leaf spot pathogen (*Fusarium culmorum*) was sprayed to run off one hour after spraying the plants with extract solution as preventive experiment. After six weeks of inoculating another batch of potted plants with spore suspension of the fungus, extract solution were sprayed to run off on diseased leaves as curative experiment. Application of 10% extract of *Zingiber officinale* before spraying with spore suspension of the fungus indicated 98.11% inhibition in disease development. In the curative application.10% aqueous extract of *Vernonia amygdalina* showed 64.14% inhibition on disease development. The biomass and tuber yields from extract treated plants were significantly (p=0.05) different from untreated plants.

**P03.075 *Candida* sp.-Quan, controlling postharvest grey mould on apples by nutrition competition and secreting anti-fungus volatiles**

*D.P. Zhang, W.C. Liu, D. Dong, C.G. Lu, H.L. Wu, T.T. Zhang and J.Y. Qiu*
*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Haidian District, Beijing, 100097, P. R. China.*
*Email: dreamstar26@126.com*

Grey mould caused by *Botrytis cinerea* is an important postharvest disease and caused huge economic losses of apple fruits. A yeast strain isolated from apples (cv. Golden Delicious) in organic orchard was demonstrated to be a new member of the genus *Candida* by sequence comparisons of 26S rDNA D1/D2 domains. Genbank accession number of 26S sequences of *Candida* sp. -Quan was JQ917720. *Candida* sp.-Quan is closely related to *C. musae* on the basis of the phylogenetic trees based on the D1/D2 regions, but has a low identification (95%). The yeast showed a strong activity against grey mould on apples. In apple juice media, the yeast at concentration of $10^8$, $10^7$ and $10^6$ cells $ml^{-1}$ inhibited the spore germination of *B. cinerea* by 100%, and on wound-inoculated apples, controlled the grey mould decay also by 100%. Co-culturing *B. cinerea in vitro* or *in vivo*, neither the inactivated cells nor the culture filtrate of the yeast had any significant effect on spore germination or germ tube elongation of the pathogens, excluding the production of secreted toxic metabolic compounds. However, in high nutrition media, the yeast showed less antagonistic activity against *B. cinerea* than that in low nutrition media, and the volatile compounds secreted by the yeast significantly inhibited the pathogen growth *in vitro*, suggesting that *Candida* sp.-Quan controlled grey mould on apples by nutrition competition and secreting anti-fungus volatiles.

**P03.076 Enhancing seedling emergence and plant growth of forage brassicas with a *Trichoderma* bio-inoculant**

*D.R.W. Kandula, A. Stewart and A. Shah*
*Bio-Protection Research Centre, PO Box 84, Lincoln University, Lincoln 7647, Canterbury, New Zealand*
*Email: diwakar.kandula@lincoln.ac.nz*

Forage brassicas occupy the largest area (250,000 ha) of cultivated crops in New Zealand (NZ) to supplement pastures both in the dairy and dryland farming systems. Forage yields are affected by poor crop establishment due to the increased susceptibility of brassica crops to *Rhizoctonia solani*, a soil-borne fungal pathogen causing damping-off and root rot. The effectiveness of a *Trichoderma* bio-inoculant (TBI) was evaluated in glasshouse experiments using potting-mix and field soils artificially inoculated with *R. solani* inoculum with kale variety "Gruner", forage rape variety "Interval" and turnip variety "Dynamo" as test crops. TBI treatment significantly increased seedling emergence of all the test crops. Regular monitoring of plant growth revealed that plants were healthier in the TBI treatment with lower incidence of wire-stem disease symptoms compared to controls. Increased seedling emergence resulted in significantly more shoot dry matter in the TBI treatment with kale and turnip plants, whereas the root dry matter was significantly higher in all the three forage brassicas tested. In the TBI treatment, visual observation of roots showed a marked increase in fine root growth in kale and forage rape plants, while in turnip, bulb growth was enhanced. On-going work is developing an appropriate seed-coating formulation that will allow TBI to be integrated into sustainable forage crop management practices in NZ.

**P03.077 Screening of rhizobium against soybean cyst nematode (*Heterodera glycines*) from soybean nodules**

*Y.X. Duan and F. Tian*
*Nematology Institute of Northern P.R. China, Shenyang Agricultural University, Shenyang, Liaoning, 110866, P.R. China*
*Email: tiantian19860713@126.com*

The soybean cyst nematode (SCN), *Heterodera glycines*, is a plant-parasitic nematode and a devastating pest of the soybean (*Glycine max*) worldwide. Chemical control with nematicides is not normally used because the economic and environmental costs are prohibitive, so biological control is a new method to control the disease. Rhizobia are well known for their ability to fix nitrogen and promote the soybean growth. In our research, we found a strain of rhizobium which could also control the SCN. 496 soil samples had been collected from 81 regions of China. Soybean root nodules had been obtained from the soil samples by using trap method. The 518 strains of endophytic bacteria had been isolated from soybean root nodules. The biocontrol rhizobium antagonistic against soybean cyst nematode had been screen from them. The strain which is effective to the soybean cyst nematode was got through bacteria suspension preliminary screening and fermentation further screening. The strain dealt with J2 for 72h was able to inhibit incubation of soybean cyst nematode, which prevention rate could reach to 82.99%, numbered as Sneb183. Sneb183 was identified as *Sinorhizobium fredii* through physiology and biochemistry and molecular biology method. The major nutrient substance of the culture medium was optimized toxicity to nematode. The best nutritional combination was choused in this research as follows: Mannitol 1.5%, yeast powder0.05%, MgSO4 0.005%, NaCl 0.01% and $CaCl_2$ 0.005%.Among the relationship of Sneb183, soybean and soybean cyst nematode, the rhizobium had the dominate position in the space competition, which could induce the soybean resistant system. Above all, Sneb183 was identified as a biocontrol rhizobium against soybean cyst nematode.

**P03.078 Antifungal mechanism of *Bacillus amyloliquefaciens* dlt3 against sugar beet root rot**

*H. Sun[1], Q. Wang[2], X.Q. Wang[1], Y.L. Li[1], B. Hu[1], J.Q. Zheng[1] and Y. Li[2]*
*[1]Institution of Beijing Plant Protection, No.9 North Third Ring Middle Road, Xicheng District, Beijing, 100029, P.R. China; [2]Department of Plant Pathology, College of Agronomy and Biotechnology, P.R.China Agricultural University, Beijing, 100193,P.R. China*
*Email: sunhai_1987@163.com*

*Bacillus amyloliquefaciens* dlt3 was isolated from the rhizosphere of sugar beet. It showed 50% inhibition of mycelia growth of *Fusarium oxysporum in vitro*. The biocontrol effect to the sugar beet root rot reached 67.8% in the field condition. The biocontrol mechanisms of strain dlt3 against sugar beet root rot were studied. The colonization of strain dlt3 maintained up to $10^6$ CFU/g in the sugar beet rhizosphere soil one month postinoculation. PCR-DGGE profile showed *B. amyloliquefaciens* dlt3 could increase the diversity of bacterial community in the rhizosphere of sugar beet. Both supernatant and crude protein extract of *B. amyloliquefaciens* dlt3 inhibited mycelia growth of *F. oxysporum* and the inhibition percentage reached 52.9% and 46.2%, respectively. The supernatant of *B. amyloliquefaciens* dlt3 inhibited the spore germination of *F. oxysporum*. The compounds in the resin extract of supernatant was found the same molecular weight as Iturin, Fengycin, Bacillomycin by HPLC-MS. PCR detection also showed that dlt3 contained genes involved in biosynthesis of antibiotics: *bamC*, *fenB* and *ituD*. Additionally, dlt3 strain was capable of producing cellulose, amylase, protease, siderophores as well as forming biofilm. In conclusion, we supposed *B. amyloliquefaciens* dlt3 increase the diversity

of bacterial community and secrete antibiotics to suppress sugar beet root rot by colonized in the rhizosphere of sugar beet.

**P03.079 Elucidation of the mechanism of plant protection against *Ralstonia solanacearum* and plant growth promotion by *Bacillus cereus* Wenshan1-18**

*H.X. Liu, J.Q. Li, C. Wang and J.H. Guo*
*Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, Key Laboratory of Integrated Management of Crop Diseases and Pests (Nanjing Agricultural University), Ministry of Education, Nanjing 210095, P.R. China*
*Email: hxliu@njau.edu.cn*

The biocontrol efficacy of *Bacillus cereus* Wenshan1-18 against bacterial wilt caused by *Ralstonia solanacearum* was 53.8% on tomato at 21 d post inoculation when Wenshan1-18 remained spatially separated from *R. solanacearum* HB10, and the increase in shoot fresh weight of tomato without pathogen inoculation reached 19.6%. There was no inhibition zone between *B. cereus* Wenshan1-18 and *R. solanacearum* HB10 in an *in vitro* antibiosis test. The activities of four defense-related enzymes (polyphenol oxidase, phenylalanine ammonialyase, superoxide dismutase and peroxidase) and the expression of the defense-related genes *PR1a* and *ETR2*, which are regulated by the Salicylic Acid (SA) pathway and the ethylene (ET) pathway respectively, increased when plants were treated with Wenshan1-18. Next, we found *B. cereus* Wenshan1-18 can produce indoleacetic acid (IAA) and utilize phosphate, and accordingly the genes associated with plant growth *ARF10* expressed stronger in plants treated with *B. cereus* Wenshan1-18 than that in control. In general, *B. cereus* Wenshan1-18 is a potential biocontrol agent that can decrease the disease severity of bacterial wilt on tomato by induced systemic resistance (ISR) through SA and ET signal pathway, and stimulate plant growth by production of IAA and utilization of phosphate.

**P03.080 Clone and sequence analysis of fusaricidin biosynthetic gene cluster in *Paenibacillus polymyxa* T99**

*H.L. Wu, D. Dong, C.G. Lu, T.T. Zhang, J.J. Li and W.C. Liu*
*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100097, P.R. China*
*Email: wuhuiling925@126.com*

Biological control using microorganisms or its metabolites to suppress soil-borne plant pathogens has been widely used in agricultural fields. Antibiotics produced by microorganisms play an important role in the suppression of the pathogens. Fusaricidins are a group of lipopeptide antibiotics produced by *Paenibacillus polymyxa* that strongly inhibits the growth of many plant pathogenic fungi such as Fusarium oxysporum and gram-positive bacteria or actinomycetes. P. polymyxa T99 isolated from a vegetable field in suburb of Beijing has a significantly inhibition against *Fusarium oxysporum* and other plant pathogenic fungi. In this study, a pair of degenerate primers designed according to the fusC of *P. polymyxa* PKB1 and SC2 were used to generate a 450bp DNA segment. BLAST analysis revealed the sequence has a high sequence identity (99%) to fusC of *P. polymyxa* SC2 but only 88% identity to fusC of *P. polymyxa* PKB1. Then, a series of primers were designed according the fusaricidin gene cluster of *P. polymyxa* SC2 to amplify the genes for fusaricidin biosynthesis in *P. polymyxa* T99. About 32-kb DNA fragments including eight ORFs were obtained. Similarity of the eight ORFs in the fusaricidin biosynthetic gene cluster of *P. polymyxa* T99 to the corresponding gene fusA-TE of the *P. polymyxa* PKBl is 90%, 93%, 91%, 85%, 83%, 89%, 88%, 82%, respectively. Then, we will identify the fusaricidins production and analysis the biosynthesis of fusaricidins in T99.

**P03.081 Fluorescent protein as the reporter for detecting the activity of erythromycin promoter in *Streptomyces lydicus* A01**

*H.L. Wu, T. Liu, D.P. Zhang, Z.F. Tian and W.C. Liu*
*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100097, P.R. China*
*Email: wuhuiling925@126.com*

*Streptomyces lydicus* A01, which was isolated from the soil of suburban vegetable field in Beijing (China), was capable of producing natamycin and proved to be a potential biocontrol agent to several plant fungal diseases. In order to establish transformation system for molecular modification and improvement of strain A01, the activity of erythromycin promoter (ermE*) was detected by labeling strain with fluorescent protein. The *egfp* 720bp and *rfp* 678bp segments were inserted into the expression vector pIB139 individually, the recombinant vector pIB139-EGFP and pIB139-RFP were successfully obtained. The recombinant vector were transfomaed into *S. lydicus* A01 strain by intergeneric conjugation and the transformants were confirmed by PCR analysis with the specific primers of *egfp* and *rfp* gene. Fluorescence observation with microscope showed that the two transformants exhibited strong fluorescence activity. This results showed heterologous or homologous gene expression could be achieved by placing the activator gene with control of ermEp*, and this provided an effective genetic transformation tool for *S. lydicus* A01.

**P03.082 High plant-growth-promoting and biocontrol activity of rhizobacteria from Chinese contaminated soils**

*L.F. Ke[1], X.F. Wang[1], D.V. Mavrodi[2], N. Zheng[1], L.S. Thomashow[3], D.M. Weller[3] and J.B. Zhang[1]*
*[1]State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, 430070, P.R. China; [2]Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430.USA; [3]United States Department of Agriculture, Agricultural Research Service, Root Disease and Biological Control Research Unit, Pullman, WA 99164-6430, USA*
*Email: zhangjb@mail.hzau.edu.cn*

By using a combination of baiting and rhizosphere colonization cycling assays with oil rape seed as host, 110, 139, 141 and 228 bacteria colonies were isolated from heavy metal polluted, recalcitrant organic contaminated and petroleum semeary and saline zone of plant rhizosphere in China, respectively, and 15.5%, 15.1%, 36.2%, 24.6% of isolates inhibited *Sclerotinia sclerotiorum* accordingly. Randomly selected 304 colonies were conducted 1-aminocyclopropane-1-carboxylic acid (ACC) Deaminase-containing rhizobacteria screening, 18.75% of selected colonies showed positive activity. We investigated 12 rhizobacterial isolates representing 3 dominant morphotypes, *Pseudomonas* sp., *Bacillus* sp., *and Micobacterium hydrocarbonoxydans*. Phylogenetic analysis of *phlD, prnD, pltC, phzF* and *acdS* genes sequences in *Pseudomonas* showed tight clustering to some known *Pseudomonas* strains Pf-5, 30-84 and Q8r1-96. All 12 strains were tested for their abilities to promote the root elongation of oil rape seed under gnotobiotic condition. All *P. brassicacearum* strains containing ACC deaminase and *P. protegens strains* increase the length of root significantly and consistently, in comparison with the negative control. Based on biocontrol assays and heavy metal test, ACC Deaminase-containing isolates showed more highly effective suppression of *S. sclerotiorum* and resisted higher concentration heavy metal. ACC Deaminase-containing PGPR *P. brassicacearum* KY5404 indicated stable inhibition of *S. sclerotiorum* in greenhouse treatment and increased oil rape seed growth significantly.

**P03.083 Characterization of a novel dsRNA mycovirus in the phytopathogenic fungus *Collectotrichum acutatum***

*J. Zhong, X.H. Lei and B.D. Gao*
*Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Hunan Agricultural University, Nongda Road 1, Furong District, Changsha 410128, P.R. China*
*Email: bdgao@yahoo.com.cn*

A novel virus contained two segments of dsRNA was detected from HNZJ001 strain of *Collectotrichum acutatum*, a phytopathogenic fungus causing anthracnose diseases in immature fruit of pepper. The complete nucleotide sequences were determined. Spherical virus-like particles in the size about 40 nm in diameter was observed from the mycelial tissue preparation. Sequence analysis showed that dsRNA-1 and dsRNA-2, which had genomes of 1760 and 1377 nucleotides, respectively, each contain a single ORF encoding proteins of 62KD and 40KD.The proteins encoded by the open reading frame in dsRNA1 was similar to RNA-dependent RNA polymerase (RdRp) of partitiviruses while the amino acid encoded by dsRNA2 has no significant similarity to any published protein in GeneBank database. Genome comparison and phylogenetic analysis indicated that the virus was found to be closely related to mycovirus in the family Partitiviridae. A RT-PCR, using the primers designed from the RDRP of the dsRNA1 segment, was proceed in the infected mycelium and 40 single spores of the progeny derivatives.The result showed that about 100% of the subcultures contain the virus and the transmission efficiency of the virus to the progeny derivatives was very high, which had been indirect confirmed by the experiment of failed to completely eliminated the virus from the host fungus.

**P03.084 Detection and sequence analysis of novel viral double strand RNAs (dsRNAs) in *Ustilaginoidea virens***

*J. Zhong and B.D. Gao*
*Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Hunan Agricultural University, Nongda Road 1, Furong District, Changsha 410128, P.R. China*
*Email: bdgao@yahoo.com.cn*

*Ustilaginoidea virens* is the causal agent of false smut disease of rice. We tested 7 isolates of *U. virens* for the presence of dsRNAs. Electrophoresis of dsRNA extracts showed that all the 7 isolates contained dsRNAs with sizes from 0.7 to 5 kb. We found 4 dsRNA bands, dsRNA 1 (5124 bp), dsRNA 2 (1711 bp), dsRNA 3 (1423 bp) and dsRNA 4 (855 bp) on agarose plate from isolate HNHS-1. Sequence analysis showed that the dsRNA1 (designated as HvRV1) contains two ORFs potentially encoding 82 KDa coat protein and a 91kDa RNA dependent RNA Polymerase (RdRp), and that dsRNA2 contains one ORF coding for another RDRP. It is noteworthy that the ORF in dsRNA 3 that encodes a 41 kDa protein showed no similarity with any other amino acid sequence in databases, and that the dsRNA4 rather surprisingly lacks any ORF. Isomeric viral particles of about 40 nm in diameter were observed under transmissive electron microscope from the mycelium tissue of isolate HNHS-1. Those 4 dsRNAs in *U. virens* HNHS-1 might belong to at least 2 mycoviruses in different families. According to the results above, HvRV1

reported here should be new mycoviruses. This is the first report on mycovirus in *U. virens*.

**P03.085 Inhibition of *Pseudoperonospora cubensis* Rostov and promotion of cucumber growth and broad-spectrum resistance by *Rhodopseudomonas* sp. S-1**

*J. Peng, D.Y. Zhang, S.B. Zhang, Y. Gao, Z. Zhang and Y. Liu*
*Key Laboratory of Pest Management of Horticultural Crop of Hunan Province, Hunan Plant Protection Institute, No 726 Second Yuanda Road, Furong District, Changsha 410125, P. R. China*
*Email: haoasliu@163.com*

The diseases caused by *Pseudoperonospora cubensis*, is destructive to many economic crops, for example, cucumber. And biological control is an increasing concern for plant diseases preventing. To this end, a bacterium,isolated from activated sludge and named strain S-1,was identified as *Rhodopseudomonas* sp. based on Morphological features, physiological and chemotaxonomic characteristics, and phylogenetic analysis of 16S rRNA. The optimal culture conditions of strain S-1 were at 30℃ and pH 7. Strain S-1 could effectively inhibit *Pseudoperonospora cubensis* Rostov, as inhibition ratio up to 78.33% at 5-fold dilution of the cell-free extractions of strain S-1 and $IC_{50}$ (half inhibition concentration) was at 23.48-fold dilution by the floating leaf disc test (FAO, 1982). Moreover, the cell-free extraction of strain S-1 could increase root-length and biomass increment, and raise producing of indoleacetic acid, amino acids and chlorophyll, and enhance activity of peroxidase of cucumber, all of this suggested it is potential for promoting the growth and enhancing broad-spectrum resistance of cucumber.

**P03.086 A novel mycovirus isolated from the hypovirulent strain BerBc-1 of *Botrytis cinerea***

*L. Yu, J. Zhang, L. Yang and G.Q. Li*
*State Key Laboratory of Agricultural Microbiology and Key Laboratory of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan 430070, P.R. China*
*Email: guoqingli@mail.hzau.edu.cn*

*Botrytis cinerea* (Teleomorph: *Botryotinia fuckeliana*) is the causal agent for grey mould disease on many economically important crops. A mycovirus designated as *Botrytis cinerea* RNA virus 1 (BcRV1) in a hypovirulent strain BerBc-1 of *B. cinerea* was characterized in the present study. The genome of BcRV1 is 8952 bp in length. It was deduced to contain two overlapping open reading frames (ORFs), ORF1 and ORF2. ORF1 and ORF2 are connected by a -1-frameshift sequence, including a shifty heptamer ($GAAAAAC_{4814-4820}$), a 7-nucleotides spacer and an H-type pseudoknot structure. ORF1 putatively encodes a 1314-amino acid (aa) polypeptide with the predicted molecular mass of 145 kDa. The function of that polypeptide remains unknown. ORF2 putatively encodes a 1338-aa polypeptide with the predicted molecular mass of 146 kDa. This polypeptide contains the RNA-dependent RNA polymerase (RdRp) domain with eight conserved motifs, which are closely related to seven unclassified dsRNA mycoviruses, including SsNsV-L, FgV3, FvV1, FvV2, PgV2, DsRV1 and PiRV3. A predicted Phytoreo_S7 domain homologous to several S7 proteins in phytoreoviruses was detected in BcRV1. Phylogenetic analysis based on the aa sequences of the RdRp domain indicated that BcRV1 and the viruses mentioned above formed a clade, compared the mycoviruses in *Totiviridae* and *Chrysoviridae*. This study suggests that BcRV1 is a novel mycovirus in *B. cinerea*. The relationship between the BcRV1 infection and hypovirulence in *B. cinerea* is under investigation.

**P03.087 Antifungal activity and compounds of essential oil of *Pelargonium graveolens***

*J.T. Wu, Y.L. Zhang, B. Zhang, L.Z. Zhao, C.S. Li and J.S. Qi*
*Institute of Plant Protection, SAAS, No.202 North Industrial Road, Licheng District, Jinan, Shandong, 250100, P. R. China*
*Email: qi999@163.com*

The increasing concern over environmental effects and food safety caused by organic synthetic pesticides has highlighted the need for alternative products. Pesticidal activity screening from plants could lead to the discovery of new pest control agents. The present thesis was focused on the study of antifungal constituents of essential oil of *Pelargonium graveolens* against plant pathogenic fungi. Oil was tested for its antifungal activities against 6 plant pathogenic fungi by mycelia growth methods to screen more effective plant protective agents. The result indicates that the oil exerted high antifungal activity against 6 fungi, providing complete growth inhibition at the concentration of 2 g/L. Oil was used for further purification, and one antifungal ingredient was purified with column chromatography of silica gel by activity direction and identified as citronellol according to 1H-NMR. The $EC_{50}$ of citronellol aganist *Botrytis cinerea* and *Fusarium graminearum* was 52.12 μg/ml and 83.30 μg/ml. Microscopic observations revealed that the mycelia of *B. cinerea* and *F. graminearum* appeared distortion, with protoplasm agglomerated and leaking of cell contents on the effect of citronellol. Results support that essential oils or some of their constituents could become natural alternatives to synthetic fungicides to control certain important plant fungal diseases.

**P03.088 Screening of medicinal plants for antifungal activity against plant pathogenic fungi**

*G.W. Zhao, B. Zhang, Y.L. Zhang, L.Z. Zhao, C.S. Li and J.S. Qi*
*Institute of Plant Protection, SAAS, No.202 North Industrial Road, Licheng District, Jinan, Shandong, 250100, P. R. China*
*Email: qi999@163.com*

Botanical fungicides have many advantages such as bounding in resources, low toxicity, environment-friendly, low cost, and low pathogen resistance, etc. So, it has already become an important route for new fungicides development. And screening of antifungal plant and structure elucidating of antifungal ingredients were the foundation of Botanical fungicides development. In this study, 37 traditional medicinal plants were used to screen fungicidal activity. Six kinds of plant pathogenic fungi, *Pythium aphanidermatum, Phytophthora capsic*, *Fusarium oxysporium*, *Fusarium solani*, *Botrytis cinerea* and *Fusarium graminearum* were used as target strains to determine the antifungal activities of acetone extracts from 37 species of medicinal plants by mycelial growth method. There are 18 medicinal plants having more than 70% fungal inhibition rate against at least one kind of the pathogenic fungi. *Eugenia caryophyllata* had 100% antifungal activity against the six kinds of plant pathogenic fungi; *Fructus mume*, *Radix stemonae* and *Radix aucklandiae* had a high fugal inhibition rate against five kinds of pathogenic fungi. *Ramulus cinnamomi* had a considerable fungal inhibition rate against *Pythium aphanidermatum*, *Phytophthora capsici*, *Botrytis cinerea*, and *Fusarium graminearum*. Spore germination method was also used to test the inhibition rates of those highly active medicinal plants against *Botrytis cinerea* conidia, and the antifungal activities of those plants tested were all above 95%. *Eugenia caryophyllata*, *Fructus mume*, *Radix stemonae*, *Radix aucklandiae*, and *Ramulus cinnamomi* had a broad antifungal spectrum with high inhibition rates, indicating that those medicinal plants contain active substances with broad-spectrum antifungal activity, which is worth of further study.

**P03.089 Antifungal potential of *Chenopodium album* against *Ascochyta rabiei***

*A.Z. Sherazi, K. Jabeen and S. Iqbal*
*Department of Botany, Lahore College for Women University, Lahore, 54000, Pakistan*
*Email: khajista_1@hotmail.com*

*Chenopodium album* L. (bathu) leaves and roots were selected to evaluate their antifungal potential against the *Ascochyta rabiei* responsible for chickpea blight that causes destructive yield looses. Extracts of *C. album* leaves and roots were prepared and their various applied concentrations viz. 1%, 2%, 3%, 4%, 5% were tested against *A. rabiei.* Leaf extracts were found more effective and showed significant antifungal activity over the root extracts. Methanolic extract *C. album* leaf was found effective in screening bioassays, so this was subjected for fractional guided bioassays. Different various organic fractions of leaf extract were isolated viz. n-hexane, chloroform, ethyl acetate and n-butanol by using separating funnel in increasing order of polarity. These isolated fractions were further serially diluted to check their Minimum Inhibitory Concentration along with a synthetic fungicide (Puslan, 72 WP) and two controls; Dimethyl sulphoxide (DMSO) and distilled water. The MIC of various concentrations from (500 mg–1.953124 mg) was recorded for each fraction in interval of 24 and 48 hours. The tested fractions showed variable antifungal activity when compared with control sets (Water and DMSO). Ethyl acetate and synthetic fungicides were found most effectual in retarding conidial germination with MIC of 1.953142 mg after 48 hrs incubation period. Chloroform fraction also inhibited the fungal growth whereas n-hexane and ethyl acetate fraction was found to be ineffective.

**P03.090 Detection of antiviral activity of protein components produced by *Streptomyces parvus* Yn168**

*Z.F. Tian, W.C. Liu, T.T. Zhang and L.Y. Hou*
*Institute of Plant & Environment Protection,Beijing Academy of Agriculture & Forestry Sciences, Beijing 100097, P.R. China*
*Email: zhaofengtian2000@yahoo.com*

Antiviral activity of protein components $I_{168}$ and $II_{168}$ from *Streptomyces parvus* strain Yn168 was identified by protoplast inoculation system and leaf-discs inoculation. In protoplast inoculation system, RANs of *Tobacco mosaic virus*(TMV) was inoculated into the protoplast of tobacco by electric shock and the antiviral substances of protein components $I_{168}$ and $II_{168}$ extracted from *Streptomyces parvus* Yn168 fermentation broth were added to the protoplast culture system 6h later. The test was carried out using sterile distilled water and antiviral antibiotics Ningnanmycin as control. The virus proliferation level was assayed by ELISA after TMV was inoculated 30h later. The results showed that the inhibition rates to TMV were 74.5% and 63.4% for active protein components $I_{168}$ and $II_{168}$ respectively. Also, the antiviral activity of the protein components was detected by leaf-disc ELISA method using 2cm diameter discs of tobacco leaf. And the inhibition rates were 65.2% and 56.5% to TMV proliferating in the following detection. Therefore, although the leaf-disc method was easy to operate, antiviral active substance was more to be absorbed into the cell interior in protoplast cultures system than on the surface of the leaf-discs so that the antiviral activity could exert the antiviral effects better.

**P03.091 Transcriptional and posttranscriptional regulation of 2,4-diacetylphloroglucinol production in biocontrol bacterium *Pseudomonas fluorescens* 2P24**

*W. Zhang, X.G. Wu, J.C. Liu, Z. Zhao, T. Tian and L.Q. Zhang*
*Department of Plant Pathology, China Agricultural University, Yuanmingyuan West Road No.2, Haidian District, Beijing, 100193, P.R. China*
*Email: zhanglq@cau.edu.cn*

*Pseudomonas fluorescens* 2P24 is a rhizospheric bacterium that aggressively colonizes the plant roots. It produces the antibiotic 2, 4-diacetylphoroglucinol (2, 4-DAPG), which plays a major role to the protection of crop plants against soil borne diseases caused by bacterial and fungal pathogens. Similar as other antagonistic Pseudomonads, the biosynthesis of 2, 4-DAPG in strain 2P24 is regulated at the transcriptional level in the expression of the *phlACBD* operon as well as at the post-transcriptional level by the Gac/Rsm signal transduction pathway. Our recent study revealed several new factors involved in the regulation of 2, 4-DAPG production. A resistance-nodulation-division (RND) efflux system EmhABC, which determines the resistance to toxic compounds in *P. fluorescens* 2P24, negatively regulates the production of antibiotic 2,4-DAPG by altering the intracellular concentration of 2, 4-DAPG and then enhancing the transcriptional feedback of *phlACBD* operon. The sigma regulator PsrA directly binds to the *phlA* promoter region and negatively regulates *phlA* transcription. At the same time, PsrA activates the expression of the sigma factor RpoS, which negatively regulates 2, 4-DAPG production by inducing the expression of the RNA-binding protein RsmA. RetS is a hybrid sensor kinase/response protein located on bacterial membrane, and modulates the phosphorylation state of another kinase GacS via a direct interaction. In *P. fluorescens* 2P24, RetS was proved to negatively regulate 2, 4- DAPG production through the Gac/Rsm pathway. Increasing number of regulatory elements reported in recent publications revealed a more complex and fine regulatory network for antibiotic 2, 4-DAPG production, and further study is needed to understand how this system works.

**P03.092 Pathogenic variation of *Ralstonia solanacearum* and it's avirulent mutants construction**

*B. Liu, X.F. Zheng, Y.J. Zhu and J.M.Che*
*Agricultural bio-resources research institute, Fujian academy of agricultural sciences, Wusi Road 247, Fuzhou 350003, P.R. China*
*Email: fzliubo@163.com*

In order to provide the development of plant vaccine with high disease reduced efficiency and stable biological characteristics strain, we constructed avirulent mutants of *Ralstonia solanacearum* through spontaneous mutagenesis, $^{60}$Co-radiated mutagenesis and EZ-Tn5 transponson. Attenuation Index (PI) was constructed to measure the pathogenicity of mutants. Results showed that three different pathogenic types (virulent, interim and avirulent) of mutants were obtained. Based on colony morphology on TTC medium, the colony of virulent strain showed irregular shape, strong mobility, humid, pink spot in the middle of colony, and big white edge. Interim strain showed immobility, humid with the surface, dark red spot in the middle of colony, narrow white edge. Avirulent strain showed round, immobility, dry, dark red spot in the middle of colony, narrow or no white edge. Based on bacterial morphological observed by transmission electron microcopy, the shape of virulent *R. solanacearum* was long oval or short rod with the ratio of cell width and length from 0.50 to 0.60, the shape of avirulent *R. solanacearum* was short oval or nearly round with the ratio of cell width and length from 0.70 to 0.80. The results of pathogenicity test showed that virulent *R. solanacearum* had high infected mortality with PI<0.41, interim *R. solanacearum* had middle high infected mortaliy with PI 0.52-0.68, avirulent *R. solanacearum* had no infected mortality with PI>0.80. High performance ion exchange chromatogramphy (HPLC) was used to analyze different virulent *R. solanacearum* strains, the results showed that three characteristic fractions of different virulences were separated, strong virulent strains formed only one characteristic peak at the retention time more than 20 min, avirulent strains also formed one characteristic peak, but the retention time was short (less than 10min), interim strains possessed with three characteristic peaks.

**P03.093 Constructe evaluation model of *Ralstonia solanacearum* avirulent mutants against tomato bacterial wilt disease**

*B. Liu, X.F. Zheng, Y.J. Zhu and J.M. Che*
*Agricultural Bio-resources Research Institute, Fujian Academy of Agricultural Sciences, Wusi Road 247, Fuzhou 350003, P. R. China*
*Email: fzliubo@163.com*

In this paper, 55 avirulent mutants of *Ralstonia Solanacearum* were used to control tomato bacterial wilt and the control efficiencies were compared. The results showed that strain FJAT1458 isolated from tomato plant was the most effective with control effect reached to 100%. It was also found that strain FJAT1458 could colonize in rhizospheric soil, root and stem of tomato plant. The colonizing density of FJAT1458 showed a tendency of 'increasing at beginning and decreasing afterward' during 1-25d after inoculation. Meanwhile, higher inoculated concentration and younger seedling age resulted in more colony amount of FJAT1458 in plant. A regression model of control efficiency of the biocontrol agent against tomato bacterial wilt was

constructed. When the inoculated concentration of FJAT1458 was bigger, the value of R was less. Therefore, the avirulent mutant FJAT1458 had good control effect on tomato bacterial wilt.

**P03.094 Beneficial effect of some yeast and bio-fungicides on peanut seeds mold fungi during storage**

M.A. Abd-AllA and R.S. El-Mohamady
*Department of Plant Pathology, National Research Centre – Giza- Egypt*
*Email: mohmednrc@yahoo.com*

*Aspergillus flavus* was a common fungus with more frequency isolated from peanut seeds during storage, followed by *Aspergillus niger* and *Aspergillus ochroecuse*. *Saccharomyces servisieae* yeast caused a highly inhibitor effect for the linear growth of *A. flavus* and *A. ochroecuse* by 62.6 and 55.0%, respectively. The bio-fungicide Rhizo-IN with recommended dose (2.5g/l) caused highly inhibitor effect against *A. niger* linear growth followed by *S. cerevisiea* by 78.3 and 63.2%, respectively. *Candida tennis* yeast and bio-fungicide Plant guard showed mediate effect against all tested mold fungi. Coating peanut seeds before storage by yeast *S. cerevisea* and *C. tennis* ( 1X108 c.f.u./ml) resulting more protective effect of peanut seeds against mold fungi incidence during storage period (45 days) , *S. cerevisea* cussed more effect than other tested treatment, it caused 71.0% , 80.0% and 87.8% protective effect against *A. flavus*, *A. niger* and *A. ochroecuse*, respectively. The results of this study showed that yeast *S. cerevisea* was a alternative safe coating method for prevent peanut seeds against mold fungi incidence which causes economic losses during transportation, marketing and storage.

**P03.095 Role of the glyoxylate cycle in growth, antagonism, root colonization and induction of systemic plant defence responses in the fungal biocontrol agent *Trichoderma atroviride***

M.K. Dubey[1], A. Broberg[2], D. Funck Jensen and M. Karlsson[1]
[1]*Uppsala BioCenter, Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, 75007 Uppsala, Sweden;* [2]*Uppsala BioCenter, Department of Chemistry, Swedish University of Agricultural Sciences, Box 7026, 75007 Uppsala, Sweden*
*Email: mukesh.dubey@slu.se*

Isocitrate lyase (ICL), a signature enzyme of glyoxylate cycle, is required for metabolism of two-carbon compounds like acetate or ethanol, and for virulence in bacteria and fungi. In the present study, we demonstrated the role of the glyoxylate cycle in the fungal biocontrol agent *Trichoderma atroviride* by generating ICL deletion (Δ*icl*) and complementation (Δ*icl*+) strains. Gene expression analysis shows that *icl* is induced in dual cultures confronting the *Botrytis cinerea* or *Rhizoctonia solani*. Phenotypic analysis of Δ*icl* and Δ*icl*+ suggested the requirement of ICL in growth, conidiation, conidia pigmentation and germination, and abiotic stress tolerance. The Δ*icl* strain displayed reduced antagonism towards *B. cinerea*. LC-MS analysis revealed that the Δ*icl* strain has reduced ability to produce the antifungal metabolite 6-pentyl-2H-pyran-2-one (6-PP) on acetate medium in comparison to the WT. Secretion and sandwich assays on acetate or PDA medium reflect the partial role of 6-PP in *B. cinerea* antagonism. Furthermore, *in-vitro* root colonization assay shows that the Δ*icl* strain retained the ability to internally colonize the *Arabidopsis thaliana* roots. However, *A. thaliana* plants with roots colonized by the Δ*icl* strain shows abolished ability for the induction of defence response gene *PAD3* in the leaves in compare to WT colonized *A. thaliana* leaves. Interestingly, root colonization by Δ*icl* strain significantly induced the expression of salicylic acid and jasmonic acid-related defence response genes in leaves of *A. thaliana* in comparison to leaves of WT colonized *A. thaliana*. These data show that the glyoxylate cycle is important for biocontrol traits in *T. atroviride*.

**P03.096 Biological control of soil-borne fungal pathogens of strawberry by *Bacillus* and *Trichoderma* strains**

A.M. Pastrana, A. Aguado, M.C. Sánchez, K. Akdi, M.D. Gomis, S. Kamah, D. Salgado and N. Capote
*IFAPA Las Torres-Tomejil. Carretera Sevilla-Cazalla km 12.2, CP 41200 Alcalá del Río, Sevilla (Spain)*
*Email: marian.capote@juntadeandalucia.es*

The antagonistic effect of two *Bacillus* strains (*B. laterosporus* and *B. megaterium*) (FUSBACT®) and *Trichoderma asperellum* T18 strain (PRODIGY®) was assessed on two soil-borne fungal pathogens of strawberry (*Macrophomina phaseolina* and *Fusarium oxysporum*) under *in vitro* and *in vivo* greenhouse conditions. Dual plate confrontation of *M. phaseolina* and *Bacillus* showed high levels of colony growth inhibition (CGI) (49.81% by *B. laterosporus*, and 30.20% by *B. megaterium*). CGI for *F. oxysporum* was 46.81% and 35.78%, respectively. In dual cultures assays of *T. asperellum* and the strawberry pathogens, the percentage of radial growth inhibition (%RGI) for *M. phaseolina* was 54.94% and for *F. oxysporum* 41.41%. High sporulation and overgrowth or competitive growth of T18 strain over the pathogens was observed. In greenhouse conditions, strawberry plants cv. Fortuna were inoculated with suspensions of *Bacillus* strains or *T. asperellum*, or both (i) 5 days before inoculation with the pathogens, as preventive treatments and (ii) 5 days after inoculation, as curative treatments. Two preventive methods of application

were also tested: pre-plant root-dip, and pre-plant watering of the pot soil with suspensions of the antagonists. Results showed that the two combined *Bacillus* species and the T18 *T. asperellum* strain were able to reduce significantly the incidence of charcoal rot caused by *M. phaseolina* and Fusarium wilt caused by *F. oxysporum* in strawberry plants. A synergistic effect between *Bacillus* species and *T. asperellum* was not observed. Preventive treatments were more effective than curative ones for the control of the fungal diseases. The pre-plant root-dip application of *B. laterosporus* and *B. megaterium* was the best treatment for the control of *M. phaseolina* and *F. oxysporum*. The use of these biocontrol agents as alternative to chemical fungicides for the control of soil-borne pathogens could be an applicable practise in integrated and ecological production of strawberries.

**P03.097 The pleiotropic regulatory gene *degQ* regulates the fengycins production and biofilm formation of *Bacillus subtilis* NCD-2**

*P.P. Wang, Q.G. Guo, S.Z. Li, X.Y. Lu, X.Y. Zhang and P. Ma*
*Plant Protection Institute, Hebei Academy of Agricultural and Forestry Sciences, IPM Centre of Hebei Province, Key Laboratory of IPM on Crops in Northern Region of North P.R. China, Ministry of Agriculture, Baoding, Hebei 071000, P. R. China*
*Email: w.peipei2009@gmail.com*

The *Bacillus subtilis* NCD-2 is the key component of the biocontrol agent against plant soil-borne diseases, such as cotton verticillium wilt and cotton damping off. Former research revealed that fengycin is the major antifungal compound produced by strain NCD-2. To clarify the genes regulatory network for fengycin production, the *degQ*, a pleiotropic regulatory gene, was deleted. Dual culture testing showed that the *degQ* deletion mutant decreased the inhibition ability against the growth of *Botrytis cinerea*. However, the inhibition ability could be restored after complementary the intact *degQ* to the mutant. The lipopeptides were extracted and analyzed by Fast Protein Liquid Chromatography (FPLC), compared to that of strain NCD-2 wild type, the *degQ* deletion mutant significantly decreased the fengycins production. Bioassay indicated that the lipopeptides extracted from the *degQ* deletion mutant not only decreased the antifungal activity against the growth of *B. cinerea in vitro*, but also significantly decreased the control effect against tomato grey mold on detached tomato leaves. The same results could be observed by using bacterial suspensions instead of lipopeptides from strain NCD-2 wild type and the *degQ* deletion mutant. We also compared the biofilm formation between the wild type and the *degQ* deletion mutant of strain NCD-2 by using crystal violet staining in 2 mL tube. The *degQ* deletion mutant significantly decreased the biofilm formation ability. In general, the pleiotropic regulatory gene *degQ* greatly affected the fengycin production and the biofilm formation of *B. subtilis* NCD-2.

**P03.098 Control efficiency of VOCs produced by *Ceratocystis fimbriata* on citrus green mold disease**

*Q. Li[1, 2], J.J. Hao[3], L.X. Luo[1, 2] and J.Q. Li[1, 2*]*
*[1]Department of Plant Pathology, China Agricultural University / Key laboratory of Plant Pathology, Ministry of Agriculture, Beijing 100193, P. R. China; [2]Beijing Engineering Research Center of Seed and Plant Health (BERC-SPH) / Beijing Key Laboratory of Seed Disease Testing and Control (BKL-SDTC), Beijing 100193, P. R. China; [3]Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA*
*Email: liq@cau.edu.cn; lijq231@cau.edu.cn*

*Ceratocystis fimbriata* is a globally distributed fungal pathogen that infects more than 30 species of woody and herbaceous plants. This fungus also produces a variety of volatile organic compounds (VOCs), which have strong bio-activities on some other plant pathogens. Inhibitory effect of the VOCs on the growth of *Penicillium digitatum*, a plant pathogen, was determined using vertical dual-culture test in a Petri plate without having physical contact. The results showed that mycelial growth and spore germination of *P. digitatum* were strongly inhibited by the VOCs. The inhibition of mycelial growth and spore germination was 80.95% and 73.62%, respectively. *Penicillium digitatum* did not produce any conidia when treated with VOCs. Morphological changes of mycelia and spores of treated *P. digitatum* were observed under scanning electronic microscope. The mycelial tip appeared intumescent and distorted, with remarkably increased branches and segmentations. To determine the effect of disease control, either 0, 3, 5, 7, or 9 plates of *C. fimbriata* cultures growing on potato dextrose agar were placed in a plastic box, where 10 oranges wound-inoculated with *P. digitatum* conidia suspension ($10^6$ canidia/mL, 10 μL) were placed. The boxes were sealed with Parafilm, and replicated three times. Disease symptom was observed at 4 days post incubation (DPI). Lesion size on orange fruit was significantly reduced; the level of reduction was positively correlated with the amount of VOCs, and the highest reduction was 97.26%. In conclusion, *Ceratocystis fimbriata* has potential to control postharvest diseases.

**P03.099 Screening, identification and development of biocontrol agent against cotton boll blight**

*X.Y. Lu, H.M. Zhou, B.Q. Li, Q.G. Guo, S.Z. Li and P. Ma*
*Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, IPM Center of Hebei Province, Key Laboratory of IPM on Crops in Northern*

*Region of North P.R. China, Ministry of Agriculture, No.437 Dongguan District, Baoding, 071000, P.R. China*
*Email:pingma88@126.com*

Cotton boll rot occurred widely in the cotton production areas of China and caused significant loses on both cotton yield and quality in recent years. Cotton boll blight, caused by *Phytophthora boehmeriae*, was one of major diseases in cotton boll stage. Cotton boll rot survey was conducted in the Yellow River and the Yangtze River cotton production areas of China in 2011 and 2012. The result showed that over 95% cotton boll rot was cotton boll blight in these areas while the other diseases of cotton boll rot were cotton boll anthracnose, and cotton boll rot caused by *Trichothecium roseum et al*. Biological control of cotton boll blight was carried out in this study. 1250 strains of rhizosphere bacteria were screened and 399 strains showed antagonism against *P. boehmeriae* by the dual culture method. Among the antagonists, Strain HMB22922 showed the most significant potential control of cotton boll blight. The control efficiency against cotton boll blight was 81.34% in growing chamber and 39.39%-58.64% in the field trials, respectively. Strain HMB22922 was identified as *Bacillus atrophaeus* based on the *gyrB* sequence determination and Biolog physiological and biochemical test. Optimal wetting agent was selected according to the wetting time test, wetting agent "885A" was the best one for strain HMB22922. Fermentation liquid of strain HMB22922 was processed into suspending preparation by adding wetting agent "885A". Properties of the processed suspending preparation were accorded with the Inspection Standard. The control efficiency of the processed suspending preparation was significantly higher than that of bacteria fermentation liquid of strain HMB22922.

### P03.100 Mechanism on *Ustilago scitaminea* sexual mating inhibited by *Pseudomonas fluorescens* strain HN58

*P.G. Xi, X. Sun, Y.Q. Zhou, H. Deng, W.K. Shen, S.Y. Liu, M.H. Li, Z.D. Jiang and L.H. Zhang*
*College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, P. R. China*
*Email: xpg@scau.edu.cn*

*Ustilago scitaminea* Syd. is one of the most serious pathogens on sugarcane, with its life cycle consisting of saprophytic and pathgenic phases. In soil, the organism proliferates in a unicellular budding mode and the haploid cells are non-infectious on sugarcane. After mating with opposite sexual haploid, the pathogen grows as diploid filamentous cells and gains the ability to infect sugarcane. Therefore, it would be interesting to test the feasibility to control the occurrence of this fungal sugarcane disease on sugarcane by blocking the fungal sexual mating. *Pseudomonas fluorescens* strain HN58 showed high inhibitory activity on sexual mating with weakly affecting haploid growth of *U. scitaminea*. Further characterization found that the active substance from strain HN58 was susceptible to protease digestion, suggesting that it is likely a peptide. Tn5 transposon insertion mutagenesis was conducted to identify the relative functional genes of strain HN58 implicated in fungal mating inhibition. From over 3100 transposon mutants, 14 mutants that lost inhibitory activity on sexual mating of *U. scitaminea* were obtained. Complementation analysis will be conducted to characterize the biocontrol mechanism of *P. fluorescens* strain HN58 against the smut sexual mating.

### P03.101 Chitinolytic enzyme from *Trichoderma harzianum:* purification, characterization and antifungal bioassay

*P. Sharma, M. Sharma, K. Sarvanan and M. Srivastava*
*Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi, 110012 India*
*Email: psharma032003@yahoo.co.in*

Endochitinase (ech42) gene (JN798187) which is involved in mycoparasitism, was isolated from taken from *Trichoderma harzianum* (Th3) cloned, sequenced and its expression profiling was carried by reverse transcription-polymerase chain reaction (RT-PCR) technique. Endochitinase gene expression was then observed for different *Trichoderma* isolates viz., *T. harzianum* (Th3 and Tv12) and *T. atroviride* (Ta5). Among the three, higher expression of endochitinase was observed in Th3and Tv12, whereas Ta5 showed lesser expression with respect to other strains. *ech-42* was purified using Ni-NTA protein coloumn.The molecular mass of the purified chitinase was estimated to be 32kDa by SDS gel electrophoresis and assayed with 1% (w/v) colloidal chitin as substrate for activity. Chitinase was optimally active at pH 5.0 and at 25℃. The enzyme was stable from pH 1-5 and upto 40℃.The in vitro assay was conducted with purified chitinase concentration ranging from 50 μg $ml^{-1}$ -200 μg $ml^{-1}$ which showed antifungal actively against the hyphal growth of all major fungal pathogens viz., *Sclerotinia sclerotiorum* , *Sclerotium rolffsii*, *Fusarium oxysporum* f. sp. *lycopersici*, *Alternaria brassicae*, *Alternaria brassicicola*, *Magnaportha grisea*, *Fusarium fujikori* etc. The zone of inhibition was visual at the outer line of the colony at 150 μg $ml^{-1}$. and above. The enzyme caused loss of cellular components, necrotic lesions, hyphal cell lysis at the concentration of 200 μg $ml^{-1}$ also analysed microscopically.

### P03.102 Field performance of *Trichoderma harzianum* (Th3*)* against major diseases and growth promotion in different crops in Rajasthan, India

*P. Sharma, P. Jambulkar, O. P. Gangwa, V. Prakasham and K. Sarvanan*

*Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012*
*Email: psharma032003@yahoo.co.in*

*Trichoderma* Technology developed at Biocontrol Laboratory, Division of Plant Pathology, IARI, New Delhi was successfully demonstrated in different districts of Rajasthan viz., Jaipur Kota and Banswara against major fungal pathogens viz., *Sclerotinia sclerotiorum, Sclerotium rolffsii, Fusarium oxysporum* f. sp. *lycopersici, Aspergillus niger, Alternaria brassicae, Alternaria brassicicola, Alternaria porri, Pythium* spp., *Rhizoctonia* spp. The objective of the study was to evaluate and understand the colonization of *Trichoderma harzianum* (Th3) in the root zone of different crops grown in different season (Rabi and Kharif) and its survival. Th3 was used in field through soil, seed, seedling and foliar application. Keeping this in view field performance of *Trichoderma harzianum* (Th3), was evaluated in terms of its rhizosphere colonization and competence, survivability (root colonization behaviour) in different crops including cereals (Rice, Wheat, Maize, Pearl millet, Barley), Legume (Chickpea), oilseed crops (Groundnut, Soybean, Mustard, Linseed), Vegetables (Cauliflower, Brinjal, Okra, Pea, Potato, Tomato, Chilli, Garlic, Onion, Coriander, Fenugreek) Ornamental crops (Rose, Marigold) and fruit (Watermelon) of both winter and summer season. Field trials on seed treatment with powdered bioformulation of *Trichoderma harzianum* (Th3) @ 4-5 g/kg seed, followed by spray of liquid bioformulation (Th3) @ 4-5 ml/l were conducted. Populations of *Trichoderma harzianum* (Th3*)* were isolated on Trichoderma Selective Medium (TSM), was not only found antagonistic to pathogen but also showed colonizing behavior in rhizosphere. The association of *Trichoderma harzianum* (Th3) with rhizosphere were measured by periodic observation of rhizosphere competance and survivability at three different stages of crops (seedling, flowering and pre-harvesting stage).

**P03.103 Phosphate starvation response regulator phoP regulates the fengycins production in *Bacillus subtilis* NCD-2**

Q.G. Guo, W.X. Dong, SH.Z. Li, X.Y. Lu, X.Y. Zhang and P. Ma
*Plant Protection Institute, Hebei Academy of Agricultural and Forestry Sciences, IPM Center of Hebei Province, Key Laboratory of IPM on Crops in Northern Region of North P.R. China, Ministry of Agriculture, Baoding, Hebei, 071000, P. R. China*
*Email: gqg77@163.com*

*phoR/phoP* two-component systems, a signal transduction system that controls the phosphate starvation response in *Bacillus subtilis*, can induce or repress Pho regulation genes transcription under the phosphate starvation conditions. PhoR is a histidine kinase and PhoP is a response regulator. In this study, the *phoP* was deleted in *B. subtilis* NCD-2, a key component of the biocontrol agent against plant soil-borne diseases. Degradation of organic phosphorus assay showed that the *phoP* deletion mutant was deprived of the degradation ability for organic phosphorus and significantly decreased the growth ability in organic phosphorus medium compared to strain NCD-2 wild type. However, capabilities of the organic phosphorus degradation and the growth could be restored after complementing the intact *phoP* to the mutant. The *phoP* deletion mutant significantly decreased the inhibition ability against the growth of *Botrytis cinerea* by dual culture assay. The lipopeptides were extracted and analyzed by Fast Protein Liquid Chromatography (FPLC), compared to that of strain NCD-2 wild type, the *phoP* deletion mutant almost lost the fengycins producing ability. Bioassay indicated that the lipopeptides extracted from the *phoP* deletion mutant not only decreased the antifungal activity against the growth of *B. cinerea in vitro*, but also significantly decreased the suppression ability against tomato grey mold on detached tomato leaves. Taken together, results indicated that the phosphate starvation response regulator PhoP greatly regulated the fengycin production in *B. subtilis* NCD-2.

**P03.104 *gacS* regulation of antifungal metabolite production in plant disease suppressive *Pseudomonas fluorescens* FD6**

Q.X. Zhang[1, 2], L. Chang[1], P.R. Harvey[2, 3] and L.Q. Zhang[4]
[1]*College of Horticulture and Plant Protection, Yangzhou University, Yangzhou, 225009, Jiangsu, P.R. China;* [2]*CSIRO Ecosystem Sciences and Sustainable Agriculture Research Flagship, Waite Campus, PMB2, Glen Osmond, 5064, SA;* [3]*Biology Research Institute of Shandong Academy of Sciences, Jinan, Shandong, 250014, P.R. China;* [4]*College of Agriculture and Biotechnology, China Agricultural University, Beijing 100193, P.R. China.*
*Email: zqx817@126.com*

*Pseudomonas fluorescens* FD6 was isolated from a canola rhizosphere in Fujian Province (China) and showed significant *in vitro* growth inhibition of the plant pathogenic fungi *Botrytis cinerea* and *Monilinia* spp. We demonstrated that strain FD6 produces the antifungal secondary metabolites pyrrolnitrin (prn), pyoluteorin (plt), 2,4-diacetylphloroglucinol (phl) and hydrogen cyanide. It was reported that the two-component regulatory system comprised of the sensor kinase, GacS, and its response regulator, GacA, is involved in regulation of secondary metabolism. In order to investigate regulation of metabolite production by the *gacS* gene, we constructed a *gacS* insertion mutant FD6-MS by homologous recombination. Production of prn, plt, HCN, proteinase and siderophores were all decreased by disruption of *gacS* function in FD6-MS. Disease suppressive efficacies of FD6-MS against *Monilinia* spp. and *B.*

*cinerea* in respective detached peach and tomato fruit bio-assays were also significantly less than those of wild type FD6. Complementation of FD6-MS with functional *gacS* to form strain FD6-S, resulted in metabolite production and disease suppressive efficacies being restored to levels comparable to those of the wild type strain. These results indicated that *gacS* regulates the production of metabolites involved in pathogen antibiosis and disease suppression by FD6. In order to isolate specific regulatory genes for prn, a promoterless *lacZ* gene was fused to the *prnA* gene to form FD6-*lacZ: prnA*, capable of expressing *lacZ* upon induction of *prnA*. Tn5 random mutagenesis was used to identify the *prnA* expression mutant FD6-ΔA, characterized by reduced production of siderophores and inability to produce prn and proteinase. Recovery of *Tn*5 mutated sequences from FD6-ΔA and complementation to form strain FD6-A restored production of these metabolites. Wild type FD6, the *prn* expression mutant FD6-ΔA and its complement FD6-A all displayed similar growth rates and *in vitro* inhibition of *B. cinerea* and *Rhizoctonia solani*. Therefore, secondary metabolites other than prn and proteinase may also feature in pathogen antibiosis by *Ps. fluorescens* FD6.

### P03.105 Development of q-PCR marker for *Trichoderma* spp. Tr905, Tr904 and LTR-2

*Q.X. Zhang*[1,2], *B.E. Stummer*[2], *R.A. Warren*[2], *H.T. Yang*[3] *and P.R. Harvey*[2,3]
[1]*College of Horticulture and Plant Protection, Yangzhou University, Yangzhou, 225009, Jiangsu, P.R. China;* [2]*CSIRO Ecosystem Sciences and Sustainable Agriculture Research Flagship, Waite Campus, PMB2, Glen Osmond, 5064, South Australia, Australia;* [3]*Biology Research Institute of Shandong Academy of Sciences, Jinan, 250014, Shandong, P.R. China*
*Email: zqx817@126.com*

*Trichoderma* spp. are known to suppress a range of disease caused by plant pathogenic fungi. Here we report on the development of quantitative strain-specific genetic markers for *T. harzianum* Tr904 and LTR2 and *T. pseudokoningii* Tr905, strains previously shown to suppress the development of cereal root diseases caused by the soil-borne pathogens *Rhizoctonia solani* and *Pythium irregulare*. Inter- and intra- specific amplified fragment length polymorphism (AFLP) analysis of *Trichoderma* genotypes was used to identify potential strain-specific fragments for LTR-2, Tr904 and Tr905. Sequences of isolated fragments were used to design primers to develop quantitative PCR (qPCR) assays for each strain. Using DNA extracted from known amounts of conidia, the *in vitro* detection limits of the optimized qPCR assays for each strain were calculated to be between 10-20 conidia. Detection limits in soil were determined via individual inoculation in alkaline sand (Warramboo, pH 8.9) and acidic loam (Temora, pH 6.0) cereal cropping soils. Inoculant survival was monitored over an 8-week period via qPCR and microbiological assessments. The lower detection limits of the qPCR assays varied between 1 $X10^4$ (Tr904, Temora) – $6\times10^5$ (Tr905, Temora) conidia per gram of soil (c $g^{-1}$ soil). Inoculant soil colonisation and persistence over the 8 week incubation period varied slightly among the 3 strains. In both soils, inoculum of LTR-2 and Tr904 increased 10 fold to around $4\times10^6$ c $g^{-1}$ soil at 4 weeks. After 8 weeks Tr904 inoculum remained unchanged, whilst that of LTR-2 had decreased to $1\times10^6$ c $g^{-1}$ soil. Tr905 inoculum increased around 100 fold ($4\times10^6$ c $g^{-1}$ soil) by 4 weeks in both soil types, maintained this level in Warramboo soil and doubled ($8\times10^6$ c $g^{-1}$ soil) in Temora soil after 8 weeks.

### P03.106 Impact of interaction of *Cercospora beticola* and *Pyrenophora teres* on their potential for survival

*R.T. Lartey*[1], *T.C. TonThat*[1], *A.O. Salami*[2], *S.L. Hanson*[1], *E. Balogh*[3] *and S. Ghoshroy*[3]
[1]*USDA/ARS, Northern Plains Agricultural Laboratory, 1500 North Central Ave. Sidney, MT 59270, USA;* [2]*Dept Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria;* [3]*The Electron Microscopy Center, Dept. of Biological Sciences. Uni. of South Carolina. Columbia, SC 29208*
*Email: robert.lartey@ars.usda.gov*

Sugar beet and barley are two commonly rotated major crops in the Northern Great Plains (NGP) of the US. Cercospora leaf spot (CLS) caused by *Cercospora beticola* and net blotch caused by *Pyrenophora teres* are major foliar diseases of sugar beet and barley, respectively, and also occur very frequently in the region. Both disease pathogens survive in infested host tissues in the soil. Rotation is highly recommended to manage both diseases by reducing inoculum potential. Experiments were conducted to study interaction of the two pathogens and their potential impact on each other's survival. In petri dish experiments, growth of *C. beticola* was significantly inhibited when it was paired against isolate of *P. teres* under dark conditions. However, *P. teres* failed to establish contact with *C. beticola* when the cultures were exposed to light. Microscopic investigation has revealed cercosporin in *Cercospora* hyphal strands prior to contacts. Hyphal strands of both fungi were without noticeable damage under darkness. However under light, significant hyphal damages were observed on *P. teres* indicating *C. beticola* -induced structural damage of *P. teres* hyphal cells prior to actual physical contact. These observations indicate that the two pathogens were able to successfully antagonize each other under specific abiotic condition (*P. teres* under darkness and *C. beticola* under light). Our results suggest that potential manipulation of abiotic condition may lead to successful management of primary inoculum of both pathogens.

**P03.107 Investigation of *Peniophora nuda* for biological control of *Cercospora beticola* and *Pyrenophora teres***

R.T. Lartey[1], A.O. Salami[2], T.C. TonThat[1], E. Balogh, S.L. Hanson[1] and S. Ghoshroy[3]
[1]USDA/ARS, Northern Plains Agricultural Laboratory, 1500 North Central Ave. Sidney, MT 59270. USA; [2]Dept. Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria; [3]The Electron Microscopy Center, Dept. of Biological Sciences. Uni. of South Carolina, Columbia, SC 29208
Email: robert.lartey@ars.usda.gov

*Peniophora nuda* (Fr.) Bres. belongs to the class Agaricomycetes under the phylum Basidiomycota and has been isolated in Europe, Great Britain, North America, Hawaii (US in Oceania) and New Zealand. Net blotch caused by *Pyrenophora teres* and Cercospora leaf spot (CLS) caused by *Cercospora beticola* are major foliar diseases of barley and sugar beet. The two diseases also occur in the Northern Great Plains (NGP) of the US and are frequently controlled by resistance selection and fungicides applications. We present first studies on potential of *P. nuda* to control *C. beticola* and *P. teres*. To investigate potential for biological control of *C. beticola* and *P. teres* by *P. nuda*, studies on standard inhibition were first conducted. In petri dish experiments, growth of *C. beticola* and *P. teres* were significantly inhibited when they were paired against isolate of *P. nuda*. Cercosporin, a broad spectrum toxin that is produced by *Cercospora* species and that is toxic to several fungi, was not able to protect *C. beticola* from being overwhelmed by *P. nuda*. In follow up studies on the basis for inhibition of the two pathogens by the potential biological control agent, electron microscopic investigations revealed structural damages of hyphae of the two pathogens after initial physical contact with *P. nuda*. Our results identified *P. teres* as a potential biological control agent to manage CLS of sugar beet and net blotch of barley.

**P03.108 *Streptomyces lydicus* JZB117: a novel natamycin-producing strain for control of plant fungal diseases**

W.C. Liu, D. Dong, H.L. Wu, C.G. Lu, D.P. Zhang and T. Liu
Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Haidian District, Beijing 100097, P.R. China.
Email: liuwich@163.com

Natamycin is a polyene macrolide antibiotic with a strong inhibitive activity against plant pathogenic fungi. Total 4 species of *Streptomyces*, *S. chattanovgensis*, *S. natalensis*, *S. gilvosporeus* and *S. lydicus*, were reported as natamycin producers to date. JZB117 was a high yield mutant bred by the complex mutation with UV ray and $LiCl_2$, and then the genome shuffling using *S. lydicus* A01 and A02 as original strains. The fermentation in flask and 30L fermentation tank with fed batch method showed that the natamycin yields of strain G117 were increased by 38.86%-55.78% and 26.59%-30.74% over that of strain A01 and A02 respectively, and the fermentation time was shortened 48 hours. Thus the total productivities of G117 were increased by 131.48%-159.62% in flask and 90.13%-110.95% in fermentation tank by comparison with the original strains. There were some changes in cultural, physiological and biochemical characteristics for G117, such as not being able of producing yellow pigment on Gause's synthetic agar medium, increasing the abilities to hydrolyze starch and use rhamnose, and reducing the abilities to use sucrose, mannitol, sorbitol and mannose. In RAPD experiment, some specific amplification bands were obtained from G117 but not form A01, A02 and other 3 natamycin-producing strains. In 16S rDNA analysis, G117 presented the high genetic similarity of 99% comparing with A01, A02 and CGMCC4.1412, the type strain of *S. lydicus*, and the relative low similarity with other 3 natamycin-producing strains. The above researches suggested that G117 was a novel natamycin-producing strain.

**P03.109 *Bacillus subtilis* SWS-11 antibacterial activity against fungi and bacteria**

H.Z. Zhou, K. Guo, Q. Chen, Y.L. Wei, J.D. Hu, J.S. Li, X.Q. Wu and H.T. Yang
Biotechnology Center, Shandong Academy of Science, Shandong Provincial Key Laboratory for Applied Microorganism, No. 19 Keyuan Road, Lixia District, Jinan, 250014, P. R. China
Email: xq_wu2008@163.com

*Bacillus subtilis* strain SWS-11 was isolated from soil and showed strong inhibitory effect of pathogenic microorganism. The data showed that SWS-11 strongly inhibited *Thielaviopsis basicola*, *Aspergillus niger*, *Bacillus cereus*, *Eschechia coli* and *Salmonella enterica* at the concentration of $10^7$, $10^7$, $10^8$, $10^6$ and $10^6$, respectively. The inhibition zone diameter was ranging from 14.5 to 22.1 mm on PDA or LB plates. The similar observation was noted using the lipopeptides isolated from the cultural supernatant of SWS-11 by the methods of hydrochloric acid precipitation and methanol extraction. The extraction effectively inhibited mycelial growth and conidia germination of a wide range of phytopathogenic fungi. The lipopeptides were stable to high temperature, wide range pH, proteinases and organic solvents. ESI-MS analysis of the lipopeptides included iturin, surfaction and fengycin. Surfactin is not only a highly active surfactant, but also exhibits anti-viral and anti-mycoplasma activity. Iturin and fengycin can obviously inhibits a broad wide of fungal pathogens. In addition, surfactin enhanced the antibiotic activity of fengycin.

This indicated the synergistic effect of surfactin on the biological properties of fengycin. The optimal composition applied by Uniform Design was 40 g Ł$^{-1}$ corn powder, 10 g Ł$^{-1}$ soybean cake powder, 5 g Ł$^{-1}$ urea, 45 mmol L$^{-1}$ $PO_4^{3-}$, 0.5 mmol L$^{-1}$ $Mn^{2+}$, 0.2mmol Ł$^{-1}$ $Mg^{2+}$, 1 μmol·L$^{-1}$ $Fe^{2+}$, 0.5 μmol·L$^{-1}$ $Ca^{2+}$, 33 ℃. The production of the lipopeptides increased from 2.1 g Ł$^{-1}$ to 3.2 g Ł$^{-1}$. In all *Bacillus subtilis* SWS-11 is a biocontrol agent for commercial applications.

### P03.110 cDNA-AFLP analysis of differentially expressed genes related to mycoparasitism in *Trichoderma harzianum* LTR-2

*X.J. Zhang[1], P.R. Harvey[1,2], R.A. Warren[2], B.E. Stummer[2] and H.T. Yang[1]*
*[1]Institute of Biology, Shandong Academy of Sciences, No.19 Keyuan Road, Jinan, 250014, P. R. China; [2]CSIRO Sustainable Agriculture National Research Flagship, CSIRO Ecosystem Sciences, PMB 2 Glen Osmond, South Australia, 5064, Australia*
*Email: yanght@sdas.org*

In the present study, cDNA-amplified fragment length polymorphism (cDNA-AFLP) analysis was used to identify differentially-expressed genes of *Trichoderma harzianum* LTR-2 during *in vitro* mycoparasitic interactions with the plant-pathogenic fungus *Rhizoctonia solani*. Changes in LTR2 gene expression were analysed by comparing expression profiles in the challenged (LTR2-*R. solani* interaction) and unchallenged (LTR2 only) control. LTR2 cDNA-AFLP experiments were optimized *in silico* using the program AFLPinSilico. Double-stranded cDNAs were digested with restriction enzymes *Pst*I and *Hae*III. Selective amplifications were performed with combinations of *Pst*I and *Hae*III primers with 1 and 2 additional bases at the 3' ends, respectively. Sixty four primer pair combinations were used in cDNA-AFLP and resolved 2,560 fragments derived from the mRNA of LTR-2 challenged with *R. solani*. A total of 255 transcription-derived fragments (TDFs) were differentially expressed (145 up-regulated and 110 down-regulated) in LTR2. To date, DNA sequence data have been obtained for 21 differentially expressed TDFs. Sequence similarities to known genes or sequences in the NCBI was found for 15 LTR2 TDFs. These differently-expressed cDNA fragments showed homologies to fungal translational repressor, kinase-activating protein, vesicle fusion factor, dipeptidyl aminopeptidase, phosphopantetheine binding protein and heavy metal exporter genes. Sequences of the remaining TDFs could not be assigned to genes with known functions. Sequence analysis of TDFs is on-going and is being used to identify genes related to mycoparasitic properties of LTR2.

### P03.111 The biocontrol characteristics of nonpathogenic *Fusarium oxysporum* strain FJAT-9290

*B. Liu, R.F. Xiao, Y.Y. Yang, Y.J. Zhu and X.F. Zheng*
*Agricultural Bio-resources Research Institute, Fujian Academy of Agricultural Sciences, Wusi Road 247, Fuzhou 350003, P.R. China*
*Email: fzliubo@163.com*

*Fusarium oxysporum*, the pathogen of *Fusarium* wilt diseases, can cause the plant wilt and induce serious economic losses on the crops. The nonpathogenic *F. oxysporum* strain FJAT-9290, isolated from tomato root, was showed to effectively inhibit activity of pathogenic *F. oxysporum*. The host range tests showed that all plants including five families (Solanaceae, Cucurbitacea, Musaceae, grass and lily) grow normally and didn't appeared wilt symptoms within 120 days when were inoculated by strain FJAT-9290. However, there were obvious differences on the colonization tests. By identifying the isolates from different plants using specific primers P12-R1/P12-F2, the nonpathogenic strain FJAT-9290 could colonized in the stem of tomato and sweet pepper, banana, melon after 5 days and 10 days inoculation, respectively. The growth rate of strain FJAT-9290 had no significant difference compared with pathogenic strains. Growing on the culture used citrus pectin or carboxymethyl-methyl cellulose sodium (CMC)as the carbon source, strain FJAT-9290 could produce five types of cell wall degradation enzyme (CWDEs): poly-galacturonic acid enzyme (PG), pectin methyl galacturonic enzyme (PMG), cellulase (Cx), poly galacturonic acid trans-eliminating enzyme (PGTE), pectin methyl-trans-elimination of the enzyme (PMTE). However, the CWDEs appeared higher activity cultured used citrus pectin as the carbon source. The activity of peroxidase (POD), polyphenol oxidase (PPO) and beta-1, 3-glucanase enzymes of the tomato plants could increase significantly after inoculating the strain FJAT-9290. Bioassay experiments revealed the growth of the potted tomato seedlings treated by the strain FJAT-9290 were promoted with higher plant height and more leaves than the seedlings in control. The control efficiency treated by strain FJAT-9290 was 76 %.

### P03.112 Biocontrol of Cucumber powdery mildew (*Sphaerotheca fuliginea*) with *Verticillium lecanii*

*Y. Zhou[1], T.T. Zhang[1], H. Yan[1] and X.X. Li[1,2]*
*[1]Institute of Plant & Environment Protection, BAAFS, No.9 Shuguanghuayuanzhonglu, Haidian District, Beijing, 10097, P.R. China; [2]Hebei University of Science and Technology, Shijiazhuang, 050018, P.R. China.*
*Email: yanhong0219@yahoo.com.cn*

Cucumber powdery mildew is one of the most important diseases in the cucumber grown region. The indoor artificial inoculation tests as well as the field tests of Cucumber powdery mildew were fulfilled, using several solutions cultured by *Verticillium lecanii* in PD liquid including spore suspension, centrifugal supernatant liquid

and crude toxin. On the conditions of the indoor artificial inoculation test, control effects on cucumber powdery mildew of the spore suspension's concentration $10^7$/ml, $5\times10^6$/ml, supernatant liquid diluted 50, 100 times, the crude toxin solution diluted 50, 100, 200 times approximate to 57.6% and 60.0% ($p>0.01$), 71.3% and 45.3% ($p<0.01$), 65.8%, 59.3%, and 83.3 % ($p<0.01$) respectively. The control effect of crude toxic solution diluted 200 times excel remarkably those of pharmaceutical Bacillus (62.0%), supernatant liquid and the spore suspension ($p<0.01$). Based on the data from field experi- ment, toxin and *Bacillus* control effect on cucumber powdery mildew are 85.35% and 91.54%($p>0.01$), both has a significant advantage over the spore suspension (52.82%) ($p<0.01$). Comparing with the control group, the results were different significantly statistically ($p<0.01$).

**P03.113 Potential of *Trichoderma atroviride* and *T. viride* to control *Botrytis cinerea*, the cause of tomato grey mold**

*Y. Wang, W.L. Wang, W. Gao, C.X. Zhang, C.Y. Liu, Y.J. Hao and J.F. Huo*
*Tianjin Plant Protection Institute, Tianjin, 300381, P. R. China*
*Email:wangyongwb@126.com*

*Trichoderma atroviride* Tr1208 and *Trichoderma viride* Tr9701 were tested for their efficacy in controlling tomato grey mold (caused by *Botrytis cinerea*) *in vitro* and in the field. Isolates of Tr1208 and Tr9701 produced both volatile and non-volatile antibiotics that suppressed mycelial growth of the pathogen. Light micro-examination showed that micro-parasitism by *Trichoderma* spp. of *Botrytis cinerea* resulted in penetration and disruption of hyphal cells, and disturbing of cytoplasm of the pathogen. The cultures and filtrates of Tr1208 and Tr9701 in wheat powder liquid medium, Gause's synthetic liquid medium, soybean powder liquid medium, corn extract liquid medium, Richard's liquid medium, Czapek's liquid medium, Martin's liquid medium, soil extract liquid and corn powder liquid medium suppressed conidial germination of the pathogen, and the germination level was negatively correlated with the duration of culture of *Trichoderma* sp.. The effect of suppressing *B. cinerea* is the best which was cultivated by wheat powder liquid medium 4 days, after the antagonists were inoculated. Re-isolation from inoculated tomato shoots demonstrated that the pathogen had been suppressed by *Trichoderma*. The ability to re-isolate the pathogen from fruits of tomato after co-inoculation and pre-inoculation with *Trichoderma* spp. was reduced by 15.5-42.3% and 22.2-47.1%, respectively. The biocontrol field trial suggested that the *B. cinerea* on tomato fruits and leaves had been efficiently controlled by the application of spore suspensions of *T. atroviride* Tr1208 and *T. viride* Tr9701. The efficacy of control by *Trichoderma* is similar to that of routine chemical control.

**P03.114 The effect of soil environment on the clubroot**

*Y.L. Luo[1,2], Y.P. Ying[1], D.W. Dong[2], X.Y. Wang[2], H. Jiang[2], Y.F. Yan[2] and Z.K. Wang[1*]*
*[1]Bioengineering College of Chongqing University, Chongqing, 40030, P. R. China; [2]Fuling Agricultural Science Institute of Chongqing, 40030, P. R. China*
*Email: luoyuanli163@163.com*

*Brassica juncea var. tumida* Tsen (Cruciferae), known as Green mustard, which is special vegetable in China. Green mustard is the raw material for one of the pickles (Fuling pickle) in the world. The picked mustard is very popular for their special flavor and nutritional value. Fuling pickle is the largest production scale, the most powerful traditional pillar industries and competitive industries in Fuling District rural economy. But in recent years, the yield of Green mustard has been seriously affected by clubroot disease caused by *Plasmodiophora brassicae*, which has caused yield losses. Although chemical measures make some progress in control of clubroot, but it cannot eliminate clubroot and cause environmental pollution to some extent. Breeding of clubroot resistance cultivars (CR) is the top priority for the disease control. But the CR is very scarce and the resistance is multigenic and may be overcome by new virulent *P. brassicae* strains. In recent years, many researchers focus on the effect of soil environment on the clubroot. We find that there is huge difference in N, P, K, organic matter content in various soil of different degree of the disease. We also construct bacterial 16S rRNA library and fungus 18S rRNA library to find difference in microbial distribution in various soil of different degree of clubroot. At present, the study is under progress. We hope to find a comprehensive control measure to reduce the yield loss caused by *P. brassicae.*

**P03.115 Identification and utilization of AM fungi in biological control**

*T.T. Wang, K. Li, Y.P. Wang and J.H. Guo*
*Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University; Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education; Nanjing 210095, P.R. China*
*Email: ypwang@njau.edu.cn*

Arbuscular mycorrhizal fungi (AMF) symbiosis confers numerous benefits to host plants, and they are affirmed to promote plant growth and improve yield and quality, control or alleviate impact of plant diseases in various plant hosts particularly diseases caused by various soil-borne pathogens. In this study, we investigated the diversity and structure AMF in the tobacco rhizosphere soil sampled from Guizhou province in southwest China. Wet-sieving and centrifugation method was combined with nested PCR and denaturing gradient gel electrophoresis (DGGE) for the diversity analysis. And four

genera and twenty AMF species were identified using the wet-sieving and centrifugation method and the most species encountered were *Glomus* species. In the following greenhouse experiments, ten AMF strains were selected to evaluate the effects of AMF on tobacco plants. The results indicated that most of the AMF strains could inoculate on the tobacco roots very well, and could form mycorrhizal symbiosis to improve tobacco growth performance. Three strains were found to control tobacco wilt disease caused by *Ralstonia solanacearum* effectively. These results indicated that AMF may be used to promote plant growth and health.

**P03.116 Production and formulation of *Trichoderma harzianum* T6C to control soilborne plant pathogens**

*J.M. Zapiola, R.A. Rojo, V. Barrera and L. Gasoni*
*Instituto de Microbiología y Zoología Agrícola, Instituto Nacional de Tecnología Agropecuaria, Los Reseros y Las cabañas, (1712) Castelar, Argentina*
*Email: gasoni@cnia.inta.gov.ar*

Large scale biocontrol agent utilization is influenced by the production process (cost, productivity, etc) and the ability to maintain a large numbers of viable and active propagules during the bioproduction cycle, from production to commercialization and utilization. The objectives where: 1) To obtain an optimized culture media for conidia production, 2) To assess the influence of additives on conidial viability up to six months, 3) To select the proper packaging to maintain conidial viability during the storage period. Media used to cultivated *T. harzianum* strain T6C was not optimized for conidial production. As a first approach, we selected four different media with variable carbon source (FC), nitrogen source (FN) and C/N ratio (CN). The best performer was selected for further analysis.. Total FC concentration without maintaining the CN ratio and trace element concentration were further optimized. Isolates of fungal strain were produced in shake flasks and then formulated by adding different compounds. Formulations were assessed up to six month of storage period at 25 ºC. Four different packages were utilized to store the formulations in proper conditions. As a result we have obtained the productive media, more economic and also a pre-selected package type to maintain the conidial viability up to six months.

**P03.117 Soil type affects efficacy of a microbial biocontrol agents in the control of cotton root rot**

*E. Dilfuza*
*Department of Microbiology and Biotechnology, Faculty of Biology and Soil Sciences, National University of Uzbekistan, Uzbekistan*
*Email: egamberdieva@yahoo.com*

Many strains of *Pseudomonas* and *Bacillus* have been shown to be potential biocontrol agents against fungal pathogens. Soil types are found to affect on biological control abilities of bacterial strains. In our experiments soil types effect on bacterial abilities to control cotton root rot caused by *F. oxysporum*. Infestation of the soil with *F. oxysporum* resulted in an increase of the percentage of diseased plants from 69 to 76 in two different soils. Priming of seedlings with the four selected bacterial strains *P. alcaligenes* PsA15, *B. amyloliquefaciens* BcA12, *B. polymyxa* BcP26 and *M. phlei* MbP18, previously isolated from salinated soil reduced this proportion to as low as 26 % in sierozem soil and 39% in cambisol soil in comparison to the non-inoculated control. However, the bacterial strains were more effective in sierozem soil than in cambisol soil. Probably the physiological adaptation of bacterial strains supported their beneficial activity much better in soil from where they have isolated. Thus our results suggest that for obtaining effective bacterial strains, they must be screened and isolated from the pool of indigenous soil bacteria, which supposedly are adapted to the particular climatic conditions of the site conditions of practical applications.

**P03.118 Biological control of damping-off of cotton caused by *Rhizoctonia solani* with rhizobacteria in saline soil**

*E. Dilfuza*
*Department of Microbiology and Biotechnology, Faculty of Biology and Soil Sciences, National University of Uzbekistan*
*Email: egamberdieva@yahoo.com*

High salinity of soils in arid and semi arid regions results in desertification and decreased crop yield. In such conditions plants become more vulnerable to diseases caused by pathogenic fungi. Under these conditions one possible solution is to use bacterial inoculants which can control diseases (biological control agents), increase plant growth, speed up seed germination, improve seedling emergence and protect plants from the deleterious effects of some environmental stresses including drought and salt. The aim of the present work was to test selected root colonising bacteria for their ability to promote cotton plant growth and control damping-off of cotton caused by *Rhizoctonia solani* in salinated soil. The bacterial strains *Pseudomonas alcaligenes*, *P. mendocina*, *P. putida*, *P. chlororaphis*, *P. extremorientalis*, fungal strain *Rhizoctonia solani* and cotton where used in this study. Soil was sampled from irrigated agricultural site of Syrdarya province, north-east of Uzbekistan. Electrical conductivity (EC) values of saline soil were 6.2 dS/m. Infestation of the soil with *R. solani* resulted in an increase of the percentage of diseased plants from 53 to 75. Selected bacterial strains, reduced this proportion to as low as 17 % and also stimulated cotton growth. The rifampicin-resistant mutants of effective strains were able to survive in the rhizosphere of cotton due to their per-

sistence in saline soil condition. These results are promising for the application of selected environmentally save microbes in protecting plants against soil-borne pathogens in saline agricultural soils.

**P03.119 Evaluation of two newly isolated *Bacillus* strains from Pakistan for their broad-spectrum antagonistic activity against multiple plant pathogens and analysis for their lipopeptide compounds**

F.U. Rajer, Y.L. Xie, H.J. Wu and X.W. Gao
*Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, Key Laboratory of Monitoring and Management of Crop Diseases and Pest Insects, Ministry of Agriculture, Nanjing, 210095, P. R. China*
*Email: faheemrajer@gmail.com*

Biological control of plant diseases using potential antagonistic microorganisms is an eco-friendly alternative in plant protection. However, selection of effective biological control agents (BCAs) is the initial step and need to be focused particularly. The aim of this study was to identify new BCAs with broad-spectrum antagonistic activity against multiple plant pathogens. A total of 44 *Bacillus* spp., were isolated from rhizosphere of tomato (*Solanum lycopersicum* Mill.) grown in Pakistan. Their antifungal and antibacterial activities and production of their lipopeptide compounds were evaluated *in vitro*. Screening of *Bacillus* spp. was carried out using dual culture and agar diffusion techniques. TM10 and TM26 out of ten isolates showed greater antagonistic activity towards *Sclerotinia sclerotiorum* and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), respectively. Strain TM10 suppressed both the pathogens, while TM26 antagonized *Xoo* only. Furthermore, MALDI TOF Mass Spectrometry analysis of TM10 resulted in the detection of bacillomycin L. We aim to apply these potential isolates as bio-pesticides in future.

**P03.121 The potency of some indigenous bacteria to inhibit the growth of *Xanthomonas oryzae* pv. *oryzae* causing rice bacterial leaf blight**

N.R. Mubarik[1], Zuraidah[2] and Y. Suryadi[3]
*[1]Departement of Biology, Faculty of Mathematics and Natural Science, Jalan Agatis, IPB Dermaga, Bogor 16680, Indonesia; [2]Department of Biology, Tarbiyah Faculty, IAIN Ar-Raniry, Ring Road Complex of IAIN Ar-Raniry, 23111 Darussalam, Banda Aceh, Indonesia; [3]Biotechnology Center for Agriculture and Genetic Resources, Tentara Pelajar 3A, Bogor 16111, Indonesia*
*Email: nrachmania@ipb.ac.id*

Bacterial leaf blight disease (BLB) caused by plant pathogenic bacteria *Xanthomonas oryzae* pv. *oryzae* (Xoo) can lead to death of rice plants. Control of plant pathogenic bacteria can be performed using biological control agents. The aim of this research was to study the inhibitory ability of eight isolates indigenous bacteria against plant pathogenic bacteria *Xoo* in greenhouse conditions. Isolates of biological agents that are consisting of: *Pseudomonas aeruginosa* (C32a and C32b), *P. fluorescens* (Pf), *Serratia marcescens* (E31), *Bacillus* sp. (I.5), *Bacillus cereus* (I.21 and II.14), and *B. firmus* (E65). The research method consists of hypersensitivity test, antagonistic to *Xoo*, and in vivo application of biological agents in the greenhouse. Hypersensitivity test on tobacco plants using inoculum C32a and C32b showed characteristics of slightly leaf yellowing but did not cause necrosis. Injection using Xoo inoculum showed necrosis on tobacco leaves. Antagonist isolates i.e. C32a, C32b, and I.5 showed inhibitory activity against Xoo, whereas other isolates did not show inhibitory activity. In greenhouse experiments by using IR 64 rice plants grown in plastic pots and sprayed with biological control agents (107cfu/ml) at 7 days, 14 days, 28 days, and 42 days after planting showed that application of C32a, Pf, C32b, and I.21 isolates could suppress the lesion length of BLB better than chemical control (Nordox).

**P03.122 Biocontrol of cantaloupe damping-off disease using formulations of antagonistic fungi**

N.M. Sallam[1], S.N. Riad[2], M.S. Mohamed[1] and A.S. El eslam[2]
*[1]Plant Pathology Research Institute, Agric. Res. Center, Giza, Egypt; [2]Plant Pathology Dept., Fac. of Agric., Assiut Univ., Assiut, Egypt*
*Email: nashwasallam@yahoo.com (Sallam, Nashwa)*

Antagonistic capability of 19 isolates of fungi isolated from rhizosphere of cantaloupe plants were tested in *vitro* against growth of *Fusarium semitectum* isolate the causal pathogen of damping-off of cantaloupe. *Trichoderma viride* (isolate no.17), *T. harzianum* (isolate no. 19) and *Fusarium concolar* (isolate no. 4) showed the greatest percentage of inhibition against *F. semitectum*. The effect of carrier formulations of antagonistic fungi (talc based powder and rice bran) on damping-off of cantaloupe were tested under greenhouse and field conditions. In greenhouse experiment, application of antagonistic fungi two weeks before planting with rice bran formulation showed the highest percentage of survival plants in pre and post damping-off (83.33 and 75, respectively), whereas application of talc based powder formulation significantly increased percentage of survival plants at the time of planting in pre and post damping-off (91.67 and 75, respectively). In field experiments, application of tested formulations of antagonistic fungi to infested soil with *F. semitectum* at time of planting caused a higher percentage of survival plants in pre and post damping-off in both tested seasons (2009 and 2010) than application two weeks before planting in the disease. The numbers of antagonistic fungal propagules

were decreased gradually by prolonging the storage period. After six months of storage period, population of fungal propagules showed high reduction in approximately 50% expect in case of *T. viride* with talc formulation showed no propagules after five months. Only propagules of *F. concolar* appeared on rice and talc formulations up to eight months.

**P03.123 Biological control of Fusarium head blight in oat and wheat**

*Z. Omer[1], J. Fatehi[2] and A.C. Wallenhammar[1]*
*[1]Rural Economy and Agricultural Society /HS Konsult AB, PO Box 412, 751 06 Uppsala; [2]Lantmännen BioAgri AB, 756 51 Uppsala, Sweden*
*Email: Zahra.omer@hush.se*

Fusarium head blight (FHB) is a destructive disease of cereals causing significant economic losses worldwide. Apart from yield reduction, FHB results in accumulation of various mycotoxins such as deoxynivalenol (DON) in cereal grains, which is toxic to humans and animals. A shift towards increased incidence of the DON-producing species *F. graminearum* has been reported in different countries including Sweden. In organic farming the control of FHB is based on cultural practices and application of fungicides in conventional farming has shown conflicting results possibly due to variations in sensitivity of *Fusarium* species to chemicals. Development of biological control as an alternative environmentally friendly approach to combat FHB disease can be of great value to cereal production worldwide. In this study we aim to investigate the potential use of yeast and bacterial isolates and a commercial biocontrol product to control FHB and to minimize production of mycotoxins. A collection of microorganisms were obtained from diverse plant and soil samples. Two different climate chamber bioassays were developed to evaluate the antagonistic effect of isolates against *F. graminearum* infection in wheat and oat plants. The infection of coleoptile in seedlings and spikes in mature plants were evaluated for the effect of treatments. The disease severity was also determined as percentage of infected seeds. The preliminary results indicate presence of potential microorganisms capable of reducing FHB disease and DON content.

**P03.124 Application of chitooligosaccharides in plant protection**

*X.M. Zhao, H. Yin, W.X. Wang, M.Y. Wang and Y.G. Du*
*Liaoning Provincial Key Laboratory of Carbohydrates, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, No. 457 Zhongshan Road, Shahekou District, Dalian, 116023, P. R. China*
*Email: zhaoxm@dicp.ac.cn*

Chitin is homopolymer of β-1, 4-linked N-acetylglucosamine and chitosan is the product of deacetylation of chitin. Chitin and chitosan have been proved to be nontoxic, biodegradable, and biocompatible. However, their high viscosity and insolubility in neutral aqueous solution restrict their uses in vivo. Chitooligosaccharides prepared by enzymatic hydrolysis of deacetylated chitosan polymer is not only water-soluble, nontoxic, biocompatible but also possesses versatile functional properties. Chitooligosaccharides could induce crop wide resistance against fungal bacterial and viral disease. The results of field trials indicate that the controlling efficacy of chitooligosaccharides to wheat scab is 60% to verticillium dahliae of cotton 71% to pepper virus 75% to tomato Late blight 70% to Chinese cabbage soft rot 85% to apple scab 57% to papaya mosaic disease 76% to TMV 84.73%. In addition, chitooligosaccharides could activate plant cold resistance. Coldness could decrease the crisp fruit setting ratio, before the coldness happened in late spring, the fruit trees treated with 75 $mg.L^{-1}$. Chitooligosaccharides expressed chilling-tolerance activity, and the trees have a high fruit setting ratio. Chitooligosaccharides mixed with some fungicide could increase controlling efficacy of fungicide and decrease some fungicide consumption.

**P03.125 Interactive proteomics between biocontrol *Chaetomium globsoum* and chilli plants against damping off pathogen *(Pythium aphanidermatum)* (Edson) Fitz.**

*R. Senthil[1], D. Saravanakumar[1], M. Raveendran[2] and T. Raguchander[1]*
*[1]Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore – 641 003, Tamil Nadu, India; [2]Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore – 641 003, Tamil Nadu, India*
*Email: sensharuk008@gmail.com*

*Chaetomium globosum* TNAU-Cg6 is studied as an effective biocontrol agent for the management of plant diseases besides a good plant growth promoter. In the current study, the biocontrol efficacy of *C. globosum* was evaluated against damping-off disease caused by *Pythium aphanidermatum* in chillies under *in-vivo* and field conditions. The results showed the greater efficacy of *C. globosum* in reducing damping off disease under glass house and field conditions. Nevertheless, the molecular study on tripartite interactions between plant-pathogen-biocontrol agent is further warranted to understand the expression of proteins. Thus, the differential protein expression studies were carried out to understand the molecular responses of chilli seedlings primed with *C. globosum* against *P. aphanidermatum*. The differentially expressed proteins were identified using two-dimensional polyacrylamide gel electrophoresis and analyzed by mass spectrometry. The study showed the differential expression of 28 proteins in chilli seedlings treated with *C. globosum* and challenge inoculated with

*P. aphanidermatum*. The differentially expressed proteins were identified as Defensin like protein, WRKY6 transcription factor, Peroxidase, Endoglucanase 3 precursor, cytochrome P450, Ethylene-responsive transcription factor, UDP-glucose dehydrogenase, annexin-like protein, nodule-specific cysteine-rich peptide, oxidoreductase, 2OG-Fe(II) oxygenase family protein, putative NBS-LRR disease resistance protein, CASP-like protein, Ribulose-bisphosphate carboxylase large chain precursor and hypothetical protein. The MATRIX analysis of proteins revealed that the differentially expressed proteins have greater role in growth promotion and enhancing defense responses in chilli plants against *P. aphanidermatum*. The biocontrol potential of *C. globosum* is further evidenced from the present study through the characterization of growth and defense related proteins.

**P03.126 Biological control of *Monosporascus cannonballus* of muskmelon by *Talaromyces flavus***

*S. Keshavarzi, A. Sarpeleh and K. Behboudi*
*Department of Plant Protection, College of Agriculture & Natural Resources, Tehran Univercity, Karaj*
*Email: s.keshavarzi@alumni.ut.ac.ir*

Root rot and vine decline disease of muskmelon, induced by *Monosporascus cannonballus*, causes severe economic losses in many arid and semi-arid production regions worldwide. However, no effective controlling measures are presently available for this pathogen. In the present study, the potential of *T. flavus* was assessed on the control of *M. cannonballus in vitro* and *in vivo* conditions. *Talaromyces flavus*, a fungal antagonist, was isolated from soil samples collected from cucumber greenhouses in Varamin district, Tehran province, Iran. The biocontrol agent inhibited mycelial growth of *M. cannonballs* in dual culture, volatile and non- volatile extracts tests that were 81% and 73.2%, 89.1% respectively. *Talaromyces flavus* was then used to treat the muskmelon seedling before and after transplanting to pot soil already infested with *M. cannonballus* and reduced disease severity to 31.1% in soil drench test and 42.2% in root dip test compared to the control (80%). To assess the efficacy of the antagonist, the presence of *M. cannonballus* in the roots using molecular tools, as well as disease severity index, fresh weight and height of root and shoot were measured after 60 days post treatment. *Monosporascus cannonballus* was detected in all replicates using specific primers. The overall results of this study show that it may be possible to manage muskmelon Root rot and vine decline disease effectively by *T. flavus*. There is the first report of *Monosporascus* biocontrol with *T. flavus*.

**P03.127 The folate precursor *para*-aminobenzoic acid elicits induced resistance against *Cucumber mosaic virus* and *Xanthomonas axonopodis***

*G.C. Song[1,2], H.K. Choi[1], Y.S. Park and C.M. Ryu[1,2]*
*[1]Molecular Phytobacteriology Laborator, KRIBB, Daejeon 305-80, Republic of Korea; 2Biosystems and Bioengineering Program, University of Science and Technology, Daejeon, 305-350, Republic of Korea*
*Email: yp272@kribb.re.kr*

The use of vitamins including vitamin $B_1$, $B_2$ and $K_3$ for the induction of systemic acquired resistance (SAR) to protect crops against plant pathogens has been evaluated previously. The use of vitamins is beneficial because it is cost effective and safe for the environment. The use of folate precursors, including *ortho*-aminobenzoic acid, to induce SAR against a soft-rot pathogen in tobacco has been reported previously. In the present study, *para*-aminobenzoic acid (PABA, also referred to as vitamin Bx) was selected owing to its effect on the induction of SAR against *Xanthomonas axonopodis* pv. *vesicatoria* in pepper plants through greenhouse screening. Dipping of pepper seedlings in a 1 mM PABA solution in field trials induced SAR against artificially infiltrated *X. axonopodis* pv. *vesicatoria* and naturally occurring cucumber mosaic virus. Expression of the *Capsicum annuum pathogenesis-related 4* gene was primed in response to pathogen infection as assessed by quantitative real-time PCR. The accumulation of cucumber mosaic virus RNA was reduced in PABA-treated pepper plants at 40 and 105 d post-treatment. Unexpectedly, fruit yield was increased in PABA-treated plants, indicating that PABA-mediated SAR successfully protected pepper plants from infection by bacterial and viral pathogens without significant fitness allocation costs. The present study is the first to demonstrate the effective elicitation of SAR by a folate precursor under field conditions.

**P03.128 Control and growth promotion of PopW to cucumber downy mildew under green-house and field conditions**

*L. Zheng[1, 4], Y.M. Luo[1], Q.Y. Xue[3], S.M. Li[2], H.X. Liu[1] and J.H. Guo[1]*
*[1]Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, Engineering Center of Bioresource Pesticide in Jiangsu Province, Key Laboratory of Integrated Management of Crop Diseases and Pests( Nanjing Agricultural University) , Ministry of Education, Nanjing 210095, P.R. China; [2]Jiangsu Key Laboratory for Eco-Agri-cultural Biotechnology around Hongze Lake, School of Life Sciences, Huaiyin Normal University, Huai'an 223001, P.R. China; [3]Institute of Plant Resources and Environment, College of Life Sciences, Nanjing Normal University, Nanjing 210046, P.R. China; [4]Chinese Academy of Tropical Agricultural Sciences Guangzhou Experimental Station; Chinese Academy of Tropical Agricultural Sciences Tropical Energy and Ecology Research Centre，Guangzhou 510140, P.R. China*

*Email: hxliu@njau.edu.cn*

The PopW, a new harpin protein, was isolated from the bacterium *Ralstonia solanacearum* ZJ3721 and showed induced resistance to various plant diseases. In this study, the biocontrol efficacy of PopW against cucumber downy mildew caused by *Pseudoperonospora cubensis* was investigated and evaluated. The PopW at the concentration of 250 mg/L obtained the best biocontrol efficacy of 42.85% at the 15$^{th}$ day after pathogen inoculation under the greenhouse conditions. Meanwhile, PopW (250 mg/L) promoted the growth of cucumber and increased the content of chlorophyll in leaves of the plant. In the field, the biocontrol efficacy reached 41.87%, 48.36%, 53.21% and 50.85% at the 8, 25, 35 and 48 d after the first treatment of PopW respectively when PopW was sprayed every 15 days and applied three times in 2010. In 2011, the biocontrol efficacy reached 47.07%, 48.17%, 50.59% and 54.48% at the day of 8, 15, 22 and 27 d after the first application of PopW respectively when PopW was applied every 10 days. The results indicated that the PopW had the potential to be an effective biocontrol agent against *Pseudoperonospora cubensis*. Furthermore, PopW increased the yield of 33.01% per individual plant and significantly improved the quality of cucumber fruit with increased content of soluble sugar, soluble protein, free amino acids and vitamin C (18.07 mg/g, 6.80 mg/g, 60.20 mg/100 g, 68.27 μg/g, in PopW treatment and 10.21 mg/g, 5.30 mg/g, 59.06 mg/100 g and 49.55 μg/ g in water treatment respectively).

### P03.129 Biological control of *Heterodera avenae* in wheat root by use of *Bacillus pumilus* 202

*H.T. Li[1,2], Y. Li[1], C.M. Zhang[2], N. Jia[2], D. Hu[2], Z.W. Wang[2] and Q.Wang[1]*
*[1]Department of Plant Pathology, China Agricultural University, Beijing, 100193, P.R. China; [2]Institute of Genetics and Physiology, Hebei Academy of Agricultural and Forestry Sciences, Shijiazhuang 050051, P.R. China*
*Email: lihongtao1314@hotmail.com; wangqi@cau.edu.cn*

The cereal cyst nematode (CCN)，*Heterodera avenae*, has caused economically significant damage to the wheat in China. So far, there is no effective way to prevent for this disease. In this study，the *Bacillus* strains 202 were isolated from wheat rhizosphere soil samples collected from middle plain region of China. The antagonistic abilities to the second stage juvenile(J2) of *Heterodera avenae*，were tested. The ferment suspension of the strain B202 showed strong nematicidal activity to J2. The strain B202 had 100% mortality to J2 in vitro test after 48 hours treatment. Through the potted experiments of wheat in greenhouse, the results of control test showed the strain B202 could suppress the formation of the galls. The control efficiency of strain B202 was 59.09% after 60 days. The strain B202 also was proved 61% control efficacy in field by three years field experiments. The strain B202 was identified as *Bacillus. pumilus* using the Biolog microbial identification system., which was further confirmed by 16S rDNA sequence analysis. So the strain B202 was name as *Bacillus. Pumilus* B202. The ability of colonization in wheat root was determined in pot experiment by labeled antibiotic mutant. The ability of colonization of the biocontrol bacteria B202 could maintain about $10^7$ CFU/g in rhizosphere of wheat above 65 days, suggesting the potential to be developed as biocontrol agents.

### P03.130 Eco-friendly management of Brown spot (*Bipolaris oryzae)* of rice and quality seed production

*H. Mahmud and I. Hossain*
*Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh and Professor, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202; Bangladesh*
*Email: mhyat81@gmail.com*

Efficacy of BAU-Biofungicide (*Trichoderma harzianum*), plant extracts: Garlic (*Allium sativum*), Neem (*Azadirachta indica*), and Bavistin (Carbendazim), Potent 250 EC (Propiconazol) were evaluated for controlling Brown spot of rice cv. BR11. Eleven treatments such as $T_1$: Garlic 1%, $T_2$: Garlic 2%, $T_3$: Neem 1%, $T_4$ : Neem 2%, $T_5$ : BAU-Biofungicide 2%, $T_6$ : BAU-Biofungicide 3%, Bavistin ($T_7$: 0.1%, $T_8$: 0.05%), Potent 250 EC ($T_9$: 0.1%, $T_{10}$: 0.05%) and control ($T_{11}$) have been sprayed at booting, heading and ripening stage under field condition to produce quality seed. Throughout the study period Potent (0.1%) showed the lowest (3.33%) disease severity where control showed highest (29.25%) and BAU-Biofungicide (3%) yielded significant result (4.50%) in controlling the disease. Potent (0.1%), BAU-Biofungicide (3%) and Garlic (2%) resulted in 34.16%, 20.42% and 12.98% significant higher yield over control. In dry inspection 78.50%, 74.33% and 70.00% of apparently healthy seed were calculated in Potent (0.1%), BAU-Biofungicide (3%), and garlic 2% respectively where the minimum (47.75%) in control. In germination test treated with above treatments Garlic (2%), BAU-Biofungicide (2%, 3%) and Potent (0.1%, 0.05%) showed the best result in germination 95%, 97%, 98% and 96%, 98% respectively whilst minimum 85% was observed in control. In normal seedling, highest (92%, 93%) was recorded in BAU-Biofungicide (2%, 3%), respectively where Potent (0.1%, 0.05%) showed poor result 64.67%, 69% respectively and 60% found in control. Seed borne fungi: *Curvularia lunata*, *Bipolaris oryzae*, *Fusarium oxysporum*, *F. moniliformae*, *Nigrospora oryzae* and storage fungi: *Aspergillus flavus*, *Penecillium* spp. *and Trichoderma harzianum* have been inspected thorough seed health test.

**N03.001 *Lysobacter enzymogenes* uses two distinct cell-cell signaling systems for differential regulations of metabolite biosynthesis**

*G.L Qian, Y. Wang, Y. Liu, F. Xu, J. Fan, B. Hu and F.Q. Liu*
*College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China/Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, P.R.China*
*Email: fqliu20011@sina.com*

*Lysobacter enzymogenes* is a ubiquitous environmental bacterium emerging as a novel biological control agent and a new source of bioactive secondary metabolites, such as the heat-stable antifungal factor (HSAF) and photo-protective polyene pigments. So far, the regulatory mechanism(s) for biosynthesis of these bioactive secondary metabolites remains largely unknown in *L. enzymogenes*. In the present study, two distinct cell-cell signaling factors, DSF (Diffusible Signal Factor) and DF (Diffusible Factor), were identified for the first time in *L. enzymogenes*. The results show that both DSF and DF played critical roles in modulating HSAF biosynthesis and exhibited accumulative effects. DSF and DF played negative and positive effects in polyene pigment production, respectively, and DF is shown to play a more important role than DSF. Interestingly, only DSF, but not DF, regulated colony morphology of *L. enzymgenes*. Furthermore, the evidence support that both DSF and DF were global regulators that modulated a wide range of gene expressions in a specific or cross-talk manner. These findings unveil for the first time the novel roles of DSF and DF signaling pathways. This knowledge will be useful in exploiting the potential of this environment-friendly biological control agent.

**N03.002 Carbendazim degradation by plant disease suppressive *Trichoderma harzianum* Tr1**

*Y.J. Huang[1], X.J. Zhang[1], P.R. Harvey[1, 2], J.S. Li[1], J.N. Wang[1] and H.T. Yang[1]*
*[1]Biotechnology Research Center of Shandong Academy of Sciences,Key Laboratory for Applied Microbiology of Shandong Province, Jinan 250014, P.R.China; [2]CSIRO Sustainable Agriculture National Research Flagship, CSIRO Ecosystem Sciences, PMB 2 Glen Osmond, South Australia, 5064, Australia*
*Email: yanght@sdas.org*

A novel *Trichoderma* isolate Tr1 was isolated from soil in Shandong Province (P. R. China) heavily contaminated with the fungicide carbendazim. Tr1 showed significant *in vitro* growth inhibition of the plant pathogenic fungi *Rhizoctonia solani*, *Phytophthora capsici*, *Verticillium dahlia*, *Phoma uvicola*, *Bipolaris sorokiniana* and *Fusarium moniliforme*. Inhibition of *R. solani* was highly effective, with colony growth reduced by approximately 80%. Tr1 was identified as *T. harzianum*, based on DNA sequencing of the-ITS1 and ITS2 regions of the rRNA gene cluster and comparison with the TrichOKey database. Strain Tr1 was able to metabolise carbendazim, evidenced by growth in liquid mineral salt medium containing 1000μg/ml carbendazim as the sole carbon source. Approximately 25% of the carbendazim was degraded following shaking incubation of the isolate at 28℃ for 14 days. Optimal carbendazim degradation was achieved between 25-30℃ at pH 6.0-6.5. The principal catabolites of Tr1-induced carbendazim catabolism were 2-amino benzimidazole (2-AB) and 2-hydro benzimidazole (2-HB). Tr1 was able to metabolise 2-AB, indicated by growth in liquid culture with this catabolite as the sole carbon source. On-going research is defining other catabolites produced by Tr1 degradation of carbendazim and the use of Tr1 as an inoculant for suppression of carbendazim-resistant plant pathogenic fungi.

**N03.003 Analysis the sclerosphere microorganism community of *Sclerotinia sclerotiorum* and evaluation of its effect on infection of sclerotia by *Coniothyrium minitans***

*X.H. Li[1], Y.Q. Lu[1], L. Yang[1], J. Zhang[1], G.Q. Li[1,2] and D.H. Jiang[1,2]*
*[1]The Key Laboratory of Plant Pathology of Hubei Province, P.R.China; [2]The National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, 430070, P.R. China*
*Email: yanglong@mail.hzau.edu.cn*

*Coniothyrium minitans* is a mycoparasite of the plant pathogenic fungus *Sclerotinia sclerotiorum*. It can parasitize sclerotia of *S. sclerotiorum*, reducing apothecial production and disease incidence. In natural soils, effect of sclerosphere microbes on infection of *S. sclerotiorum* sclerotia by *C. minitans* is poorly understood. This study focused on the ecology of the sclerosphere microorganism community of *S. sclerotiorum*, used molecular techniques and isolation techniques. Results showed that the amount of bacteria and fungi in sclerosphere are significantly higher than those in sclerotia-free soils. Base on bacterial 16S rDNA and fungal internal transcribed spacer of ribosomal DNA sequences, we identified *Pseudomons*, *Bacillus* and *Archrobacter* as the dominant genera in sclerosphere. *Penicillium* was dominant genus in sclerosphere. Suppression of mycelia growth of *C. minitans* by 86 bacteria strains and 43 fungal strains isolated from sclerosphere was observed. Seven bacteria strains and seven fungal strains could strongly suppress infection of sclerotia by *C. minitans in vitro.* This result suggests that sclerosphere microorganisms may play an important role in suppression of the infection of sclerotia by *C. minitans* in field. We also make an effort to screen microorganism chaperone of *C. minitans*, which ecological function complementation with *C. minitans*, and promotion the infection sclerotia of *S. sclerotiorum* by *C. minitans.*

**N03.004 Effect of *Aspergillus niger* strain Y-1 on infection of sclerotia of *Sclerotinia sclerotiorum* by *Coniothyrium minitans***

*D. Yang[1], X.P. Li[1], L. Yang[1], J. Zhang[1], G.Q. Li[1,2] and D.H. Jiang[1,2]*
*[1]The Key Laboratory of Plant Pathology of Hubei Province, P.R.China; [2]The National Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, 430070, P.R.China*
*Email: yanglong@mail.hzau.edu.cn*

The mycoparasite *Coniothyrium minitans* is a promising biocontrol agent of *Sclerotinia sclerotiorum*. It can infect sclerotia of *S. sclerotiorum* in soil. Our previous study showed that sclerosphere bacteria can suppress infection of sclerotia by *C. minitans*. We plan to screen a microorganism chaperone of *C. minitans*, which can functionally complement *C. minitans* leading to promoting infection sclerotia by *C. minitans.* Strain Y-1 of *Aspergillus niger* isolated from sclerosphere of *S. sclerotiorum* was able to secrete large amount of organic acids. Moreover, the PDB cultural filtrate of strain Y-1 strongly suppressed mycelia growth of *S. sclerotiorum*. The objective of this study was to evaluate the effect of *A. niger* strain Y-1 on infection of sclerotia by *C. minitans.* Results showed that *A. niger* strain Y-1 is an antagonist inhibitory to sclerosphere bacteria. Meanwhile, no negative effects of *A. niger* strain Y-1 on conidial germination and mycelia growth of *C. minitans* were observed. Furthermore, *A. niger* strain Y-1 could weakly parasitize sclerotia. It could assist *C. minitans* to infect and degrade sclerotia of *S. sclerotiorum*. Simultaneous application of *A. niger* strain Y-1 and *C. minitans* significantly reduced the number of apothecia produced by *S. sclerotiorum* in the potting experiment. This result suggests that such combined application can results in the synergistic interaction with enhanced and more consistent reduction of carpogenic germination of sclerotia. Further studies utilizing large field plots are required for evaluation of the efficacy of application of *A. niger* strain Y-1 combined with *C. minitans* for control of *S. sclerotiorum*.

## Concurrent Session 4-Biosecurity and Plant Quarantine

### O04.001 Plant biosecurity: what is at stake and why does it matter?

*J.P. Stack, Y. Jin and S. McKirdy*
*Kansas State University, Department of Plant Pathology, 4024 Throckmorton Plant Sciences Center, Manhattan, KS 66506-5502, USA*
*Email: jstack@ksu.edu*

Plant biosecurity may very well become one of the most significant challenges confronting humans in the 21st century. Plant systems reside at the center of the debates over biodiversity, water resource management, land-use change, and public health. The direct and indirect connections between plant health and human health and wellbeing are not obvious to most people and the dependence of plant health on plant biosecurity is of little concern to the general populations of most countries. It is difficult to imagine how we will develop productive and profitable food, feed, and fiber production systems and healthy natural and managed ecosystems without a more comprehensive and coordinated approach to plant biosecurity. The rush to global market systems that connect countries with well-developed plant biosecurity infrastructure to countries with poorly developed and ineffective plant biosecurity infrastructure will continue to have consequences that unless managed well will lead to the inability of many plant systems to support the nine billion people that will rely on those plant systems for food, feed, fiber, fuel, and shelter. At the center of this challenge to sustain the plant systems upon which humans depend is the disconnect among scientists whose perspectives on sustainable ecosystems is in direct conflict. Will we protect some plant systems (e.g., endangered species) at the expense of other plant systems (e.g., crop plants)? To develop strategies and policies necessary to protect all plant systems, we need cross discipline communication and a concerted effort to educate policy makers as to the consequences of poor policy.

### O04.002 Strategies for tackling the impact of global change on biosecurity

*K.A. Garrett*
*Department of Plant Pathology, Kansas State University, Manhattan, KS, 66506, USA*
*Email: kgarrett@ksu.edu*

Global change factors, such as climate change and changing transport and communication networks, are likely to have important impacts on biosecurity, for better and for worse. As the 'playing field' for biosecurity shifts, a mechanistic understanding of how mitigation activities support biosecurity will be needed for adaptation of strategies. An 'impact network analysis' (INA) can be implemented to evaluate the integrated effects of research results and new technologies, communication networks, decision networks, and ecological/transport networks. In the biosecurity context, an INA can be used to understand mechanisms and evaluate the likely outcomes of different strategies for management pre- and post-introduction of a pathogen. Just as communication networks have been growing rapidly, our ability to measure and predict the effects of communication has seen its own expansion. Decision networks, determining decisions within individuals or groups of managers, are becoming a more commonly studied feature of plant pathology as interdisciplinary links with sociology and the psychology of decision making grow. The driving factors of epidemic networks may vary widely from one system to another, whether movement is by human transport, vectors, wind, rain, or other means. But if the general structure of an epidemic network is known, it can be used to identify key locations for sampling and mitigation, as well as being incorporated in an INA. Examples of the application of an INA to stored grain networks and smallholder farm seed systems will be discussed.

### O04.003 Statistical design of a surveillance system for non-indigenous species on Barrow Island, Australia

*K. Mengersen, P. Whittle and F. Jarrad*
*Mathematical Sciences, Queensland University of Technology, GPO Box 2434, Brisbane 4001, Australia*
*Email: k.mengersen@qut.edu.au*

Biosecurity programs for surveillance must be designed to meet multiple criteria including budget constraints, practical considerations and statistical power of detection. These designs are challenging for the complex surveillance problems common in biosecurity, such as prioritizing detection among multiple invasive species, specifying risk over a heterogeneous landscape, combining multiple sources of surveillance data, and resource management. Confirmation of statistical power of a surveillance program results in the protection and conservation of environmentally important areas with statistical confidence. We present an approach taken to meet these challenges in a comprehensive and statistically powerful surveillance design for non-indigenous terrestrial plants on Barrow Island, a high conservation nature reserve off the Western Australian coast where the possibility of incursions is increased due to construction and expanding industry on the island. The design is flexible in the choice of surveillance components such as traps and passive surveillance, includes cost considerations, prioritises sampling in locations with greater risk, and is adaptable to other sites. It also provides the opportunity for scenario assessment regarding alternative sampling designs based on different risk evaluations and choices of surveillance components.

More general issues involved in statistical modelling and design of surveillance programs, including the elicitation and use of expert information and the choice of statistical models, will also be discussed in the presentation.

### O04.004 The role of disease and pest management in USAID's "Feed the Future" Initiative

*J. Bowman*
*USAID Bureau for Food Security, 1300 Pennsylvania Ave. NW, Washington, DC, 20523, USA*
*Email: jobowman@usaid.gov*

In 2010, the United States government (USG) launched a multi-billion dollar global hunger and food security initiative known as "Feed the Future". This presidential initiative uses a "whole of government" approach, whereby the resources of many USG agencies are partnered to alleviate poverty, hunger and malnutrition in over twenty impoverished countries. The U.S. Agency for International Development (USAID) leads the agricultural components of the initiative. Agricultural research and development projects under Feed the Future are designed to have a high degree of impact on themes such as diet diversity, gender balance, and environmental sustainability. USAID employs a range of environmentally sustainable disease and pest management strategies in order to ensure agricultural productivity. Integrated pest management research implemented by U.S. land grant universities focuses on the use of cultural and biological control. Breeding for disease resistance is implemented through investments with the Consultative Group for International Agricultural Research (CGIAR) in partnership with U.S. universities and private sector companies. In close collaboration with the United States Department of Agriculture, (USDA) many of USAID's implementing partners are working in the crucial area of mycotoxin mitigation. USAID and USDA have also invested in a sanitary and phytosanitary (SPS) capacity building program for Africa, Latin America, and Asia which raises awareness and harmonizes regulations for crop diseases and pests of quarantine significance. USAID combines shorter term disease and pest control interventions through commodity-driven value chain projects, with longer term research strategies to ensure high crop yields, diverse diets, food safety, and environmental sustainability for its small holder farmer clients.

### O04.005 Modern plant quarantine: opportunities and challenges

*S.F. Zhu, X. Pan, H. Xu, Y. Yu and J. Zhang*
*Institute of Plant Quarantine, Chinese Academy of Inspection and Quarantine Huixinli 241, Beijing 100029, P.R. China*
*Email: zhushf020420@gmail.com*

Plant quarantine is very important for preventing the exotic invasive species. It is necessary to rethink and promote the modern plant quarantine under the context of trade globalization and climate change. Here we recall the history of plant quarantine and species conservation, and the development of plant quarantine system first. Then we describe several critical questions and problems which we meet in the practices. In order to solve these issues, we look back the evolution regularity of alien species and biodiversity effected by them in the world, and find that the mechanism of species colonization and epidemiology of pests are key points in the field of plant quarantine. And the challenges include risk early warning, pest risk analysis, policy management, border detection and monitoring, and quarantine treatment. New information centre at national border are proposed, integrating the dynamic distribution of pests in the world, rapid identification of intercepted species, early warning and emergency system, and the cooperation between the public and government. We also introduced these development tendencies of modern plant quarantine in China.

### O04.006 National risk registers for plant health: lists, priorities and performance

*J.D. Mumford, J. Holt, A.W. Leach, M.M. Quinlan*
*Centre for Environmental Policy, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, United Kingdom*
*Email: j.mumford@imperial.ac.uk*

National plant protection organisations need to describe the plant health risks they face, set priorities on proportionate risk mitigation actions, and evaluate their performance in managing the risks for which they or others are responsible. Plant health risk analyses are complex and time consuming. A risk register identifies "what could go wrong" and allows potential risks to be catalogued in less detail before full risk analyses are initiated. Common dimensions to consider for risk profiles are agents, pathways and receptors, so that similar or generic risks, management measures and responsible stakeholders can be grouped where applicable. Risk registers should be consistent with the principles of ISPM 11 to quantify likelihood of entry and establishment and the magnitude of spread and impact on defined temporal and spatial scales. This ensures diverse risks can be compared within a common framework. Risk registers should include descriptions of management measures available to reduce the likelihood or magnitude of risks, with estimates of their efficacy, feasibility and cost (including uncertainties). The performance of plant health services should be measured in terms of how efficiently risk is addressed, rather than that actions such as inspection or control are carried out. A risk register, incorporating a baseline risk distribution, could allow comparison with the managed risk profile, which should be regularly

reevaluated against evidence from actual biosecurity challenges and responses. Beyond the known risks identified in a risk register, a significant source of uncertainty in performance still arises from unknown risks.

**O04.007 Identifying uncertainties and knowledge gaps with risk assessment and epidemiological modelling of quarantine plant pathogens**

*G. Stancanelli, R. Baker, E. Ceglarska, D. Makowski, O. Mosbach-Schulz, M. Pautasso, T. Rafoss, J. Schans, V. Rossi, A. Vicent Civera, W. van der Werf* and *M. Jeger*
*European Food Safety Authority, Scientific Panel on Plant Health, Via Carlo Magno 1A, 43126 Parma, Italy*
*Email: plh@efsa.europa.eu*

The risk assessment of exotic plant pathogens for new areas highly depends on accurate prediction of their potential for establishment and for causing damages into these areas outside their current distribution. Generic models or specific epidemiological models can be used to predict the establishment and eventually the potential impact of new pathogens, however when assessing the risk it is necessary to carefully consider the uncertainties of such models. Key uncertainties are often due to knowledge gaps on model parameters, which can only be filled by further plant pathology research. Sensitivity analyses show which parameters, e.g. related to the environmental requirements of the organism, have the strongest influence on the model outputs and thus provide guidance on which traits and responses of the pathogen should be targeted to carry out experiments to estimate these parameters more accurately. Model outputs have been shown to be highly sensitive to the spatial and temporal scales of the climatic input data. The consequences for risk assessment are discussed with reference to case studies on fungal pathogens from the scientific opinions of the EFSA Plant Health Panel. The models discussed include a simple generic infection model for foliar fungal plant pathogens, models for pseudothecia maturation and ascospore release and CLIMEX.

**O04.008 Genomics-based detection of bacterial plant pathogens; Implications for quarantine and trade policy**

*B. Rodoni, R. Mann, G. Smith, T. Chapman, M. Bellgard and J. Stack*
*Biosciences Research Division, Department of Primary Industries, AgriBio, 5 Ring Road, La Trobe University, Bundoora, Victoria, 3083, Australia*
*Email: brendan.rodoni@dpi.vic.gov.au*

The detection of closely related but non-pathogenic *Erwinia* species (e.g. *E. tasmaniensis*) using poorly validated PCR tests during the 1997 incursion of *Erwinia amylovora* into Australia has been well documented and highlighted the importance of validated robust molecular detection tools for bacterial species and pathovars. A genomics approach to develop diagnostic markers for the detection and differentiation of phytopathogenic bacterial species and pathovars is becoming more feasible with the advances in Next Generation Sequencing (NGS) technology. Over 20,000 bacterial genomes have been sequenced providing a tremendous resource for the design of diagnostic markers. NGS is a cultivation-independent strategy that enables sequencing of not only the target species but also closely related and usually undescribed bacterial species that may generate false positive test results. This strategy has recently been reported for the detection of *Xanthomonas oryzae* pv. *oryzicola, Erwinia amylovora* and *Bacillus anthracis*. An additional challenge for plant biosecurity policy makers generated by the cultivation-independent sequencing of microbial communities is the resultant discovery of novel bacterial species. A recent study of the apple flower identified 1600 bacterial species; 26% were unidentified species (Shade et al. 2013). Since plant quarantine and trade policy is based on the presence of a pathogen, how do biosecurity agencies determine whether these newly discovered bacterial species are plant pathogens or have the potential to cause disease? The implications of bacterial species discovery will be discussed. It is recommended that accurate identification of bacterial pathovars is based on a combination of molecular, morphological, pathological and epidemiological data.

**O04.009 Biosecuring Indian agriculture from plant viruses: Role of serological and molecular diagnostics**

*V. Celia Chalam, D.B. Parakh, A.K. Maurya and P.C. Agarwal*
*Division of Pant Quarantine, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India*
*Email: mailcelia@gmail.com;celia@nbpgr.ernet.in*

Viruses are one of the most important yield-reducing factors in various crops, of which many are seed-transmitted. Germplasm including transgenics of different crops is imported into India every year for crop improvement programmes and a number of viruses can be introduced into the country through import of seeds. The challenges in virus detection include availability of antisera, viral genome sequences in GenBank, detecting an unknown/ exotic virus etc. Attention is now given to issues like developing an antisera bank for exotic viruses, database on sequences of virus-specific primers, Multiplex RT-PCR, Real-time RT-PCR, LAMP, HAD, etc. Adopting a workable strategy such as post-entry quarantine (PEQ) growing in PEQ greenhouses/ containment facility, electron microscopy, ELISA, RT-PCR, 34 viruses have so far been intercepted, which includes 11 viruses not yet reported from India viz., *Barley stripe mosaic virus, Bean pod mottle virus, Broad bean stain*

*virus, Cherry leaf roll virus, Cowpea mottle virus, Cowpea severe mosaic virus, Maize chlorotic mottle virus, Pea enation mosaic virus, Raspberry ringspot virus, Tomato ringspot virus* and *Wheat streak mosaic virus*. Besides, 15 viruses not known to occur on particular host(s) in India were intercepted. The infected plants were incinerated and harvest from virus-free plants was released for crop improvement programmes. The risk of introduction of 34 seed-transmitted viruses or their strains into India was thus eliminated. Adopting the appropriate technique and the right strategy for virus detection would go a long way in ensuring the biosecurity of Indian agriculture from transboundary introduction of plant viruses.

**O04.010 Australia assists Timor Leste to monitor banana threats**

*J. D. Ray, V. Quintao, L. Jones, R. James, K. Grice and J. B. Rossel*
*Department of Agriculture, Fisheries, and Forestry (DAFF), Northern Australian Quarantine Strategy (NAQS), 1 Pederson Rd, Eaton NT, Australia, 0820*
*Email: jane.ray@daff.gov.au*

Collaborative plant health surveys in Timor Leste have been conducted regularly since 2000 by the Timor Leste Ministry of Agriculture and Fisheries (TLMAF) and the Australian Department of Agriculture Fisheries & Forestry (DAFF). In addition to these surveys, capacity building activities have included training in surveillance, specimen collection, sample processing and identification using traditional microscopy, Enzyme-linked immunosorbent assay (ELISA) and molecular techniques. The activities are designed to increase the knowledge on Timor Leste's plant health status and to provide early detection of any incursions of exotic plant pests. During these surveys many banana samples have been collected from across Timor Leste and diseases identified include black Sigatoka (*Mycosphaerella fijiensis*), yellow Sigatoka (*Mycosphaerella musicola*), cordana leaf spot (*Cordana musae*), black cross (*Phyllachora musicola*), freckle (*Phyllosticta cavendishii*) and rust (*Uredo musae*). However, these surveys have also demonstrated the absence of some of the most serious banana diseases such as fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*), blood disease (*Ralstonia solanacearum* Phylotype IV), eumusae leaf spot (*Mycosphaerella eumusae*) and bunchy top (*Banana bunchy top virus*, BBTV). Absence from such devastating diseases highlights the importance of ongoing pest surveillance and maintaining good quarantine systems.

**O04.011 Biosecurity implications of plant pathogens in irrigation water**

*R.E. Zappia, D. Hüberli, G.E. Hardy and K.L. Bayliss*
*Plant Biosecurity Cooperative Research Centre, LPO Box 5012, Bruce ACT 2617, Australia, and School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch, WA, 6150, Australia*
*Email:K.Bayliss@murdoch.edu.au*

Water used for the irrigation of plants has the potential to act as both a source and a means of spread of plant pathogens, yet little research is conducted within this field. This study was conducted to increase our understanding of water-borne plant pathogens in an open irrigation system in Western Australia, particularly in the context of plant biosecurity. It was determined that various Oomycetes and fungal pathogens are present including *Phytophthora* (multiple spp.), *Pythium* (multiple spp.), *Colletotrichum gloesporioides, Fusarium solani*, *Fusarium* sp., *Giberella* sp., *Leptosphaeria* sp., *Mortierella* sp., *Saprolegnia* sp., *Paecilomyces lilacinus*, and various unknown species. The pathogenicity of some of these species has been confirmed on crops grown in the region. There are significant gaps in our knowledge of how these plant pathogens survive and spread in the irrigation system, and very limited information on their ability to cause disease when contaminated water is applied to crops in the region. This study has highlighted the need for new research on the epidemiology and pathogenicity of water-borne plant pathogens, to inform biosecurity risk assessments and develop mitigation strategies.

**O04.012 A new and emerging wilt disease of bananas associated with phytoplasmas**

*R.I. Davis, F.T. Tsatsia, C. Fanai, P. Kokoa, J. Henderson, L.M. Jones and J.B. Rossel*
*Northern Australia Quarantine Strategy (NAQS), Department of Agriculture, Fisheries and Forestry (DAFF), PO Box 96, Cairns International Airport, Qld 4870, Australia*
*Email: richard.davis@aqis.gov.au; richard.davis@daff.gov.au*

An indicative relationship found between phytoplasma presence and a unique set of external and internal wilt symptoms in cooking banana plants (*Musa* sp. ABB genome) in Papua New Guinea (PNG) was revealed using nested PCR. This became the first record of a phytoplasma associated with a banana wilt disease, worldwide. Phytoplasmas were detected in banana pseudostem vascular tissue samples using primer pairs P1/P7 and R16F2n / m23SR. Sequence analysis of the 16S rRNA gene, 16S-23S spacer region and a part of the 23S rRNA gene and the ribosomal protein S19, L22, and S3 genes indicated that phytoplasmas from diseased banana plants from five separate locations across PNG were most closely related to a phytoplasma in the 16SrIV group, recently associated with a new lethal disease of coconuts in PNG. Phylogenetic analyses suggested that this phytoplasma may be a distinct species or

a new taxonomic group and it was named the Banana wilt associated phytoplasma. Subsequent plant health surveys in the neighbouring Solomon Islands discovered cooking banana plants showing identical external and internal symptoms on one island near the border with PNG. Samples were collected and these were also positive in nested PCR tests for phytoplasma presence. The identity of these and their relationship to phytoplasmas found in these and other banana plants in PNG, together with results of suspect insect vector collections is discussed.

**O04.013 Should quarantine action be based on DNA sequence rather than species?**

*W.P. Roberts*
*Plant Biosecurity Cooperative Research Centre, LPO Box 5012, Bruce, ACT 2617, Australia*
*Email: b.roberts@pbcrc.com.au*

Under the International Plant Protection Convention countries have an obligation to develop and publish lists of pest species of quarantine concern. These lists allow countries to consider pest management measures that may be required to allow exports of plants and plant products to other countries and are the focus of quarantine negotiations between exporting and importing countries. In addition to formal bilateral arrangements many countries routinely inspect or test imported plants and plant products for species of quarantine concern. Where pests are found action such as chemical treatments or destruction of the imported goods may be undertaken. The quarantine protection provided by this approach is highly dependent on the quality of the identification and the understanding of the full range of pests that may be of concern. Modern molecular methods can greatly assist in identification but taking action based on species identification can fail to provide quarantine protection where organisms are not listed of quarantine concern or are not already known to be a pest. Rapidly increasing knowledge about the molecular basis of pathogenicity combined with the rapidly decreasing cost of DNA sequencing provides an opportunity to discard the conventional approach of identifying pest species in favour of sequencing organisms of quarantine concern and taking action on the basis of the sequences found.

**O04.014 On-the-spot diagnosis and detection of plant pathogens**

*X. Li, J. Nie, H. Arsenault, H. Xu and S.H. De Boer*
*Canadian Food Inspection Agency, Charlottetown Laboratory, 93 Mt Edward Road, Charlottetown, PE, Canada, C1A5T1*
*Email: sean.li@inspection.gc.ca*

On-the-spot or in-the-field diagnostic strategies in plant science are adapted from point-of-care concepts in the biomedical industry for human disease diagnoses. Many of the test strategies being developed in medical research are also applicable to on-the-spot agricultural applications. One such test is the commercially available lateral flow device (LFD) for detection of specific plant pathogens on the basis of immunochromatographic or nucleic acid-based techniques. In immunochromatographic assays, generally a monoclonal antibody at the core of the LFD recognizes a specific epitope found in the target pathogen, and thereby gives an effective method for screening of plant samples for the presence of the pathogen. In nucleic acid-based assays, a taxon-specific probe hybridizes with target-specific DNA/RNA, with or without prior amplification, to detect the target pathogen. A particularly useful technique is loop-mediated isothermal amplification (LAMP) in which specific DNA is amplified at a constant temperature, obviating the need for thermal cycling. In our study LAMP assays targeting three bacterial and two fungal plant pathogens, as well as two reverse-transcription LAMP assays targeting viral pathogens were evaluated for use in rapid on-the-spot detection. Because a high concentration of amplified DNA is generated in the LAMP assay, this technology is particularly suitable for integration into hand-held devices. The advanced techniques based on portable LAMP assays, lab on-a-chip technologies, and portable PCR and real time PCR machines are just starting to become available in diagnostic laboratories. Their practical application for on-the-spot application by growers, extension agents, and regulators in the foreseeable future is predicted.

**O04.015 Detection of *Albugo tragopogonis* by a quantitative real-time fluorescent PCR method**

*X.L. Zhang, B. Liu, C. Wang, J.G. Zhang*
*Xinjiang Entry & Exit Inspection and Quarantine Bureau, No.116 North Nanhu Road, Urumqi, 830063, P. R. China*
*Email: xl6479@163.com*

A TaqMan tamra probe ATH-X and a pair of primers ATHF/ATHR were designed and synthesized according to the specificity of the rDNA ITS sequences of *Albugo tragopogonis*. A method of quantitative real-time fluorescent PCR was consequently developed and optimized to detect *A. tragopogonis*. It was used in detection of *Albugo* spp., other Oomycetes fungi and some sunflower seeds & leaves. The results indicated that *A. tragopogonis* could be differentiated from other eight tested pathogens by quantitative real-time fluorescent PCR assay with the prob ATH-X and a pair of primers ATHF/ATHR. The *A. tragopogonis* could also be detected from the sunflower seeds & leaves tested. The method could be used for detection the *A. tragopogonis* to entery-exit sunflower seeds and diseased sunflower plants in fields.

**O04.016 Methods for the early detection of new invasive forest pathogens**

*A.J.M. Hopkins, C. Castanyo, J.B. Boberg and J. Stenlid*
*Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, PO Box 7026, 750 07 Uppsala, Sweden*
*Email: anna.hopkins@slu.se; jan.stenlid@slu.se*

New pathogens in forest ecosystems have been detected at an increasing rate over the past century, primarily as the result of increasing global trade and population mobility. Climate change also threatens well established tree species, making forest ecosystems more vulnerable to disease. The early detection of newly introduced pathogens is a critical component in reducing the impact of new disease threats. This study examines effective aerial spore trapping methods for the detection of new invasive forest pathogens. Two types of active (Burkard-style) trap and one passive (rain gauge) spore trapping method were examined for their effective use in combination with 454- pyrosequencing. Some species-specific PCRs were also undertaken to look for pathogens of interest not amplified by the fungal primers used e.g. *Phytophthora* species. Traps were located at two forest sites in central and southern Sweden and one high-use port in western Sweden and trapping was carried out for up to one year at each location. Sequencing was successfully carried out on samples from all trap types. Many forest fungi were detected including a large number of wood-decay species and several common forest pathogens. Algae and yeasts were also amplified by the primers used. Different trap types captured different fungal species indicating that choice of traps for monitoring will depend on the target species and required outcomes. This study illustrates the potential for this system to be used as the basis for a comprehensive monitoring network to facilitate early detection of new forest pathogens and help to prevent potentially damaging disease outbreaks.

**P04.001 Sugarcane quarantine in the South African sugarcane industry**

*T. van Antwerpen*
*South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe, 4300*
*Email:Tania.vanantwerpen@sugar.org.za*

In order to broaden the genetic base for breeding, sugarcane clones are imported from a number of countries into South Africa for their specific genetic traits. While this is an integral part of the Plant Breeding programme at the South African Sugarcane Research Institute (SASRI), the risk of introducing exotic diseases into the South African sugar industry is increased. Sugarcane importation into South Africa falls under the terms and conditions of the Agricultural Pests Act, 1983 (Act No. 36 of 1983). The Directorate of Plant Health is ultimately responsible for plant quarantine in South Africa but the technical management of sugarcane quarantine has for many years been delegated to pathologists at SASRI. Imported cane is maintained in the quarantine glasshouse for approximately two years. During this period, the sugarcane is inspected regularly and undergoes intensive testing for diseases. Molecular tests such as polymerase chain reaction (PCR), reverse transcriptase polymerase chain reaction (RT-PCR) and real-time polymerase chain reaction (qPCR) are used for the detection of *Sugarcane yellow leaf virus* (SCYLV), *Sugarcane mosaic virus* (SCMV), *Fiji disease virus* (FDV), *Maize streak virus* (MSV), *Sugarcane streak mosaic virus* (SCSMV), *Sorghum mosaic virus* (SrMV), *Peanut clump virus* (PCV), *Xanthomonas albilineans* (Leaf scald) and phytoplasmas, while immunofluorescence microscopy and tissue blot enzyme immunoassays are used to detect *Leifsonia xyli* subsp. *xyli* (Ratoon stunt) and SCYLV. Pathogen elimination is achieved by means of hot water treatment and apical meristem tip culture before varieties are released to a post quarantine area for further assessment. Popular local N varieties used for export to sugarcane industries worldwide are maintained in the quarantine glasshouse and disease-indexed before transfer. SASRI also renders a quarantine service for neighbouring countries.

**P04.002 Detection of *Pantoea ananatis* on *Dracaena reflexa* in post entry quarantine**

*M.K. Romberg[2], R.K. Taylor[1] and B.J.R. Alexander[1]*
*[1]Plant Health and Environment Laboratory, Ministry for Primary Industries, PO Box 2095, Auckland 1140, New Zealand; [2]Current address: USDA-APHIS, Beltsville, MD, United States*
*Email: robert.taylor@mpi.govt.nz*

Dracaenas are ornamental plants with shrub-like growth and are popular for their decorative foliage, low maintenance requirements, and tolerance of a wide range of growing conditions. Currently, cuttings and whole *Dracaena* spp. plants (including roots, stems, and leaves) may be imported into New Zealand under precise phytosanitary requirements and are required to spend 3 months of active growth in post entry quarantine (PEQ) where they are inspected for pests and diseases. Leaf blight and vascular wilt symptoms were observed on a number of consignments in PEQ of *Dracaena reflexa* plants imported from Central America. Yellow bacterial colonies were consistently isolated from plant tissue exhibiting leaf blight and vascular wilt symptoms. *Pantoea ananatis* was identified on the basis of biochemical and molecular tests and confirmed as the causal agent by fulfilling Koch's postulates. Isolates of *Pantoea ananatis* caused onion slices to rot and results of pathogenicity tests on *Dracaena* will be presented. *P. ananatis* has been reported to affect other crops such as maize, onion and rice; however, to the best of our knowledge this is the

first report of *Pantoea ananatis* associated with leaf blight and vascular wilt on *Dracaena reflexa*.

**P04.003 Development of rapid assay method using PCR-based marker for the detection of plant quarantine pathogen, *Phoma destructive***

H.J. Kim[1], S.Y. Kim[1], W.C. Chung[1], I.H. Hyun[1], N.Y. Heo[2] and H.G. Kim[3]
[1]*Experiment & Analysis Division, Animal, Plant and Fisheries Quarantine & Inspection Agency;* [2]*Plant Quarantine Technology Center, Department of Animal and Plant Health Research, Plant and Fisheries Quarantine & Inspection Agency;* [3]*Department of Agricultural Biology, Chungnam National University, Republic of Korea*
*Email: hjkim0805@korea.kr*

*Phoma destructiva* Plowright is known as plant pathogenic fungus causing leaf spot or fruit rot of pepper, tomato, and potato in many areas of the world. In Korea, this fungus was defined as the regulated pest by plant quarantine's law. Therefore, We aim to develop tools as the species-specific primers (PD-F, PD-R) based on β-tublin and actin region of the fungus to detect the this pathogen on seeds, fruits, and trees for quarantine issues and phytosanitary measures. As a consequence, a rapid technique for detection of *P. destructiva* has been well developed using polymerase chain reaction (PCR). A single 193-bp DNA amplification product was consistently obtained from primers that was specific for *P. destructiva* but were not produced from other *Phoma* spp., *Phyllosticta* spp., or from other soilborne pathogens including *Rhizoctonia* sp., *Fusarium* sp., *Pythium* sp., or *Phytophthora* sp. Also, A 193-bp amplification product was detected from DNA product of seeds, leaves, and root tissue known to be artificially infected with *P. destructiva* in a humidity condition. Overall, we suggest that this PCR assay method can be usefully applied in a rapid and accurate inspection tool for the disease diagnosis by *P. destructive* for the plant quarantine purpose.

**P04.004 *Leptosphaeria* spp. and Phoma stem canker on oilseed rape in China**

X. Zhang[1,2], R.P. White[3], M. Jedryczka[4], R.M. Lange[5], Z.Q. Li[6], Y.J. Huang[1], A.M. Hall[1], G. Zhou[7], Z. Wang[8], X. Cai[9], P. Skelsey[10] and B.D.L. Fitt[1]
[1]*University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB, UK;* [2]*Southwest University, Chongqing, 400715, P. R. China;* [3]*Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK;* [4]*Institute of Plant Genetics, Polish Academy of Sciences, ul. Strzeszynska 34, 60-479 Poznan, Poland;* [5]*Alberta Innovates-Technology Futures, Post Office Bag 4000, Vegreville, AB T9C IT4, Canada;* [6]*Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot 010031, P. R. China;* [7]*Shanghai Entry-Exit Inspection and Quarantine B ureau, Shanghai, 200135, P. R. China;* [8]*Hubei Entry-Exit Inspection and Quarantine Bureau, Wuhan, Hubei, 430050, P. R. China;* [9]*Huazhong Agriculture University, Wuhan, Hubei, 430000, P. R. China;* [10]*James Hutton Institute, Dundee, Scotland DD2 5DA, UK*
*Email: b.fitt@herts.ac.uk*

In China, the incidence of phoma stem canker observed in pre-harvest surveys from 2005 to 2012 was greater on winter oilseed rape in provinces along the Yangtze River (in May) than on spring oilseed rape in north China (in August). In all cases when the causal pathogen was isolated from stem cankers, it was identified as *Leptosphaeria biglobosa* by morphology in culture and/or by species-specific polymerase chain reaction. Both *L. biglobosa* and *L. maculans* were detected on crop debris and seed in shipments of oilseed rape seed imported into China through Shanghai or Wuhan ports in 2009-2011. Descriptions of the observed spread of *L. maculans* into areas previously colonised by *L. biglobosa* across a spring oilseed rape growing region (Alberta, Canada, westwards, 1984-1998) and across a winter oilseed rape growing region (Poland, eastwards, 1984-2004) were used to estimate the potential westward spread of *L. maculans* in China across spring oilseed rape growing regions (north China) and winter oilseed rape growing regions (provinces along the Yangtze River in central China), respectively. The rates of spread were estimated as 47 km per year across spring oilseed rape in north China and 70 km per year across winter oilseed rape in central China. Dispersal modelling suggested that the rate of spread of *L. maculans* across Alberta, Canada (*c.* 17 km per year) could be explained by wind-borne dispersal of ascospores.

**P04.005 Coniferous rust fungi of Phylogeography and international quarantine**

M.M. Chen
*University and Jepson Herbaria, 100 Valley Life Sciences Building, University of California Berkeley, Berkeley, CA 94720*
*Email: momeichen@berkeley.edu*

In the Northern Hemisphere, the primary forest disease is coniferous rust fungi, a group of heteroecious host specific pathogens that have coevolved with their hosts. Tree rust are among the most complicated taxa in the world with diverse geographic patterns. Recently, I was identified 3 major tree rust species, 12 pine species and 24 *Ribes* species of white pine blister rust in the in regions of North America, Europe, and Asia, and 27 pine species and 28 oak species susceptible to pine-oak rust in North America and East Asia. In Forest Rust *Phytogeography*, presents an overview on white pine blister rust, pine-oak rust and limb rust including their taxonomic position, host range and geographic distribution

patterns respectively. This wealth of tree rust information can help further control its spread and specifically help the International Quarantine. Phylogeography is a new field of advanced science (Avise 2000); By studying the phylogeography of tree rust, we can better explain the geographic patterns that may result in speciation. It has been suggested that white pine blister rust was introduced to the US from an external source but, evidence of lineage is lacking and suggests that occurrences of rust floras in virgin forests demonstrate separate changes in evolution based the regional ecosystem. The phylogeograhy of forest tree rust heavily depends on information gathered from molecular and evolutionary genetics, natural history, population biology, palaeontology, historical geography, and speciation analysis.

**P04.006 Metagenomic assembly of *Ca. Liberibacter solanacearum* haplotype A from sources in New Zealand and USA suggests significant genome plasticity in the species**

*S.M. Thompson[1,2], C.P. Johnson[3], M.W.E.J. Fiers[1], A.R. Pitman[1,2], I.A.W. Scott[1], G.R. Smith[1,2] and N.C. Gudmestad[3]*
*[1]The New Zealand Institute for Plant & Food Research Limited, Gerald Street, Lincoln 7068, New Zealand; [2]Plant Biosecurity Cooperative Research Centre, Canberra, ACT 2617, Australia and [3]Department of Plant Pathology, North Dakota State University, Fargo 58108, USA*
*Email: grant.smith@plantandfood.co.nz*

Four haplotypes of *Ca. Liberibacter solanacearum* (CLsol), the putative causal agent of Zebra Chip disease of potato, have been described. The genome of CLsol haplotype B has been published revealing a genome of approximately 1.25 Mbp. Draft genome assemblies of haplotype A from two independent New Zealand sources (a single tomato potato psyllid (*Bactericera cockerelli*)) from a colony originally from tamarillo (*Solanum betaceum)* and a commercial tomato plant (sequenced on Illumina), and one USA source (Ion torrent) revealed significant changes between haplotypes A and B in the genome organisation of this fastidious phloem-limited bacterium. The three haplotype A draft genomes show significant colinerality with the exception of the prophage regions, where the two NZ domains are similar to each other, and divergent from the USA prophage sequence. All three haplotype A genomes also show a substantial number of SNPs relative to the haplotype B genome. The origin of CLsol in New Zealand is believed to be via incursions of the tomato potato psyllid (*Bactericera cockerelli*) from the US. The genetic similarity of the current assemblies of the two NZ CLsol genomes (from diverse solanaceous sources) may suggest a limited incursion of the genetic diversity of this bacterium into NZ: this preliminary hypothesis will require analysis of further NZ CLsol genomes to validate. Differences between the NZ and the US haplotype A genome suggest that there is significant genome plasticity in the species: the role of the prophage domains in genome reorganisation and diversity is unknown at this time.

**P04.007 Development of single step immuno-chromatography assay for rapid detection of quarantined karnal bunt (*Tilletia indica*) disease of wheat**

*M. Singh, D. Pandey and A. Kumar*
*Department of Molecular Biology & Genetic Engineering, College of Basic Sciences & Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar-263145, Uttarakhand, India*
*Email: manojmanu.biotech@gmail.com*

Karnal bunt (KB) disease of wheat (*Triticum aestivum*), incited by fungus *Tilletia indica* is an economical important disease and pathogen is placed as a quarantine pest. Contamination of wheat lots infected with Karnal bunt has emerged as a serious non-tariff barrier in the international trade of wheat. However, seed health testing using newer on-site and modern diagnostics is highly advocated as an integral component of KB management. In this context, for on-site testing of KB, a competitive format based lateral flow immunodipstick assay (LFID) was developed using nano-gold and anti-teliospore antibodies. For development of LFID, mono-dispersal colloidal gold was synthesized and conjugated with anti-teliospore antibodies which were further characterized under optimal condition by UV-VIS spectroscopy and transmission electron microscopy (TEM). It was found the peak showing maximum O.D. at 520 nm which indicate the synthesis of colloidal gold of optimum size and shifting of absorption maxima is an indication of efficient coupling. In order to determine the optimal conditions for conjugation of antibody and colloidal gold, the pH of the prepared colloidal gold solution was determined to be 8.5 and the minimal antibody concentration to stabilize colloidal gold was 20 µg/ml. Competitive format was used after immoblization of teliosporic protein on test zone of strip. In the absence of teliosporic proteins, the binding of the immunogold-labeled antibody with the solid-phase teliosporic proteins at test point gave a strong red band. In contrast, when the test solution contained teliosporic protein at a level above 1.5 µg/200 µl), the appearance of red colored band was found to be almost negligible. The minimum teliosporic protein concentration, which made the colour of detection line disappear was 2 µg/200 µl, but was a strong red band at control line and hence > 10 ng/µl of teliosporic protein was found to be detection limit of visual test of immuno- chromatographic strip. The anti-teliospore antibodies raised against intact teliospores showed the genera-specific reactivity. However, species specific detection is yet to be achieved by the generation of monoclonal antibodies against diagnostic

antigen identified in our lab (28 kDa teliospore wall's protein). The assay could serve as a rapid screening methodology for visual screening of KB infected wheat samples within 7-8 minutes of analysis time. The developed nanogold based immuno-chromatographic systems not only accelerate the analytical procedure but also provide a means for performing the test without the handling of reagents, allowing a one-step assay.

**P04.008 *Impatiens necrotic spot virus* infecting orchids in Yunnan, China**

*Q. Zhang, Y.M. Ding and M. Li*
*Yunnan Entry-Exit Inspection and Quarantine Bureau, No.429 Dianchi Road, Kunming，650228, P. R. China*
*Email: dingdym2008@yahoo.com.cn*

Leaves of *Phalaenopsis* and *Dendrobium* orchid plants in a nursery in Yunnan Province showed large chlorotic/ necrotic ringspot symptoms and were sampled and tested for *Impatiens necrotic spot virus* (INSV), *Tomato spotted wilt virus* (TSWV), *Watermelon silver mottle virus* (WSMoV), *Groundnut bud necrosis virus* (GBNV), *Tomato chlorotic spot virus* (TCSV), and *Groundnut ringspot virus* (GRSV) with double-antibody sandwich (DAS)-ELISA kits. All samples were positive for INSV and negative for TSWV, WSMoV, GBNV, TCSV, and GRSV. Reverse transcription (RT)-PCR was carried out with specific primers to the INSV N gene (ZI2F, 5′-GTT TAGCCTTACCAAT-3′ and ZI2R, 5′-TACCAACAAC CGTGAA-3′), designed from a sequence of GenBank Accession No. AB109100. All ELISA-positive samples yielded an amplification product of the expected 539 bp. Three clones from each isolate were sequenced and two N gene consensus sequences of the isolates from *Phalaenopsis* and *Dendrobium* were determined (GU289904 and GU289905, respectively). Nucleotide sequences of these two Chinese orchid isolates were 98 to 99% identical with sequences of isolates from the Netherlands, United States, Italy, and Japan. This is the first report of INSV infecting *Phalaenopsis* and *Dendrobium* in Yunnan Province, although INSV has been reported in *Oncidium* in Yunnan Province previously. A few thrips were found in the orchid nurseries and the orchids were imported from Taiwan and reproduced by tissue culture and it is possible that INSV may be from the infected source plant and was not eradicated completely through tissue culture.

## Concurrent Session 5-Biotechnological Applications in Plant Disease Control

### O05.001 Pattern recognition receptors: discovery, function and application in crops for durable disease control

*C. Zipfel*
*The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, United Kingdom*
*Email: cyril.zipfel@tsl.ac.uk*

Microbes and pests place major constraints on food security. Repeated agrochemical applications are the most common means of controlling diseases. Pesticides and fungicides are increasingly being banned over concerns to human health and environment. New plant protection products must meet stringent criteria to reduce adverse health and environmental impacts. Thus, more sustainable solutions for agricultural intensification are required. One way to improve plant disease resistance is to enhance the capability of the plants' own innate immune system. The first layer of plant innate immunity relies on the recognition of microbes via the perception of pathogen-/microbe-associated molecular patterns (PAMPs/MAMPs) by surface localized receptors called pattern recognition receptors (PRRs). Plant PRRs are either receptor kinases or receptor-like proteins. Loss of PRR function often leads to enhanced disease susceptibility to both adapted and non-adapted pathogens, while PAMP treatment increases disease resistance. PAMP perception is also sufficient to induce systemic-acquired resistance. Recently, it has also been shown that transgenic expression of PRRs could be used to engineer broad-spectrum disease resistance. However, the number of PAMPs and corresponding plant PRRs currently known is still limited. Here, I will illustrate our efforts, as part of collaborative European and international consortia, to document PTI responses in crops, to identify novel PAMPs and their corresponding PRRs, and to engineer and transfer PRRs in crops.

### O05.002 A cytoplasmic kinase gene provides resistance against major bacterial and fungal pathogens in *Arabidopsis* and rice

*S. Maeda[1], S. Sugano[1], J.G. Dubouzet[1], N. Yokotani[2], C.J. Jiang[1], K. Oda[2], M. Matsui[3], H. Hirochika[1], H. Takatsuji[1] and M. Mori[1]*
*[1]National Institute of Agrobiological Sciences, Tsukuba, 305-8602, Japan; [2]RIBS Okayama, Okayama 716-1241, Japan; [3]RIKEN, Plant Science Center, Yokohama, 230-0045, Japan*
*Email: morimasa@affrc.go.jp*

Broad-spectrum disease resistance against two or more types of pathogen species is desirable for crop improvement. In rice, *Xanthomonas oryzae* pv. *oryzae*, the causal bacteria of rice leaf blight, and *Magnaporthe oryzae*, the fungal pathogen causing rice blast, are two of the most devastating pathogens. To isolate rice genes conferring broad-spectrum disease resistance, the rice-FOX (Full-length cDNA Over-eXpressor) *Arabidopsis* transgenic lines that overexpressed 13,000 rice full-length cDNAs in *Arabidopsis* were used. Approximately 20,000 of the rice-FOX *Arabidopsis* lines were screened for disease resistance to a bacterial pathogen, *Pseudomonas syringae* pv *tomato* DC3000 (*Pst*DC3000), and a fungal pathogen, *Colletotrichum higginsianum*. A total of 85 and 112 rice-FOX *Arabidopsis* lines were selected by screening for resistance against *Pst*DC3000 and *C. higginsianum*, respectively. Of these, 18 lines showed strong resistance to both pathogens. The rice cDNAs in the resistant lines were identified and introduced into rice for overexpression to evaluate the disease resistance phenotype. One of the genes encoding a BIK1 like receptor-like cytoplasmic kinase, which we designated *BROAD-SPECTRUM RESISTANCE 1* (*BSR1*), conferred resistance to both *X. oryzae* pv. *oryzae* and *M. oryzae* when overexpressed in rice. Furthermore, overexpression of *BSR1* conferred resistance to *Pst*DC3000 in tomato (Micro-Tom). To investigate salicylic acid (SA) dependency of the *BSR1* dependent resistance, we crossed *BSR1*-overexpressing lines to transgenic rice lines overexpressing bacterial *NahG* gene, in which endogenous SA levels are undetectably low, and examined blast resistance. Results indicated that the disease resistance in *BSR1*-overexpressing rice is largely independent of the SA pathway.

### O05.003 Programmed cell death: insights into engineering pathogen resistance in banana and sugarcane

*M. Dickman[1], H. Khanna[2], J.Y. Paul, M. Faion[1], M. Molina[1] and J.L. Dale[2]*
*[1]Texas A&M AgriLife Research, Borlaug Center, College Station, Texas 77843 United States; [2]Centre for Tropical Crops and Biocommodities, Brisbane, Australia*
*Email: mbdickman@tamu.edu*

We have generated transgenic plants that express plant (BAG) as well as animal genes (CED-9) that negatively regulate apoptosis. These genes have the potential to generate effective disease resistance/stress tolerance in economically important crop plants. For example, the vast majority of bananas grown today have not undergone improvement through conventional breeding. The major reason for this is that most cultivars are essentially sterile. An alternate strategy is to improve current cultivars through genetic modification. We have found that *A. tumefaciens* exposure induces a programmed cell death in banana cell suspensions. More than 90% of embryogenic banana cells died after exposure to *A. tumefaciens* and cell death was accompanied

by a subset of features associated with apoptosis in mammalian cells, including DNA laddering, fragmentation, and formation of apoptotic-like bodies. Importantly, these cellular responses were inhibited in cells expressing anti-apoptosis genes. In addition, these plants were drought tolerant and resistant to the two major fungal pathogens of banana. In analogous studies, we will discuss sugarcane improvement efforts via transformation primarily for cold tolerance in an effort to expand the potential growing region of elite sugarcane varieties. Field studies are underway for both crop plants. Time permitting, we will also discuss bioinformatic efforts to identify plant genes predicted to function in an apoptotic-like manner, serving as candidate genes for crop improvement.

**O05.004 Approaches to enhance fungal disease resistance in transgenic carrots**

*Z.K. Punja, O. Wally and J. Jayaraj*
*Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada*
*Email: punja@sfu.ca*

Diseases caused by fungi and bacteria are among the most important constraints to carrot production worldwide. In this study, transgenic expression of antifungal genes was investigated to enhance tolerance in carrots The *Arabidopsis* ubiquitin promoter and *Cauliflower mosaic virus* 35S promoter showed high-level β-glucuronidase (*uidA*) gene expression in root tips, leaves and tap roots. Using an *Agrobacterium*-mediated transformation system, a thaumatin-like protein, chitinase, glucanase and peroxidase genes were expressed in carrot tissues. Leaf tissues were challenged with the fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea.* The most significant reduction was observed in the peroxidase-expressing lines, with 75-90% disease reduction. One chitinase-expressing line and 2 TLP-expressing lines had a 15-50% reduction in disease but no glucanase-expressing lines showed significant disease reduction. In a subsequent series of experiments, the Arabidopsis *Non-Expressor of Pathogenesis Related Proteins 1* (*AtNPR1*), a key regulatory gene of the SA-mediated SAR in Arabidopsis, was expressed in carrot plants under control of the 35S promoter. Two primary regenerated lines (I and XI) were challenged with the following pathogens: three necrotrophs - *S. sclerotiorum*, *B. cinerea*, and *Alternaria radicina*; a foliar-infecting bacterium - *Xanthomonas hortorum* pv. *carotae*; and a biotrophic fungus - *Erysiphe heraclei*. Greater than 50% disease reduction was observed in the transgenic lines against the necrotrophic pathogens, 80% reduction against *Xanthomonas* infection, and 90% reduction against *Erysiphe*. Experiments with transgenic carrot suspension cultures constitutively over-expressing *AtNPR1* showed that the enhanced resistance was not correlated with a constitutive induction of defense-related genes or SAR but rather with a more intense and longer induction of specific defense genes when induced with the SA analogue INA or *Sclerotinia* cell wall extracts. In particular, PR-1 and PR-2 expression was enhanced by 15-50 –fold within 3-9 hr after treatment. Peroxidase and *NPR1* gene over-expression appear to hold the greatest promise for broad-spectrum resistance disease resistance in carrots.

**O05.005 Development of transgenic sweet potato (*Ipomoea batatas* (L.) Lam.) with broad virus resistance in the Republic of South Africa**

*B.J. Sivparsad and A. Gubba*
*Discipline of Plant Pathology, School of Agricultural, Environmental and Earth Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa*
*Email: 201297209@stu.ukzn.ac.za*

Multiple virus infections of *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato virus G* (SPVG) and *Sweet potato mild mottle virus* (SPMMV) can cause a devastating synergistic disease complex of sweet potato in RSA. In order to address the problem of the multiplicity and synergism of sweet potato viruses in RSA, this study aimed to develop transgenic sweet potato cv. Blesbok with broad virus resistance. Untranslatable coat protein (CP) gene segments of SPFMV, SPCSV, SPVG and SPMMV in a sense orientation were fused to a silencer DNA, the middle half of the nucleocapsid (N) gene of *Tomato spotted wilt virus* (TSWV), and used as a chimeric transgene to induce gene silencing in the transgenic sweet potato. *Agrobacterium tumefaciens* strain LBA4404 harboring a modified binary vector pGA482G carrying the expression cassette was used for transformation of apical tips. A total of 24 putative transgenic plants were produced. Polymerase chain reaction (PCR) and Southern blot analysis showed that six of the 24 putative transgenic plants were transgenic, each with two insertion loci of the transgene and that all plants were derived from the same transgenic event. The six transgenic sweet potato plants were challenged by graft inoculation with SPFMV, SPCSV, SPVG and SPMMV-infected *Ipomoea setosa* Ker. All transgenic plants displayed delayed and milder symptoms, of chlorosis and mottling in the lower leaves when compared to the untransformed control plants. These results lay the foundation for sustainable control of virus diseases in sweet potato.

**O05.006 The plant defensin NaD1 acts synergistically with other molecules to inhibit fungal growth in vitro**

*J.A. McKenna, N. van der Weerden, M.A. Anderson and R.L. Heath*
*Department of Biochemistry, La Trobe University,*

*Australia; School of Botany, The University of Melbourne, Australia*
*Email: j.mckenna@latrobe.edu.au*

Plant defensins are small, basic, cysteine-rich proteins that are members of the innate plant defense mechanism. Several plant defensins have potent antifungal activity *in vitro* and are greater than 30 publications have reported that the transgenic expression of defensins in plants can enhance resistance against fungal pathogens. The plant defensin NaD1 was isolated from the floral tissues of ornamental tobacco (*Nicotiana alata)* and inhibits a range of filamentous fungi *in vitro*. NaD1 inhibits fungal growth by destabilizing and permeabilizing the fungal plasma membrane. With this property in mind, we have investigated the potential of NaD1 to enhance the activity of other antifungal molecules such as fungicides. Members of the triazole and strobilurin families are highly valued as agricultural fungicides. Strobilurins inhibit fungal growth by blocking mitochondrial respiration and the production of ATP. Triazoles inhibit growth by blocking the production of ergosterol leading to the incorporation of unusual sterols in the fungal membrane, thus altering permeability and fluidity. Together these classes of fungicide are the dominant form of chemical protection against fungal pathogens of agricultural crops worldwide. We have demonstrated that combinations of NaD1 and fungicides act synergistically to inhibit the *in vitro* growth of *F. graminearum*, *F. oxysporum*, *V. dahliae* and *C. heterostrophus* dramatically reducing the concentration of fungicide and NaD1 required for fungal control.

### O05.007 Increased *Fusarium* resistance of transgenic wheat by pathogen induced expression of autoactivated resistance proteins

*K. Schmidt, C. Klapprodt, M. Pflugmacher, A. Maeser, H. Keunecke, B. Holtschulte and D. Stahl*
*KWS SAAT AG, Grimsehlstraße 31, D-37555 Einbeck, Germany*
*Email: dietmar.stahl@kws.com*

Plant diseases caused by fungal infections result in serious yield and quality losses of crops. We report the pathogen induced over expression of autoactivated resistance proteins of the CC-NBS-LRR type as a new strategy to increase the fungal resistance of wheat. In order to circumvent the detrimental effect of a constitutive expression of R genes in transgenic plants the R genes were combined with pathogen inducible synthetic promoters. Synthetic pathogen-inducible promoters exclusively containing well defined regulatory elements of PR genes were identified which showed a strong induction after fungal infection of the crop and a low basic activity in uninfected tissue. The promoters were activated by different wheat pathogens like *Fusarium graminearum* and *Blumeria graminis* f. sp. *tritici*, indicating that the promoters are feasible for the development of a broad spectrum disease resistance. The ability of R-genes to trigger a hypersensitive reaction (HR) was further improved either by mutations in the NBS domain or by the singular expression of a new DAE subtype of the CC domain. Resistance assays were performed in the greenhouse and revealed an enhanced resistance of the wheat heads against *Fusarium graminearum* and *Fusarium culmorum*. Beside a reduction of head blight symptoms a reduction of fungal biomass and of the toxin deoxynivalenol was shown by qPCR and ELISA. Our results demonstrate that the controlled activation of plant defence reactions by genetic elements of plant origin is a promising strategy for a sustainable improvement of disease resistance.

### O05.008 High-throughput screening of antimicrobial peptide genes and antimicrobial activities analysis

*J.Y. Huang[1], T. Ke[2], J. Huang[1,3], C.H. Dong[1], X.D. Ma[3] and S.Y. Liu[1]*
*[1]Oil Crops Research Institute of CAAS, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Wuhan 430062, P.R. China; [2]Department of Life Science and Technology, Nanyang Normal University, Wo long Road, Nanyang 473061, P.R. China; [3]College of Life Science, Hubei University, Wuhan 430062, P.R. China*
*Email: liusy@oilcrops.cn*

Antimicrobial peptides (AMPs) are widely distributed in nature and play a critical role in the innate immunity of host defense systems. The number of described AMPs has increased over recent decades; however, the recent generation of huge amounts of genomic, transcriptomic, proteomic data open an opportunity to discover new or novel AMPs. In this study, 1308 AMP genes were predicted from ESTs and genome data of oilseed rape using bioinformatics tools, in which, 40 AMP genes were cloned and analyzed their antimicrobial activities. The average length of candidate AMP genes is 135bp, and they encode about 45 amino acids. Using the overlap PCR, the 40 AMP genes were synthesized artificially and cloned into pET30a - EDDIE - GFP expression vector. His-EDDIE-AMP fusion proteins were expressed in *Escherichia coli*. Purified His-EDDIE-AMP inclusion bodies were diluted in optimized refolding buffer and incubated to enable self-cleavage to occur. Antimicrobial activity of 40 recombinant AMPs was detected, and the result showed that 32 recombinant AMPs had specific bactericidal activities against Gram positive bacteria or Gram negative bacteria. The antifungal activities of AMPs from oilseed rape are being analyzed now. In conclusion, the method based on bioinformatics tools and the described vector-screening is useful and high- throughput for discovery of AMPs.

**P05.001 CAPS markers TAO1 and TG105 in the identification of *I2* resistant gene in Nigerian accessions of tomato, *Lycopersicon esculentum* Mill**

A.R. Popoola[1], M.R. Ercolano[2], F. Ferriello[2], P.D. Kaledzi[3], S.A. Ganiyu[1], O.F. Owolade[4], I.A. Kehinde[5], A.A. Fajinmi[1], C.G. Afolabi[1], H.K. Dapaah[6], D.K. Ojo[7], D.A. Adegbite[8], Y. Falana[9] and O.B. Adedibu[1]

[1]*Department of Crop Protection, Federal University of Agriculture, P.M.B. 2240, Alabata Road, Abeokuta, Ogun State, Nigeria. 110001;* [2]*Department of Soil, Plant, Environmental and Animal Production Sciences, University of Naples, Via Universita, 100 80055 Portici, Italy.;* [3]*Department of Horticulture, Kwame Nkrumah University of Science and Technology, KNUST, Kumasi, Ghana.;* [4]*Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan, Nigeria.;* [5]*Department of Microbiology, Federal University of Agriculture, P.M.B. 2240, Alabata Road, Abeokuta, Ogun State, Nigeria.;* [6]*Department of Crop and Soil Sciences, University of Education, Winneba, Ghana.;* [7]*Department of Plant Breeding and Seed Technology, Federal University of Agriculture, FUNAAB, P.M.B. 2240, Alabata Road, Abeokuta, Ogun State, Nigeria.;* [8]*Department of Agricultural Economics and Farm Management, Federal University of Agriculture, FUNAAB, P.M.B. 2240, Alabata Road, Abeokuta, Ogun State, Nigeria.;* [9]*Agricultural Technical Services Department, Ekiti State Agricultural Development Programme, Ikole-Ekiti, Ekiti State, Nigeria*
*Email: popoolaar@funaab.edu.ng; docakinpopoola@yahoo.com*

*Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) is a soil-borne fungus that inhabits most tomato-growing regions worldwide, causing vascular wilt disease. In Nigeria, the disease constitutes close to 40% loss in tomato yield annually. Cleaved Amplified Polymorphic Sequence (CAPS) markers TAO1 and TG105 were developed elsewhere to identify tomato genotypes possessing the *I2* gene, which confers resistance to *Fol* race 2. In this work, the two CAPS markers were used to screen fifty accessions of Nigerian tomato, *Lycopersicon esculentum* Mill, for resistance to *Fol*. The restriction enzymes *Hinf1* and *Fok1*, for TG105 and TAO1 respectively, produced fragments corresponding to presence or absence of *I2* resistant gene in the tomato accessions. Restriction fragments from the two markers indicated that 17 accessions had no *I2* gene, indicating susceptibility to Fusarium vascular wilt, while 2 accessions had fragments suggesting presence of *I2* gene and resistance status. The combined effect of the two markers enhances precision in the identification of tomato accessions with resistance status to *Fusarium* vascular wilt.

**P05.002 Interaction between *Helicoverpa zea* damage with incidence and severity of *Fusarium* sp. and *Ustilago maydis* on genetically modified corn in Sinaloa Mex.**

L. A. Aguirre, G.A .Fr ús, A. Hern ández, M. Flores, E. Cerna, J. Landeros and J. Valenzuela
*Universidad Aut ónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo Coah, Mexico, 25315*
*Email: luisaguirreu@yahoo.com.mx*

Genetically modified (GM) corn with the Cry1Ab protein of *Bacillus thuringiensis* var. *kurstaki (Bt)* and its respective isohibrid as check with and without chemical control for corn earworm were used in this research. *Fusarium* corncob damage was lower on GM corn with (12.5%) and without (25.7%) insecticide treatment as compared with conventional hybrid that had 48.3 and 83.1% of damaged corncobs with and without chemical control, respectively. Corn smut cob damage was also lower on GM corn with (3.2%) and without (6.3%) insect control compared with 15.5 and 49.7% damage with and without insecticide treatment, respectively. *Fusarium* sp. conrcob rot was also lower on GM corn with 5.7 and 9.5% whereas a 24.6 and 63% rot was observed on the isohibrid with and without insecticide control, respectively. *Ustilago maydis* severity was also lower on *Bt* corn finding 0.07% and 0.25% damage on treatments with and without insect control as compared with the conventional hybrid that showed an 11.6 and a 41.4% smut rot with and without insecticide treatment, respectively. It is concluded that GM corn resistant to *H. zea* prevents damage by the pest, eliminating the entrance pathway for *Fusarium* sp. and *Ustilago maydis.*

**P05.003 FTIR microspectroscopy for rapid identification of plant growth promoting gram-positive rhizobacteria**

M. Sompong[1], K. Thumanu[2], D. Athinuwat[3], S. Prathuangwong[4] and N. Buensanteai[1]

[1]*School of Crop Production Technology, Institute of Agriculture Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand*; [2]*Synchrotron Light Research Institute (public organization), Ministry of Science and Technology, Nakhon Ratchasima, 30000 Thailand*; [3]*Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, Pathumthani, 12121 Thailand*; [4]*Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900 Thailand*
*Email: natthiya@sut.ac.th*

*Bacillus* species *are* the most common plant growth promoting rhizobacteria, enhanced growth and induced resistance in several economic crops around the world, especially Thailand. In this study, we developed a novel strategy for the rapid identification of *Bacillus species* based on Fourier transform infrared microspectroscopy (FTIR microspectroscopy). Two reference strains of *B. subtilis* and *B. amyloliquefaciens* and 5 isolates of gram-

positive rhizobacteria isolated from soil of cassava field were used in this study. The isolates were identified according to the guidelines of bacteriology. The plant growth promoting gram-positive rhizobacteria were further identified by 16S rDNA and sequencing. A standardized experimental protocol was established, and FTIR spectral database containing more than 200 infrared spectra was investigated. FTIR microspectroscopy identification system consisted of two hierarchical levels. The top-level FTIR network allowed the identification of *B. subtilis* and an identification success rate more than 95%. The second-level network was developed to differentiate the two most relevant species of *B. subtilis* and *B. amyloliquefaciens*, with a correct identification rate more than 95%. Our results demonstrate the high degree of reliability and strong potential of FTIR spectrum analysis for the rapid identification of plant growth promoting gram- positive rhizobacteria suitable for use in routine *Bacillus* species diagnosis.

**P05.004 Gene expression of *Fusarium graminearum* during Fusarium head blight disease on wheat**

*K. Altaweel, A. Br ûl ébabel and W.G. Dilantha Fernando*
*Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada*
*Email: Dilantha.fernando@ad.umanitoba.ca*

*Fusarium graminearum* strains cause head blight of wheat and produce trichothecene mycotoxins. Mycotoxins such as deoxynivalenol (DON) affect plant and animal health, and cause significant reductions in quantity and grain quality. With the goal of understanding fungal gene expression related to pathogenicity, two cDNA libraries were created; a cDNA library was constructed, and a subtracted cDNA library was synthesized by suppression subtractive hybridization using "Sumai3" wheat heads inoculated with a highly aggressive strain and mock-inoculated samples. Fifty one fungal genes expressed during disease development were sequenced and identified through GenBank database. We present in this study the transcriptome profiling of eight fungal genes induced for the "Sumai3" wheat cultivar (resistant) and "Caledonia" (susceptible) at 6, 12, 24, 36, 48, 72 and 144 hours after inoculation (hai) using quantitative real-time PCR (qPCR). Overall, we have seen greater induction of the fungal genes in the resistant cultivar compared to the susceptible one.

**P05.005 *Fusarium oxysporum* f. sp. *cubense* resistance in transgenic lady finger and cavendish bananas**

*J.Y. Paul, B. Namukwaya, H. Khanna, A.P. James, R.M. Harding and J.L. Dale*
*Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, Queensland, Australia*
*Email: jy.paul@qut.edu.au*

*Fusarium oxysporum* f. sp. *cubense* (Foc) "Tropical" Race 4 (TR4) infects virtually all banana cultivars including the major export Cavendish complex. Foc is extremely difficult to control as there are no effective fungicidal treatments, it remains in infested soil for decades and is easily spread with contaminated equipment and planting material. TR4 has been recorded in Indonesia, Malaysia, southern China, the Philippines and the Northern Territory in Australia and is now considered a serious threat to banana production worldwide. Host resistance is considered the best approach to control Foc but low fertility and a long regeneration time has limited the development of such cultivars by conventional breeding. Biotechnology offers opportunities to develop Foc resistance while retaining the elite agronomical characteristics of Cavendish cultivars. We have previously demonstrated that anti-apoptosis genes are effective against Foc Race 1 in the susceptible cultivar Lady Finger (LF) under greenhouse conditions. We have now developed genetically modified Cavendish bananas containing these candidate resistance genes which are being assessed for resistance against TR4 in field trials in northern Australia. Additionally, plant derived stress tolerance genes have now been isolated and assessed in LF against Foc Race 1 under glasshouse conditions with some transgenic lines showing high levels of resistance. These genes have also been transformed into Cavendish varieties for field-testing against Foc TR4.

**P05.006 Structural response of *Heliopsis longipes* extract on the tomato- *Fusarium oxysporum* f. sp. *lycopersici* pathosystem**

*S. Gonz ález, H. P érez, A. Benavides, E.M. Rodr íguez and A. Flores*
*Universidad Aut ónoma Agraria Antonio Narro. Calzada Antonio Narro #1923, Buenavista, Saltillo, Coahuila, M éxico CP.25315. Departamento de Parasitolog á Agr ícola. Mexico*
*Email: aflooli@uaaan.mx; aflooli50@gmail.com*

Tomato (*Solanum lycopersicum*) is one of the most important crops from the economic and productive point view. The vascular wilt caused by *F. oxysporum* f. sp. *lycopersici* is the main disease that cause problems in tomato plants, reducing the 60% of yield, and affecting the quality of the product. In its effort to counter the attack of pests and maximize production, modern agriculture is highly dependent on inputs and synthetic pesticides. This has prompted the search for new alternatives for the management of agricultural pests and diseases, exploring the current potential of secondary metabolites and advances in systemic induced or acquired resistance. The histological effect of *H. longipes* extract on tomato plants susceptible to *F. oxysporum* f. sp.

*lycopersici* was studied. The extract of *H. longipes* was applied by spraying tomato plants, subsequently inoculated by immersion roots in a spore solution of *F. oxysporum* f. sp. *lycopersici*. Twenty days after inoculation, was determined the severity of symptoms, which was reduced 70% on plants sprayed with the *H. longipes* extract. On agronomic traits showed 56% increase in plant height, 84% in root length, 186% leaf dry weight, dry weight 177% and 502% of stem dry weight of roots in plants sprayed with the extract. In the histological analysis of stem and root of sprayed plants, it increased the area of cortex and reduced the spinal area with *H. longipes* extract.

**P05.007 Exogenous siRNA-mediated protection of plants from *African cassava mosaic virus* infection**

A. Mohamed[1, 3], T. Osman[1, 3] and Y. Hong[1,2]
[1]School of Life Sciences, University of Warwick, Coventry UK; [2]Research Centre for Plant RNA Signalling, Hangzhou Normal University, Hangzhou, P.R. China; [3]Department of Botany, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt
Email: mmm_atef@yahoo.com; amm12@fayoum.edu.eg

RNA silencing is an adaptive antiviral defence mechanism in plant. The unifying feature of RNA silencing is the production of small interfering (si) RNAs of 21-25 nucleotides that are generated from the viral genome. Moreover, profiles of viral (v) siRNAs suggest that certain regions, namely hotspots, of the viral genome are more prone to RNA silencing-mediated degradation. We hypothesised that direct targeting of viral genome by synthetic hotspot siRNAs could confer protection of plants from virus infection. To test this idea, we obtained a high resolution profile of ACMV vsiRNAs using northern blotting and high-throughput deep sequencing. One hotspot and one coldspot on each DNA component of ACMV, a ssDNA virus causing a massive destruction to cassava crop production, a staple food for more than 700 million people all over the world, were selected and in vitro transcribed to produce viral sense and anti-sense small RNAs. To test the turnover of these synthetic siRNA on ACMV infection, co-inoculation of double-stranded (ds) hotspot siRNA onto *Nicotiana benthamiana* prevented ACMV infection, in which viral DNA replication was almost undetectable and the plants remained healthy. However, neither the sense, anti-sense sRNA of hotspot vsiRNA or cold spot vsiRNA had less or no impact on ACMV infection or disease severity. Furthermore, the systemically acquired resistance by applying exogenous hotspot siRNA has a threshold effect and requires a functional *RDR6*. These data show that hotspot vsiRNAs bear a functional significance on antiviral RNAi, suggesting that they may have the potential as an exogenous biological agent for controlling destructive viral diseases such as cassava mosaic disease.

**P05.008 Overexpression of rice chitinase gene (Os-Chi II) in cotton (*Gossypium hirsutum*) confers resistance to two fungal pathogens**

M. Ganesan[1] and N. Jayabalan[2]
[1]Plant Cell Engineering, Department of Biosciences, Presidency University, Kolkata 700073, West Bengal, India; [2]Plant Biotechnology Unit, Department of Plant Science, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India
Email: markganesh@gmail.com

The present investigation describes a simple and reproducible protocol for transgenic cotton regeneration and characterization of chitinase (Chi II) gene expression against two different fungal pathogens in cotton**.** Transgenic cotton (*Gossypium hirsutum* cv. SVPR2) plants were produced by pCambia-bar-Chi II (13.8 kb) under the control of the CaMV 35S promoter, harbored in the strain LBA 4404 *Agrobacterium tumefaciens* by using shoot tip explants. Finally, from the 10 experiments, 21.8% of transformation frequency was recorded. Segregation ratio of 3:1 was recorded in the $T_0$ plant seeds. Polymerase chain reaction and southern blotting analysis were used to confirm the integration of Chi II transgene in the $T_0$ plants genome of putative transgenics. Quantitiave and qualitative (SDS-PAGE) analyses were also carried out to confirm the expression of chitinase enzyme in $T_0$ plants. Further, randomly selected transgenic plants ($T_0$) were analyzed for disease tolerance by evaluating them with spores of *Fusarium oxysporum* and *Alternaria macrospora*. All the selected PCR positive plants showed enhanced disease resistance against Fusarium wilt. The plants selected randomly showed an enhanced survival rate compared with the control when they were grown in earthen pots inoculated with $1\times10^5$ spores $100^1$ g of soil mixture. Another four randomly selected plantlets were sprayed with spores of *Alternaria macrospora* in order to test their tolerance to Alternaria leaf spot disease. After 20 days of culture, the number of lesions per leaf and the lesion length per leaf spot in non-transferred leaves increased. In the case of transgenic plantlets, lesion formation was completely absent. The disease resistance against Fusarium wilt and Alternaria leaf spot in cotton strains would serve as good breeding materials for producing fungal disease resistant cotton varieties.

**P05.009 The target gene 5' RACE validation and function prediction of soybean miR2118**

Z.Q. Luo[1,2], L.J. Qiu[1] and B.Y. Xie[2]
[1]Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R. China; [2]Institute of Vegetables and Flowes, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R. China
Email: lzhqin@163.com

MicroRNAs (miRNAs) are a new class of approximately 21 nucleotide, endogenous, small noncoding single-strand RNAs that play critical regulatory roles in plants growth and development, as well as response to biotic and abiotic stresses. In these studies, 5 classes target genes were validated by a modified RNA ligase-mediated 5′ rapid amplification of cDNA ends (5′RLM-RACE), which encoded TIR-NB-LRR class R protein, peptidase S24-like, suppressor of gene silencing 3-like (GSG3-like) protein, serine/threonine protein kinase (Ser/Thr PK) and an unkown protein. And all these target genes were mapped in some anti-disease QTL loci. In addition, expression analysis of miR2118 using Northern blotting indicated that miR2118 were upregulated by drought stress，the earliest responsive tissue was root, the leaf latest in soybean. And miR2118 didn't response to high salinity and low temperature. According to the above results, we predicted that miR2118 participated in the regulation of disease and drought resistance in soybean.

**P05.010 Transcriptional analysis of sclerotial morphogenesis in rice sheath blight pathogen *Rhizoctonia solani* Kühn AG-1 IA by cDNA-AFLP technique**

*C.W. Shu, J.L. Chen, J.Y. Chen, S. Si and E.X. Zhou*
*Department of Plant Pathology, South China Agricultural University, Guangzhou 510642, P.R. China*
*Email: exzhou@scau.edu.cn*

Sheath blight of rice, caused by the necrotic soil-borne basidiomycete fungus *Rhizoctonia solani* Kühn AG-1 IA (telemorph: *Thanatephorus cucumeris* (Frank) Donk), is one of the major diseases of rice (*Oryza sativa* L.) worldwide. Despite the mechanisms of formation and development of the sclerotium of *R. solani* had been reported, there is still lack of the knowledge on the molecular mechanisms of sclerotium formation of *R. solani*. By means of the cDNA amplified fragment length polymorphism (cDNA-AFLP) detection technique, 256 combinations of *Eco*RI and *Mse* I primers were used to identify differentially expressed genes during three stages (initial, developing and mature stages) of sclerotium morphogenesis in *R. solani*. So far, 44 primer sets, each of which could amplify 40 transcript-derived fragments (TDFs), were used. After sequencing and BLASTx analysis of 45 TDFs, 27 of which had significant homology sequences with those in GenBank database and their functions are as follows: basic and secondary metabolism, cellular biogenesis, transcriptional regulation, signal transduction transportation etc. The majority (75%) of these TDFs were observed to be differentially expressed during in the mature stage, indicating that sclerotial morphogenesis undergo a complex developmental progress. Therefore, the findings provide interesting information for the further understanding of the molecular mechanisms of sclerotial morphogenesis in *R. solani*.

**P05.011 Identification of genes important for rice blast resistance by application of the VIGS system**

*H. Xie[1], Z. Liu[1], H. Wang[2] and D. Stahl[2]*
*[1]School of Life Sciences and Technology, Tongji University, 1239 Siping Road, YangPu, Shanghai, 200092, P.R. China; [2]KWS SAAT AG, Grimsehlstrasse 31, D-37555 Einbeck, Germany*
*Email: hyx.step@gmail.com*

Fungal diseases are significant challenges to crop production, and the traditional management involved conventional plant breeding and the application of agrochemicals. Virus induced gene silencing (VIGS), as a powerful tool to study the function of plant genes, can specifically knock down the genes-of-interest. Application of silencing provides a promising new avenue to identify genes which may be relevant of plant protection. We have successfully adapted Brome Mosaic Virus (BMV) as VIGS system for rice as shown by an efficient down-regulation of the phytoene desaturase (PDS) gene expression. We further show that transient silencing of the EDR1 gene is associated with the up regulation of PR-1a gene expression and an enhanced resistance to the fungal pathogen *Magnaporthe oryzae*, the causal agent of rice blast. Our datas show that the VIGS system could be used as a high throughput technique for the identification of genes which are relevant and suitable for crop protection.

**P05.012 Molecular characterization and the transcriptional profiling of *Pi50 (t)* candidates suggests their putative functions in rice blast resistance**

*J. Su, J.L. Han, S. Chen, W.J. Wang, C.Y. Wang, L.X. Zeng and X.Y. Zhu*
*Institute of Plant Protection Research, GDAAS, No.7 Jin-ying Road, Tian-he District, Guangzhou, Guangdong, 510642, P.R China*
*Email: bsujing@hotmail.com*

Rice blast, caused by *Magnaporthe oryzae*, is one of the devastating rice diseases in the worldwide, and broad-spectrum resistance genes are highly desirable for disease control and prevention. We have identified a novel broad-spectrum blast resistance gene *Pi50 (t)* which belongs to *Pi2/9* multigene family, from a resistance donor Er-ba-zhan (EBZ) in South China. We further revealed that the resistance spectrum and race specificity of *Pi50 (t)* allele were different from other known *Pi2/9* carriers. To probe the functional candidates of *Pi50 (t)*, we sequenced the region of *Pi50 (t) loci* in EBZ. We found that there are 7 NBS-LRR type *R* gene candidates (*Nbs 1-7*) clustered in this locus, which share 44% to 100% identity in amino acid sequence compared with the cognate NBS-LRR sequences from related rice varieties in Genbank. Phylogenetic analysis of the multiple alleles based on the amino acid sequence

revealed that two out of seven *Pi50 (t)* candidates are grouped into the same clade which contains the functional *Pi2*, *Pi9* and *Piz-t*. However, low similarity among them indicating a high variation in that clade, may also explaining the difference of their race specificity. Furthermore, the transcriptional profiling of these two *Pi50 (t)* candidates in the response to rice blast suggests that they are the functional candidates in EBZ cultivar.

**P05.013 Soil metagenome-derived hydrolase reduce the exopolysaccharide production in *Ralstonia solanacearum***

*M.H. Lee[1], W. Tao[1], N.H. Kim[1], E.C. Hwang[1], J.W. Lee[2] and S.W. Lee[1]*
*[1]Department of Applied Biology, Dong-A University, Busan 604-714, Republic of Korea; [2]Department of Chemistry, Dong-A University, Busan 604-714, Republic of Korea*
*Email: loveve1980@hanmail.net*

An autoinducer 3-hydroxypalmitic acid methyl ester (3-OH PAME), which is an unique quorum sensing signal molecule, regulates the production of exopolysaccharide (EPS) in *Ralstonia solanacearum*. Previously, four soil metagenomic clones hydrolyzing 3-OH PAME were selected from metagenomic library constructed in *Escherichia coli* with industrial waste contaminated soil metagenome. Hydrolysis of 3-OH PAME by *E. coli* carrying the metagenomic clones was confirmed by gas chromatography (GC). Deduced amino acid sequences of four 3-OH PAME hydrolyzing enzymes revealed that these enzymes may encode novel family of lipase/esterase. The over-expressed and purified proteins displayed high catalytic activity towards the short chain *p*-nitrophenyl acylesters, suggesting that all of them are esterase. GC analysis of four enzyme reactions confirmed that all four enzymes hydrolyzed 3-OH PAME effectively. To investigate function of 3-OH PAME hydrolase in quorum sensing network system in *R. solanacearum*, *elp86*, *elp96*, *elp104* and *estDL33* genes were introduced into *R. solanacearum*. EPS production of four *R. solanacearum* carrying the 3-OH PAME hydrolase genes were significantly reduced in the *R. solanacearum*. These results indicated that the novel 3-OH PAME hydrolases were isolated from industrial waste contaminated soil metagenome libraries and the enzymes suppress EPS production in *R. solanacearum* by hydrolysis of autoinducer 3-OH PAME. The 3-OH PAME hydrolases could be a potential source to be introduced into susceptible host plants of *R. solanacearum* to prevent bacterial wilt occurrence.

**P05.014 Establishment of a field disease nursery for identification of resistance to *Sclerotinia sclerotiorum* in oilseed rape**

*X.H. Cheng, Y.Y. Liu, J.Y. Huang and S.Y. Liu*
*Oil Crops Research Institute of CAAS, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Wuhan 430062, P.R. China*
*Email: liusy@oilcrops.cn*

The disease caused by *Sclerotinia sclerotiorum* is one of the most important diseases in oilseed rape in China. Use of resistance varieties to the disease is the most effective measure for control of the disease. It is significant to develop a sensitive, precise and reliable method for resistance identification. In this study, a high efficient method for resistance identification in fields was developed in which two major measures were taken: one is to bury sclerotia in soil in a given way and the second to equip spray system and spray during the flowering time at a regular time interval each day. The tested winter rapeseed lines were those in the China National Regional Trial in 2005-2012. A total of 1080 lines were identified. With Zhongyou 821 as a resistance control, the lines were classified into groups of from high resistance and high susceptibility in 6 scales. The result showed that the disease pressure of more than 15% (percent diseased plants of susceptible variety) is required for a valid experiment in terms of both statistics and resistance difference distinguishment. Percent diseased plants and disease index (DI) were correlated significantly. The significant correlations of the two disease parameters between replicated and between the experiments indicated the method reliable. The wide resistance scale variation between the lines indicated the method sensitive, precise and useful. Resistance consistency of each set of the lines between the years indicated the method reproducible and reliable.

**P05.015 Antifungal activity of chitinases (ChiA, ChiC and ChiB) produced by *Serratia marcescens* CFFSUR-B2 against *Mycosphaerella fijiensis* causal agent of black sigatoka in banana (*Musa* spp.)**

*M.I. Gutiérrez-Román, M. Dunn, R. Tinoco-Valencia, F. Holguín-Meléndez, K. Guillén-Navarro and G. Huerta-Palacios*
*Departamento de Agricultura, Sociedad y Ambiente, El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km. 2.5, C.P. 30700. Tapachula, Chiapas; Mexico, +52 (962) 6289800, Fax +52 (962) 6289806*
*Email: mgutierrez@ecosur.mx*

Genes encoding chitinases ChiA, ChiB and ChiC were cloned from *Serratia marcescens* CFFSUR-B2, overexpressed as 6His-Sumo fusion proteins in *Escherichia coli*, and purified by affinity chromatography. Following affinity tag removal, chitinolytic activity of the recombinant proteins was evaluated individually and in combination using colloidal chitin as substrate. Also, antifungal activity of different concentrations (0, 3.0. 10 y 30 ppm) of ChiA and ChiB ChiC was evaluated *in vitro*

against *Mycosphaerella fijiensis* ascospores. Our results of chitinolytic activity using colloidal chitin indicated that ChiB and ChiC were highly active while ChiA was essentially inactive. The evaluation *in vitro* of chitinases showed that ChiA and ChiB were most efficient (6.84 and 3.50 ppm, respectively) in reducing 50% germinative tube of ascospores of *M. fijiensis* being Chi C (38.32 ppm) the least active enzyme. These results contribute to our understanding of the mechanism of action of the chitinases produced by strain CFFSUR-B2, which may serve in nutrient capture and hence competition with other organisms, knowing these, successful strategies for biological control of this disease at the level of field can be generated.

## Concurrent Session 6-Breeding Strategies for Plant Resistance

### O06.001 How can we achieve durable disease resistance in agroecosystems? Increase diversity!

*B.A. McDonald*
*Plant Pathology, Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland*
*Email: bruce.mcdonald@usys.ethz.ch*

Disease resistance fails because pathogens evolve. Thus the path toward durable resistance should be based on an understanding of pathogen evolution. We proposed a strategy for resistance breeding based on knowledge of pathogen evolutionary potential in 2002. An update is warranted. The root of the problem of pathogen evolution is the lack of genetic diversity in agroecosystems. Genetic diversity declined steadily in agroecosystems globally over 1000s of years and accelerated during the last 100 years to increase the efficiency of food production. Durable resistance will not be achieved unless we increase diversity at the farm and regional scales, but this must be accomplished without losing the advantages that came with increasing genetic uniformity. Many strategies can increase spatial and temporal diversity, including crop rotations, intercropping, smaller fields, new crop species, and increasing diversity in existing crop species. Breeding strategies that increase diversity include cultivar mixtures, multilines, major gene resistance (MGR) pyramids, quantitative resistance (QR) and mixing MGR and QR into the same cultivar. QR used alone is likely to erode over time as a result of pathogen evolution. It is likely that pyramids of QR and MGR will also fail eventually. A dynamic turnover of MGR and QR pyramids will be more likely to provide durable resistance. Advanced genetic technologies can enable this solution by engineering cassettes composed of MGR and QR clusters, imitating the solution that plants created over millions of years of coevolution with their pathogens.

### O06.002 Durable control of rice blast disease with resistance

*S. Koizumi*
*National Agricultural Research Center, Tsukuba International Center, Japan International Cooperation Agency, Koyadai, Tsukuba, Ibaraki 305-0074, Japan*
*Email: Koizumi-Shinzo@jica.go.jp*

Although many blast resistance genes including cloned ones and QTLs have been mapped on rice chromosomes and rice cultivars with durable resistance to blast have been reported, durable control of rice blast with resistance has not been sufficiently achieved. For stable use of race-specific and qualitative complete resistance (CR), mixtures of isogenic lines (ILs) or cultivars with different resistance, use of CR genes with broad-spectrum resistance and their pyramiding have been conducted, and accumulation of CR and quantitative partial resistance has been proposed. However, multilines have been cultivated with laborious blast race monitoring in Japan, and possibility of CR breakdown still exists for CR genes with broad-spectrum resistance. On the other hand, to durably control blast with partial resistance, several near-isogenic lines and cultivars with single partial resistance genes including cloned *pi21* and *Pb1* have been developed, and their pyramiding has been proposed, while the partial resistance genes *Pi34*, *Pi35* and *Pif* show isolate-specific resistance. Furthermore, genomic analyses have been conducted to identify QTLs conferring broad-spectrum resistance, and accumulation of candidate defense genes, which enhance quantitative resistance level, is planning. Nevertheless, there is still no sufficient evidence to decide durable blast control methods with resistance, because we have not enough knowledge on interaction between blast resistance genes and blast fungus in both individual and population levels to decide them. For durable control of rice blast disease with resistance, accumulation of knowledge on the interaction is required.

### O06.003 Engineering rice broad-spectrum resistance to diseases: gene resources and strategies

*S. Wang*
*National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research (Wuhan), Huazhong Agricultural University, Wuhan 430070, P.R. China*
*Email: swang@mail.hzau.edu.cn*

Rice crops are severely damaged by diseases caused by bacterial, fungal, and viral pathogens. Application of host resistance to these pathogens is the most economical and environmentally friendly approach to solve this problem. Broad-spectrum resistance to two or more types of pathogen species or the majority of races of the same pathogen species is desirable for rice improvement. Quantitative resistance conferred by quantitative trait loci (QTL) is a valuable resource for the improvement of rice broad-spectrum resistance. Recently years, great progress has been made in identifying the genes contributing to broad-spectrum disease resistance quantitatively. The characterization of these genes suggests that different breeding strategies should be selectively applied for efficiently using different types of resistance QTLs for rice improvement.

### O06.004 Research highlights on breeding for soybean disease resistance in the United States

*S.X. Li[1], P.Y. Chen[2] and D.R.Walker[3]*
*[1]United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Crop Genetics and*

*Production Research Unit, Stoneville, MS 38776, USA; [2]University of Arkansas, Fayetteville, AR 72701, USA; [3]USDA-ARS, Soybean/Maize Germplasm, Pathology and Genetics Res. Unit and University of Illinois, Urbana, IL 61801, USA*
*Email: shuxian.li@ars.usda.gov*

Soybean pathogens and pests in the USA annually cause economic losses by reducing seed yield and quality. The Midwest and North Central, Mid-Atlantic, Mid-South, and Southeast production regions each have a major pathogen and pest complex that can be best managed by combining genetic resistance with other methods. Breeding to improve disease resistance in soybean involves a variety of approaches and methods, ranging from traditional to molecular technology. Deliberate selection for resistance to pathogens like *Phomopsis longicolla*, a causal agent of Phomopsis seed decay (PSD), can be carried out phenotypically using controlled assays and fortuitous natural infections, or by using marker-assisted selection. Screening soybean germplasm for resistance to PSD in Southern and Midwest states has recently identified novel sources of resistance. Breeding for durable resistance to soybean rust, an important foliar disease, is particularly challenging due to the highly variable pathogenicity among populations of the pathogen. A strategy has been pursued to pyramid pairs of *Rpp* genes into adapted genetic backgrounds. Breeding for resistance to soybean virus is accomplished by incorporating single dominant genes or pyramiding several resistance loci in elite lines. Selections are done by artificial inoculation, serological assays, or gene-specific marker screen. A GBS (genotyping by sequencing) approach has been used to identify and map resistance genes for Sclerotinia stem rot. A brief overview of research on breeding soybean for resistance to these and other important diseases will be presented.

### O06.005 Taming infectious eBSV alleles for breeding new banana hybrid varieties

*J.P. Pichaut[1], B. Farinas[1], M. Umber[1], M. Chabannes[2], N. Laboureau[2], P.O. Duroy[2], L. Bonheur[1], F. Salmon[1], C. Jenny[1], M.L. Iskra-Caruana[2] and P.Y. Teycheney[1]*
*[1]CIRAD, UMR AGAP, F-97130 Capesterre Belle-Eau, Guadeloupe, France; [2]CIRAD, UMR BGPI, F-34398 Montpellier Cedex 5, France*
*Email: teycheney@cirad.fr*

Banana streak viruses (BSV) infect bananas and plantains worldwide. They are naturally transmitted by mealybugs; however infections can also occur in the absence of vector-mediated transmission, through the activation of infectious endogenous BSV sequences (eBSVs). Infectious eBSVs are present in the genome of *Musa balbisiana* spp, which are important progenitors for breeding improved banana varieties. Once activated by biotic or abiotic stresses, these viral sequences cause spontaneous infection in both natural and synthetic interspecific hybrids harbouring the *M. balbisiana* genome, denoted B. Therefore, the presence of infectious eBSVs within B genomes is currently the main constraint for breeding banana and plantain interspecific hybrids and for exchanging *Musa* germplasm. The sequence and organization of eBSVs in the diploid *M. balbisiana* genitor Pisang Klutuk Wulung (PKW) was elucidated for the three BSV species Obino l'Ewaï (BSOLV), Goldfinger (BSGFV) and Imové (BSImV). This work showed that integration of infectious eBSGFV and eBSOLV is di-allelic, with one infectious and one non-infectious allele, whereas that of infectious eBSImV is monoallelic. Taking advantage of the development of allele-specific molecular markers, eBSV signatures were established for all *M. balbisiana* genitors of the CIRAD Guadeloupe *Musa* collection, unveiling important differences between accessions. All combinations of infectious and non-infectious alleles were observed for all three BSV species, as well as complete and uncomplete integrants when compared to those described in PKW. Breeding improved *M. balbisiana* progenitors devoid of infectious eBSGFV and/or eBSOLV alleles was undertaken through self-pollination and chromosome doubling of haploid lines. Both approaches successfully lead to *M. balbisiana* cultivars devoid of infectious eBSOLV and/or eBSGFV resulting from the segregation of eBSOLV and eBSGFV alleles. Improved lines of one particular *M. balbisiana* cultivar, cv. Honduras, originally free of eBSImV, were shown to be free of infectious eBSV. These results pave the way to the safe use of *M. balbisiana* in breeding programs, and open new perspectives for breeding improved banana and plantain hybrid varieties.

### O06.006 Spinach downy mildew – resistance to a globally important pathogen

*J.C. Correll, C.D. Feng, S.T. Koike and K.E. Kammeijer*
*Plant Pathology, University of Arkansas, Fayetteville Arkansas USA; Univerisity of California Cooperative Extension, Salinas, California USA*
*Email: jcorrell@uark.edu*

Spinach downy mildew disease, caused by the oomycete pathogen *Peronospora farinosa* f. sp. *spinaciae* (*Pfs*), continues to be a major production constraint for commercial spinach (*Spinacia oleracea*). A total of 14 races of Pfs have been reported and a number of deviating isolates have been described. Based on the disease reactions of a diverse group of open pollinated spinach cultivars and hybrid spinach cultivars, six downy mildew resistance loci, designated RPF1 to RPF6, have been hypothesized. In order to determine the genetics of resistance to *Pfs*, a consortium project, involving a number of spinach seed companies, was initiated to introgress each of the hypothesized loci into a common susceptible genetic background (Viroflay) to develop Near Isogenic

Lines or NILs. Although this work is still underway, efforts with RPF1, RPF2, and RPF3 have shown that each segregates as a single locus and the resistance is dominant. Interestingly, each of the six hypothesized loci controls resistance to multiple races including some of the newest races identified. No races have been found that defeat all six of the hypothesized resistance loci. Molecular markers have been developed for RPF1 and RPF2 and additional markers are being developed for RPF3-6.

**O06.007 *R* gene-mediated resistance in *Brassica napus* that limits asexual sporulation but allows sexual sporulation by *Pyrenopeziza brassicae* (light leaf spot)**

*E.F. Boys[1,2], J.S. West[1], C.P. Werner[3], G.J. King[4], P.S. Dyer[2] and B.D.L. Fitt[5]*
*[1]Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK; [2]School of Biology, University of Nottingham, University Park, Nottingham, NG7 2RD, UK; [3]KWS UK Ltd., Thriplow, Nr Royston, Herts, SG8 7RE, UK; [4]Southern Cross Plant Sciences, Southern Cross University, Lismore, NSW 2480, Australia; [5]University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB, UK*
*Email: b.fitt@herts.ac.uk*

The phenotype of a form of *Brassica napus* (oilseed rape) *R* gene-mediated resistance against the hemibiotrophic plant pathogen *Pyrenopeziza brassicae*, cause of light leaf spot, was investigated. Using a doubled haploid *B. napus* mapping population that segregated for resistance against *P. brassicae*, development of visual symptoms was characterised and asymptomatic growth was followed using quantitative PCR and scanning electron microscopy on leaves of resistant/susceptible lines inoculated with suspensions of *P. brassicae* conidia. A new phenotype of resistance was observed in lines derived from *B. napus* cv. Imola. Initially, in controlled environment experiments growth of *P. brassicae* was unaffected; then from 8 days post-inoculation (dpi) some epidermal cells collapsed ('black flecking') in green living tissue of cv. Imola and from 13 to 36 dpi there was no increase in amount of *P. brassicae* DNA and no asexual sporulation (acervuli/pustules). By contrast, during this period there was a 300-fold increase in *P. brassicae* DNA and extensive asexual sporulation in leaves of susceptible cv. Apex. However, when leaf tissues senesced, there was a rapid increase in amount of *P. brassicae* DNA in the resistant but not susceptible cultivar and sexual sporulation (apothecia) was abundant on senescent tissues of both. These results were supported by results of controlled environment and field experiments with lines from the mapping population that segregated for this resistance. Analysis of results of both controlled environment and field experiments suggested that the resistance was mediated by a single *R* gene located on chromosome A1.

**O06.008 Pathogen-based resistance: a strategy for the protection of plants against Fusarium head blight and mycotoxins**

*H.P. Li[1,2], J.B. Zhang[1], T. Huang[1], W. Cheng[1], P. Yang[1], Q.S. Yuan[1] and Y.C. Liao[1,3]*
*[1]Molecular Biotechnology Laboratory of Triticeae Crops, Huazhong Agricultural University, Wuhan 430070, Hubei, P.R. China; [2]College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, P.R. China; [3]College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, P.R. China*
*Email: yucailiao@mail.hzau.edu.cn*

Pathogen-based resistance (PBR) has been proposed to describe antibody-mediated disease resistance and similar phenomena in plants. The concept is based on the fact that pathogen-specific antibodies are generated by using pathogens as targets for selection and only antibodies that specifically bind to the pathogens can be isolated. This strategy has been used to protect plants against *Fusarium graminearum*, a toxigenic pathogen responsible for Fusarium head blight of wheat and other cereals in China and many other countries. Innate resistance for FHB is inadequate and investigation of antibodies with resistance roles could provide an alternative strategy for breeding FHB-resistant cultivars. Cell wall-bound proteins from a representative strain of *F. graminearum* in China were used to generate antibodies. Recombinant antibodies specific to antigens displayed on the *Fusarium* cell surface were isolated from a pooled immunocompetent phage display library. The isolated antibodies inhibited fungal growth *in vitro* when fused to antifungal peptides. Expression of the antibody fusion proteins *in planta* conferred a high level of protection against *Fusarium* pathogens, with a significant reduction in initial infection, fungal spread and mycotoxin production. Envisaged as a promising resistance germplasm, the resistant antibodies were further improved *in vitro*, generating 11- to 15-fold higher affinities for FHB pathogens and conferring even higher resistance to FHB. Thus, PBR with defined specificity has potential to improve resistance in plants, especially in pathosystems that lack natural resistance.

**P06.001 *Sclerotinia sclerotiorum* (Lib.) de Bary culture filtrate in developing resistance through tissue culture techniques in rapeseed–mustard and biochemical basis of resistance**

*N. Mehta and S. Watpade*
*Department of Plant Pathology, CCS Haryana Agricultural University, Hisar – 125004 Haryana, India*
*Email: nareshmehta282@gmail.com*

Rapeseed-mustard assumes the significance in Indian economy by occupying the second position next to groundnut and consider as a 'cash crop' in dry land

agriculture. The relatively susceptibility of this crop to *Sclerotinia sclerotiorum* in India is one of the limiting factor in productivity in addition to others biotic and abiotic factors. Eleven prominent genotypes/varieties were inoculated artificially at stem portion and genotype *B. carinata* cv. HC-9002 escape the infection whereas all other species had disease intensity more than 50% and these were considered as highly susceptible. In tissue culture studies, callogenic response to culture filtrates of *Sclerotinia sclerotiorum* added in MS medium supplemented with NAA 1 $mg^{-}1$+ BAP 1 $mg^{-}1$ was more prominent in resistant/tolerant genotype *B. carinata* cv. HC-9002 than in other *Brassica* species. *S. Sclerotiorum* culture filtrate at 15% concentration can be applied in *in vitro* screening for selection of tolerant species. Total soluble sugars and reducing sugars were high after selection with fungal culture filtrate in calli of *B. napus* cv. GSH-1 and *B. carinata* cv. HC-9002 while susceptible species showed low sugar contents. Increased level of culture filtrate in callus medium increases total phenol, flavanol and total soluble protein as well as polyphenol oxidase, peroxidase and catalase enzymes. PPO and peroxidase activity was higher in *B. carinata* cv. HC-9002 which was less in susceptible than other species, while catalase activity was maximum in *B. juncea* cv. RH-30 which was highly susceptible to this disease.

### P06.002 High level resistance to *Sclerotinia sclerotiorum* and *Pseudocercosporella capsellae* in introgression lines derived from hybridization between *Brassica carinata* and the oilseed crop *B. napus*

*M.P. You[1], S.S. Banga[2], S.K. Banga[2], M.B. Uloth[1], N. Gunasinghe[1], P.M. Finnegan[1], P.A. Salisbury[3] and M.J. Barbetti[4]*

*[1]School of Plant Biology, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, W.A. 6009, Australia; [2]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, 141004 Punjab, India; [3]Melbourne School of Land and Environment, University of Melbourne, Victoria 3010 Australia; [4]School of Plant biology, The Institute of Agriculture, Faculty of Science, The University of Western Australia, Crawley, Western Australia 6009 Australia*

*Email: mingpei.you@uwa.edu.au*

Sclerotinia stem rot (*Sclerotinia sclerotiorum*, SSR) is a serious threat to oilseed rape (*Brassica napus*) production in regions of Asia, Europe, North America and Australia. White Leaf Spot (*Pseudocercosporella capsellae*, WLS) is also an important disease worldwide. There is keen interest to identify sources of resistance, in particular to SSR, but also to WLS, especially where the two coincide creating a need for high levels of SSR + WLS resistance. Using field stem inoculation at 50% flowering with *S. sclerotiorum*, and sequential conidial inoculations with *P. capsellae* during the growing season, 54 *B. napus* genotypes carrying *B. carinata* introgressions were tested in the field within a nylon mesh insect-proof tent. SSR severity was assessed as stem lesion length 3 weeks post inoculation, while WLS severity was assessed on a 0 to 10 scale separately for both incidence and severity throughout the season. A range of varietal reactions occurred among the tested genotypes in response to inoculation with either pathogen, ranking from highly susceptible (lesion length ≥36.5mm SSR; score > 5 for severity of WLS) to highly resistant (lesion length ≤ 10mm SSR; score ≤2 for severity of WLS). Fourteen of the tested genotypes showed combined resistance to both SSR (lesion length ≤ 10mm) and WLS (severity score ≤ 2), providing the first combined high level resistance against both diseases within the one genotype, offering new opportunities for oilseed *Brassica* breeding programs to rapidly develop new varieties with combined SSR + WLS resistance.

### P06.003 Diverse cruciferous species offer potential for developing new *Brassica* crops highly resistant to *Sclerotinia sclerotiorum*

*M.B. Uloth[1], M.P. You[1], P.M. Finnegan[1,5], Y. Huang [2], P.A. Salisbury[3,4] and M.J. Barbetti[1,5]*

*[1]School of Plant Biology, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia.; [2]Institute of Oil Crops Research, China Academy of Agricultural Science, Wuhan 430062, P.R. China; [3]Melbourne School of Land and Environment, University of Melbourne, Victoria 3010, Australia; [4]Department of Primary Industries Victoria, VABC, 1 Park Drive, La Trobe R and D Park, Bundoora, Victoria 3083, Australia; [5]The Institute of Agriculture, Faculty of Science, The University of Western Australia, Crawley, Western Australia 6009, Australia*

*Email: ulothm@hotmail.com*

Sclerotinia stem rot (SSR) is a serious threat not only to *Brassica napus*, *B. juncea* and other *Brassica* oilseed production in regions of Asia, Europe, North America and Australia, but also to vegetable *Brassica* production worldwide. As most commercial cruciferous oilseed and horticultural cultivars are highly susceptible to SSR, there is keen interest in identifying sources of resistance that could be utilised to develop cultivars with effective resistance. Genotypes from Brassicas *B. carinata, B. fruticulosa, B. juncea, B. napus, B. oleracea, B. cretica, B. incana, B. insularis, B. montana, B. nigra, B. oxyrrhina, B. parachinensis, B. rapa and B. tournefortii*, and representatives from genera *Carrichtera, Diplotaxis, Eruca, Hirschfeldia, Raphanus, Rapistrum, Sinapis* and *Sisymbrium*, were tested in the field within a nylon insect-proof-mesh covered house by inoculating stems with agar plugs colonised by *S. sclerotiorum*. Lengths of stem lesions were measured 3 weeks post inoculation. Mean lesion length across tested genotypes ranged from

less than 3mm to more than 150mm. Stem lesion length within multiple selections of *B. carinata* and *B. oleracea* varied widely, highlighting the need to test sufficient genotypes when determining the potential of a species as a source of resistance to SSR. Genotypes with stem resistance identified in this study are of high value for developing new disease-resistant cultivars of oilseed and vegetable Brassicas.

**P06.004 Breeding of Czech malting barley cultivars for disease resistance**

*P. Matušinsky[1], L. Tvarůžek[1], M. Balounová[1] and T. Sedláček[2]*
*[1]Agrotest Fyto, Ltd., Havlickova 2787/121, 767 01 Kromeriz, Czech Republic; [2]Research Centre SELTON, Ltd., Stupice 24, 250 84 Sibrina, Czech Republic*
*Email: matusinsky@vukrom.cz*

The Czech malting industry produces 500,000 tons of malt a year for which approximately 180,000 ha of barley are necessary to be planted in fertile regions. In recent years, 50 - 60 % of spring barley area has been sown with Czech cultivars. This has considerably been encouraged by attaining a protected geographical indication (PGI) "Czech Beer". The barley cultivars for production of beer with the PGI "Czech Beer" are characterized by lower levels of proteolytic and cytolytic modification, and lower apparent attenuation limit causing the presence of residual extract. Another essential precondition is a high level of resistance to fungal diseases. In spring barley these are especially powdery mildew (*Blumeria graminis*), leaf rust of barley (*Puccinia hordei*), net blotch (*Pyrenophora teres*), scald (*Rhynchosporium secalis*), spot blotch (*Cochliobolus sativus*), ramularia leaf spot (*Ramularia collo-cygni*) and others. Molecular markers rank among selection tools exhibiting the highest use potential. They are useful to identify resources of resistance to fungal diseases such as powdery mildew (allele *mlo-11*, Piffanelli et al. 2004), leaf rust (genes *Rph7* and *Rph16*, Brunner et al. 2000, Ivandic et al. 1998), net blotch (gene *Rpt5*, Manninen et al. 2000, Friesen et al. 2006) and others. Effective alleles of these genes are introduced in barley lines intended for breeding cultivars for beer production with the PGI "Czech Beer".

**P06.005 Understanding interactions between quantitative resistance and qualitative resistance for sustainable management of Phoma stem canker (*Leptosphaeria maculans* ) in oilseed rape (*Brassica napus*)**

*Y.J. Huang, G.K. Mitrousia and B.D.L Fitt*
*School of Life and Medical Sciences, University of Hertfordshire, Hatfield, Herts, AL10 9AB, UK*
*Email: y.huang8@herts.ac.uk*

Effective control of phoma stem canker (*Leptosphaeria maculans*) relies on use of host resistance. Two types of resistance to *L. maculans* have been identified; major resistance (*R*) gene-mediated qualitative resistance and quantitative resistance (QR). *R* gene-mediated resistance is race-specific and often rendered ineffective due to pathogen population changes from avirulent to virulent. QR is race non-specific and is considered durable. To investigate the interactions between *R* genes and QR, eight cultivars with different resistance were used in field experiments at 13 different sites in the 2010/2011 and 2011/2012 growing seasons. Severity of phoma leaf spots in autumn and severity of phoma stem canker in summer were assessed. Results showed that there were effects of background quantitative resistance on the effectiveness of an *R* gene. The severity of stem canker on DK Cabernet (*Rlm1* + QR) was less than on Capitol (*Rlm1*), suggesting that *Rlm1* is more effective when it is introduced into a host background with QR than in one without QR. Similarly, less severe stem canker on Adriana (*Rlm4* + QR) than on Bilbao (*Rlm4*) suggested that *Rlm4* is more effective when it is introduced into a host background with QR than in one without QR. Interestingly, cultivars Roxet and Excel both carry *Rlm7* but Excel developed less severe phoma leaf spots and stem canker at most experimental sites than Roxet. It is not clear whether this was due to difference between them in host background QR because avirulent *AvrLm7* was predominant in *L. maculans* populations at UK sites.

**P06.006 Development of homozygous pepper (*Capsicum annuum* L.) lines carrying *Potato virus Y* (PVY) resistance genes (*pvr2[1]* and *pvr2[2]*) using marker-assisted selection (MAS)**

*V. Moodley, R. Naidoo and A. Gubba*
*Discipline of Plant Pathology, School of Agricultural, Environmental and Earth Sciences, University of KwaZulu-Natal, Pietermaritzburg, Republic of South Africa*
*Email: 201501239@stu.ukzn.ac.za*

Pepper (*Capsicum annuum* L.) is an important vegetable crop grown and consumed worldwide. *Potato virus Y* (PVY) is a highly destructive, globally distributed pathogen which significantly reduces the yield and quality of cultivated peppers. Chemical and cultural methods have proven ineffective in controlling PVY; therefore the development of resistant varieties is the best alternative to manage PVY diseases on pepper. Several alleles at the *pvr2* locus are known to control recessive resistance to PVY in pepper. In this study, homozygous pepper lines were developed from local germplasm carrying PVY resistance genes (*pvr2[1]* and *pvr2[2]*) using marker assisted selection (MAS). $F_1$ progeny were obtained by crossing a *pvr2[1]* (resistant) 'Double Up' cultivar with a heterozygous susceptible 'Benno' cultivar carrying *pvr2[+]* (susceptible) and *pvr2[2]* (resistant) alleles. $F_1$ and

$F_2$ generations were assessed for the presence of PVY resistance alleles (*pvr2*$^+$/*pvr2*$^1$/*pvr2*$^2$/*pvr2*$^3$) at the *pvr2-elF4e* locus using the tetra primer amplification refractory mutation system–polymerase chain reaction (ARMS-PCR) procedure. Negative selection was carried out using markers to detect the *pvr2*$^+$ (susceptible) allele. All $F_1$ progeny displaying the *pvr2*$^+$ allele were eliminated from further study. A total of 302 plants belonging to 29 $F_2$ families expressing homozygous recessive traits were mechanically inoculated to test for their response to PVY infection. Resistance to PVY was confirmed in all selected families based on symptomatology in greenhouse screens. These results show that ARMS-PCR can be used to screen pepper genotypes for alleles that confer PVY resistance thereby contributing to the improvement of pepper production.

**P06.007 Reaction of tomato lines carrying different *Ty*-gene combinations to leaf curl viruses in Taiwan**

W.S. Tsai, P. Hanson and L. Kenyon
*AVRDC – The World Vegetable Center, 60 Yi-min Liao, Shanhua, Tainan 74151, Taiwan, China*
*Email: wen-shi.tsai@worldveg.org*

Leaf curl diseases caused by begomoviruses (whitefly-transmitted geminiviruses) are important constraints to tomato production in many parts of the world. The deployment of resistant cultivars is considered as an efficient and sustainable measure for managing such diseases. We compared the reaction of tomato lines carrying different combinations of leaf curl resistance (*Ty*) genes to whitefly-mediated inoculation in the net house with either *Tomato leaf curl Taiwan virus* (ToLCTWV) or *Tomato yellow leaf curl Thailand virus* (TYLCTHV), the predominant tomato-infecting begomoviruses in Taiwan. Symptom development on the test plants was observed for 11 weeks following exposure to viruliferous whiteflies (*Bemisia tabaci*) and virus infection was confirmed by polymerase chain reaction (PCR) with universal and specific begomovirus primers. For lines carrying *Ty-1*/*Ty-3* at least 69% of the plants became infected with either ToLCTWV or TYLCTHV. Tomato lines carrying *Ty-2* (a gene first identified in Taiwan using ToLCTWV) alone showed good resistance to ToLCTWV (<24% infected), but were susceptible to TYLCTHV (>95% infected). Three tomato lines carrying both *Ty-2* and *Ty-1*/*Ty-3* showed good resistance to both ToLCTWV and TYLCTHV (<5% infected), and one *Ty-2* + *Ty-1*/*Ty-3* combination line presented no infection by either ToLCTWV or TYLCTHV. The results indicate that combining *Ty* genes can create an additive effect, but it may be necessary to identify molecular markers more tightly linked with these genes and to take greater account of the background in which the genes are deployed.

**P06.008 Pathogenicity of *Leptosphaeria biglobosa* to oilseed rape (*Brassica* napus) cultivars differing in resistance against *Leptosphaeria maculans***

G.K. Mitrousia, Y.J. Huang, A.M. Hall and B.D.L. Fitt
*School of Life and Medical Sciences, University of Hertfordshire, Herts, AL10 9AB, United Kingdom*
*Email: g.mitrousia@herts.ac.uk*

Field and controlled environment experiments were done to examine the pathogenicity of *L. biglobosa* on cultivars with good resistance (carrying the *Rlm7* gene) or moderate resistance (carrying the *Rlm4* gene) against *L. maculans*, causal agents of phoma stem canker on oilseed rape. Leaves with phoma leaf spots were sampled from established winter oilseed rape field trials at three representative sites in the UK. The proportions of lesions caused by *L. maculans* and *L. biglobosa* were scored on cultivars carrying the *Rlm7* gene (Excel and Roxet) or the *Rlm4* gene (Adriana and Bilbao) and the susceptible cultivar Drakkar. The proportion of *L. biglobosa* leaf spotting was greater on the cultivars that carrying *R* genes than on Drakkar. Leaf spotting caused by *L. biglobosa* was greater in the south than the north of England for all the cultivars. The aggressiveness of *L. maculans* and *L. biglobosa* isolates obtained from Excel (LmExc, LbExc) and Drakkar (LmDr, LbDr) was tested on Drakkar, Excel and Roxet. LbExc was more aggressive than LbDr on the three cultivars. There was no difference between the three cultivars in aggressiveness of LbExc and LbDr. LmDr was more aggressive on Roxet than on Excel. There was no difference between Excel and Roxet in aggressiveness of LmExc. LmExc was less aggressive than LmDr on Drakkar. These results indicate that breeding for resistance against *L. maculans* affects the susceptibility of the cultivars to *L. biglobosa.*

**P06.009 Resistance associated to several pathogens in tomato: a meta-QTLs analysis**

L. Balzano-Nogueira[1], E.A. Carbonell[2], W.E. Fry[3] and J.R. Demey[1]
[1]*Laboratorio de Biometría y Estadística. Instituto de Estudios Avanzados IDEA. Valle de Sartenejas, Caracas 1080, Venezuela;* [2]*Unidad de Biometría. Instituto Valenciano de Investigaciones Agrarias IVIA. Valencia, Spain;* [3]*Department of Plant Pathology and Microbe-Biology, Cornell University, Ithaca, USA*
*Email: lbalzano@idea.gob.ve*

*Lycopersicon esculentum* is one of the crops majorly planted worldwide for its different uses and nutritional value. This crop is sensitive to be attacked from various pathogens that cause significant economic losses. Many strategies of disease control are applied against each pathogen; however, the most appropriate control method involves the use of intrinsic defense mechanism of selected plants. The selection of resistant plants is usually due to recurrent marker-assisted selection (MAS). To

accomplish resistant plant selection, researchers have correlated genotypic with phenotypic characteristics using several statistical and quantitative genetics methods, generating different loci resistance-related QTLs. We perform a meta-analysis from nine (9) studies reporting several resistance-related QTLs, in order to generate a consensus map of tomato QTLs by integration study-characteristics like: crossing size and type, LOD score and additivity, with marker chromosome maps obtained from sol genomics network. We obtained 24 meta-QTLs in regions associated to resistance to *Phytophtora infestans* (92%), *Alternaria solani* (46%), *Botrytis cinerea* (13%), *TYLCV* (8%), *Xantomonas perforans* (4%) and *Xantomonas campestris* (4%). These results can be used to distinguish other resistant lines through detection of these regions in other tomato genomes, contributing to increase culture-resistance by selecting more resistant progeny after each sowing process.

**P06.010 New DNA markers (RAPDs) to identify rust resistant and susceptible genotypes of sugarcane**

*K. Muhammad[1,2], Y. Que[3], H. Ahmad[2] and J. Iqbal[1]*
*[1]Department of Genetics, Hazara University Mansehra, 21300, Pakistan; [2]School of Biological Sciences, University of the Punjab, Lahore, 54590, Pakistan; [3]Sugarcane Research Institute / Key Lab of Sugarcane Genetic Improvement, Fujian Agriculture and Forestry University Fuzhou MOA, 350002, P.R. China*
*Email: khushisbs@yahoo.com*

Present study set out to screen and identify rust resistance elite genotypes of sugarcane on the basis of agronomical impression and PCR based DNA markers. The RAPD (Random Amplified Polymorphic DNA) technique was used for potential estimation of genetic relatedness among commercially grown sugarcane genotypes of Pakistan. In this study, six sugarcane genotypes were characterized on the basis of rust resistance and susceptibility under natural inoculation in the field and divided into two discrete phenotypic classes, three rust resistant genotypes RR and three rust susceptible genotypes RS, for identification of RAPD markers. Initially, 260 decamers were subjected against the genomic DNA of six commercially grown sugarcane genotypes. After screening, 12 of 260 decamers were picked on the basis of polymorphism and specificity, which are the most important application of DNA markers. Nine of 12 produced 13 genotype specific loci. We were also able to trace specific loci in RR and RS discrete classes of sugarcane commercially grown genotypes in Pakistan. From 12 primers, 3 generated 3 specific loci, 2 loci in RS group and 1 locus in RR group. Primer L-04 and L11 generated 800 bp and 900 loci in rust susceptible group of genotypes (RS) respectively. But, only one primer L15 amplified 1400 bp locus in rust resistant group of genotypes (RR). This study will be useful to provide powerful tool to explore molecular basis of disease resistance in sugarcane. Moreover, these markers could be converted into more specific, stable, reliable and reproducible SCAR (Sequence Characterized Amplified Region).

**P06.011 Pathogenecity of *Sclerotinia sclerotiorum* isolates to *Brassica juncea* carrying introgressed resistance**

*P.S. Sandhu[1], C. Atri[1], M.J. Barbetti[2] and S.S. Banga[1]*
*[1]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141 004, India; [2]School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA, 6009, Australia*
*Email: jaisandhu@rediffmail.com*

Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* is one of the most destructive diseases of rapeseed-mustard worldwide. Genetic resistance is the only reliable and sustainable tool for disease management. We had previously reported high level of resistance in the *Brassica juncea* introgression lines. *B. juncea* lines carrying genomic segments from *Erucastrum cardaminoides*, *Diplotaxis tenuisiliqua* and *B. fruticulosa* had much higher levels ($P < 0.001$) of resistance in comparison to the standard check germplasm. Experience with this pathogen showed that identifying sources of resistance is a challenging task due to varied aggressiveness of the pathogen isolates. To study such varied responses, thousands of field grown plants were stem inoculated to confirm the resistance, and its functionality against four isolates representing key mustard growing areas of North-west India. These included: Bharatpur (2), Ludhiana (1) and Bawal (1). These isolates were selected mostly on the basis of geographic diversity, and sclerotia morphology. Bharatpur 1 was the most pathogenic isolate followed by the one from Ludhiana. Significant differences were observed among the isolates ($P \leq 0.001$) in relation to their pathogenecity as well as among the germplasm ($P \leq 0.001$) for their responses to the different isolates. Many introgression lines were resistant to all the four isolates. The frequency of resistant reaction to four, three, two and only one isolate was 23.8, 30.9, 28.6 and 16.6, respectively. The sources of resistance identified in this study are highly valuable and are being genotyped to initiate marker assisted breeding for resistance in oilseed Brassica.

**P06.012 Interspecific blackleg (*Leptosphaeria maculans*) resistance transfer into oilseed rape (*Brassica napus*)**

*H. Winter, M. Mosch, F. Marthe, H. Peterka, O. Schrader and H. Budahn*
*Department of Biology–Molecular Biotechnology, Technische Universitaet Dresden, D-01062 Dresden, Germany*
*Email: he_winter@gmx.de*

Blackleg caused by the ascomycete *Leptosphaeria maculans* (*Phoma lingam*) is the most significant disease affecting oilseed rape (*Brassica napus*) worldwide. Considering climate change, it is expected to become even more relevant in future. To widen the narrow base of oilseed rape resistance, offspring derived from somatic hybrids *B. oleracea* (+) *B. nigra* and *B. oleracea* (+) *B. carinata*, respectively, are currently characterised and developed towards the *B. napus* karyotype (genome AACC, 2n=38). The focus of this study is on blackleg resistance behaviour of selected selfing and backcross offspring produced using embryo rescue techniques. Adult plant resistant individuals of different generations, along with susceptible genotypes, were examined cytologically, e. g. by genomic *in situ* hybridisation (GISH). Furthermore, the most promising genotypes were self pollinated again and backcrossed with *B. napus* to obtain resistant plants with an AACC background. Moreover, a complete set of nine disomic *B. napus-Raphanus sativus* addition lines ($2n=38_{AACC}+2_{(R)}$), originally developed for nematode resistance transfer, has been examined in blackleg resistance tests. One of these lines showed adult plant resistance similar to *R. sativus*. GISH results are compared with those obtained earlier from blackleg resistant addition and putative recombination lines derived from interspecific, sexual hybrids between *B. napus* and *Sinapis arvensis*, *Coincya monensis* and *B. juncea*, respectively.

**P06.013 Resistance of Polish cultivars of potato to the most relevant pathotypes of *Synchytrium endobioticum* (Schilb.) Perc.**

*J. Przetakiewicz*
*Plant Breeding and Acclimatization Institute – National Research Institute, Department of Plant Pathology, Laboratory of Quarantine Organisms, Radzikow, 05-870 Blonie, Poland*
*Email: j.przetakiewicz@ihar.edu.pl*

*S. endobioticum* is one of the most known soil-borne pathogen of cultivated potato. Although *S. endobioticum* originated from Andean zone in South America, thanks to the popularity of the potatoes is distributed world-wide and occurs almost around the world. The geographical distribution of this pathogen includes all EPPO region, Asia, North and South America as well as Oceania. The most widely is distributed pathotype 1(D1) of *S. endobioticum* but in 1941 the first reports of race diversity of *S. endobioticum* were observed on potato resistant cultivars. During the next decades of the last century 43 different pathotypes of *S. endobioticum* were described in Europe. The only strategies to confine the disease are strict quarantine and phytosanitary measures as well as the cultivation of resistant cultivars of potato. According to the Polish low all cultivars of potato have to be resistant to pathotype 1(D1) of *S. endobioticum*. Among of them there was possible to find cultivars which were resistant to European virulent pathotypes [2(G1), 6(O1), 8(F1) and 18(T1)] of *S. endobioticum*. Eleven of them: Adam, Bzura, Cekin, Gandawa, Ibis, Igor, Ikar, Kuba, Wiarus, Wist and Zagloba were resistant to all tested pathotypes of *S. endobioticum*. Cv. Gawin and Bosman were resistant to all pathotypes except 8(F1) for Gawin and 2(G1) for Basman. Cv. Jutrzenka, Sleza, Legenda and Ruta were resistant to three pathotypes: Jutrzenka and Legenda to 1(D1), 6(O1) and 18(T1); Sleza and Ruta to 1(D1), 2(G1) and 6(O1). Cultivar Czapla was resistant to pathotype 1(D1) and 2(G1) of *S. endobioticum*.

**P06.014 Resistance levels of on-farm selected cocoa accessions to Phytophthora pod rot, Vascular-Streak Dieback and Cocoa Pod Borer in Sulawesi, Indonesia**

*A. Purwantara[1,2], H.B. Purung[3], P.J. McMahon[4], S. Mulia[5], N. Laila[5], K. Fauziah[5], S. Lambert[3], A.W. Susilo[6], E. Sulistyowati[6], S. Sukamto[6], A. Iswanto[2], D.I. Guest[1] and P.J. Keane[4]*
*[1]Faculty of Agriculture and Environment, University of Sydney; [2]Biotechnology Research Institute for Estate Crop; [3]Mars Inc., Jln Kima 10, Makassar, Sulawesi; [4]Department of Botany, La Trobe University; [5]Balai Pengkajian Teknologi Pertanian Sulawesi Selatan; [6]Indonesian Coffee and Cocoa Research Institute*
*Email: david.guest@sydney.edu.au*

Phytophthora pod rot (PPR) and Vascular-Streak Dieback (VSD) are the most important diseases of cocoa in Indonesia. Along with Cocoa Pod Borer, these diseases and pest cause significant losses of cocoa in all growing areas. Therefore resistance to these major diseases and pest is becoming an increasingly important criterion for selection of new cocoa cultivars/clones in Indonesia. Since 2000, Indonesian Coffee and Cocoa Research Institute (ICCRI) and Mars Inc, under Australian Centre for International Agricultural Research (ACIAR) project, have been selecting new cocoa accessions with direct involvement of farmers. Farmers were visited and their mother trees with high yield potential, low PPR and VSD incidence and/or with low CPB pest infestation were collected as on-farm accessions. These local accessions, along with 3 ICCRI crosses and two Malaysian clones (PBC123 and BR25), were used in the trials in two locations, in Polewali-Mandar District, West Sulawesi and in Pinrang District, South Sulawesi. During the first two years of harvest, the average annual dry bean production (estimated per ha) was between 196 kg (Nasir Rauf) and 702 kg (KW617). For the two years, 2 local accessions showed lowest incidence of PPR (Nasir Rauf 2.3% and Husbitori 4.3%), whereas the highest was in PBC123 (12.8%). In both years, incidence of PPR was below 16% in any clone. The accessions demonstrating highest VSD resistance were Gene J and M05, at similar level to PBC123. Cocoa pod borer infestation (average % pods infested for the two years)

indicated the high level of tolerance in Husbitori (21.9%), but high susceptibility in Gene J (63.5%), KW523 and KW617 (48% in each) and M01 (46.9%). Some clones indicated useful characteristics but were either highly susceptible to CPB or VSD (e.g. Gene J and Husbitori), or had poor quality beans (e.g. M05). Two selections, Muhtar and Ilham, were unproductive and excluded from the evaluations. The results of this study showed the usefulness of farmers' participation in the identification of trees resistant to PPR, VSD and CPB. It is recommended that the genetic diversity identified through participatory selection of promising mother trees in farmers' fields be further exploited in breeding to obtain new hybrid or cocoa clones with high yield and good quality, and low incidence of PPR and VSD, and low CPB damage.

**P06.015 Screening of deshi chickpea (*Cicer arietinum* L.) germplasm resistant to Botrytis gray mold in Bangladesh**

*M.H. Rashid[1], M.K. Shahadat[1], M.A. Quddus[2], M.R. Hasnat[3] and M. A. Hossain[4]*
*[1]Regional Agricultural Research Station, Bangladesh Agricultural Research Institute (BARI), Rahmatpur, Barisal, Bangladesh ; [2]Pulse Research Substation, BARI, Madaripur ; [3]Instructor, Agricultural Training Institute, Faridpur, Bangladesh; [4]Pulses Research Centre, BARI, Gazipur, Bangladesh*
*Email: md_harunor_rashid@yahoo.com*

An experiment was carried out at the Pulse Research Sub-Station (PRSS), Madaripur, Bangladesh during three subsequent years of 2008-2009, 2009-2010 and 2010-2011 to screen out the suitable high yielding chickpea varieties that performed best against Botrytis gray mold under natural epiphytotic condition. Thirty three kabuli chickpea lines/varieties collected from ICARDA, Syria along with check BARIchhola-5 were used under this experiment. The experiment was conducted in RCBD with three replications. Plant debris and *Botrytis cinerea* inocula ($10^5$ conidia/ml) were applied in this nursery at the flowering stage for ensuring the high disease pressure. The severity of BGM was recorded at flowering stage, pod formation stage and maturity stage. Out of 34 variety/lines 10 germplasms showed Resistant (R) reaction and 24 lines showed Susceptible (S) reaction to *Botrytis cinerea*. The line FLIP03-45C showed 99% germination in three subsequent years. The line FLIP03-141C was found early maturity (120 days). The tallest plant was found in line FLIP98-206C and the shortest plant was recorded in BARIchhola-5. The highest mean number of branch (8) was recorded in ILC-1929 and the lowest number (4) in lines FLIPO2-47C and FLIPO3-36C. The lowest number of pods (12) was recorded in FLIPO3-45C and the highest number of pods was recorded in BARIchhola-5. The highest 100 seed (33.67g) weight was recorded in FLIP03-141C and the lowest (12.88g) weight was recorded in BARIchhola-5. The maximum yield (1961) was recorded in FLIP03-141C and the minimum yield was recorded in line FLIP03-36C.

**P06.016 Selection of coffee genotypes with resistance to coffee leaf rust and Ceratocystis canker from interspecific hybrids (*Coffea arabica x Coffea canephora*)**

*B.L. Castro[1], H.A. Cortina[2], J. Roux[1] and M.J. Wingfield[1]*
*[1]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Hatfield, Pretoria, 0028, South Africa; [2]Plant Breeding Discipline, Centro Nacional de Investigaciones de café (CENICAFE), Chinchiná, Colombia*
*Email: Berthal.castro@cafedecolombia.com*

Coffee leaf rust (CLR), caused by *Hemileia vastatrix* and Ceratocystis stem canker (Cc), caused by *Ceratocystis* spp. are the most important diseases of coffee in Colombia. They cause substantial losses to commercial varieties of *Coffea arabica*, which are highly susceptible to both pathogens. The aim of this study was to identify resistant genotypes against these pathogens in progenies from interspecific crosses of *C. arabica* var. 'Caturra' and *C. canephora* backcrossed (BC) to 'Caturra'. Twenty-three ($F_3BC_1$) progenies were evaluated, including *C. arabica* var. Caturra and var. 'Colombia' as controls. Field experiments were established and CLR evaluations were made based on natural infections, using a rating scale (0 to 9). For Cc, artificial inoculation studies with an isolate of *C. colombiana* were used. Inoculation results were assessed after one year based on the (%) width (WSA) and length (cm) of lesions (LL) on the stems of inoculated plants. Agronomic and bean characteristics were also evaluated. Resistance to both pathogens was found in 12 progenies, however, acceptable agronomic and bean characteristics were found in only eight of these progenies and these will be suitable for future coffee propagation.

**P06.017 Screening of lentil germplasm for resistant to *Stemphylium* blight (*Stemphylium botryosum*)**

*M.A. Razzak[1], M.H. Rashid[2], H. Rahman[1], M. Riazuddin[2] and A. Sarker[3]*
*[1]Department of Plant Pathology, Patuakhali Science and Technology University, Patuakhali, Bangladesh; [2]Regional Agricultural Research Station, Bangladesh Agricultural Research Institute (BARI), Rahmatpur, Barisal, Bangladesh; [3]Regional Coordinator, South Asia and China, ICARDA, Delhi, India*
*Email: md_harunor_rashid@yahoo.com*

An investigation was carried out at Regional Agricultural Research Station (RARS), Bangladesh Agriculture

Research Institute (BARI), Rahmatpur, Barisal, during November/2011 to April/2012 for screening of lentil germplasms against Stemphylium blight under natural epiphytotic condition. At maturity stage, out of 102 lines/varieties, 40, 52 and10 lines were showed Moderately Resistant (MR), Moderately Susceptible (MS) and Susceptible (S) reaction, respectively. Early flowering was recorded in BD-3927 and BARImasur-4. The highest 50% flowering days was recorded in BD-4024, BD-4053, BD-4069, BD-4087, BD-4105, BD-4115, BD-5982, BD-5983 and BD-5991. Lowest 50% flowering was recorded in BD-3948.Long maturation period of 111.5 days was recorded in BD-4024 and that of short was recorded in BD-3924 and BD-3927.The plant height maximum was observed in BD-3974 and shortest was in BD-4127. Number of branch per plant maximum was observed in BD-3936 and BD-4024, minimum was found in BD-4097. Maximum number of pod was recorded in BD-3922 and minimum pod was in 4053. The lowest number of seed per pod was recorded in BD-4053. The highest 100 seed weight was recorded in BD-5986 and lowest of that was observed in BD-4127. Highest biological yield was recorded in BD-4053 and lowest was in BD-5989.The highest and lowest grain yield was in BD-5983 (MR) and BD-4024, respectively.

### P06.018 Virulence comparison of isolates of *Magnaporthe oryzae* in rice fields of the southern USA and Jilin Province, China

*J.C. Wang* [1], *Y. Jia* [2], *J.W. Wen*[1] *and W.P. Liu*[1]
[1]*Institute of Plant Protection, Jilin Academy of Agricultural Sciences, 130033, Changchun, P.R China;* [2]*U.S. Department of Agriculture - Agricultural Research Service, Dale Bumpers National Rice Research Center, 2890 Hwy 130E, Stuttgart, AR 72160, USA*
*Email: wangjichun1972@yahoo.com*

Rice blast disease caused by the ascomycetes fungus *Magnaporthe oryzae* is one of the most damaging crop diseases worldwide. This disease is managed with a combination of the use of resistant cultivars, fungicides and cultural practices. Among them, deployment of resistant cultivars is the most economical and environmentally sound approach to control blast. Understanding isolate virulence and spectra of resistance genes is critical for guiding resistant breeding. In this report, 44 isolates of *M. oryzae* from Jilin Province, and 14 races/ isolates of *M. oryzae* from the southern USA were inoculated under greenhouse conditions onto international rice monogenic lines (MLs) carrying 24 major blast resistance genes – *Pia*, *Pib*, *Pii*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pik-s*, *Pish*, *Pit*, *Pita*, *Pita-2*, *Piz*, *Piz-t*, *Pi1*, *Piz-5*, *Pi3*, *Pi5(t)*, *Pi7(t)*, *Pi9*, *Pi11(t)*, *Pi12(t)*, *Pi19* and *Pi20* – and the susceptible recurrent parent, Lijiangxintuanheigu. The percentage of virulent reactions of MLs to the 44 Chinese isolates was found ranging from 8.3% to 79.2%, the frequency of resistant reactions of the MLs carrying *Pi9*, *Pi19*, *Piz* and *Piz-5* were 94.2%, 84.1%, 81.8% and 81.8%, respectively. All 14 US races/isolates were virulent to 8 or more MLs. The frequency of pathogenicity ranged from 33.3% (race IG1) to 87.5% (race IE1). The ML carrying *Pi9* and *Pita-2* demonstrated broad spectrum resistance to 14 representative USA races or isolates, and their resistance frequency was 92.9% and 78.6%, respectively. These findings suggest that resistant germplasm from USA can be used to prevent blast disease in Jilin province of China.

### P06.019 Production of resistant pine plantlets against the pine wilt disease through tissue culture techniques

*L.H. Zhu, X.Q. Wu, J.R. Ye and X.F. Chu*
*Institute of Forest Protection, College of Forest Resources and Environment, Nanjing Forestry University, Nanjing, 210037, P. R. China; Jiangsu Key Laboratory for Prevention and Management of Invasive Species, Nanjing, 210037, P. R. China*
*Email: lhzhu@njfu.com.cn*

An efficient micropropagation system for pine wilt disease resistant *Pinus densiflora* was developed. Cotyledon-hypocotyl explants obtained from 28-day-old aseptic seedlings of 7 disease-resistant families of *P. densiflora* were first cultured on GD medium supplemented with 4.0 mg $l^{-1}$ 6-BA in combination with 0.2 mg $l^{-1}$ NAA to stimulate the formation of axillary buds. Induced axillary buds were subcultured on DCR basal medium supplemented with 0.1% (w/v) activated charcoal for elongation. Mass shoot proliferation was achieved by cutting elongated axillary shoots into stem segments and subculturing on GD medium containing 2 mg $l^{-1}$ 6-BA and 0.2 mg $l^{-1}$ NAA. Roots were formed in 70% of shoots when transferred to half-strength WPM medium containing 0.2 mg $l^{-1}$ NAA for 4 weeks. To investigate the resistance ability of regenerated microcuttings against the pine wilt disease, 70 six-month-old microcuttings derived from 7 clones of *P. densiflora* were inoculated under aseptic conditions with aseptic pine wood nematodes (virulent AMA3c1, 200 nematodes/microcutting). Ten aseptic microcuttings derived from resistant species *P. elliotii* were inoculated in the same manner as a control. The result of 18 days after inoculation was observed as follow: wilting ratio for all clones ranged from 20 to 100%. Clone 6-4 showed highly susceptible with a wilting ratio of 100%, clone 1-B and 8-4 showed high resistance with the wilting ratio of 20%, while the control *P. elliotii*, with a wilting ratio of 90%. Nematodes were recovered from all of wilted microcuttings. None of microcuttings inoculated with sterile water wilted.

### P06.020 Mapping and identification of QTLs for resistance to *Sclerotinia sclerotiorum* and its interaction with other trait-related QTLs in *Brassica napus* L.

*F.Q. Zhang, J.Y. Huang, X.H. Cheng, C.B. Tong, Y.Y. Liu, C.H. Dong, L.Y. Liu and S.Y. Liu*
*Oil Crops Research Institute of CAAS, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Wuhan 430062, P. R. China*
*Email: liusy@oilcrops.cn*

Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is a major yield-limiting factor in canola production, and annually causes yield loss ranging from 5 to 30% with incidence of 5%-80%. Breeding for and use of resistant varieties is a main measure for control of the disease. Therefore, exploiting resistance-related molecular markers, cloning resistance-related genes and finding its interaction with other traits is urgent at present. In this study, we identified the resistance of more than 200 RILs, which derived from a cross between 888-5 (susceptible) and M083 (resistant), using artificial inoculation, artificial disease nursery and sclerotia-seeded semi-natural field evaluations under 3 different environments in 2010-2011 and 2011-2012. The results of disease identification showed that significant differences were observed ($P$<0.01) among the RILs responsive to *S. sclerotiorum*. Flowering time, plant height, the first branching height also showed obvious difference ($P$<0.01) in the population. We found that correlation between disease index and flowering time or plant height was significantly ($P$<0.01) negative, respectively. Based on the reference *Brassica* genome sequences and the variety re-sequencing information, *B. napus* infinium iSelect®HD BeadChip containing about 60K loci have been developed by the Illumia company and used for genotyping of the RILs. The results indicated that there were 12K homozygous SNP loci between the parents. The population genotyping analysis is being performed. The next work is to construct high density linkage mapping, identify the QTLs or gene related to the resistance to *S. sclerotiorum* and their interactions with other trait QTLs or genes in *B. napus*.

**P06.021 Detection of infected watermelon seeds and resistant evaluation of watermelon varieties to Fusarium wilt**

*L.X. Kong, L.J. Ji, C.X. Wang and Y. Xiao*
*Plant Protection Institute, Hebei Academy of Agricultural and Forestry Sciences, IPM Center of Hebei Province, Key Laboratory of Integrated Pest Management on Crops in Northern Region of North China, Ministry of Agriculture, Baoding 071000, P. R. China*
*Email: konglingxiao@sohu.com*

Watermelon Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *niveum* was one of the most serious diseases in watermelon production. The disease occurred widely and the area was about 250,000 hectares in China each year. The use of resistant varieties was the most important control measure against Fusarium wilt. More than 3000 watermelon seeds of 66 different varieties collected from 14 provinces were detected by putting them on PDA media. The result showed that average seed infection rate by fungi was 50.4% on seed surface, and 60.9% inside seeds. Further investigation showed that *F. oxysporum* was detected on surface of watermelon seeds collected from Shandong, Jiangsu and Xinjiang province with the infection rate of 5%-20%. *F. oxysporum* could be detected inside seeds collected from Jiangsu and Xinjiang province with the infection of 5%-15%. Therefore, it was confirmed that watermelon Fusarium wilt could be spread by the infected seeds. Seed treatment would be efficient to reduce the primary infection source. Resistant of 76 watermelon varieties from 12 provinces to Race 1 of the pathogen were evaluated in the greenhouse. High resistant varieties were 25 （32.9%）with the disease incidence less than 20%. Moderate resistant varieties were 15 (19.7%) with the disease incidence from 20.1% to 50%. Light resistant varieties were 10 (13.2%) with the disease incidence from 50.5% to 80%. Susceptible varieties were 26 (34.2%) with the disease incidence higher than 80%. The resistant varieties mainly came from Henan, Hebei, Xinjiang, Shandong province and susceptible varieties came from Beijing, Fujian, Xinjiang, Hubei, Heilongjiang province where should be paid more attention to the disease control.

**P06.022 Molecular mapping of the new blast resistance gene *Pi49* in the durably resistant rice "Mowanggu"**

*Z.Z. Zheng, P.Y. Sun, L.Wei, G.L. Wang and L.Y. Dai*
*Hunan Provincial Key Laboratory of Crop Germplasm Innovation and Utilization and College of Bio-safety Science and Technology, Hunan Agricultural University, Changsha, Hunan 410128, P.R. China*
*Email: daily@hunau.net*

Breeding the rice cultivars possessing broad-spectrum and enduring resistance against blast is the key point and thorny point of study on rice blast. Mowanggu is a rice japonica cultivar with durable and broad-spectrum resistance to populations of *Magnaporthe oryzae* (the causal agent of blast) in Yunnan Province of China. To understand the molecular mechanism of its resistance to blast, we developed an F2 population and 280 F8 recombinant inbred lines (RILs) from a cross between Mowanggu and the highly susceptible indica cultivar CO39. A linkage map with 145 SSR and SFP markers over 12 chromosomes was constructed using the 280 RILs. The resistance evaluation of the F2 and F8 populations showed that a single dominant gene controls blast resistance in Mowanggu. The dominant resistance gene, named *Pi49*, was mapped on chromosome 11 with genetic distance of 0.16 and 0.16 cM from CAPS marker NBS-195 and SSR marker NBS-7352, respectively. The physical distance between NBS-195 and NBS-7352 is

about 84 kb in the Nipponbare genome. BAC library of Mowanggu was screened using this pair markers and got two candidate single clones for sequencing. Now we are trying to construct the resistance-like genes with NBS-LRR domain in this 84 kb region directly. Our study not only identified tightly linked markers for introgression of *Pi49* into elite rice cultivars via marker-aided selection but also provides a starting point for map-based cloning of the new resistance gene.

**P06.023 Mapping QTL for resistance to stripe rust in spring wheat PI 192252 and winter wheat Druchamp**

*Y. Lu, L. Hou, M.N. Wang, J.X. Jing and X.M. Chen*
*USDA-ARS and Washington State University, 361 Johnson Hall, WSU, Pullman, WA 99164-6430, USA*
*Email: xianming@wsu.edu*

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is an important disease of wheat worldwide. High-temperature adult-plant (HTAP) resistance has proven to be durable, but may not be adequate. Spring wheat PI 192252 and winter wheat Druchamp have high-levels of HTAP resistance. To elucidate the genetic basis of HTAP resistance in these genotypes, molecular mapping studies were conducted using an $F_5$ recombinant inbred line (RIL) population for PI 192252 and an $F_8$ RIL population for Druchamp using multi-year and multi-location stripe rust data and simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers. We identified two significant quantitative trait loci (QTL) for HTAP resistance in PI 192252, collectively explained 74.2 % of the total phenotypic variation. The QTL on the long arm of chromosome 4B explained 40-60% and the one on the short arm of chromosomes 5B explained 22.1-27.4% of the phenotypic variation depending upon the environment. The flanking markers, *Xgwm251* and *IWA1923*, of the 4BL QTL were highly polymorphic across various wheat genotypes. We mapped four QTL for HTAP resistance in Druchamp on chromosomal arms 1BL, 1DS, 4BL, and 5AL, which explained 9-34, 21-38, 11-37, and 12-19% of the phenoltypic variation, respectively depending upon the year and location. In addition, three genes for race-specific all-stage resistance were mapped on chromosomal arms 1BS, 5BS, and 6AL in Druchamp. The tightly linked molecular markers should be useful for incorporating the HTAP resistance QTL into commercial cultivars with durable and high level of resistance.

**P06.024 Identification of Liaoning Province main rice cultivars to rice blast resistance genes**

*Y. Wang [1], W.J. Zheng[2], S.W. Wang[1], B.H. Zhao[1] and Z.H. Liu [1]*
*[1]Shenyang Agricultural University, Shenyang, 110161, P. R. China; [2]Liaoning Academy of Agricultural Sciences, Shenyang, 110161, P.R. China*
*Email: zwj27@126.com*

Rice blast is one of the main diseases in our country's rice areas, even in the whole world. The application of resistant cultivar is the effective measures in prevention and control of the plant disease. In China, there is rich resource of rice cultivars which contain various genes of traits, which is the material basis of breeding. Therefore, identification of genotype helps breeding experts understanding the resistance genes' distribution in different cultivars and reducing the blind choice on the varieties of breeding. In this experiment, primers are designed according to eighteen cloned resistance genes' CDS, and then main twenty-three cultivars of rice from Liaoning Province are amplified by using PCR, PCR products are tested through electrophoresis and sequenced. Then sequence result is compared with the known sequence. Genotype identification results show that: The rice variety of Liaonong 979 is a high resistant material polymerized for five resistance genes, five susceptible cultivars such as Liaoxing 1 carry only 1 resistance genes, but each variety contained resistance genes *pib*, *pikh*, *pi5* and *pita* respectively. It indicates that four genes are not major resistance genes to the current popular physiological race. Sequencing results show that: Eighteen resistance genes in different varieties have different gene mutation. The gene *pib*, *pita*, *pid3*, *pit*, *pikp-2* and its allele gene *pikm-2* and *pik-2* have maximum genetic differences between species, gene *pb1*, *pi36* have minimum genetic differences.

**P06.025 Screening for resistant sources of powdery mildew in pea landraces**

*Z.Y. Wang, C.X. Duan and Z.D. Zhu*
*Institute of Crop Science, The National Key Facility for Crop Gene Resources and Genetic Improvement, CA AS, 12 Zhongguancun South Street, Haidian District, Beijing, 100081, P. R. China*
*Email: zhuzhendong@caas.cn*

Pea powdery mildew, caused by *Erysiphe pisi*, is an important disease on pea in the world. Use of resistant pea cultivars is the most economical and effective method for controlling the disease. Up to now, two recessive resistance genes (*er1* and *er2*) and one dominant resistance gene *Er3* have been identified in *Pisum* germplasm. Since *er1* is a durable gene that offers complete resistance to the powdery mildew pathogen by preventing pathogen penetration, it has been widely used in the Europe, northern America, Australia, and India. We recently evaluated resistance of 82 pea landraces originated from some provinces to two powdery mildew isolates, and 8 accessions collected from Yunnan province were found to are immune or highly to both isolates. Four molecular makers closely linked to pea powdery resistance gene *er1* were used to genotype the 8 immune or high resistance accessions, and 7

marker haplotypes at *er1* locus were identified. The results indicated the resistance sources for pea powdery mildew are effective and have diversity in Yunnan province.

**P06.027 Identification and mapping of a hidden resistance gene in tetraploid wheat using laboratory strains of *Magnaporthe oryzae* produced by backcrosses**

*C.J.R. Cumagun[1], V.V. Anh[2], T.T.P. Vy[2] and Y. Tosa[2]*
*[1]Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna 4031 Philippines; [2]Laboratory of Plant Pathology, Graduate School of Agricultural Sciences, Kobe University, Nada, Kobe 657-8501, Japan*
*Email: christian_cumagun@yahoo.com*

*PWT3* is a gene involved in the avirulent reaction of *Avena* isolates Br58 of *Magnaporthe oryzae* on wheat. Molecular mapping using $BC_1F_1$ population derived from the backcross 73Q2 and the *Triticum* isolate Br48 revealed that *PWT3* locus is located on chromosome 6 and completely linked to an SSR marker MoSSR6-1. White and black colonies segregated in a 1:1 ratio using $BC_3F_1$ population, suggesting that colony color is controlled by a single major gene. The progeny are considered color mutants because both parental cultures are black. Colony color is perfectly linked with virulence of the $BC_3F_1$ population on common wheat cultivar *Triticum aestivum* 'Norin 4' (N4). A cross between a moderately resistant tetraploid cultivar *Triticum dicoccoides* 'KU109' (Tat4) and susceptible tetraploid cultivar *Triticum paleocolchicum* 'KU196' (Tat14) inoculated with white $BC_3F_1$ cultures produced $F_2$ and $F_3$ seedlings which segregated in a 3:1 ratio and a 1:2:1 ratio, respectively, suggesting that resistance is also controlled by a single major gene. This gene was designated as *RmgTat4* and is considered a hidden resistance gene because it was not detected with Br58, $F_1$, $BC_1F_1$ and $BC_2F_1$ isolates. Molecular mapping using $F_3$ lines derived from the cross Tat4 and Tat14 showed that *RmgTat4* is located on chromosome 7B. Cytological analysis revealed that the moderate resistance controlled by *RmgTat4(t)* produced hypersensitive reaction of mesophyll cells upon inoculation with a $BC_3F_1$ isolate.

**P06.028 Carrot collection screening for powdery mildew resistance**

*B.M. Amirov, Z.S. Amirova, U.A. Manabaeva and K.R. Zhasybaeva*
*The Kazakh Research Institute of Potato and Vegetable Growing, No 1, Nauryz st., Kainar, Karasai District, Almaty Region, 040917, Kazakhstan*
*Email: bamirov@rambler.ru*

The studies were conducted on the experimental field of the Kazakh Research Institute of Potato and Vegetable Growing on furrow irrigated clay loamy soils. Over three seasons carrot collection samples were screened visually for powdery mildew (PM) resistance. Field evaluation of carrot leaf infection by PM was visually scored: 0 - disease signs are absent; 1 – less susceptible (1-10% of foliage); 2 – moderately susceptible (11- 25% of foliage); 3 - susceptible (26-50% of foliage); 4 - highly susceptible (more than 51% of foliage). For the assessment of carrot plants resistance to leaf infection by PM the coefficients of weighted-mean infection, intensity of distribution and degree of infection development were calculated. Of the 98 carrot collection samples screened visually, collection number CR096 demonstrated no foliar lesions of PM infection, 7 carrot samples displayed the least susceptibility to PM infection (0,1-1,0 points), 22 accessions were considered less susceptible (1,1-2,0 points), 41 selection numbers were moderately susceptible (2,1-3,0 points) to PM. The rest of the carrot collection numbers showed high points of susceptibility to PM. No correlations were revealed between the PM infection rates, on the one hand, and the yield and commodity characteristics of studied carrot collection numbers, on the other hand (R= -0,029-0,222).

**P06.029 Potato breeding for resistance to *Fusarium solani***

*G.L. Ligai, B.A. Yertayeva and B.M. Amirov*
*Kazakh Research Institute of Potato and Vegetable Growing, No 1, Nauryz st., Stlm. Kainar, Karasai District, Almaty Region, 040917, Kazakhstan*
*Email: bamirov@rambler.ru*

In Kazakhstan, the loss of potato yield from dry rot, caused by the pathogen *Fusarim solani*, in the field may reach 20-25 percent and in storage -15-20 percent. In potato breeding to induce resistance to *F. solani*, we used cellular selection technology. As a selective agent we used pathogenic filtrate (PF) of pure culture *of F. solani* isolated from the infected potato tubers of variety Sante. Fresh callus masses of potato variety Sante, susceptible to *F. solani*, and of variety Tamasha, relatively resistant to it, were produced for further research. In order to increase somaclonal variability, the calli were treated with nitrosoethyl urea (NEU). The received NEU-treated fresh calli were placed on a liquid nutrient medium for suspension culture. During the second passage to the culture medium selective agent of PF was added. Viable cell masses were floated on fresh medium, which was incubated in a thermostated shaker for 10 days, then transferred to solid growth medium. There were obtained 47 regenerants, which were tested for resistance to PF of *F. solani* in *in vitro*. Mortality of the regenerants was 58 percent. Survived regenerants were field tested on the provocative background with *F. solani*. As a result of the test, three somatic clones with no signs of damage to *F. solani* were obtained.

**P06.030 Molecular marker development of stripe rust resistance gene *Yr26* and application in marker assistant selection (MAS)**

*D.J. Han[1,3], X.J. Zhang[1, 2], Q.L. Wang[1, 2], L.L. Huang[2] and Z.S. Kang[1, 2]*

*[1]State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China; [2]College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China; [3]College of Agronomy, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China*

*Email: Kangzs@nwsuaf.edu.cn*

Stripe rust, caused by fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most destructive foliage diseases of common wheat (*Triticum eastivum* L.) worldwide. Marker-assisted selection (MAS) has been incorporated into breeding programs to improve the efficiency of differentiating, selecting, pyramiding, and integrating disease resistance genes. Resistance gene *Yr26*, which confers resistance to all current Chinese *Pst* races, has been used for wheat stripe rust resistance widely in China. It was previously mapped on the short arm of wheat chromosome 1B, further it was assigned to deletion bin C-1BL-6-0.32. To efficiently manipulate *Yr26* gene using MAS, 196 $F_2$ plants and corresponding $F_3$ progenies derived from Avocet S (AvS) × 92R137 were inoculated with Chinese *Pst* race CYR32 and used as mapping population, and wheat expressed sequence tags (ESTs) that mapped in the *Yr26* region were used to develop closely linked EST-STS (sequence tagged site) markers. As a result, eight newly developed EST-STS markers were found closely linked with *Yr26*, and three markers of them were co-segregated with the resistance gene in the $F_2$ population. Near-isogenic lines (NILs) with different *Yr* genes, twelve wheat cultivars with known *Yr* genes, and 74 wheat cultivars, collected from Sichuan province of China, were used to verify the closely linked EST-STS markers. The results indicated that three of them, *STS-CD77*, *STS-BQ74* and *STS-CD33* can be used for marker assistant selection of *Yr26* gene. These markers will facilitate the selecting and pyramiding of *Yr26* in wheat breeding program.

**P06.031 State of resistance level of wheat cultivars to stripe rust in China**

*Q.D. Zeng, Q.L. Wang, D.J. Han, X.J. Wang and Z.S. Kang*

*State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P.R. of China*

*Email: kangzs@nwsuaf.edu.cn*

Stripe rust, caused by *Puccinia striifomis* f. sp. *tritici* (PST), is one of the most destructive diseases of wheat in China. Understand resistance levels and the genetic basis of resistance in different epidemic regions wheat cultivars to PST in China could provide useful information to enhance the breeding and regional cooperation for deployment of resistance genes. 494 of wheat entries, including 224 elite cultivars and 270 advanced lines were tested with four Chinese *Pst* races (CYR23, CYR29, CYR32 and CYR33) and one pathotype CH42 on seeding stage in greenhouse, and on adult plant stage under the field condition. The *Yr*-genes were postulated by DNA marker combining with analysising pedigree. Among the 494 cultivars or advanced lines, there were only 55 (11.1%) cultivars resistant to the four test races CYR23, CYR29, CYR32, CYR33 at seedling stage, and 121 (24.5%) had the adult plant resistance. While considered the new pathotype of CH42, which was virulent to *Yr26*，there were only 23 (4.7%) entries, resistant to all test race; and 92 (18.6%) of 494 cultivars had the adult plant resistance. Combine with molecular markers only two cultivars carrying *Yr5* gene, 13 (26%) cultivars carry *Yr26* gene. There are 134 (29.4%), 45 (9.1%), 10 (2%) cultivars out of 494 entries carrying *Yr9*, *Yr17*, *Yr18* gene, respectively. The results showed that 'Guinong' and '92R' series of resistance sources to stripe rust have been frequently used in currently wheat breeding programs. The continuing widespread use of Guinong series and *Yr26* is a major concern.

**P06.032 Molecular mapping of stripe rust resistance gene *Yrpd34* in wheat cultivar Pindong 34**

*X.L. Zhou, Z.S. Kang, D.J. Han, L.L. Huang, S.J. Guo, L. Rong, Q.L. Wang, G.M. Zhan, Q.D. Zeng and F.P. Yuan*

*State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P.R. China*

*Email: kangzs@nwsuaf.edu.cn*

Several new races of the stripe rust pathogen have established throughout the wheat growing regions of the China in recent years. These new races are virulent to most of seedling resistance genes limiting the resistance sources that can be used for effective stripe rust control. It is urgent to identify new genes for diversification and for pyramiding different types of resistance genes in order to achieve more durable resistance. We report here the identification of a new resistance gene, designated as *Yrpd34*, in wheat cultivar Pindong 34. A mapping population of 208 $F_2$ plants and 128 derived $F_{2:3}$ lines in a cross between Mingxian 169 and Pindong 34, evaluated for seedling stripe rust response. A genetic map consisting of seven RGAP and three SSR markers was constructed. *Yrpd34* was located on chromosome 7BS 3.6 cM distal to RGAP marker *Xgpw5467*. SSR markers *Xgwm400*, *Xcfa2106* and *Xwmc76* were 5.0, 9.1 and 12.2 cM proximal to the resistance locus. The flanking markers could be used for marker-assisted selection of Yrpd34 in breeding programs.

## Concurrent Session 7-Cereal Diseases

### O07.001 The agroecosystem's effect on the epidemiology of rust diseases

J. Yuen[1], A. Berlin[1], K. Gillen[1] and Y. Jin[2]
[1]Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, SE 750 07 Uppsala, Sweden; [2]USDA-ARS Cereal Disease Laboratory, University of Minnesota, St Paul, MN, USA
Email: Jonathan.Yuen@mykopat.slu.se

The availability of molecular-based tools and markers has enabled better, more comprehensive studies of the epidemiology of rust diseases. Our studies of stem rust (caused by *Puccinia graminis* f. sp. *avenae* and *P. graminis* f. sp. *secalis*, Pga and Pgt), as well as ongoing studies of yellow rust (caused by *P. striiformis*) are made possible by a number of molecular tools, but the interpretation of the data is also dependent on understanding the cropping system and other factors that affect the pathosystem on the relevant time scales. One striking conclusion from a comparison of these diseases is that stem rust seems to almost exclusively rely on sexual reproduction by the pathogen, and that the oat and rye crops are infected by aeciospores that originate from barberry plants in the spring. On the other hand, there does not seem to be any direct evidence of sexual reproduction in *P. striiformis*, and the yellow rust pathogen appears to survive primarily as clonal lineages, although there does seem to be some variation as well as the appearance of novel genotypes. While the wheat cultivars grown in Sweden possess few effective resistance genes to either pathogen, stem rust (caused by *Puccinia graminis* f. sp. *tritici*, Pgt) on wheat is relatively rare, despite the widespread occurrence of barberry. This is likely due to a lack of Pgt in the rust flora. One difference between the two pathosystems could be that *P. graminis* has difficulty in surviving the Swedish winters, whereas the winter is less challenging for *P. striiformis*. The green bridge (a result of fall sown wheat crops) that is present can function as a survival refuge for *P. striiformis*, but not for *P. graminis*. This, in turn, is confounded by the fact that oats are only spring-sown in Sweden, thus making it nearly impossible for Pga to survive the winter on this crop, whereas this possibility technically exists for the pathogens of the fall sown crops (Pgs, Pgt, and *P. striiformis*). The possible existence of two sorts of inocula (either from immigrant genotypes or aeciospores or from clonal lineages that survive the winter on fall-sown crops), and their relative competitive ability, will affect the pathogen population structure. Thus, new genotypes of *P. striiformis*, whether a result of immigration or sexual reproduction, would be extremely difficult to detect before they had multiplied and attained the status of a clonal lineage.

### O07.002 The genetic basis of pathogen adaptation to agroecosystems

B.A. McDonald
Plant Pathology, Institute of Integrative Biology, ETH Zurich, 8092 Zurich, Switzerland
Email: bruce.mcdonald@usys.ethz.ch

The wheat pathogen *Zymoseptoria tritici* is a leading model to understand the genetic basis of host specialization and ecosystem adaptation in fungal plant pathogens. *Z. tritici* emerged as a wheat pathogen from several closely related species during the domestication of wheat in the Fertile Crescent around 11,000 years ago. Comparative and population genomic analyses identified genomic regions that have evolved most quickly, leading to candidate genes involved in host specialization and speciation. Population genetic studies using neutral markers showed that all field populations exhibit high levels of gene and genotype diversity, with significant recombination and regional gene flow by airborne ascospores. Analysis of the global distribution of quantitative trait diversity, including thermal adaptation, virulence, and fungicide sensitivity showed significant population subdivision consistent with local adaptation for most traits. Mutations giving fungicide resistance can arise many times de novo and spread rapidly in European populations where fungicide use is high. Virulence is mainly quantitative in this pathosystem. A significant correlation was found between virulence and fungicide resistance, indicating that some genes may be shared for these traits. A rigorous evolutionary and transcriptional analysis of plant cell wall degrading enzymes (PCWDEs) identified PCWDEs that were most likely involved in host specialization. The majority of PCWDEs were shown to exhibit life cycle specialization, with different members of the same CAzY class expressed during biotrophic, necrotrophic and saprotrophic phases of the life cycle. QTL mapping is underway to identify genes underlying virulence, fungicide resistance and thermal tolerance.

### O07.003 Regulation of the biosynthesis of trichothecene mycotoxins in *Gibberella zeae*

C.F. Wang[1], R. Hou[1], Q. Zheng[1] and J.R. Xu[1, 2]
[1]Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, P.R. China; [2]Department of Botany and Plant Pathology, Purdue University, USA
Email: jinrong@purdue.edu

Wheat is one of the most important cereal crops worldwide. Fusarium head blight (FHB) or scab primarily caused by *Gibberella zeae*, is a devastating diseases of wheat, barley, and other small grains in many countries. In 2013, there was a nationwide outbreak of FHB in China. FHB infested cereals are reduced in yield and

grain quality, and often contaminated with trichothecene mycotoxins, such as deoxynivalenol (DON), which is monitored in finished wheat products in many countries. DON is also an important virulence factor for plant infection. Although the *TRI* genes responsible for trichothecene biosynthesis have been well characterized, there are only limited studies with environment and host factors that affect DON production. Both *TRI6* and *TRI10* are transcription factor genes in the *TRI* clusters. In *G. zeae*, unlike in *Fusarium sporotrichioides*, *TRI6* plays more a more important role than *TRI10* in the regulation of *TRI* gene expression. The promoters of *TRI6* and *TRI10* contain sequences identical to the conserved PacC binding sites. Functionally characterization of these PacC binding sites will determine which one is be responsible for induced DON production under acidic conditions and inhibition of DON synthesis at alkaline pH. The *TRI6* promoter also contains several putative AreA-binding sites. Deletion analysis revealed that at least one of them is important for the regulation of DON synthesis by nitrogen metabolism. In addition, we have characterized three ammonium permease genes to characterize their roles in ammonium sensing and regulation of secondary metabolism. The functional relationship between the ammonium sensor and downstream signaling pathways also are being investigated to determine their regulatory role in DON production.

**O07.004 Exploitation of necrotrophic effectors to improve crop protection**

*R. Oliver*
*Department of Environment & Agriculture, Curtin University, Perth, Australia*
*Email: richard.oliver@curtin.edu.au*

The role of necrotrophic effectors in promoting virulence can be exploited as a way to select more resistant germplasm. Resistance to necrotrophic diseases was typically found to be partial, in contrast to the major gene resistance noted in some cases for biotrophic pathogens. This has meant that breeding for disease resistance is much more demanding and explains why necrotrophic pathogens have grown in importance whilst progress in controlling biotrophic diseases was often rapid (until the emergence of the next mutant pathogen race). However the identification and production of cloned and expressed effectors of necrotrophic pathogens allows breeders to select introgressions that are insensitive. Effectors from both *Stagonospora nodorum* and *Pyrenophora tritici-repentis* have been expressed in microbial systems and used to identify germplasm that is insensitive to the effector Thus, in the case of multi-effector systems like *P. tritici-repentis* and *S. nodorum*, selection of cultivars insensitive to each effector allows breeders to improve disease resistance in an incremental, step-wise fashion.

**O07.005 Stem rust resistance in barley: unraveling the prehaustorial response**

*R. Brueggeman*
*Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA*
*Email: Robert.Brueggeman@ndsu.edu*

Comparison of the flax rust pathogen (*Melampsora lini*) - flax (*Linum usitatissimum*) and the wheat stem rust pathogen (*Puccinia graminis*) - barley (*Hordeum vulgare*) pathosystems show dichotomy in resistance mechanisms. It was demonstrated in flax rust resistance that AVR proteins are translocated from the haustoria into the plant cell where direct protein–protein interaction occurs between NBS-LRR R-proteins and AVR proteins. However, the two stem rust resistance mechanisms characterized in barley, *Rpg1* and *rpg4*/*Rpg5*, appear to be prehaustorial resistance mechanisms. Pathogen recognition in the barley *Rpg1*-mediated resistance mechanism occurs at the cell surface and two distinct AVR proteins expressed in the spore are recognized, followed by phosphorylation of the protein kinase domain of RPG1. Preliminary expression analysis data also shows that there is an early response to the pathogen in the *rpg4*/*Rpg5* resistance mechanism with *Rpg5* up regulation within 12 hours of pathogen inoculation. We identified three genes at the complex *rpg4/Rpg5* locus required for the resistance response, including an NBS-LRR-protein kinase, a second NBS-LRR gene and an actin depolymerization factor, common protein domains of the *Rps5* resistance mechanism speculated to guard components of the FLS2-mediated PAMP-triggered immunity (PTI) against *Psuedomonas syringae* in Arabidopsis. It appears that barley rust resistance may represent prehaustorial and possibly PTI-like resistance responses. The characterization of the limited resistance sources and the mechanisms determining pathogen recognition in the barley-stem rust interaction indicates that barley may be a recent host to stem rust and the resistance mechanisms present in barley are holdovers of non-host resistance mechanisms.

**O07.006 In planta stage-specific fungal gene profiling elucidates the molecular strategies of *Fusarium graminearum* growing inside wheat coleoptiles**

*X.W. Zhang, L.J. Jia, Y. Zhang, G. Jiang, X. Li, D. Zhang and W.H. Tang*
*Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, P.R China*
*Email: whtang@sibs.ac.cn*

The ascomycete *Fusarium graminearum* is a destructive fungal pathogen of wheat. To better understand how this pathogen proliferates within the host plant, we tracked pathogen growth inside wheat coleoptiles and then

examined pathogen gene expression inside wheat coleoptiles at 16, 40 and 64 hours post-inoculation (hpi) using laser capture microdissection and microarray analysis. We identified 344 genes that were preferentially expressed during invasive growth in planta. Gene expression profiles for 134 putative plant cell wall degrading enzyme genes suggest that there was limited cell wall degradation at 16 hpi and extensive degradation at 64 hpi. Expression profiles for genes encoding reactive oxygen species (ROS)-related enzymes suggest that *F. graminearum* primarily scavenges extracellular ROS before a later burst of extracellular ROS is produced by *F. graminearum* enzymes. Expression patterns of genes involved in primary metabolic pathways suggest that *F. graminearum* relies on the glyoxylate cycle at an early stage of plant infection. A secondary metabolite biosynthesis gene cluster was specifically induced at 64 hpi and was required for virulence. Our results indicate that *F. graminearum* initiates infection of coleoptiles using covert penetration strategies, and switches to overt cellular destruction of tissues at an advanced stage of infection.

### O07.007 Gene discovery in wheat against Fusarium head blight through metabolomics

*A. Kushalappa, R. Gunnaiah and S. Hukkeri*
*Plant Science Department, McGill University, Ste. Anne de Bellevue, QC, Canada H9X3V9, Canada*
*Email: ajjamada.kushalappa@mcgill.ca*

Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the most destructive global diseases of wheat, barley and other cereals. Besides yield losses, it deteriorates the grain quality by contaminating with trichothecene mycotoxins, such as deoxynivalenol (DON), which poses serious health risks in animals and human beings. Breeding for resistance is a reliable and environment friendly approach for FHB management. FHB resistance in wheat is quantitatively inherited and more than 100 FHB resistance QTLs have been mapped. However, these QTLs contain several genes, and thus, breeding is very challenging (Trends in Plant Science, 2013). *Fhb1,* the most consistent and largest effect FHB resistance QTL in wheat was functionally characterized by non-targeted metabolomics of two sets of near isogenic lines (NILs), with resistant and susceptible *Fhb1* alleles. From Nyubai, the resistant alleles of *Fhb1* was associated with a greater deposition of hydroxicinnamic acid amides (HCAAs), which increased the cell wall thickening, thus preventing the pathogen spread within the plant. From Sumai-3, the susceptible alleles of *Fhb1* was associated with DON induced programmed cell death. Following pathogen inoculation, a gene coding for histidine rich $Ca^{2+}$ binding protein, localized at *Fhb1* region, was activated by $H_2O_2$, which in turn increased the cytoplasmic $Ca^{2+}$ levels, leading to cell wall disintegration in susceptible genotypes, but not in resistant. Following validation through gene silencing this gene can be used to enhance resistance in wheat to FHB. *Fhb5*, the next best QTL was associated with cell wall thickening, mainly through deposition of HCAAs. The candidate genes are being sequenced.

### O07.008 Resistance in South African maize inbred lines to *Fusarium verticillioides* and fumonisin contamination

*M. Mouton, L. Rose, B.C. Flett and A. Viljoen*
*Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*
*Email: marilim@sun.ac.za*

*Fusarium verticilliodes* causes Fusarium ear rot (FER) of maize worldwide. Apart from reducing yield and grain quality, *F. verticillioides* produces fumonisins, a group of mycotoxins which are harmful to both human and animal health. The planting of resistant cultivars can be an efficient approach to reduce ear rot diseases and minimize the risk of economic losses and mycotoxin accumulation in maize. The objective of this study, therefore, was to evaluate 18 genetically diverse maize inbred lines as potential sources of resistance to FER and fumonisin contamination under different production systems and environmental conditions. Inbred lines were artificially inoculated with *F. verticillioides* isolate MRC 0826, GCI 316 and GCI 790 during field trials at Potchefstroom, Vaalharts, Cedara, Makhatini and Buffelsvlei in 2011 and 2012. Following harvest in 2011, trials were evaluated by visual rating of disease severity, and fungal concentration and fumonisin content as quantified by means of qPCR and LC-MS/MS, respectively. The individual inbred lines differed considerably in FER severity and fumonisin accumulation, with lines CML 390, CML 444, US254 W, RO 544 W, RO 424 W and VO617 Y-1 consistently showing low disease incidence and accumulated fumonisin levels less than 2 mg/kg in all the locations. Samples collected from the field trials during the 2012 growing season are currently being analysed. Maize inbred lines with durable resistance to *F. verticillioides* will provide the South African maize industry with opportunities to develop maize cultivars with resistance to the pathogen and the fumonisins it produces.

### O07.009 Mechanisms regulating grain contamination with trichothecenes translocated from the stem base of wheat (*Triticum aestivum* L.) infected with *Fusarium culmorum*

*M. Winter, B. Koopmann, K. Doell, P. Karlovsky, U. Kropf, K. Schlüter and A.V. Tiedemann*
*Plant Pathology and Crop Protection Division, Department of Crop Sciences, Faculty of Agriculture, Georg-August University Göttingen, Grisebachstraße 6, D-37077 Göttingen, Germany*

*Email: mwinter@gwdg.de*

Factors limiting trichothecene contamination of mature wheat grains after fusarium infection of plants are of major interest in crop production. Beside ear infection, systemic translocation of deoxynivalenol (DON) may contribute to mycotoxin levels in grains after stem base infection with toxigenic *Fusarium* species. Winter wheat infected at the stem base with *F. culmorum* in climate chamber experiments, was analysed for the transfer of DON into maturing grains following systemic translocation in the plant. Fungal DNA was only found in the infected stem base tissue while DON and its derivative DON-3-glucoside (D3G) were detected in upper plant parts. Surprisingly, this systemic translocation of DON was not associated with contamination of mature grains. Although infected stem bases contained more than 10,000 µg DON per kg DW and mean levels of DON in the ear rachis and husks reached 1,900 µg DON per kg DW, no DON or D3G were detectable in mature grains. D3G quantification revealed that DON detoxification mainly took place in the stem base, where up to 50% of DON was metabolized into D3G. Histological studies demonstrated that the vascular transport of DON labelled with fluorescein as a tracer from the peduncle to the grain was compromised by a barrier zone at the interface between grain and rachilla, formerly described as 'xylem discontinuity'. Such effective control of trichothecene uptake into the young grain at the rachilla-seed interface appears a general mechanism which minimizes the risk of systemic contamination of wheat grains from infection with toxigenic fusaria at lower plant parts.

### O07.010 A splicing factor is essential to pathogenicity and efficient splicing of short introns in *Magnaporthe oryzae*

*J. Yang, W. Wang, L. Kong, Z. Li, X. Chen, W. Zhao, M. Xue, X. Zhou, D. Wang, J. Sun, R. Wang, X. Xu, Y. Zuo, J.R. Xu, Y. Zhang and Y.L. Peng*
*State Key Laboratory of Agrobiotechnology and Ministry of Agriculture Key Laboratory of Plant Pathology, China Agricultural University, Beijing, P.R China*
*Email: pengyl@cau.edu.cn*

The spliceosomes of higher eukaryotes contain some additional components that are absent in *Saccharomyces cerevisiae*. However, few of them have been functionally characterized. By insertion mutagenesis and gene knockout, we identified a novel gene named *PCG1* that was essential to pathogenicity of *Magnaporthe oryzae*. *PCG1* encodes a nuclear protein that is constitutively expressed during the entire infection stages. With the protein pull-down, co-immunoprecipitation and yeast two-hybrid, Pcg1 was found to associate with dozens of components of the spliceosome, and notably physically interacted with certain components of the Prp19-associated complex, including Cwc2, the sole component of the complex for RNA binding. With the RNA-seq, it was found that deletion of *PCG1* resulted in reduced splicing efficiency for approximately one-third intron-containing genes in the pathogen, including 62 previously functionally characterized genes. Interestingly, 55 of the 62 genes were required or important for pathogenicity. Pcg1 was also important for efficient splicing of genes those have introns with 50 to 110 base pair in length. Deletion of *FgPCG1*, the ortholog in the wheat scab fungus, also resulted in loss of pathogenicity. Introduction of *FgPCG1* and the human ortholog *hCCDC12* could completely and partially rescue the defects caused by the *PCG1* deletion, respectively. Thus, Pcg1 and its orthologs in higher eukaryotes as component of the Prp19-associated complex are important for splicing short introns and safeguarding efficient intron splicing of pathogenicity genes in fungal pathogens. This study also suggests that interaction of Pcg1 with Cwc2 may be important for regulating splicing of short inrons.

### O07.011 The biology and control of the barley pathogen, *Ramularia collo-cygni*

*J.M. Fountaine, P. Hoebe, M. Kaczmarek, M. Piotrowska and N.D. Havis*
*Crop and Soil Research Group, Scotland's Rural College (SRUC), King's Buildings, Edinburgh, EH9 3JG, UK*
*Email: james.fountaine@sruc.ac.uk*

The fungus *Ramularia collo-cygni* is the major biotic agent involved in Ramularia Leaf Spot on barley. Although it was first described in 1893 it was only initially identified on spring barley crops in Scotland in 1998. Since then it has increased in its importance in barley growing areas. Research over the last decade has allowed a greater understanding of the economic impact of the disease and the development of optimal disease control programmes. Advances in both areas have in turn, lead to an increase in fungicide use and this remains the only reliable control method available to growers. However, the threat of fungicide resistance and the future limitation of pesticide groups in Europe, means that alternative control methods will have to be developed in the near future. Advances in our knowledge of the developmental biology and epidemiology of *R. collo-cygni* have also lead to the development of an accurate risk forecast system and a recognition of the impact and role of seed infection on subsequent disease epidemics. Research into breeding resistant lines is still in its infancy and currently there are no fully resistant cultivars for this disease. The entire *R. collo-cygni* fungal genome has been fully sequenced and this data is now being used for comparative genetic studies to address the biology of the pathogen in areas such as population genetics, fungicide resistance and pathogenicity. These advances should assist in the development of environmentally

sound strategies to control this important disease in barley production systems.

**O07.012 Map-based cloning of rice resistance genes based on next-generation sequencing**

*Y. Yang, J. Zhou, X.M. Wang, C.L. Yu, X.Y. Cheng, Y. Cheng, C.Q. Yan and J.P. Chen*
*State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control, Ministry of Agriculture Key Laboratory of Biotechnology in Plant Protection, Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, P. R. China*
*Email: jpchen2001@yahoo.com.cn*

Map-based cloning has been widely used to isolate and characterize many important plant resistance genes. However, most mapping strategies based on different molecular markers are very time-consuming and then make gene cloning difficult. Here we present a useful method for rapidly cloning rice resistance genes based on the connection of map-based strategy and next-generation genomic sequencing. In this method, the rice resistance mutant is indica/japonica crossed to another rice line, and then two small bulked segregant populations each including extremely resistant and susceptible individuals are selected from the $F_2$ population. Restriction-site associated DNA sequencing of the bulked DNA provides the SNPs information for primary mapping the resistance gene in a certain chromosome region. The resistance mutant is then back-crossed to its wild-type line. Whole-genome resequencing of the wild-type parent and the bulked resistance $F_2$ population is subsequently performed to screen the target gene in the primary mapping region. In this present work, we identified four rice EMS mutants that showed high resistance to both rice bacterial blight (BB) and rice blast. Using the new map-based cloning strategy mentioned above, we cloned the four resistance genes and studied their possible role in rice resistance to diseases. Moreover, we introduced the trait of BB resistance from a wild rice *Oryza meyeriana* into cultivated rice using asymmetric somatic hybridization and obtained the hybrid line with high resistance. A *meyeriana*-derived quantitative trait locus (QTL) controlling BB resistance was also identified by using mapping method based on whole-genome resequencing.

**O07.013 Identification of wheat susceptibility factors to *Fusarium graminearum***

*C. Chetouhi, L. Bonhomme, P. Lecomte, F. Cambon, D. Biron and T. Langin*
*CNRS, UMR 6023, Laboratoire Microorganismes Genome et Environnement, F-63120 Aubiere, France; INRA, UMR1095 GDEC, F-63039 Clermont-Ferrand, France;Université Blaise Pascal, UMR1095 GDEC, F-63039 Clermont-Ferrand, France*
*Email: cherif.chetouhi@clermont.inra.fr*

Among the most damaging fungal pathogens of wheat, *Fusarium graminearum*, main causal agent of Fusarium head blight (FHB), reduces significantly yield worldwide, and affects grains quality by mycotoxins contamination. During the past years, numbers of FHB resistance QTL with moderate effects against this pathogen have been identified in very genetically diverse wheat collections. However the lack of diagnostic markers as well as detrimental linkage drag associated with some of these QTL (QTL governing the yield and grain quality) limit breeding for resistance. Understanding the molecular basis of FHB susceptibility can provide alternative tools to improve the control of this disease in fields. To decipher the molecular crosstalk involved during compatible interaction between *F. graminearum* and its host, and to gain information about susceptibility host factors, we analyzed a time course infection of the susceptible French wheat cultivar Recital by *F. graminearum* through transcriptomics and proteomics approaches. Microarray data showed that 1,453 genes exhibit differential accumulation between *F. graminearum*-infected and mock-inoculated plants whereas a total of 74 proteins displayed contrasting abundances on 2DE gels. All these host genes/proteins identified were classified into three functional groups: (*i*) plant defense, (*ii*) primary, secondary and energy metabolism and (*iii*) regulation and signaling. These results strongly suggest that *F. graminearum* manipulates its host during a compatible interaction. Its infection strategy relies on the suppression of basal plant defense and subtle changes in nutrient availability related-processes. A detailed picture of the potential host pathways involved during susceptibility will be described along with interesting targets for improved resistance.

**O07.014 MoLys2 is necessary for growth, conidiogenesis, lysine biosynthesis and pathogenicity of *Magnaporthe oryzae***

*Y. Chen, H.F. Zhang, X.B. Zheng and Z.G. Zhang*
*Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, and Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Nanjing 210095, P. R. China*
*Email: zhgzhang@njau.edu.cn*

We previously showed that the transcription factor MoAp1 is required for pathogenicity in *M. oryzae* by mediating the oxidative stress response. A transcription factors MoGti1 which was regulated by MoAp1 played an important role in conidiogenesis, appressorial formation, and virulence. To better understand the regulation

mechanism of MoGti1, many potential interaction proteins including MoLys2 were identified by affinity purification assay. MoLys2 was a very conservative protein and widely existence in fungi. In *Saccharomyces cerevisae*, *ScLYS2* coding an Alpha aminoadipate reductase protein, which catalyzes the reduction of alpha-aminoadipate to alpha-aminoadipate 6-semialde- hyde and is the fifth step in biosynthesis of lysine. Lys2 have crucial roles in growth and development of *S. cerevisae*. However, little was known about their functions in phytopathogenic fungi. Here, we characterized the functions of MoLys2, which is a homology of ScLys2, in the rice blast fungus *Magnaporthe oryzae.* We found that Δ*Molys2* mutant have defects in developing aerial hyphae on MM, OM, and SDC media and block conidial production. Exogenous lysine could restore the defects of growth and conidiogenesis. However, conidia which produced by adding exogenous lysine only developed tiny and restricted lesions on rice and barley leaves. Infection assay showed the invasive hyphae of Δ*Molys2* mutant that added exogenous lysine were mostly restricted to the primary infected leaf sheath cells. Our results indicated that MoLys2 is necessary for growth, conidiogenesis, lysine biosynthesis and pathogenicity of *Magnaporthe oryzae*.

**O07.015 Crown rot complex of wheat, an increasingly serious wheat disease in China**

*H.L. Li[1], H.X. Yuan[1], Z.H. Feng[1], Y. Yang[1], X.P. Xing[1], B.J. Sun[1] and W.H. Tang[2]*
*[1]Department of Plant Pathology, Henan Agricultural University, Zhengzhou, 450002, P.R. China; [2]Department of Plant Pathology, China Agricultural University, Beijing, 100094, P.R. China*
*Email: honglianli@sina.com*

Crown rot complex of wheat caused by *Fusarium* spp. has become an important problem in recently years in Huang-Huai floodplain of China, for on surviving residue of wheat and maize being returned to the field, especially under irrigated conditions. The pathogens of this disease are quite complex and variable in different areas in China, including *F. pseudograminearum*, *F. graminearum*, *F. culmorum*, *F. tricinctum*, *F. equiseti*, *F. avenaceum*, and *F. oxysporum. F. graminearum* is the most common and widespread pathogen of crown rot in most areas of China, and also causes serious head blight of wheat in the Yangtze River valley and Huang-Huai flood plain in the winter wheat area of China. *F. pseudograminearum* is a new pathogen of wheat. It was first reported in Qinyang county, Henan province of China in 2012, but it also exists in Hebei Baoding, Henan Yuanyang and Xingyang counties based on the soil DNA detection results provided by SARDI (South Australian Research and Development Institute). *F. culmorum* is the major pathogen of crown rot of wheat in many countries, but we are still not aware of the importance of this pathogen in China. In fact,it is widespread in the main wheat production provinces in China(Henan, Hebei, Shandong and Jiangsu)as judged from DNA detection data obtained by SARDI. In over 40% of soil samples, the pathogen DNA content was at a moderate or high risk level, from a total 167 samples, based on the SARDI results. *F. tricinctum*, *F. avenaceum*, *F. oxysporum* and *F. equiseti* are also common pathogens of wheat causing stem base browning and crown rot in some areas of China but with weaker pathogenicity. It is considered that the damage severity of crown rot is lower than with sharp-eye spot (*Rhizoctonia cerealis*) and take-all (*Gaeumannmyces graminis* var. *tritici*) in most areas in China at present. But the rapid rise of crown rot causing obvious yield loss in some important wheat provinces is an indisputable fact. In Henan, the crown rot complex of wheat caused by *Fusarium* spp. has become a major disease of wheat in many counties, and led to a mass of whiteheads in the field in recent years. Some field yield losses are over 30% in Qinyang county and Xuchang county, Henan province. The field test results showed that seed treated by carbendazim, difenoconazole, tebuconazole and triticonazole has a certain control effect on crown rot of wheat, with the whitehead rate declining significantly.

**O07.016 The genome and pathogenic mechanisms of the rice sheath blight pathogen**

*A.P. Zheng, R.M. Lin, D.H. Zhang, Y. Chen and P. Li*
*State Key Laboratory of Hybrid Rice, Rice Research Institute of Sichuan Agricultural University, Huimin Road 211, Wenjiang, Chengdu, 611130, P.R. China*
*Email: apzh0602@gmail.com*

*Rhizoctonia solani* is a major fungal pathogen of rice (*Oryza sativa* L.) that causes great yield losses in all rice-growing regions of the world. Here we report the draft genome sequence of the rice sheath blight disease pathogen, *R. solani* AG1 IA, assembled using next-generation Illumina Genome Analyser sequencing technologies. The genome encodes a large and diverse set of secreted proteins, enzymes of primary and secondary metabolism, carbohydrate-active enzymes, and transporters, which probably reflect an exclusive necrotrophic lifestyle. We find few repetitive elements, a closer relationship to Agaricomycotina among Basidiomycetes, and expand protein domains and families. Among the 25 candidate pathogen effectors identified according to their functionality and evolution, we validate 3 that trigger crop defence responses; hence we reveal the exclusive expression patterns of the pathogenic determinants during host infection.

**P07.001 Autophagy-inducing condition during infection process of *Magnaporthe oryzae***

*K. Inoue, H. Nakayashiki and K. Ikeda*

*Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai, Nada, Kobe 657-8501, Japan*
*Email: kanakomushi2004@yahoo.co.jp*

*Magnaporthe oryzae* is known as a causal agent of blast disease on various gramineous crops. This fungus shows distinct patterns of infection-related differentiation depending on physical and chemical properties of contact surface. Upon infection of leaf surfaces, fungal penetration of host cell walls is mediated by a specialized infection structure, appressorium, formed at the tip of the germ tube. In contrast, on the root surface, the germ tube differentiates into hyphopodia to invade host cells. Recent studies suggested that autophagy played an important role in forming functional appressoria by facilitating the metabolism of storage substance in spores. However, to date, no cytological observation of autophagosome, a typical feature of macroautophagy, was reported during appressorium formation. Very little information is also available about the relationship between hyphopodium formation and autophagy. In this study, we performed cytological examination of infection-related morphorogenesis of *M. oryzae* on hydrophobic, hydrophilic and root surfaces with a focus on autophagy. Transmission electron microscopy (TEM) observation at 12 hours post inoculation (hpi) revealed that many autophagosome-like vesicles were accumulated at the periphery of vacuoles only in spores producing appressoria on hydrophobic surface. At 24 hpi, most organelle disappeared in the spores germinating on hydrophobic surface. Such autophagosome-like vesicles and organelle disintegration were never observed in spores germinating on hydrophilic or root surface. These results suggested that autophagy is required for appressorium formation but not for hyphopodium differentiation, and thus, could be a key determining element on organ specific pathogenicity in *M. oryzae*.

### P07.002 Different soil management technologies and severity of Fusarium head blight of wheat

*P. Matušinsky[1], M. Váňová[1], D. Spitzerová[1], I. Polišenská[1], M. Janeček[2], P. Hlaváček[2] and V. Smutný[3]*
*[1]Agrotest fyto, Ltd., Havlickova 2787/121, 767 01 Kromeriz, Czech Republic; [2]Crop Research Institute, Prague 6 – Ruzyne, Czech Republi; [3]Mendel University in Brno, Zemedelska 1, Brno, Czech Republic*
*Email: matusinsky@vukrom.cz*

Soil tillage practices involving various depth, intensity, and different methods of loosening the soil and treatment with plant residues have changed significantly in recent years and have spread also due to technical advance. Diseases that can be potentially of importance in relation to the soil management practice are Fusarium head blight. In current study in year 2011 in Ivanovice na Hane (Czech Republic) four tillage practices were set-up: (i) tillage 22 cm, (ii) tillage 15 cm, (iii) no tillage (iv) chiseling 10 cm. Our experiments did not demonstrate an increased demand for protection against Fusarium head blight, but the preceding crop corn increased significantly deoxynivalenol (DON) concentration. Level of mycotoxins permitted in cereal grains is limited, and in the case of the mycotoxin DON, in particular, it is 1,250 μg $kg^{-1}$. That limit was not exceeded in any of the examined samples. The highest value measured during experiment was 235 μg $kg^{-1}$.

### P07.003 Morphological characteristics and mating populations of *Fusarium* species in Section Liseola associated with stalk rot disease of corn in Indonesia, Malaysia and Thailand

*Darnetty[1] and B. Salleh[2]*
*[1]Department of Plant Pests and Diseases, Faculty of Agriculture, Andalas University, Limau Manis 25163 Padang, West Sumatra, Indonesia; [2]School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia*
*Email: Darnetty_06@yahoo.com*

Fusarium stalk rot is one the most important diseases of corn plants worldwide. Several species of *Fusarium*, mostly in Section Liseola, have been reported causing the disease. The disease reduces the quantity and quality of corn yield and affects animal and human health through ingestion of mycotoxin-contaminated corn products infected by the pathogens. So far, the researches on the disease have not been carried out intensively in tropical countries including Indonesia, Malaysia, and Thailand, where corn also is widely planted. The objectives of the studies were to isolate and identify the species of *Fusarium* in Section Liseola based on morphological characteristics and to determine their mating populations (MPs). A total of 112 strains of *Fusarium* in Section Liseola were isolated from corn plants showing typical stalk rot symptoms in Indonesia, Malaysia, and Thailand, and cultured on potato dextrose agar (PDA) and carnation leaf pieces agar (CLA) for morphological identification. For MP, the strains of *Fusarium* were crossed with nine standard tester strains on carrot agar (CA). Five species of *Fusarium* were morphologically identified as *F. verticillioides* (79 strains, 70.54%), *F. proliferatum* (21 strains, 18.75%), *F. subglutinans* (4 strains, 3.57%), *F. konzum* (2 strains, 1.79%), and *F. miscanthi* (6 strains, 5.36%). Three mating populations were identified as MP-A, *G. moniliformis* (69 strains, 61.61%), MP-D, *G. intermedia* (14 strains, 12.50%), and MP-E, *G. subglutinans* (3 strains, 2.68%). MP-A (*F. verticillioides*) was the most dominant species associated with stalk rot disease of corn in this region. This is the first report on the presence of MP-A, MP-D and MP-E on stalk rot-infected corn in Indonesia, MP-A and MP-E in Malaysia, and MP-A in Thailand. The occurrence of *F. konzum* and *F. miscanthi* on stalk rot-infected corn plants are new records in this region.

**P07.004 Identification of new sources of resistance in pearl millet mini core to multiple pathotypes of *Sclerospora graminicola***

*R. Sharma, H.D. Upadhyaya, V.L. Gate and S. Sharma*
*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India*
*Email: r.sharma@cgiar.org*

Downy mildew (DM) caused by *Sclerospora graminicola* (Sacc.) Shroet. is a major biotic constraint to pearl millet production. The pathogen is heterothallic and frequent recombination leads to genotypic diversity and evolution of new virulent populations. Identification of resistance to new virulent pathotypes is a prerequisite for resistance breeding. Therefore, pearl millet mini-core comprising 238 accessions which represent most of the useful variation in the 21,594 pearl millet accessions conserved at ICRISAT gene bank was evaluated in a greenhouse against eight pathotypes (Sg 409, Sg 384, Sg 445, Sg 457, Sg 510, Sg 519, Sg 526 and Sg 542) of *S. graminicola* being maintained at ICRISAT, India. Twenty accessions exhibited resistance (≤10% DM incidence) to 3-7 pathotypes. None of the accessions was resistant to all eight pathotypes; however, IP 14537 was resistant to seven pathotypes and had moderate resistance (≤20% DM) to Sg 409. Six accessions (IP 14542, IP 14599, IP 21244, IP 9645, IP 11930 and IP 11943) exhibited resistance to six pathotypes; whereas five accessions (IP 6193, IP 14522, IP 21187, IP 21201 and IP 12374) were resistant to five pathotypes. These twenty accessions were further evaluated in a downy mildew sick plot. Most of these accessions except three (IP 21187, IP 21201 and IP 21244) were found resistant in the field screen. The resistant lines originated from seven African countries. Multiple pathotype resistance identified in the germplasm accessions from different agro-ecologies will be very useful in breeding program to develop downy mildew resistant pearl millet hybrids.

**P07.005 Effect of seed treatment based on SDHI fungicide on the control *Rhizoctonia* spp. in winter wheat**

*Z. Sawinska, T. Kosiada and R. Głowicka-Wołoszyn*
*Poznan University of Life Sciences, Dojazd 11, 60-632 Poznań, Poland*
*Email: zuza@up.poznan.pl*

Succinate dehydrogenase inhibitors (SDHIs) block the fungal respiration process by binding to the ubiquinone reduction site of complex II of the respiratory chain also known as succinate dehydrogenase (SDH) or succinate: ubiquinone oxido-reductase (SQR). Field and laboratory trials were conducted in winter wheat in 2009-2011 at Brody Research Station of the Poznan University of Life Science, Poland on a soil classified as Albic Luvisols developed on loamy sands overlying loamy material. In the experiment we used Sedaxan this is a new, broad-spectrum seed treatment fungicide with focus on control of seed- and soil-borne diseases in a broad range of crops. Its activity spectrum covers soil-borne fungi *Rhizoctonia solani, R. cerealis.* Under field and laboratory conditions, sedaxane showed high levels and consistent protection *Rhizoctonia* spp. Under field conditions, efficacy against *Rhizoctonia* spp. resulted in increased yield compared with the untreated check.

**P07.006 Virulence factors of *Puccinia triticina* on wheat and effectiveness of Lr genes for leaf rust resistance in Ardabil**

*S.A. Safavi[1] and F. Afshari[2]*
*[1]Agricultural and Natural Resources Research Center of Ardabil, Ardabil, Iran; [2]Department of Cereal Research, Seed and Plant Improvement Institute, Karaj, Iran*
*Email: Safaralisafavi@yahoo.com; Safavi_502002@yahoo.com*

Rust diseases of wheat (*Triticum aestivum* L.) are major constraints to production in most wheat growing regions of the world. Leaf rust, caused by *Puccinia triticina* Erikss, is one of the most important diseases on wheat in Iran, second to yellow rust. Control of this disease has often been achieved through the use of race-specific resistance genes. However, these types of genes have been quickly overcome by new virulent pathotypes (races) of pathogen. Hence, the knowledge of effective resistance genes in the region will enable breeders to target those useful genes in their breeding programs. From 2007 to 2012 in order to determine of effective resistance genes in Ardabil, northwest of Iran, virulence patterns of wheat leaf rust were studied under the field conditions by planting of wheat genotypes and near-isogenic lines. The results showed that leaf rust resistance genes *Lr17*, *Lr18*, *Lr19*, *Lr22a*, *Lr23+*, *Lr25*, *Lr28*, *Lr35*, *Lr36* and the combination genes of *Lr13*, *Lr27*, *Lr31* and *Lr34* with together/other resistance genes in wheat genotypes Noroeste, Opata 85, Anahuac 75, Genara 81, Babax1, Babax2, Super seri 2, and Parula were effective during study periods. The genotypes having resistance genes *Lr22b*, *Lr1*, *Lr2b*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr3bg*, *Lr9*, *Lr10*, *Lr11*, *Lr12*, *Lr13*, *Lr14b*, *Lr16*, *Lr20*, *Lr23*, *Lr24*, *Lr29*, *Lr30*, *Lr32*, *Lrb*, *Lr37* showed susceptible reaction and were found ineffective. The Genes found effective against leaf rust under natural conditions may be deployed singly or in combinations with durable resistance genes to develop high yielding resistant wheat cultivars in wheat growing areas in which leaf rust races have the same virulence profile to the prevalent race/s of Ardabil.

**P07.007 Seedling and adult plant reaction of some promising wheat lines to yellow rust**

*S.A. Safavi*

*Agricultural and Natural Resources Research Center of Ardabil, Ardabil, Iran*
*Email: Safaralisafavi@yahoo.com; Safavi_502002@yahoo.com*

Yellow (stripe) rust caused by *Puccinia striiformis* f. sp. *tritici* is an important disease that threatens wheat production around the world. Host resistance is the most economical way to manage wheat stripe rust. For this purpose, 18 promising wheat lines of moderate area were evaluated to yellow rust in Ardabil in order to determine their resistance level. The seedling reaction was evaluated in greenhouse by using race 6E150A+, *Yr27*. Adult plant resistance was also evaluated by measuring of final rust severity (FRS) and coefficient of infection (CI) under natural infection conditions with two times artificial inoculations. Artificial inoculation was carried out by yellow rust inoculum having virulent genes against *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr22*, *Yr23*, *Yr24*, *Yr25*, *Yr26*, *Yr27*, *YrA*, and *YrSU*. Field evaluation was conducted based on randomized complete block design with three replications during 2011-2012 cropping season at Ardabil Agricultural Research Station (Iran). Results showed that lines M-90-13, M-90-15, M-90-18 along with susceptible check (Bolani) had the highest values of FRS and CI. The lines M-90-2, M-90-4, M-90-5, M-90-7, M-90-8, M-90-13 and M-90-17 were susceptible at the seedling stage and had low level infection at the adult plant stage. Consequently, these lines had different levels of durable resistance based on the results of this investigation and their pedigree information. The remaining lines that had low level infection at the seedling and adult plant stages, were selected as moderately resistant or resistant lines.

**P07.008 *Rhizoctonia solani* (AG8): Surviving the summer after various winter crops**

*D. Huberli, M. Connor, S. Miyan and W. MacLeod*
*Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth, WA, 6151, Australia*
*Email: daniel.huberli@agric.wa.gov.au*

The effect of crop rotation and management practices on Rhizoctonia solani root disease and inoculum survival over two summers was examined in a 2-year paddock trial. In the first year (2011), there were treatments of barley, wheat, canola and chemical fallow, and in the second year (2012), barley plots of untreated, seed dressing (Dividend), in-furrow application of an unregistered fungicide or tilling to a depth of 10 cm below the seeding depth. Inoculum survival in five paddocks (two canola, two wheat and one barley) was monitored over summer (February-June) 2013. In the 2-year trial, inoculum levels increased for cereals in the 2011 growing season, while for canola and fallow, they declined. Rhizoctonia root disease on barley roots in 2012 were highest following barley. Inoculum levels in cereals were significantly higher over summer 2012 compared with those for the canola and fallow plots. Inoculum in the latter two plots had declined to below detection levels at sowing in June 2012, while barley plots were still significantly higher compared to the other plots. For the five paddocks, inoculum levels over summer are postulated to be affected by the previous crop and significant summer rainfall events. A break crop of canola or a chemical fallow in paddocks with severe Rhizoctonia bare-patch, may reduce inoculum levels and reduce disease in the following cereal crop. Barley exacerbates disease substantially compared to the other crops, so in paddocks with high levels of R. solani inoculum it is recommended that another crop is sown.

**P07.009 Dashboard "*Septoria tritici* blotch of wheat"**

*T.Z. Ibragimov and S.S. Sanin*
*All-Russia Scientific Research Institute of Phytopathology, Bolshie Vyazemy, Moscow region, 143050, Russia*
*Email: tibragimov@vniif.ru*

Dashboard - the software (application) for visually displaying the most important information needed to achieve one or more objectives, and data are combined and arranged on a single screen so the information can be monitored at a glance. For the development the dashboard of disease management of *Septoria tritici* blotch (STB) of wheat (*Mycosphaerella graminicola* (*Septoria tritici*)) was used software Xcelsius 2008. One of the main features of the dashboard is a dynamic visualization of the source data (phytosanitary data) and the results of their processing and analysis (forecast phytosanitary situation and safety recommendations) online. For STB, the risks are determined by several factors (resistant varieties, predecessor, tillage, weather conditions, etc.). Predictive phytosanitary situation is determined by summing the corresponding risk. Recommendations are issued according to the phytosanitary situation and forecast the planned yield. The interface consists of five containers:

- The name of the system;
- The entering phytosanitary information;
- The displays the forecast phytosanitary situation;
- Recommendations on possible courses of action, depending on the forecast of the phytosanitary situation. It also contains the module "Control of *Septoria tritici* blotch of wheat" which contains the text information;
- The fifth consists of auxiliary units of the system. Blocks "*Septoria tritici* blotch of wheat" - text information on the biology of a pathogen, "*Mycosphaerella graminicola (Septoria tritici)*" let you jump to a web page dedicated to the pathogen in the database of the International Mycological Association. Visualization can publish it by exporting to Adobe PDF.

**P07.010 *Fusarium* spp. associated with head blight of wheat in South Africa**

G.J. van Coller, A.L. Boutigny, L. Rose, T.J. Ward, S.C. Lamprecht and A. Viljoen
*Directorate of Plant Science, Western Cape Department of Agriculture, Private Bag X1, Elsenburg 7607, South Africa, and Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*
*Email: gertvc@elsenburg.com*

Fusarium head blight (FHB) of wheat is caused by numerous *Fusarium* species, including trichothecene-producers. In South Africa, FHB is mostly associated with irrigated wheat rotated with maize. Twenty symptomatic wheat heads were collected from four cultivars each in irrigated fields in the Northern Cape, KwaZulu-Natal (KZN), the Bushveld and Free State (2008 and 2009) and under dry land conditions in the Western Cape (2009 and 2012). *Fusarium* species (1323 isolates) were isolated from kernels, identified morphologically and molecularly, and chemotyped. Fifteen *Fusarium* species were isolated, with the *F. graminearum* species complex (FGSC) most dominant. Other *Fusarium* spp. included *F. avenaceum*, *F. brachygibbosum*, *F. cerealis*, *F. chlamydosporum*, *F. culmorum*, *F. incarnatum-equiseti*, *F. lunulosporum*, *F. oxysporum*, *F. poae*, *F. pseudograminearum*, *F. solani*, *F. tricinctum*, the *Gibberella fujikuroi* species-complex and an unknown *Fusarium* species. *Fusarium pseudograminearum* was dominant at one location in the Free State and Western Cape. Selected FGSC and other trichothecene-B producing isolates were identified with MLGT analyses. FGSC members included *F. graminearum s.s.* (85.2%), *F. boothii* (8.3%), *F. meridionale* (3.6%), *F. acaciae-mearnsii* (1.4%), *F. cortaderiae* (1.1%), and *F. brasilicum* (0.4%). The 15-ADON chemotype was most common in 2008 and 2009, the 3-ADON chemotype in the Western Cape in 2009 and at one location in the Free State (2008 and 2009), and the NIV chemotype was most common at one site in KZN in 2009. This extensive survey reported *F. lunulosporum* for the first time on wheat worldwide and identified production areas of concern in South Africa regarding mycotoxin contamination.

**P07.011 Mating type diversity of *Setosphaeria turcica* isolates in Thailand**

S. Kasam[1,3], P. Chaijuckam[1], J. Yhamsoongnern[2], S. Prathuangwong[1,3] and W. Bunkoed[1, 2, 3]
[1]*Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand;* [2]*National Corn and Sorghum Research Center, Nakhon Ratchasima, 30320, Thailand;* [3]*Center for Advanced Studies in Tropical Natural Resources, Kasetsart University, Bangkok, 10900, Thailand*
*Email: ijswpb@ku.ac.th*

*Setosphaeria turcica*, a cause of northern leaf blight is one of the major diseases of corn worldwide including Thailand. In 2011 growing season, a sexual fruiting structure pseudothecium formed by *S. turcica* was firstly found in the production areas around the country. The 225 isolates collected from 9 different locations were studied for morphology, vegetative compatibility, and molecular characterization. They revealed near mating type equilibrium that 104 and 121 isolates were represent in mating type A and mating type a respectively. Both mating types required indirect contact for pseudothecium production via vitro test indicating *S. turcica* is a heterothallic fungus. Inter simple sequence repeat (ISSR) marker analysis and pathogenicity test of these isolates distinguishing the same group (A or a) generally clustered with one another demonstrating that sexual recombination could be genetically more diversed. Isolates of pathogen type A and a recovered from culture media were virulent on plant assay by 73 and 78% suggesting they were genetically close as an aggressive isolates and however, not a characteristics of all isolates of these 2-mating type pathogen. We also found that the pseudothecia could be survived on PDA or Sachs' agar plus corn leaves at room temperature for at least 3 months and likely longer, providing evidence to promote pseudothecia responsible for implication of disease management. As far as we know, this is the first report of sexual state formation under natural infection by this target fungus.

**P07.012 Greenhouse and field aggressiveness of *Fusarium verticillioides* from corn in Laguna province, Philippines**

C.J.R. Cumagun, M.L.D. Vargas and A.N. Alviar
*Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños (UPLB), College, Laguna 4031, Philippines*
*Email: christian_cumagun@yahoo.com*

*Fusarium verticillioides* is one of the economically important pathogens of corn worldwide. Sixteen isolates of *F. verticillioides* from Laguna province, Philippines were tested for aggressiveness expressed as total and main lesion length on 'Super Sweet' corn IPB variety 1 under field conditions across two trials using the toothpick inoculation method. Furthermore, other aggressiveness traits such as inhibition of seedling emergence, decrease of seedling height, fresh and dry weight were also determined in two greenhouse trials. All isolates were pathogenic to corn seedlings and mature plants compared to noninoculated control. Significant genotypic variation was observed ($P = 0.01$) in trial and isolate-trial interaction for all traits across two greenhouse trials and that aggressiveness was highly influenced by the trial conditions. High correlation was found between seedling height, fresh weight, and dry weight results ($r = 0.81$, 0.83 and 0.74, respectively) and germination

percentages. Correlation between greenhouse and field aggressiveness is moderate (r=-0.40). Implications of these findings in the population genetics of *F. verticillioides* and resistance breeding are discussed.

**P07.013 Assays for phytotoxin of *Bipolaris sorokiniana* in wheat seedlings by leaf infiltration with culture filtrates**

S.P. Banu[1] and B.J. Deverall[2]
[1]Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh; [2]The University of Sydney, Australia
Email: salinapbanu@yahoo.com

This study investigated the techniques of infiltration for phytotoxicity assays in wheat seedlings against *Bipolaris sorokiniana*. Primary leaves of wheat seedlings were infiltrated with culture filtrates at two-leaf-stage through a Hagborg device. Leaves of wheat seedlings expressed sensitivity to toxin after 24-48 hours from infiltration with shake culture. Symptoms were not observed with culture filtrates from still culture. Toxicity in wheat seedlings was greater with culture filtrates from complete medium with mycological peptone than complete medium with casein hydrolysate and Czapek-Dox medium. The severity of symptoms was higher with 6-day-old culture filtrates than 9- and 12-day-old culture filtrates in all media. Variation in sensitivity was found with serial dilutions X4 and X8 in respect of number of seedling affected and severity of differential symptoms obtained as chlorosis, necrosis and leaf bending. All cultivars/lines were sensitive to toxins from B. sorokiniana. Variation among isolates and wheat cultivars/lines was obtained corresponding to the number of seedlings showing toxicity symptoms, and to the severity of differential symptoms observed after infiltration with serial dilution X4 and X8 of culture filtrates. Results of this study suggested that toxic culture filtrates appeared to contain a pathogenicity factor and toxin assays could be an effective tool for selecting sensitivity of potential wheat cultivars/lines. This simple bioassay would be beneficial for initial screening of wheat germplasm.

**P07.014 Virulence diversity of *Pyricularia oryzae* (Cavara) on differentials, monogenic lines**

M.K.P. Kumar[1], H.B. Mahesh[2], S. Hitalmani[2] and M. Gowda[3]
[1]Zonal Agricultural Research Station, V.C. Farm, Mandya, India; [2]Deparment of Genetics and Plant Breeding, GKVK, UAS, Bangalore, India; [3]C-CAMP, National Centre for Biological Sciences, GKVK Campus, Bangalore, India
Email: babu_prasanna@rediffmail.com

Rice blast is economically important disease in Karnataka (India) affecting yield significantly. The pathogen is highly variable in different geographical location of the state. Thirty two monogenic lines with Lijiangxintuanheigu (LTH) background along with twenty six international differentials were used to study virulence and pathotype difference in two ecosystems (plains and hilly regions) of the state during 2010, 2011 & 2012. Two years study revealed that none of the monogenic lines showed resistant reaction to *Pyricularia oryzae* in both the locations. This could be attributed to presence of mixed population of races/pathotypes of P. oryzae. To check the seasonal variability, Friedman Two-way Analysis Of Variance (ANOVA) by Ranks (Friedman, 1937) with the assumption that test statistic distributed approximately as chi-square with degrees of freedom (df) k-1 when 'k' is number of seasons. The analysis showed that the response to pathogen variability in all three seasons is not variable at 5% significance level ($\alpha$= 0.05). This stability of the genotypes with different genetic background imparted the broad-spectrum resistance to pathogen variability. Whereas, monogenic lines showed differential reaction in each seasons. Since all the monogenic lines are in the LTH background the variable pathosystem in each season would have been different and conferred variable reaction to pathogen. From this study we can anticipate the importance of hot spot with high blast pressure and pathogen diversity, to identify durable resistance genes and combinations, and to identify changes in genetic structure and virulence in the pathogen population that may cause major threat to cultivars.

**P07.015 Fusarium head blight and *Fusarium* spp. occurring on small grain cereals in Russia**

T.Y. Gagkaeva, and O.P. Gavrilova
All-Russian Institute of Plant Protection (VIZR), Sh. Podbelskogo 3, 196608, St.-Petersburg – Pushkin
Email: t.gagkaeva@yahoo.com

*Fusarium* infection of small grain cereals results in reduced seed quality and yield, but the toxins which may accompany the disease are often a more serious problem. The genus *Fusarium* contains a number of species, some of which are more broadly adapted to environmental variability (*F. poae, F. sporotrichioides*) than the other (*F. graminearum, F. culmorum, F. cerealis*). Assess the diversity of *Fusarium* species reveals the existence of different phylogenetic species in the complex. *F. graminearum sensu stricto* belongs to geographically restricted fungi and detected in Russian Far East and South-European area. Some new phylogenetic species of *F. graminearum* species complex (*F. ussurianum, F. vorosii*) are associated with the same geographical regions, but this does not preclude their detection in other regions of the world. Only *3-AcDON- and 15-AcDON chemotypes* detected among *F. graminearum* sens. str. isolates together with the 3ADON chemotype of *F. ussurianum* and the 15ADON chemotype of *F. vorosii*. NIV chemotype of *F. graminearum* was not found on the observed

territory. *F. sporotrichioides* has detected on all cereal production territory. *F. langsethiae* has been predominantly found on the *territory* of *European* part of *Russia and has* one of the most *prevalent positions* among *Fusarium* pathogens of grain. Surveys performed clearly show the potential risk for *Fusarium* mycotoxins in cereals. The investigations were supported by the contract No. 14.518.11.7067 of the *Ministry of Education and Science* of the *Russian Federation*.

**P07.016 QTLs for small brown planthopper resistance in wild rice (*Oryza officinalis*)**

*C.Q. Yan[1], W.L. Zhang[1,2], Y. Dong[1,3], Y. Yang[1], C.G. Qian[4], L. Yang[1,2], B.J. Ma[1,2], C.S. Li[1], F.D. Huang[1] and J.P. Chen[1]*
*[1]State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control, Ministry of Agriculture Key Laboratory of Biotechnology in Plant Protection, Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, P. R. China.; [2]College of Chemistry and Life Science, Zhejiang Normal University, Jinhua 321004, P. R. China; [3]College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, P. R. China; [4]Jiaxing Vocational Technical College, Jiaxing 314036, P. R. China*
*Email: jpchen2001@yahoo.com.cn*

Host-plant resistance is the most practical and economical approach to control the planthoppers. However, up to date, few germplasm accessions that resistant to the all three kinds of planthoppers (1) brown planthopper-BPH (*Nilaparvata lugens* Stål), (2) small brown planthopper-SBPH (*Laodelphax striatellus* Fallen), and (3) whitebacked planthopper-WBPH (*Sogatella furcifera* Horvath) has been conferred, consequently, seldom genetics of the useful trait has been studied and resistance gene(s)/ QTL(s) has been identified. Here, one wild species, *Oryza officinalis*, was detected showing resistance to the all three kinds of planthoppers. Based on WBPH and BPH resistance in *O. officinalis* has been documented, attention mainly focuses on the SBPH resistance in the *O. officinalis*. The SBPH resistance gene(s) for rice was (were) introduced into a cultivated rice via asymmetric somatic hybridization. Using the $F_2$ population derived from a cross between 9311 and Pf9279-4, three QTLs for SBPH resistance detected by the SSST method were assigned to chromosomes 3, 7 and 12, and accounted for 55.3% of the phenotypic variance. *Through* detection the position and length of chromosomal segments that introgressed from *O. officinalis* into *O. sativa*, the three QTLs were confirmed and further narrowed into no more than 3.2 Mb interval, respectively. Information on such source of three kinds of planthoppers resistance in a variety and the genetics and QTLs of resistance in the variety is useful to breeders in deciding on a breeding methodology and breeding strategies to be adopted for incorporation of host-plant resistance to planthoppers into elite rice cultivars.

**P07.017 Rice false smut is a stamen filament-infected disease**

*D.W. Hu, Y.X. Tang, and M.L. Yong*
*The State Kay Laboratory for Rice Biology, Institute of Biotechnology of Zhejiang University, Hangzhou 310029, Zhejiang, P.R China*
*Email: hudw@zju.edu.cn*

Rice false smut is caused by the pathogen, *Ustilaginoidea virens*, and had become one of the most important diseases in rice-growing areas throughout the world. The pathogen infection route had remained unclear for long time. Recently we found the pathogen infected specifically rice stamen filaments at the booting stage (Tang *et al*., 2013). Here barley cultivar X151 was inoculation by injecting the conidia of *U. virens* at early booting stage. The results showed that the pathogen infected specifically barley stamen filaments intracellularly. The ultrastructural observation released that the cellulose microfibrils of cell walls in the epidermal and cortex cells in barley stamen filaments deposited very sparsely at the early booting stage. The cell gaps between the cortex cells were very much wide. The stamen filaments infected was destroyed greatly and always broken before barley anthesis, but inoculated spikes grained normally.

**P07.018 Susceptibility of European winter wheat cultivars to *Rhizoctonia cerealis* (sharp eyespot) and *R. solani***

*G. Lemanczyk*
*University of Technology and Life Sciences, Department of Entomology and Molecular Phytopathology, Kordeckiego 20, 85-225 Bydgoszcz, Poland*
*Email: grzegorz.lemanczyk@utp.edu.pl*

In the field study period from 2005 to 2012, the incidence and severity of sharp eyespot were recorded on 147 cultivars of winter wheat. Five localities in Poland were included: Chrzastowo, Debina, Konczewice, Minikowo and Radostowo. Susceptibility of the seedlings of 143 cultivars of wheat to *R. cerealis* (AG-D subgroup I) and *R. solani* (AG-5) was studied under laboratory conditions, while the susceptibility of 132 wheat cultivars was examined in the field trial in Mochelek in the 2012-2013 cropping season. There was much variation in incidence and severity of sharp eyespot between years and locations. The disease was most intense at Chrzastowo. At this location, mean percentage of diseased stems was 1.06-43.0, and mean disease index was 0.3-17.3, with the lowest and highest values in 2006 and 2007, respectively. In Debina and Radostowo, disease was least intense. The most infected cultivars

were Cubus (Chrzastowo, Debina), Tonacja (Konczewice), Dekan (Minikowo) and Rywalka (Radostowo). Culture analysis on PDA medium and PCR assay confirmed that *R. cerealis* was the main causal organism; *R. solani* was detected only sporadically. There was a wide variation in the susceptibility of wheat cultivars to both *Rhizoctonia* species. Cultivars Adequat and Adler showed low, while cv. Turkis - high susceptibility to *Rhizoctonia* species. No cultivar was resistant to *R. cerealis* and *R. solani*. Most symptoms developed on coleoptiles, but also leaves and roots were infested.

**P07.019 Influence of *Rhizoctonia* spp. infestation on volatile production by wheat plants**

*G. Lemanczyk, D. Piesik and A. Baturo-Ciesniewska*
*University of Technology and Life Sciences, Department of Entomology and Molecular Phytopathology, Kordeckiego 20, 85-225 Bydgoszcz, Poland*
*Email: grzegorz.lemanczyk@utp.edu.pl*

Pathogen infestation to vegetative tissues can induce volatile organic compounds (VOCs) production, which can provide defensive functions to injured and uninjured plants. In our studies 'Jenga' wheat plants were infected by one of four *Rhizoctonia* species (*R. cerealis, R. solani, R. zeae, R. oryzae*). The soil was inoculated by the pathogens during the sowing or shoots had been inoculated at stage BBCH 33. VOCs emission was investigated after 3, 7 and 11 days following the inoculation by tested pathogenes. In our studies, we confirmed that several green leaf volatiles (GLVs; (Z)-3-hexenal, (E)-2-hexenal, (Z)-3-hexen-1-ol, (Z)-3-hexen-1-yl acetate), terpenes (β-pinene, β-myrcene, Z-ocimene, linalool, β-caryophyllene), and shikimic acid pathway derivative (indole) were positively induced from wheat plants infected by *Rhizoctonia* spp. Infested palnts by *Rhizoctonia* spp. emitted the greatest amounts of (Z)-3-hexenal, (Z)-3-hexen-1-yl acetate, linalool. VOCs released by the plants after *R. cerealis* (AGD I) and *R. solani* (AG 5) infestation were larger to compare to *R. zeae* (WAG-Z) and *R. oryzae* (WAG-O). β-caryophyllene was emitted in considerably larger amounts by plants *R. cerealis* infested plants, but (Z)-ocimene by *R. solani* infested wheat. The quantities of induced VOCs were higher at 7 days and 11 days than 3 days post-infection and greater when plants were infected with *Rhizoctonia* on stem base than through soil.

**P07.020 One generation propagation distance of wheat powdery mildew and the influence of intercropping on development of the disease**

*N. Liu[1], G.S. Gong[1], M. Zhang[1], Y. Zhou[1], X. Wang[2], Y. Lei[1], X.L. Chang[1], X.B. Qi[1], J.Z. Yang[1], H.B. Chen[1] and P. Zhang[1]*
*[1]Agricultural College, Sichuan Agricultural University, Chengdu 611130, P. R. China.; [2]College of Resources and Environment, Sichuan Agricultural University, Chengdu 611130, P. R. China*
*Email: guoshugong@126.com*

Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *tritici*, an obligate biotrophic pathogen, is an important atmospheric spread disease. Studies have shown that several reasons are responsible for increase and spread of this disease, such as the close planting cultivation techniques, the rise of fungi resistance, and so on. One generation propagation distance as a key parameter for disease development is helpful to control the disease repeated infection. Improvement of farming cultivation system is presently accepted as the most simple, safe and effective prevention and control measures of agriculture to reduce the disease in actual production. Therefore, this paper focuses on studies of one generation propagation distance of wheat powdery mildew and the influence of intercropping. The field experiments were performed in Ya'an in 2011 and 2012. The results showed that the pathogen spread as far as 527 cm in 2011 and up to 534 cm in 2012 from the disease center through one generation. Different intercropping affected the prevalence rate, severity and disease index, which of wheat-broad bean intercropping (98.39%, 38.19% and 37.58, respectively) and wheat-garlic intercropping (100%, 26.63% and 26.63, respectively) were all lower than wheat monocropping (100%, 53.13% and 53.13, respectively). In addition, the wheat yields of sole cropping were about 30% lower than intercropping with broad bean and garlic. To compare these two intercropping system, wheat-garlic system was a little better than wheat-broad bean. Through these experiments, it can be clearly seen that the intercropping system is a useful planting method to prevent the disease dispersal in a certain distance range.

**P07.021 Distribution and development of gray leaf spot of maize and genetic diversity of the causal organism**

*G.S. Gong[1], M. Zhang[1], S.A. Shen[1], H.Yuan[1], J.Z. Yang[1], H.B. Chen[1], J.K. Zhang[2], X.L. Chang[1], X.B. Qi[1], Y.L. Chui[3],Y. Zhou[1] and N. Liu[1]*
*[1]Agricultural College, Sichuan Agricultural University, Chengdu 611130, P. R. China; [2]Ya'an Municipal Bureau of Agriculture, Ya'an 625014, P. R. China; 3Sichuan Provincial Institute of Natural Resource Science, Chengdu 610015, P. R. China*
*Email: guoshugong@126.com; yalanmin@126.com*

Gray leaf spot of maize is an important worldwide disease. It has become the serious foliar disease in Sichuan of China. The objectives of this study were to investigate the distribution and development of gray leaf spot, and determine the genetic diversity of the causal organism. Occurrence and severity of gray leaf spot were positive correlated with altitude based on our investigation

in 101 different locations, and the explosive areas were about at an altitude of 700 m and above in Sichuan Province. The development of gray leaf spot was investigated in Ya'an, in Sichuan (about at an altitude of 1500 m). The disease-progress curve (the progress of the epidemic over time) showed a S type, and the disease was polycyclic. The exponential phase was from the end of June to early July. The logistic phase was from early July to early August. And the decline phase was from the mid-August to the end of maize growth. The epidemic rate(*r*) was 0.14 units per day in the region. 126 samples were collected in Sichuan and the causal organism on lesions was identified as *Cercospora zeae-maydis* Tehon & Daniels. 49 representative strains were used to analyze the genetic diversity by RAPD markers. The results showed that 38 bands were polymorphic stripes among 52 bands which were amplified by 6 primers, and the rate of polymorphism was 73.08%, which revealed abundant genetic polymorphisms. Cluster analysis showed that 49 strains could be divided into three groups but had no significant correlation with cultivars, altitudes and geographic areas.

**P07.022 Identification of a new gene *PmH962* conferring resistance to powdery mildew in wheat assisted by RNA-Seq**

*J. Huang[1,2,3], Z.H. Zhao[1,4], X.M. Wang[1], H.X. Xu[3], D.G. An[3] and H.J. Li[1]*
*[1]Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China; [2]Biotechnology Institute, Guilin Medical University, Guilin 541004, P. R. China; [3]Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang 050021, P. R. China; [4]College of Life Science and Technology, Hebei Normal University of Science and Technology, Changli 066600, P. R. China*
*Email: lihongjie@caas.cn*

RNA-Seq is a revolutionary tool for transcriptomic analysis, which can efficiently detect differential expressed genes on a global pattern in plants. To determine the usefulness of RNA-Seq in mapping genes in a plant species with complicated genome, a pair of hexaploid wheat residual heterozygous lines, H962R and H962S, with different reactions to powdery mildew (caused by *Blumeria graminis* f. sp. *tritici*, *Bgt*), were subjected to RNA-Seq analysis. Using an $F_2$ population and $F_{2:3}$ families derived from H962R×H962S, genetic analysis indicated that a single dominant gene, designated *PmH962*, was responsible for resistance of H962R to isolate E09. Out of ~1500 SSR markers tested, only *Xgwm783* proved to link to *PmH962* with genetic distance of 4.5 cM. Polymorphisms of EST markers based on differentially expressed genes between H962R and H962S were examined, resulting in 5 EST-STS markers that flanked *PmH962* with genetic distances ranging from 1.8 cM (*X27*) to 10.1 cM (*X8*). All these EST-STS markers, together with the SSR marker *Xgwm783*, were located on the distal bin 7BL10-0.78-1.00 of chromosome 7BL. This indicates that *PmH962* resides on this chromosome region on 7BL, which shares the same region with *Pm5* locus. However, the precise marker locations, different inheritance modes, and reaction patters to an array of 42 *Bgt* isolates indicated that *PmH962* was most likely different from the genes on *Pm5* locus. Results in this study provide an example for use of RNA-Seq technique in mapping of genes for disease resistance in the crops with complicated genomes.

**P07.023 Identification and effects of four biocontrol strains to cereal cyst nematode of wheat**

*J. Zhang, H.L. Li ,B.J. Sun, X.P. Xing and H.X.Yuan*
*Department of Plant Pathology, Henan Agricultural university, NO.95 Wenhua Road, Jinshui District, Zhengzhou, 450002, P. R. China*
*Email: honglianli@sina.com*

Cereal cyst nematode (CCN) has become an important disease in Huanghuai wheat production area of China. Effective control measures are desperately needed. In order to seek for biocontrol agents to this disease, four strains (the fungus 08F04, the actinomycetes S07, the bacterium 09B18 and 09X01) were isolated from the cyst in the diseased fields. The fungus 08F04 was identified as *Beauveria bassiana* by morphology and rDNA-ITS sequence analyses. The biocontrol strain of S07, 09B18 and 09X01 were identified as *Streptomyces anulatus*, *Bacillus cereus* and *Alcaligenes faecalis* respectively by morphology, physiological biochemical character and rDNA-16S sequence analyses. In 2012, their control efficiency and the influence on the growth of wheat plant were tested in the greenhouse and diseased fields. The cysts decreasing rates of the treatment with 08F04 agent, S07 agent, 09B18 agent and 09X01 agent in pots were 50.95%, 62.78%, 44.21% and 61.47% respectively. They were 58.49%, 45.09%, 37.54% and 35.55% in Xuchang diseased fields successively. Also, the actinomycetes strain S07 and the bacteria 09B18 can increase the fresh weight of wheat plant obviously. Above all, the four strains can be supposed as potential biocontrol agents of cereal cyst nematode of wheat.

**P07.024 A novel dominant gene *Pigy1*(t) controls the durable resistance of Gangyuan 8 against the rice blast**

*H. Zhang, W. Zhao, C. Liu, W. Fang and Y.L. Peng*
*State Key Laboratory of Agrobiotechnology and Ministry of Agriculture Key Laboratory of Plant Pathology, China Agricultural University, Beijing, P.R China*
*Email: pengyl@cau.edu.cn*

Rice blast, caused by ascomycete fungi *Magnaporthe oryzae* and causing 10~30% yield loss of the annual rice harvest, is a destructive disease in rice growing areas worldwide. Breeding and application of resistant cultivars is the most effective and economic strategy to control rice blast. Gangyuan 8, a local japonica rice cultivar in Liaoning province are cultivated annually on 70,000 hectares of land, has shown durable resistance and no susceptible lesion was observed in the field since its extension in 2005. To identify the resistance genes controlling durable resistance in this cultivar, $F_1$, $BC_1$ and $F_2$ generations were made by crossing the susceptible variety Lijiangxintuanheigu (LTH) and Gangyuan 8 and tested by inolculating with the incompatible isolate RB2 of *M. oryzae*. The phenotype of $F_1$ plants was in accordance with that of Gangyuan 8, and the segregations of the $BC_1$ and $F_2$ population fitted to theoretical ratio of 1:1 and 3:1, respectively, showing that there is a dominant gene in Gangyuan 8. The gene named *Pigy1*(t) was preliminary mapped on short arms of chromosome 12 by SSR markers, and then finely mapped between RM27947 and RM27954 using more than 1,000 $F_2$ individual susceptible plants. The two markers span a region about 300 kb in long relative to Nipponbare genome. Furthermore, a BAC genomic library of Gangyuan 8 was constructed and three BAC clones covering the region between RM27947 and RM27954 were screened out and confirmed overlapping each other. By sequence analysis of the three BAC clones, candidate genes were cloned and being subjected to functional complementation assay.

### P07.025 Infection processes of *Ustilaginoidea virens* by artificial inoculation in rice panicles

*M.L. Hu [1, 2], L.X. Luo [1, 2] and J.Q. Li [1, 2]*
*[1]Department of Plant Pathology, China Agricultural University / Key laboratory of Plant Pathology, Ministry of Agriculture; [2]Beijing Engineering Research Center of Seed and Plant Health (BERC-SPH) / Beijing Key Laboratory of Seed Disease Testing and Control (BKL-SDTC), Beijing, 100193, P. R. China*
*Email: maolin522612@126.com*

The rice false smut disease, caused by *Ustilaginoidea virens*, is one of the most damaging diseases of rice (*Oryza sativa* L.) in China recently. However, the infection process of *U. virens* was not comprehensive studied. In this study, developmental processes of *U. virens* in rice panicles were illustrated by using of green fluoresce protein (GFP) labeled strain. During rice booting stage, the mixture of hyphae and conidia of *U. virens* were inoculated in rice panicles by leaf sheath injection in greenhouse condition. The spikelet samples were collected 12, 24, 36, 84, 134, 168, 192, and 216 hours post inoculation (hpi) for developmental progress observation by confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). The results showed that the inner spikelets were infected by hyphae at 24 hours post inoculation (hpi) by CLSM and SEM observations. The hyphae reached the highest peak at 168hpi before rice heading stage. The primary colonization site of *U. virens* is on the base of filaments. The pathogen extended in the inner spikelets upward to anther apex from basal filaments. The hyphae expanded mainly in anther clearance, then wrapped all floral organs and produced a velvety smut ball.

### P07.026 Epiphytic characteristics of rice false smut pathogen, *Ustilaginoidea virens*

*J. Fan[1], X.Y. Guo[1], L. Li[1], Y. Lei[1], Y.Y. Huang[1], Y. Li[1], F. Huang[2], Y.F. Liu[3] and W.M. Wang[1]*
*[1]Rice Research Institute, Sichuan Agricultural University, Chengdu 611130, P.R China; [2]Department of Plant Pathology, College of Agronomy, Sichuan Agricultural University, Chengdu 611130, P.R China; [3]Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, P.R China*
*Email: fanjing7758@126.com*

Rice false smut is becoming from a minor rice disease to one of the major diseases in China. It not only causes the reduction of rice yield, but also affects the grain quality due to the mycotoxins produced by the false smut pathogen, *Ustilaginoidea virens* (*Uv*). However, the disease cycle and the alternative hosts of *Uv* remain largely unclear. Here, we inoculated a GFP (green fluorescent protein)-labeled strain of *Uv* on leaves of various plants belonging to different families, some of which are weeds common to paddy field, including *Poacea* (*Oryza sativa*, *Echinochloa crusgalli* [Linn.] Beauv. and *Digitaria sanguinalis* [Linn.] Scop.), *Brassicaceae* (*Arabidopsis thaliana*) and *Solanaceae* (*Nicotiana benthamiana*). Using fluorescence microscopy and confocal microscopy, we observed that on these leaves *Uv* could complete a life cycle, including conidia germination, hyphal growth and sporulation. Generally, conidia germinated at 1 dpi (days post-inoculation); at 3 dpi, lots of secondary conidia were produced from conidiophores. Water and high humidity were essential for this process. But no obvious disease symptoms and infection sites were observed on the inoculated leaves, and no invading structures such as appresorium and haustorium were detected. Our data imply that *Uv* can epiphytically colonize on leaves of many plants and produce large number of spores, which could contribute greatly to the disease cycle of *Uv* by providing plenty of initial inoculums and explain why the disease is prevalent when rice booting and heading stages are in rainy days.

### P07.028 Genetic diversity and trichothecene chemotypes of the *Fusarium graminearum* clade isolated from maize in China

*L.J. Ji, L.S. Wang, Q.S. Li and L.X. Kong*

*Plant Protection Institute, Hebei Academy of Agricultural and Forestry Sciences, IPM Center of Hebei Province, Key Laboratory of Integrated Pest Management on Crops in Northern Region of North China, Ministry of Agriculture, Baoding 071000, P. R. China*
*Email: konglingxiao@sohu.com*

In large area of China, semi-annual cropping of winter wheat followed by summer maize is the most common agricultural production system. Wheat-maize rotation for long time has been accompanied by an increase in maize ear rot and also FHB. A total of 229 *Fusarium graminearum* species complex (FGSC) isolates was obtained from maize kernel samples affected by *Gibberella* ear rot collected from Hebei, Henan and Gansu province in China. Polymerase chain reaction (PCR) assays and sequencing of the elongation factor 1-α (EF-1α) gene were used to characterize FGSC species and trichothecene mycotoxin genotypes [3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON) and nivalenol (NIV)]. We found that *Gibberella* ear rot of maize in China is associated with a complex of species of the FGSC - mainly *Fusarium graminearum* sensu stricto (133 isolates) and *Fusarium boothii* (89), but also *Fusarium asiaticum* (5) and *Fusarium meridionale* (2). Nearly all of the isolates studied (220/229) were of the 15-ADON genotype. Six of the isolates were of the 3-ADON genotype. The NIV genotype was not observed. *Fusarium boothii* was the dominant species, accounted for 94.7% of the total isolates in Gansu province. *Fusarium graminearum* showed the highest isolation frequency in Hebei and Henan with 76.1% and 66.7%, respectively. This study indicates that 15-ADON producing *Fusarium graminearum* and *Fusarium boothii* are important pathogens in the etiology of *Gibberella* ear rot of maize in China. Whereas the predominant pathogen in regions of China may be different.

### P07.029 Fine mapping of a new blast resistance gene *Pi58*(t) in *Oryza sativa* subsp. *japonica* cultivar, Yunxi 2

*L.Y. Dong, Q. Wang, S.F. Liu, X.D. Li, F.P. Zheng and Q.Z. Yang*
*Agricultural Environment & Resources Research Institute, Yunnan Academy of Agricultural Sciences, Kunming, Yunnan province, 650205, P. R. China*
*Email: qzhyang@163.com*

A new blast resistance gene was identified in *Oryza sativa* subsp. *japonica* cultivar Yunxi 2 of Yunnan. Through genetic and linkage analysis using $F_2$ population derived from susceptible cultivar Lijiangxingtuanheigu and resistant cultivar Yunxi 2, this gene was mapped on the long arm of chromosome 1 flanked by 2 SSR markers RM2527 and RM11757. This gene was designated tentatively as *Pi58*(t). Using $F_2$ mapping population consisting of 500 resistant and 736 susceptivle individuals, *Pi58*(t) gene was finally mapped in an interval of ca. 108 kb, flanked by 2 markers, STS42-8 and STS42-4, and cosegregated with STS marker STS42-7. According to the gene prediction results based on the genomic sequences of Nipponbare flanked by STS42-8 and STS42-4, four genes belonging to NBS-LRR class encoded by most plant disease-resistant genes was identified in this region. These four genes are considered as the candidate genes of *Pi58*(t), named as *Pi58*(t)-1, *Pi58*(t)-2, *Pi58*(t)-3 and *Pi58*(t)-4, respectively. Comparison of allele sequences of candidate genes among susceptible cultivars and resistant cultivar Yunxi 2 indicated that candidate genes *Pi58*(t)-1 and *Pi58*(t)-2 from Yunxi 2 are identical to those from susceptible cultivars; *Pi58*(t)-3 is absent in Yunxi 2; *Pi58*(t)-4 gene from Yunxi 2 has 6 amino acids difference to that from susceptible cultivars, it suggests that *Pi58*(t)-4 gene would be considered as the candidate gene of new blast resistance gene *Pi58*(t). This result provided an important information for further cloning and function analysis of *Pi58*(t) gene.

### P07.030 Postulation of all-stage leaf rust resistance genes in 96 Chinese wheat cultivars

*T.G. Liu, X.L. Ren, B. Liu, L. Gao and W.Q. Chen*
*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R. China*
*Email: tgliu@ippcaas.cn; wqchen@ippcaas.cn*

Leaf rust, caused by the fungus *Puccinia triticina* Eriks., is one of the most important foliar diseases of wheat (*Triticum aestivum* L.). Host resistance is the most economical and safest method of controlling the disease. Knowledge of the leaf rust resistance genes present in lines can greatly improve the efficiency of utilizing resistant cultivars and pyramiding resistant genes. Gene postulation helps to identify the probable leaf rust resistance genes (*Lr* genes) present in a large number of wheat cultivars at a time. The objective of this study was to identify the race-specific *Lr*-genes present in 96 commercial wheat cultivars from China. Leaf rust infection types (ITs) produced on the cultivars and lines by 11 *P. triticina* races were compared with the ITs produced on a standard set of 'Thatcher' near-isogenic lines that differed for single leaf rust resistance genes. Molecular markers that are closely linked or co-segregated with wheat leaf rust resistance genes *Lr9*, *Lr10*, *Lr19*, *Lr20*, *Lr21*, *Lr24*, *Lr26* and *Lr29*, were used for further identification of the 96 Chinese winter wheat cultivars. Thirteen *Lr* genes *Lr1*, *Lr3*, *Lr3bg*, *Lr3ka*, *Lr11*, *Lr13*, *Lr14a*, *Lr21*, *Lr26*, *Lr27*, *Lr30*, *Lr31* and *Lr32* were postulated to be present both in combinations in Chinese wheat cultivars. Thirty seven cultivars may carry unknown resistance genes to leaf rust and *Lr9*, *Lr20*, *Lr21*, *Lr24*, *Lr25* and *Lr29* were not present in the tested 96 accessions. The *Lr*-genes present in some wheat cultivars

could not be postulated because of non-matching virulence spectra by comparison with any of the NILs. The results of this study show that most of the wheat cultivars tested do not have adequate resistance for leaf rust, indicating the need for incorporating more effective genes into the target wheat cultivars.

**P07.031 A novel endornavirus detected in *Rhizoctonia cerealis*, the cause of sharp eyespot in wheat**

*W. Li, T. Zhang, H.Y. Sun, Y.Y. Deng, A.X. Zhang and H.G. Chen*
*Institute of Plant protection, Jiangsu Academy of Agricultural Sciences, No.48 Zhongling Street, Xuanwu District, Nanjing, 210014, P.R. China*
*Email: lw0501@jaas.ac.cn*

*Rhizoctonia cerealis* Van der Hoeven is the causal pathogen of sharp eyespot in wheat. And in China, sharp eyespot has been one of the most economically important diseases of wheat. In this study, a large double-stranded RNA (dsRNA) virus was detected in all *R. cerealis* strains tested, and the dsRNA did not appear to cause obvious disease symptoms. The linear genome of one isolate from *R. cerealis* strain R0959 were sequenced and compared. Sequence information indicates that the dsRNA genome consists of more than 17 kbp and potentially encodes a single polyprotein. This polyprotein contained conserved motifs of putative viral methyltransferase (MTR), helicase 1 (Hel-1) and RNA-dependent RNA polymerase. This dsRNA is more similar to *Phaseolus vulgaris* endornavirus 2 (PvEV-2), hence this virus, for which the name *Rhizoctonia cerealis* endornavirus (RcEV) is proposed, is a distinct species in the genus Endornavirus (family Endornaviridae). Endornaviruses have unique plasmid-like properties that differ markedly from those of other conventionally encapsidated viruses. This is the first report that the Endornavirus was detected in *R. cerealis*.

**P07.032 Research and exploration on rice false smut**

*W. Peng[1], X.L. Tang[1], X.X. Ran[1] and X.Q. Xu[2]*
*[1]Sichuan Tourism University, Hongling Road, Longquanyi District, Chengdu, Sichuan, 610100, P. R. China; [2]The Plant Protection and Quarantine Station of Zitong County, Mianyang, Sichuan, 622150, P. R. China*
*Email: 1574116931@qq.com*

Rice false smut (hereinafter referred to as RFS) has turned to be a severe kind of fungal disease from secondary disease in recent years. The discovery and nomenclature, the domestic and overseas occurrence, distribution and damage status of RFS, the pathogenic morphological characteristics, the typical symptom, the toxicity and the occurrence regularity of RFS and the chemical control experimental demonstration are discussed in the paper. The author focuses on the occurrence regularity of RFS by analyzing the first and second infection sources, the biological characteristics of RFS pathogen, the infection and damage of RFS and the epidemic regularity of RFS. He ends the article by proposing possible approaches to the comprehensive treatments of RFS. First of all, the resistant rice species are suggested to be chosen and disinfecting the seeds before sowing are required; then, disposal of the rice piles, the plant debris and possible intermediate hosts is a necessary step for prevention; keeping rational close planting, favorable humidity, lighting permeation and good ventilation are key principles when sowing for pest control, so is the rational fertilization and watering during the booting to blooming stages; chemical control during 5-10 days before blooming in serious damaged regions is effective to restrict the prevalence of RFS and maintain good yield and quality of rice.

**P07.033 Molecular identification of the gene effective against powdery mildew in the wheat cultivar Liangxing 99**

*Z.H. Zhao[1,2], H.G. Sun[1,2], W. Song[1,2], J. Huang[1,3], X.M. Wang[1] and H.J. Li[1]*
*[1]Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China; [2]College of Life Science and Technology, Hebei Normal University of Science and Technology, Changli 066600, P. R. China; [3]Biotechnology Institute, Guilin Medical University, Guilin 541004, P. R. China*
*Email: lihongjie@caas.cn*

Liangxing 99, one of the most widely grown cultivars of wheat (*Triticum aestivum*) in the winter and facultative wheat producing regions in northern China, exhibited a broad spectrum resistance to many *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolates. Genetic analysis on the $F_2$ populations and $F_{2:3}$ families derived from Liangxing 99 × Zhongzuo 9504 demonstrated that a single dominant gene, designated *MlLX99*, was responsible for the resistance of Liangxing 99 to the *Bgt* isolate E09. The results of genetic analysis indicated that this gene was located on chromosome 2BL and flanked by SSR marker *Xgwm120* and EST-STS marker *BE604758* with genetic distances of 2.9 cM and 5.5 cM, respectively. Since the flanking markers were previously mapped to the bin 2BL2-0.36-0.50, *MlLX99* might also be located in this chromosome region. *MlLX99* had a different reaction pattern against an array of *Bgt* isolates from other known genes *Pm6*, *Pm33*, and *PmJM22*, which were all mapped to chromosome 2BL but differed in positions. Because of its unique position on chromosome 2BL and reaction pattern to the *Bgt* isolates, *MlLX99* is most likely a new resistance gene to powdery mildew. Liangxing 99 has shown superior yield performance and wide adaptation to different agricultural conditions, which makes it a promising wheat cultivar in agriculture.

**P07.034 Factors affecting oat leaf spot caused by *Drechslera avenae***

*X.Y. Zhang, H.Y. Zhou, X.M. Sun and X.F. Gu*
*College of Agronomy, Inner Mongolia Agricultural University, Huhhot 010019, P. R. China*
*Email: hongyouzhou2002@yahoo.com.cn*

Oat leaf spot caused by *Drechslera avenae* (Eidam) is a major disease in most oat production areas in China, threatens oat production and reduces the yield and quality. During the growing season of 2009-2011, field trials were conducted in Wuchuan county of Inner Mongolia to determine the effects of oat variety, sowing time, irrigation, plant density, nitrogen and phosphate fertilizer by orthogonal design of $L_{25}(5^6)$. The oat varieties Yanke 1, Baoluo, Caoyou 1, Conventional variety and Keyan 1 were selected. Sowing time were May 2nd, 9th, 16th, 23th, 30th. Planting density were 300000, 350000, 400000, 450000, 500000 plants per hectare. Five irrigation methods were used, No irrigation, Water once in jointing stage, Water once in heading stage, Water twice in jointing and heading stage, Water three times in jointing, heading stage and ratooning buds. Each water $50m^3/667m^2$. 5 kinds of nitrogen and phosphorus levels were used. Repeat 2 times and each plot was $30m^2$. The statistical analysis was conducted with spass17.0. The results showed that the varieties resistance was the most important factor affecting, the conventional variety was more resistant and had low severity. The second important factor was sowing time in that late sowing was favorable for disease development, and the disease occurred more seriously in plots sowed on May 30th than in other plots. Another important factor was irrigation. The more irrigation water, the higher was the disease incidence. Planting density also affected the incidence of oat leaf spot. The higher density, the more serious was the disease. P and N ratios in the fertilizer didn't show significant effects on oat leaf spot. Therefore sowing early, planting with appropriate density, controlling humidity can reduce the severity of oat leaf spot.

**P07.035 The expression of carbohydrate active enzymes of wheat sharp eyespot pathogen, *Rhizoctonia cerealis***

*X.R. Ren[1, 2], W.Li[1], H.Y. Sun[1], H.S. Yu[2] and H.G. Chen*
*[1]Institute of Plant Protection, Jiangsu Academy of Agriculture Science, 210014, P. R. China; [2]College of life Sciences, Nanjing Agricultural University, Nanjing 210095, P. R. China*
*Email: huaigu@hotmail.com*

Enzymes involved in carbohydrate metabolism include Carbohydrate esterases (CE), Glycosidehydrolases (GH), Glycosyltransferases (GT), and Polysaccharide lyases (PL), commonly referred to as carbohydrate active enzymes (CAZymes). Many fungal pathogens produce CE, GH, and PL superfamilies known as cell wall degrading enzymes (CWDE) to disintegrate the plant cell wall. *Rhizoctonia cerealis* is soilborne pathogen, which causes wheat sharp eye spot with significant impacts on crop yield and grain quality. Transcriptome (mRNA) of *R.cerealis* strain 0301 were sequenced using Illumina Genome Analyzer sequencing technology (BGI-Shenzhen, China) and then assembled the short reads to unigene. In *R. cerealis* transcriptome, there were 95 GHs, 34 GTs, 21 CEs and 16 PLs. Then we compared the expression pattern of CAZymes of *R. cerealis* on potato sucrose agar (PSA), medium with wheat cell wall as carbon source (CW) and in plant. Expression level of GH17, GH16, GT4 and GT17 was higher when *R. cerealis* infected wheat seedlings than on CW. We suggested that some CAZymes of *R. cerealis* may play important role in the infection.

**P07.036 Occurrence of rice sheath blight and it's integrated control in Fengxian district, Shanghai**

*J.Z. Jiang, S.F. Tao, W.Y. Zhang and J. He*
*Fengxian District Agro-technology Extension Service Center, Shanghai 201400, P. R. China*
*Email: shfxjiang@126.com*

Rice sheath blight, one of the most destructive rice diseases is widely prevalent in Fengxian district, Shanghai. It occurred in almost all the rice fields, and is getting more and more serious, with an increased disease index from 2.01 in 2003 to 9.07 in 2009. Meanwhile, the disease outbreak occurred earlier. Based on the occurrence characteristics of these years, factors causing occurrence of this disease were analyzed as follows: (1)Ignoring agricultural control increased the pathogen quantity in fields year by year; (2)Climactic conditions were conducive to disease outbreak;(3)Lacking of plant varieties resistant to the disease; (4) Low control efficiency caused by increased resistance to conventional pesticides, for example, the consumption of 5% valid amycin aqueous solution increased from 200 ml to 600 ml per $666.7m^2$ while the control effect was decreased from 80%-90% to 40%-50%. To control this disease efficiently, we proposed several integrated control measures. Firstly, reduce pathogens by removing diseased plant debris. Secondly, apply scientific management, including suitable compact planting, water and fertilizer management method. Finally, spray pesticides in time and use new pesticides such as Armure and Pulsor, if possible.

**P07.037 A uninucleate *Rhizoctonia* sp. infecting maize plants in China**

*S.Y. Zhou, Y. Liu, Z. Ji, M. Zhang and B.D. Li*
*College of Agronomy and plant Protection, Qingdao Agricultural University, Qingdao, Shandong 266109, China; Key Lab of Integrated Crop Pests Management of Shandong Province, Qingdao, Shandong 266109, P. R. China*

*Email: zhoushanyao@126.com*

Plant-pathogenic *Rhizoctonia* spp. reported are mainly consisted of a majority of multinucleate and a minority of binucleate strains. The uninucleate *Rhizoctonia spp.* pathogens were only reported on Norway spruce and Scots pine and on turf-grasses, to our knowledge. Here a uninucleate *Rhizoctonia* sp. infecting maize plants was obtained by tissue isolation method in China. The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (rDNA) of the isolate was amplified using the universal primers ITS-1F and ITS4. Analysis of the ITS sequences showed there were two kinds of sequence which shared 95% and 99% homology with ITS sequence of YR-270 in GenBank (Accession No. HQ 636470), respectively. The pathogenicity of the isolate was tested on maize plants in growth chamber under the condition of 28℃ and about 80% relative humidity. The results showed infection of the isolate formed typical necrotic lesion on sheath of susceptible maize plants, while formed primary slight brown lesion which expanded little on sheath of resistant maize plants and the control plants remained healthy. These results indicated though the pathogenicity of the uninucleate isolate was weak, it could infect maize plants as pathogen.

**P07.038 Infection dynamics of two species of cereal cyst nematode in Henan Province**

*H.T. Yan, H.L. Li, H.X. Yuan, B.J. Sun and X.P. Xing*
*Department of Plant Pathology, Henan Agricultural university, No.95 Wenhua Road, Jinshui District, Zhengzhou, 450002, P. R. China*
*Email: honglianli@sina.com*

The cereal cyst nematode has become one of the serious diseases of wheat in the recent years in China。It is a basic work for the disease control and prevention to find out the biological characteristics of pathogen nematodes, especially the infection dynamic. A study has been conducted under field conditions on the infection dynamics of the two species of cereal cyst nematode, *Heterodera avenae* and *H. filipjevi.* The result indicates that the wheat roots were invaded by the second stage juveniles 10 days after the emergence of seedling, and 20 days later, a few of the second stage juveniles develop into the third stage, and the peak of the number of the second stage juveniles comes 40 days after the emergence, at the same time a few of the fourth stage juveniles appear. 20 days later when the temperature gets lower, the number of the larvaes of different stages remains stable. 120 days after the emergence, with the temperature rising ,the number of the second stage juveniles starts to increase and reaches to the second peak 30 days later ,but this time ,the number of junvenile which invade the wheat roots is apparently less than the previous one .after that the larvaes gradually developed into white females and the peak of the number of it comes 180 days after the emergence, The third stage, fourth stage of the juveniles and the white female of the *H. filipjevi* appear one week before that of *H. avenae*.

**P07.039 Construction and identification of physiological race library of *Magnaporthe oryzea* in western Hubei Province**

*X. Xu, Z.W. Long, X.Q. Liu and C.T. Wang*
*Key Biotechnology of State Ethnic Affairs Commission, College of Life Science, South-Central for Nationalities, Wuhan, 430074, P. R. China*
*Email: wangchuntai@mail.scuec.edu.cn*

Rice blast, caused by the ascomycete fungus *Magnaporthe oryzae*, is one the most devastating diseases of this crop worldwide. While *M. oryzae* is known for its complex population structure and high variability in field conditions, which causes frequent loss of host resistance. Therefore, analysis the genetic structure and variation of *M. Oryzae* can provide an important basis for the prediction and forecasting of disease and the layout of resistant varieties. The Western Hubei province belonging to Wuling mountainous area has special ecological conditions and is marked as the disaster area of rice blast. The aim was to construct and initially identify the physiological race library of *M. oryzea* in western Hubei Province. *M. oryzea* infected rice straws were collected from 7 rice main production regions of western Hubei Province from 2010 to 2012. A physiological race library was constructed by single spore isolation, purification and identification of colony morphology. Cluster analysis of this library was conducted by RAPD and REMAP. The results showed that a physiological race library of 182 isolates was constructed. Cluster analysis showed that population structure of *M. oryzea* demonstrated diversity and complexity, and there was no corresponding relationship between genetic lineages and colony morphology. The study provides a basis for predicting the trend of changes in *M. oryzea* population and breeding new resistant varieties in western Hubei Pro vince.

**P07.040 The *GSK3* glycogen synthase kinase is important for plant infection and DON production in *Fusarium graminearum***

*J. Qin[1], C.F. Wang and J.R. Xu[1, 2]*
*[1]Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, P.R. China; [2]Department of Botany and Plant Pathology, Purdue University, USA*
*Email: qqinjun@gmail.com*

Fusarium head blight caused by *Fusarium graminearum* is one of the most important diseases on wheat and barley worldwide. In addition to causing severe yield losses during epidemics, the pathogen produces harmful my-

cotoxins, such as deoxynivalenol (DON) and zearalenone, in infested kernels. In a previous study of systematic characterization of protein kinase genes, a number of them, including the *GSK3* glycogen synthase gene, were found to be important for hyphal growth, conidiogenesis, and pathogenesis in *F. graminearum*. *GSK3* glycogen synthase kinase functions in various cellular and developmental processes, including cell fate specification, cytoskeleton movements, and programmed cell death. Although *GSK3* orthologs are well conserved in filamentous fungi, none of them have been functionally characterized in plant pathogens. In this study, we aimed to determine the functions of *GSK3* in conidiation and plant infection in *F. graminearum*. The *gsk3* deletion mutant was characterized in details for its defects in hyphal growth, conidiation, DON production, and plant infection. Glycogen synthesis and DON production was significantly reduced or limited in the *gsk3* mutant, which was non-pathogenic in infection assays with flowering wheat heads. In self-cross plates, the mutant was blocked in the production of ascospores and perithecia. Expression of the *GSK3*-GFP fusion construct in the *gsk3* mutant complemented its phenotypes and GFP signals appeared localized to the cytoplasm. Stress responses of the *gsk3* mutant and the functional relationship between *GSK3* and other Gsk3-related protein kinases are being characterized. Overall, our results indicate that *GSK3* is important for hyphal growth, conidiogenesis, DON production, and pathogenesis in *Fusarium graminearum*.

### P07.041 Resistance in wild emmer wheat (*Trtiticum dicoccoides*) from the fertile crescent to yellow rust in China

*Y. Liang, Z.Y. Zhang, Y.L. Peng and J.H. Peng*
*Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, and Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, Hubei, 430074, P.R. China*
*Email: ly318318@126.com*

Wild emmer wheat (*Triticum dicoccoides*) is the progenitor of domesticated wheat and harbors a valuable pool of resistance genes that can be transferred into cultivated wheat. However, little is known about their response to Chinese stripe rust population of *Puccininia striiformis* f. sp. *tritici*, which has caused severe yield lost and huge input of pesticides in to the environment. In this study, 311 *T. dicoccoides* genotypes representing 31 populations from the Fertile Crescent were planted in the disease nursery located at Yanting, Sichuan Province of China. We used these genotypes to investigate the virulence composition of the fungal population and the infection types on wild emmer wheat at the seedling and adult stages. The main virulence types of the pathogen at the disease nursery were CY32 and CY33, the most virulent and prevailing race in China. Newly emerged mutant virulent to *Yr24* was also detected at a low frequency of 2.17%. Among the 311 genotypes tested, 17 genotypes out of 7 populations of expressed whole stage resistance to yellow rust. Fifty-six genotypes out of 19 populations were susceptible at the seedling stage but with expressed resistance at the adult stage. Water and temperature variables at the origin of each population were found to be correlated with the Infection types at the disease nurseries. Therefore, wild emmer wheat is a valuable resistance source for China wheat breeding programs against the yellow rust disease.

### P07.042 Role of grasses acting as green-bridge on occurrence of wheat stripe rust in China

*J. Zhao[1], Z.Y. Wang[2], W. Chen[2], L.L. Huang[1] and Z.S. Kang[1]*
*[1]State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yanling, Shaanxi 712100, P. R. China; [2]College of Plant Protection, Northwest A&F University, Yanling, Shaanxi 712100, P. R. China*
*Email: kangzs@nwsuaf.edu.cn*

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), has been one of the most destructive diseases on wheat worldwide. Historically, huge yield losses caused by the rust fungus reached up to at least hundreds millions of kilograms in each of serious epidemics of the disease in China. Our surveys showed some grass species including *Poa pratensis* L., *Elymus nutans* Griseb., *Elymus excelsus* Turcz., and *Agropyron cristatum* (L.) Gaertn. distributed widely in Southern Gansu province where has been thought as “hot-spot” and important over-summering areas of *Pst*, and seriously infected by stripe rust and other rusts. Stripe rust collections from the four grasses were used for inoculating wheat cv. Mingxian 169 (MX 169) susceptible to *Pst*, indicating different infection types (IT) on MX 169. Some isolates expressed IT 0, 1, or 2 on susceptible wheat cv., showing resistant. Other ones showed susceptible to MX 169 with IT 3, or 4. Importantly, isolates with low IT (1, or 2), especially IT 2, changed to be ones with high IT (3, or 4) on MX 169 after re-inoculating, showing susceptible from initial resistance to wheat. Remarkable diverse in virulence were found based on identification of the isolates on a set of differential host consisting 19 genotype wheat cultivars. Therefore, besides volunteer wheat, graminaceous grasses acting as accessory hosts of *Pst*, was critical for survival of the rust fungus in summer, and provide inoculums to give rise to autumn-sown wheat infection in cool areas.

### N07.001 *MoCTA1*, encoding a calcium transporting P-type ATPase, is required for conidium morphogenesis and pathogenicity in *Magnaporthe oryzae*

*C. Peng, J.W. Wang, H.L. Chen and S.W. Guo*
*Institute of Food Crops, Jiangsu High Quality Rice R & D Center, Institute of Agro-Biotechnology, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, P.R. China*
*Email: gogow126@126.com*

$Ca^{2+}$-dependent signaling plays important roles in mycelial growth and appressorium morphogenesis in the rice blast fungus, *Magnaporthe oryzae*, which causes the most serious disease of rice. However, the information of molecular and physiological characteristics is known very little for the $Ca^{2+}$-dependent signaling. Here we reported a calcium transporting gene, belonging to P-type ATPase family, *MoCTA1* in *Magnaporthe oryzae*, which regulated spore formation and pathogenicity of the fungus. Targeted gene replacement confirmed that *MoCTA1* was required for aerial mycelium growth, spore formation, and pathogenicity of the fungus, especially for its spore morphogenesis. *ΔMoCTA1* mutant was growing more slowly than Guy11, and could not produce conidium. Pathogenicity assay indicated that the *ΔMoCTA1* mutant could not infect both the rice and barley leaves effectively.

**N07.002 Diagnostic and pathogenicity assay for the corn sheath rot in China**

*Z.Y. Cao, P.P. Li, K. Wang, K.K. Cao, L.H. Zhang, J.L. Zhang and J.G. Dong*
*Mycotoxin and Molecular Plant Pathology Laboratory, Agricultural University of Hebei, Baoding, 071001, P.R. China*
*Email: dongjingao@126.com*

Corn sheath rot was a new disease which was firstly discovered in China in 2008 and widespread rapidly at northeast and other corn planting regions. It was reported that the purple sheath disease of corn occurred in American in 2005, which took place during the phase of corn silk and speculated sheath rot may be caused by *Fusarium* spp. or bacterium. There was little knowledge on the disease occurrenced mechanism and absent effective control measures by now. The morphological characterizations and molecular identification were conducted on pathogens isolated from each sample. *Fusarium* spp. cultures were identified from all diseased tissues. The analysis of nucleotide sequences of the complete rDNA ITS region showed 94% to 100% identities to *F. proliferatum*. But *F. moniliforme* and *F. graminearum* were also separated from part of regions. The results of pathogenicity test showed that the incidence and disease index caused by *F. proliferatum* were higher than that of *F. moniliforme* and *F. graminearum.* With further study on the pathogenic mechanisms of those pathogens, we found that the activities of extracellular cell wall degrading enzymes and the cell membrane damage level caused by different cell wall-degrading enzymes (CWDEs) produced from *F. proliferatum* were higher than those from *F. moniliforme* and *F. graminearum.* It was initial cleared that corn sheath rot was caused by *F. proliferatum* mainly.

**N07.004 MoPyr5 is required for growth, cell wall integrity, and pathogenicity of *Magnaporthe oryzae***

*L.W. Li, Z.Q. Qi, H.F. Zhang, X.B. Zheng and Z.G. Zhang*
*Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, and Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Nanjing 210095, P.R. China*
*Email: zhgzhang@njau.edu.cn*

Orotate phosphoribosyl transferase (OPRTase) plays important role in de novo and salvage pathways of nucleotide synthesis, catalysing the transformation of orotate to Orotidine 5'-phosphate (OMP) in *Saccharomyces cerevisiae*. The function of the enzyme in plant pathogens is not clear. To determine the function of *MoPYR5* in *Magnaporthe oryzae*, here, we identified a homolog to Ura5 in *Saccharomyces cerevisiae*, we cloned the *MoPYR5* gene and generated specific mutants using gene knock-out strategy. Disruption of *MoPYR5* resulted in reduced ability of penetrating the host, and only produced tiny necrosis spots. Cytorrhysis assay suggested that the turgor pressure of appressorium of Δ*Mopyr5* was decreased, and the rate of the protoplast release was accelerated than the wild type, indicating the mutant displayed cell wall integrity defects. Moreover, DAB strain assay suggested that the deletion mutant triggered the defense response, and was unable to scavenge the accumulated ROS produced by the plant tissues. Furthermore, the Δ*Mopyr5* mutant was involved in colony surface hydrophobicity. Complementation of the mutated gene restored its ability to cause typical lesions, demonstrating that *MoPYR5* is required for fungal pathogenicity. These results indicate that *MoPYR5* functions as a pathogenicity factor that involved to the cell wall integrity, the formation of turgor pressure, scavenging of ROS, and colony surface hydrophobicity.

**N07.005 The bZIP transcription factor MoHac1 modulates growth, conidiogenesis, and pathogenicity of the rice blast fungus *Magnaporthe oryzae***

*W. Tang, H.F. Zhang, X.B. Zheng and Z.G. Zhang*
*Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, and Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Nanjing 210095, P. R. China*
*Email: zhgzhang@njau.edu.cn*

The bZIP transcription factor MoAp1 and MoAtf1 were

reported to have crucial roles in growth, conidiogenesis, pathogenicity of *Magnaporthe oryzae* in our previously studies. Here, another bZIP transcription factor family member MoHac1, which played an important role in the unfolded protein response (UPR) was investigated. Our results showed that the Δ*Mohac1* mutant had a severe growth defect with thinner and lacked aerial hyphae. Moreover, *MoHAC1* deletion led to a significant reduction in conidiation and pathogenicity on both barley and rice leaves. Microscopic observation found that the Δ*Mohac1* mutant could form appressoria on the barley leaf surface but failed to penetrate into the cells. In addition, the mutant was more sensitive to ER stress induced by the tunicamycin (TM) and dithiothreitol (DTT) treatment, which consistent with the situation in *Saccharomyces cerevisiae*, suggesting MoHac1 was involved in the UPR signaling pathway. Similar to the *S. cerevisiae* Hac1, a 23 bp conventional intron was also identified in the *MoHAC1* mRNA, indicating the UPR was involved in the unconventional splicing of *MoHAC1* mRNA. Further analysis revealed that MoHac1 was localized in the nucleus during the development stages. Together, we concluded that the bZIP transcription factor MoHac1 is critical for growth, conidiogenesis, and pathogenicity of the rice blast fungus *Magnaporthe oryzae* and have roles in the UPR signaling pathway.

## Concurrent Session 8-Chemical Control of Plant Diseases

### O08.001 Present and prospects for chemical control of soybean rust in Brazil

*C.A. Forcelini and R. Roherig*
*University of Passo Fundo, P.O. Box 611, Passo Fundo, RS, 99001-970, Brazil*
*Email: forcelini@upf.br*

Soybean crop is planted over 27 million hectares in Brazil and its production accounts for 83 million tons a year. After Asian rust (*Phakopsora pachyrhizi*) introduction in 2001, this disease soon became the most important threat to soybeans. In the following season yield reductions were reported to be above 70% in some fields, and in the 2003 season the disease impact (yield losses + control costs) was estimated in 1.6 billion dollars. Because most soybean cultivars are susceptible to *P. pachyrhizi*, disease management largely relies on chemical control, which used to take up to seven fungicide sprays, from early vegetative stages to late pod filling. Most of these applications have used single DMI fungicides (triazoles) because of their efficacy and low cost. Commercial mixes of DMIs + QoIs (strobilurins) used to account for only two sprays per season. After 2008 the pathogen population shifted towards resistance to DMIs, and today spray programs are totally based on three applications of DMIs + QoIs starting at early flowering. The next step is the substitution of DMIs for SDHIs in mixes with QoIs. The most promising SDHIs are solatenol (®Syngenta) and fluxapyroxad (®Basf), which have proved to be highly efficacious to control rust and to protect plants longer. Another important rust management strategy in Brazil is based on a free host period in the winter to reduce inouclum for summer plantings. Since the establishment of this strategy in 2006 fungicide sprays to control rust in V stages are usually unnecessary.

### O08.002 Current status and prospect of fungicide innovation in China

*J.L. Liu, N.G. Si, L. Chen, J.Z. Zhou and Z. Kang*
*State Key Laboratory for Discovery and Development of Novel Pesticides, Shenyang Research Institute of Chemical Industry Co., Ltd., 8 ShenLiao Dong Road, Tiexi District, Shenyang, Liaoning Province, 110021, P. R. China*
*Email: liujunli@sinochem.com*

The history of fungicide innovation in China dated back to late 1940s, when China Agricultural University (CAU) and Nankai University (NKU) initiated new pesticide research and development programs. By 1970, novel Carbendazim and Validamycin were developed by Zhang Shao-Ming of Shenyang Research Institute of Chemical Industry (SYRICI) and Shen Yin-Chu of Shanghai Pesticide Research Institute, respectively. Since 1993, the implementation of the new patent law of China has accelerated the establishment of a pesticide innovation system. Six national pesticide innovation centers were founded by the end of 2000, which include SYRICI, NKU, Hunan Research Institute of Chemical Industry et al. In addition, other institutions such as Shanghai Institute of Organic Chemistry, East China University of Science and Technology, Guizhou Universityet al. were brought into the pesticide R&D system through various collaborations channels and funding mechanisms. As of today, more than 20 innovative fungicides which were discovered and developed by Chinese institutions have been registered, with more lead candidates are at the advanced phases of development in the pipeline. These registered fungicides can be divided into four categories: (1) fungus inhibitors, such as Flumorph, fenamistrobin et al. (2) bactericides, such as Zn thiazole, and No.6 Zhongke; (3) virus inhibitors like Dufulin; and (4) microbial antibiotics such as Ascomycin, and phenazino-1-carboxylic acid. The evolving process of pesticide R&D in China could be characterized by three changes, i.e. from 'me too' to 'me better', and in turn to 'me first'. Furthermore, the funding source has been shifted from government grants to more and more enterprise investment; and the approach has also evolved from empirical to employing multidisciplinary tools and techniques. Looking ahead, China plant protection requires convergence of continuous chemical pesticide innovation and the tools of modern genetic engineering.

### O08.003 Benefit of chemical seed treatments on crop yield and quality

*C. Watrin, M. Scheller, R. Zeun and G. Olaya*
*Syngenta Seeds, Inc 11055 Wayzata Boulevard Minnetonka, MN 55305, USA*
*Email: clifford.watrin@syngenta.com*

The use of seed treatments has increased in the last 10 years and has become an integral component for the control of many diverse soil and seed-borne pathogens, nematodes and insect pests. The benefits of chemical seed treatments are well documented by their improvement of general seed health and limitation of the spread of harmful organisms. The benefit of such treatments results in improved seed germination, stand establishment, uniform growth of the crop and high yields. The application of chemical seed treatments is safe and provides consistent results across different environmental conditions, target pathogens and pests. Due to the low application rates per hectare seed treatments are an effective way of limiting exposure of the environment to chemicals. Seed treatment is the fastest growing business in the agricultural market. The increasing use of genetically modified seeds and their high cost to the

grower is driving the high demand and use of such treatments. Worldwide the use of chemical seed treatments is increasing on all crops, including corn, soybean and cereals. On a land area basis fungicides are the most broadly used chemical seed treatment compared to insecticides and nematicides. Protectant and systemic fungicides from different fungicide classes have been developed as seed treatments, following an extensive battery of seed safety and handling evaluations. Plant protection companies are innovating every day the seed treatment solutions to the farmers. Products like Avicta® nematicide and Vibrance™ fungicide to control nematodes and soil-borne fungal diseases have been introduced recently into the market.

**O08.004 Contribution of plant defense activators to rice production in Japan**

*K. Umemura*
*Agri&Vet Researh Labs, Meiji Seika Pharma Co. Ltd. 760 Morooka, Kohoku, Yokohama, 222-8567 Japan*
*Email: kenji.umemura@meiji.com*

Acquired disease resistance is known to be induced by specific chemical compounds, called plant defense activators (PDAs). Among PDAs, probenazole (3-allyloxy-1,2-benzisothiazole-1,1-dioxide) was developed as a first PDA by Meiji Seika (present Meiji Seika Pharma) Co. Ltd. in 1975. Since then, it has been used to protect rice against rice blast, the most devastating disease in Japan. Apart from probenazole, other PDAs have been recently developed. Utilization of PDAs is an important prerequisite for the expected yield in rice production. Our company launched Oryzemate® containing probenazole as an active ingredient at the beginning. When Oryzemate® granule is applied to paddy fields, probenazole is absorbed by the roots of rice, then systemically transferred to whole plants, resulting in almost controlling the leaf blast. In 90's we put on sale a new version of probenazole formulation. Dr. Oryze ® under our brand name has been applied to the culture box before transplantation of rice seedlings. The application of such agrochemicals is an established laborsaving approach to control blast disease and some insects in Japan. In 2010 First-Oryze® was launched as a formulation applicable before sowing seed in the culture box. In recent years probenazole product has been used in about 30% of paddy fields. Since probenazole has been presumed to activate an innate immune system of plants, we have mainly investigated the responses in rice plants by probenazole in order to examine the defense mechanism in the rice-blast fungus pathosystem. Here I discuss the results of the studies conducted on disease responses in rice plants.

**O08.005 Fungicide resistance in cereal pathogens**

*G. Stammler and M. Semar*
*BASF SE, Agricultural Center, 67117 Limburgerhof, Germany*
*Email: gerd.stammler@basf.com*

Various fungal pathogens in cereals have significant impact on yield and quality. In wheat, *Mycosphaerella graminicola, Blumeria graminis* f.sp. *tritici, Puccinia triticina, Oculimacula* spp., *Pyrenophora tritici-repentis* and *Fusarium* spp. are important and in barley *B. graminis* f. sp. *hordei, Rhynchosporium secalis, Pyrenophora tritici* and *Ramularia collo-cygni*. Fungicides for cereal disease control include Qo inbibitors (QoIs), sterol biosynthesis inhibitors (SBIs) and succinate-dehydrogenase inhibitors (SDHIs). QoIs were first introduced in 1996 and in countries with intensive QoI use, resistance has developed in *M. graminicola, B. graminis, P. tritici-repentis* and *R. collo-cygni*, which is conferred by mutation G143A in the cytochrome *b* gene. This mutation has so far not been detected in *P. triticina, P. teres* and only once in 2008 in *R. secalis* and these pathogens are still controlled by QoIs. SBIs have been used for more than 30 years to control cereal diseases and changes in SBI-sensitivity have been described for most target pathogens. Resistance development follows a stepwise mode with low to moderate resistance factors. Mutations in the *cyp51* gene are the main mechanism for reduced sensitivities; interestingly, in some cases, SBIs are differentially affected. Nevertheless, SBIs are still the backbone in cereal disease control programs. More recently, the new generation of SDHI fungicides has been introduced worldwide against many cereal diseases. From laboratory mutants of *M. graminicola* it is known that mutations in different SDH-subunits which make up the binding site of ubiquinone and SDHIs influence binding affinity thus reducing sensitivity. Extensive monitoring programs are running to identify sensitivity changes.

**O08.006 Research progress in fungicide resistance in China**

*Z.H. Ma, Y. Yin* and *Y. Chen*
*Institute of Biotechnology, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, P. R. China*
*Email: zhma@zju.edu.cn*

Although application of fungicide is a key component in the integrated management of many plant diseases, the appearance of resistance has become an important factor in limiting the efficacy and lifetime of fungicides developed at increasingly higher costs. In the last few years, extensive studies have been conducted in monitoring fungicide resistance, and in investigating mechanisms and management strategy of fungicide resistance in China. In this presentation, I will review some of our recent advances in i) monitoring fungicide resistance in China; ii) assessing the resistance risk for novel compounds; iii) resistance mechanisms of phytopathogenic

fungi to several major classes of fungicides including benzimidazoles, demethylation inhibitors, Qo respiration inhibitors, and dicarboximides, succinate dehydrogenase inhibitors and carboxylic acid amides at molecular levels; and iv) management strategies for fungicide resistance in China.

**O08.007 SDHI resistance in *Alternaria alternata* and complications in managing late blight of pistachio in California**

*T.J. Michailides, H.F. Avenot, D.P. Morgan and H. van den Biggelaar*
*University of California-Davis, Kearney Agricultural Research and Extension Center, Parlier, California 93648, USA*
*Email: tjmichailides@ucanr.edu*

Alternaria late blight (ALB) caused by *Alternaria alternata* is a major disease of pistachio. The disease causes premature defoliation and severe staining of the nuts lowering the value of the crop. Repeated fungicide sprays during the summer are required to control ALB. In the early 2000s strobilurin fungicides were registered and used successfully for a few seasons until strobilurin resistance developed and became widespread. In 2003, a mixture of boscalid and pyraclostrobin (Pristine) was registered and used for the first time to reduce the strobilurin resistant strains of *A. alternata.* Pristine gave highly effective control of ALB, even in orchards with QoIs resistance. However within 2 years from its registration, boscalid resistant strains of *A. alternata* were detected. Subsequently, failures in controlling the disease were reported in several orchards. A survey done 3 year later showed an increase in the incidence of SDHI resistance in *A. alternata* populations (100% resistant to boscalid strains in some orchards). PCR-RFLP and allele specific PCR assays revealed five amino acid alterations in subunits AaSdhB, AaSdhC, and AaSdhD conferring resistance in *A. alternata.* In 2012, two more SDHI fungicides (penthiopyrad and fluopyram) were registered in pistachio, with the latter used in mixture with a strobilurin or a DMI fungicide. When a large number of *A. alternata* were analyzed, boscalid-resistant isolates of *A. alternata* from pistachio showed cross resistance to penthiopyrad but lack of cross resistance to fluopyram. Double resistance to strobilurins and SDHIs and different levels of cross resistance among SDHIs in *A. alternata* populations pose major difficulties in controlling ALB of pistachio.

**O08.008 Fungicide resistance in flower, foliar, and fruit pathogens of tree fruit and nut crops in California**

*J.E. Adaskaveg and H. Forster*
*Department of Plant Pathology and Microbiology, University of California, Riverside, CA, 92521, U.S.A.*
*Email: jim.adaskaveg@ucr.edu*

Stone fruit and nut crops are major commodities in California agriculture. Numerous fungal diseases need to be managed routinely and thus, fungicide use is common. Although awareness of fungicide stewardship increased in recent years, over-use and mis-use of some fungicide classes in several host-pathogen systems has resulted in resistance that is associated with crop losses in most cases. MBC (FRAC 1) resistance developed in the 1970s and usage of this class has since declined with widespread resistance in *Monilinia* species causing of brown rot of stone fruits and in *Fusicladium carpophilum*, a major pathogen of almond. Resistance is also widespread in *Alternaria* spp. from almond and pistachio against QoIs (FRAC 11; first detected in 2003/04) and several of the SDHI (FRAC 7) sub-groups (first detected in 2007), as well as in *F. carpophilum* against QoIs (first detected in 2003). Alternative fungicide classes and management programs are developed to control these diseases. Anilinopyrimidine (FRAC 9) resistance was found locally in *M. fructicola* and *M. laxa* in several prune and almond orchards since 2007. Resistance can also occur naturally (in absence of selection pressure) in a pathogen. For example, at many locations isolates of *F. carpophilum* with reduced sensitivity to DMI (FRAC 3) or SDHI fungicides were present before their commercial use. Currently, newly registered DMIs and SDHIs still effectively manage the disease. The limited number of fungicide classes available for managing particular diseases mandates resistance management strategies to prevent further spread and increase of resistance in these valuable food crops.

**O08.009 Solatenol™, the second generation benzonorbornene SDHI carboxamide with outstanding performance against cereal diseases**

*E. Guicherit[1], A.J. Leadbeater[1], S.M. Dale[1], J.U. Haas[2] and H. Walter[2]*
*[1]Syngenta, Schwarzwaldallee 215, CH-4002, Basel, Switzerland; [2]Syngenta, Schaffhauserstrasse, CH-4332, Stein, Switzerland*
*Email: eric.guicherit@syngenta.com*

Solatenol™ is a new broad spectrum foliar fungicide discovered and developed by Syngenta. It is the third, Syngenta succinate dehydrogenese inhibitor (SDHI) carboxamide and the second belonging to the benzonorbornene amide subclass. Solatenol™ has very high intrinsic activity on key pathogens at remarkably low rates. Solatenol™ is classified as a pyrazole carboxamide (FRAC C2). The very high affinity to succinate dehydrogenese results in its high intrinsic activity. This, together with strong binding to the plant's wax layer, from where it slowly penetrates into the tissue, results in the long lasting disease control. In research, the focus of the Solatenol™ project was in finding compounds with high intrinsic activity on soybean and cereal diseases, in particular rusts and Septoria leaf blotch (*Septoria tritici*).

In addition to the outstanding activity against these targets, Solatenol™ is also highly active against several other major pathogens, with rates between 30 and 75g/ha providing excellent control of soybean rust (*Phakopsora pachyrhizi*), apple scab (*Venturia inaequalis*), *Sclerotium* and *Rhizoctonia* and other economically important targets. On rusts Solatenol provides long lasting control, even when applied under high disease pressure conditions. Solatenol™ is highly active against *Septoria tritici* at rates as low as 75g/ha. On apple scab Solatenol™ sets new standards with 3.5g/hl giving outstanding control. Solatenol™ has early curative effect on fungal development but the compound should be applied preventatively in order to ensure best disease control and longest lasting effect. Solatenol™ is safe to the crop when applied alone and when mixed with DMI and QoI compounds.

### O08.010 Genus-specific *CYP51C* of *Fusarium graminearum* identified as a novel CYP51 rather than azole fungicide target sterol 14α-demethylases

*J. Fan, M. Urban, J.E. Parker, H.C. Brewer, S.L. Kelly, K.E. Hammond-Kosack, B.A. Fraaije, X. Liu and H.J. Cools*
*Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: fanjieru1981@gmail.com*

The recent expansion of publically available fungal genome sequence data has revealed many ascomycete fungi carry more than one copy of the cytochrome P450 sterol 14α- demethylase gene (*CYP51*, syn. *ERG11*), encoding the target for azole fungicides. In several *Fusarium* species, including pathogens of both humans and plants, three paralogous *CYP51* genes (*FgCYP51A*, *FgCYP51B*, *FgCYP51C*) have been identified. *FgCYP51C* is unique to the genus *Fusarium*. Currently, the functions of these three genes and the rationale for their conservation within the *Fusaria*, is unknown. In this study, by expression in *Saccharomyces cerevisiae*, we demonstrate that both *FgCYP51A* and *FgCYP51B* can complement yeast *CYP51* function, whereas *FgCYP51C* cannot. By generating both single (*ΔFgCYP51A, ΔFgCYP51B*, and *ΔFgCYP51C*) and double (*ΔFgCYP51AC* and *ΔFgCYP51BC*) *CYP51* gene deletion mutants we demonstrate the intrinsically low sensitivity of *F. graminearum* to some azole fungicides is conferred by the *FgCYP51A* paralogue. We show ascospores formation is blocked in the *ΔFgCYP51B* and *ΔFgCYP51BC* mutants, even though superficially normal perithecia develop. Specific accumulation of eburicol, a CYP51 substrate in filamentous fungi, and two 14-methylated sterols occurred in the *ΔFgCYP51B* and *ΔFgCYP51BC* mutants, which suggests *FgCYP51B* is the primary sterol 14α-demethylase. Various bioassays revealed the *ΔFgCYP51C* mutants, unlike *ΔFgCYP51AC* and *ΔFgCYP51BC* mutants, are phenotypically indistinguishable from wild-type *in vitro* and during *Arabidopsis* infection. However, on host wheat ears, *FgCYP51C* is required for full virulence and DON production. This is the first example of a fungal *CYP51* gene with a function supplementary to primary sterol biosynthesis.

### O08.011 Chemical control of white mold (*Sclerotinia sclerotiorum*) on soybean in Brazil

*M.C. Meyer, H.D. Campos, J. Nunes Junior, W.S. Venancio and C.V. Godoy*
*Brazilian Agricultural Research Corporation, Embrapa Soybean, 75375-000, Santo Antonio de Goias, GO, Brazil*
*Email: mauricio.meyer@embrapa.br*

Brazil produces almost 24% of the soybean in the world. White mold caused by *Sclerotinia sclerotiorum* is one of the most important soybean diseases in Brazil, causing losses up to 70% and affecting about 5 million hectars in the country. To control the disease efficiently in infested fields is necessary to adopt some integrated measures, like mulching with grass crops, biological and chemical control, seed treatment with fungicides, crop rotation with non hosts, choosing cultivars with scarce canopy and short flowering period, avoiding high plant populations and cleaning machinery and vehicles. A network of trials for evaluating fungicides efficiency by foliar sprays has been carried out since 2008 in several Brazilian regions. Crboxamides, benzimidazoles, phenylpyridinamine, dicarboximides and mixture of carboxamide with strobilurine were tested. The fungicides with highest levels of control were fluazinam and procymidone sprayed alone or in combination with carbendazim or thiophanate-methyl, ranging from two to four sprayings at intervals of 10 days, starting at the R1 stage of plant development (early flowering). Fluopyram and boscalid+dimoxystrobin were as efficient as fluazinam and procymidone but are still under registration for use in Brazil.

### O08.012 Oxathiapiprolin: The first member of a novel class of Oomycete fungicides

*J.A. Bruhn, T.H. Carski, J.L. Genet, L.E. Hoffman, B. Perotin, C. P. Shepherd and J.A. Sweigard*
*DuPont Crop Protection, Stine-Haskell Research Center S200/122C, 1090 Elkton Rd., P.O. Box 30, Newark, Delaware, 19714-0030, USA*
*Email: Jack.A.Bruhn@Dupont.com*

Oxathiapiprolin (ISO) is a novel fungicide recently discovered by DuPont and is the first member of a new class of isoxazoline fungicides. It acts at a unique site of action in Oomycete pathogens. High intrinsic efficacy, an effect on virtually all stages of pathogen development plus trans-laminar and acropetal systemic movement in plants allow oxathiapiprolin to provide robust and reliable disease control even under the most severe conditions.

Product development is focused on crops where Oomycete pathogens limit agricultural productivity and profitability including potatoes, grapes, vegetables and other specialty crops. At use rates 5-100X lower than current commercial fungicides, oxathiapiprolin is highly effective for the control of important Oomycete pathogens, such as; *Phytophthora infestans*, *P. capsici*, *Plasmopora viticola* and *Psuedoperonospora cubensis*. Its new mode of action makes oxathiapiprolin a valuable option for fungicide resistance management strategies, while safety to key beneficials confer a strong fit within IPM programs. The remarkably favorable toxicity profile of oxathiapiprolin, combined with low use rates, provides large margins of safety for consumers, agricultural workers and the environment.

**O08.013 Metabolomics: a novel bioanalytical tool in pesticide research and development**

*K.A. Aliferis and S. Jabaji*
*McGill University, Department of Plant Science, 21111 Lakeshore Rd., Sainte-Anne-de-Bellevue, QC H9X 3V9, Canada*
*Email: konstantinos.aliferis@mcgill.ca; suha.jabaji@mcgill.ca*

The increasing food demand and concerns over consumer and environmental health, the development of pest and pathogen resistance to pesticides, and climate change, represent major challenges to the agrochemical industry. The development of metabolomics protocols could serve as a valuable bioanalytical tool for pesticide research and development (R&D), and largely contribute to overcoming the abovementioned challenges. Here, we describe metabolomics protocols for the study and discovery of the mode(s)-of-action (MoA) of bioactive compounds, their toxicological and eco-toxicological assessment, the study of unintended effects of genetic modified organisms (GMOs), and the development of new or alternative crop protection agents. The discovery of the MoA of natural or synthetic compounds can be accelerated by the construction of metabolomics models, which is of great importance for the discovery of crop protection agents with improved characteristics compared to the already existing ones. The development of powerful analyzers and bioinformatics software within the context of metabolomics have facilitated the comprehensive monitoring of metabolomes and their fluctuations in response to biotic and abiotic stimuli, which is significant for studies related to the assessment of unintended effects of GMOs, or *in vivo* studies on the mechanisms of metabolism of bioactive molecules. Furthermore, the dissection of host-pathogen pathosystems applying metabolomics could provide insights on the regulation of such interactions, which in turn could lead to new crop protection agents. Although the application of metabolomics in pesticide R&D is still in its infancy, it holds the premise of becoming one of its key components in the near future.

**O08.014 Multiple fungicide resistance, population dynamics, and genetic diversity of *Botrytis* spp.**

*C. Plesken[1], M. Leroch[1], F. Kauff[1], G. Scalliet[2] and M. Hahn[1]*
*[1]Department of Biology, University of Kaiserslautern, P.O. Box 3049, 67653 Kaiserslautern, Germany; [2]Syngenta Crop Protection Münchwilen AG, Schaffhauserstr. 215, 4332 Stein, Switzerland*
*Email: hahn@biologie.uni-kl.de*

Repeated fungicide treatments are common to protect fruit and vegetable crops against grey mould. *Botrytis* populations in German strawberry fields show high resistance frequencies against all botryticides. A novel, stronger efflux-mediated multidrug resistance phenotype, called MDR1h was discovered, caused by a small deletion in a drug efflux regulator. MDR1h strains often contained multiple target resistance mutations and seriously compromised fungicide control efficiency. Population studies indicated that all MDR1h isolates are derived from a single founder cell, and that they rapidly invade fungicide treated fields. Multiple gene sequencing and whole genome sequencing revealed a surprising genetic diversity of *Botrytis* strains on strawberries. All MDR1h strains belong to a novel genetic clade, called *Botrytis* group Sa, closely related to *B. cinerea sensu stricto* and *B. fabae*. PCR markers were developed for rapid identification of the main grey mould species infecting dicots (*B. cinerea*, *B. fabae*, *B. pseudocinerea*, *B. calthae*), and three newly discovered clades. Different patterns of occurrence of each *Botrytis* species or clade were found, depending on host plant, fungicide treatments, time of season, and geographic region. While *B. cinerea* is common on many hosts, *Botrytis* group Sa strains are largely absent from vineyards, but found worldwide in strawberry fields and selected by fungicide treatments. In conclusion, grey mould populations are mixtures of of genetically diverse genotypes which undergo dynamic changes in the field. These results have practical implications and will be used for a better prediction of fungicide resistance frequencies and the development of anti-resistance management strategies against *Botrytis.*

**O08.015 Detection of copper and antibiotic resistant bacteria from pome and stonefruit blossoms**

*S.C. Gouk[1], E. Mace[1] and L. Byrne[2]*
*[1]Department of Primary Industries, Biosciences Research Division, Bundoora, Victoria 3083, Australia. [2]Department of Primary Industries, Future Farming Systems, Tatura, Victoria 3616, Australia*
*Email: chin.gouk@dpi.vic.gov.au*

Long term use of copper fungicides and antibiotics in

orchards has been known to exert selection pressure for development of resistant microflora. Although antibiotics are not used in Australian orchards, genetic linkage of copper and antibiotic resistance may enhance co-selection of antibiotic resistant bacteria in an orchard ecosystem. More than a thousand bacterial isolates from pome and stonefruit blossoms collected from orchards from five eastern states of Australia were tested for resistance to copper and antibiotics. Copper resistance was screened using casitone-yeast-extract-agar impregnated with copper sulphate concentrations ranging from 0 to 360 ppm. Sensitivity of bacteria to twelve medically important antibiotics was tested using disc-diffusion assays. Copper and antibiotic resistant *Pseudomonas* spp. were detected for the first time from apple, pear and cherry orchards. The highest frequency of resistance was against ampicilin and penicillin, of the chemical group Penicillin; and also to erythromycin, rifampicin and vancomycin. Low frequency of resistance was against gentamycin, kanamycin, and chloramphenicol, the former two are of the chemical group, aminoglycosides. *P. syringae* isolates showed moderate frequency of resistance to streptomycin and low frequency of resistance to tetracycline. Whilst both antibiotics are used in the USA, streptomycin is only permitted for emergency use should there be an incursion of the exotic bacterial disease, fire blight. The findings indicate risks of copper and antibiotic resistance exist and more in depth understanding of the mechanism of resistance would be beneficial.

### O08.016 Resistance to multiple fungicides including fludioxonil and mechanisms of resistance in *Botrytis cinerea* from Blackberry in the Southeastern United States

*X.P. Li, D. Fernandez-Ortuno, A. Grabke, S.N. Chen and G. Schnabel*
*School of Agricultural, Forest & Environmental Sciences, Clemson University, Clemson, South Carolina, 29634, USA*
*Email: xingpel@clemson.edu*

*Botrytis cinerea* causes blossom blight and fruit rot on many crops, including blackberry. Between 2010 and 2011, 202 fruit from blackberry fields were collected from North and South Carolina and single-spore colonies were obtained. Isolates were subjected to cyprodinil, fenhexamid, fludioxonil, pyraclostrobin, boscalid, thiophanate-methyl and iprodione sensitivity evaluations using germination assays and several different fungicide resistance phenotypes were discovered. The resistance mechanisms for fenhexamid, pyraclostrobin, boscalid and thiophanate-methyl were examined. Of 22 isolates resistant to fenhexamid, 18 had the F412S mutation and 4 had the F412I mutation in the Erg27 gene. All of the 93 isolates resistant to pyraclostrobin had the G143A mutation in *SdhB* gene. Boscalid resistant isolates had the H272Y or the H272R mutation in the *CYTB* gene. Among 69.8% of the isolates resistant to thiophanate-methyl, 87.5% contain the E198A mutation and the rest contained the E198V mutation. For three fludioxonil resistant isolates, detached fruit assays confirmed its pathogenicity after fludioxonil treatment and isolates were found without fitness penalty. Isolates resistant to fludioxonil were sensitive to NaCl and contained a 5bp deletion in the intron of the *BOS2* gene that was not present in sensitive isolates. Our study shows that resistance to multiple fungicides is widespread in *B. cinerea* populations from blackberry.

### P08.001 Molecular characterization and management of metalaxyl resistant populations of *Phytophthora infestans* in India

*T.S. Thind, R. Kaur, S. Goswami and A. Bala*
*Department of Plant Pathology, Punjab Agricultural University, Ludhiana, 141004, India*
*Email: thind_ts@yahoo.co.in*

Management of late blight disease of potato largely depends on the use of fungicides as host resistance to *Phytophthora infestans* is highly unstable. Metalaxyl-based formulations are commonly used by the farmers to get post-infection control of the disease under congenial weather conditions. Monitoring of sensitivity levels of *P. infestans* populations from various locations in Punjab State revealed the prevalence of metalaxyl resistance to varying extent. Characterization of resistant strains based on pathological and molecular parameters was carried out. Among 48 sporangial populations of *P. infestans* studied, 8 populations showed resistant response at 100 µg/ml of metalaxyl, while 2 populations showed highly resistant response and could infect potato leaves (cv. Kufri Chandramukhi) treated with 200 µg/ml of metalaxyl with resistance factor of 60. Resistant strains were found to be highly pathogenic and exhibited competitive fitness in mixture with sensitive strains. There was no cross resistance to novel action fungicides such as mandipropamid, cymoxanil, benalaxyl, previcur, fluopicolide, azoxystrobin and multisite contact fungicides. Molecular markers were identified for resistant strains using RAPD primers and cluster analysis. Metalaxyl resistance was effectively managed under field conditions through application of novel action fungicides such as Infinito 68.75 SC (fluopicolide+ propamocarrb chloride), Amistar 25 SC (azoxystrobin), Mandipropamid 250 SC, Acrobat 50 WP (dimethomorph) and Curzate M-8 72WP (cymoxanil + mancozeb). Development of metalaxyl resistance in *P. infestans* can be retarded by using combination of fungicides with different modes of action so as to ensure sustainable management of late blight.

### P08.002 Screening of chillies germplasm against anthracnose and its managemen

*I.U. Haq, A. Siddique, S.A. Khan, H. Abbas and M. Kamran*
*Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan*
*Email: imran_1614@yahoo.com*

The present investigation was carried out to screen the varieties of chillies obtained from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan against anthracnose disease in order to find out the resistant source by two methods of inoculations viz. pin prick and spray. In vitro studies were conducted to evaluate the efficacy of different fungicides; Dithane M-45, Alliet, Carbendazim, Acrobit and Antracolagainst *C. capsici* at different concentrations after three time intervals. The results indicated that there was no immune and resistant variety. Four varieties: Sanam, C-19, 5-2010, 28-2010 were moderately resistant, GolaPeshawari, C-33, C-68, 15-2010, 18-2010 were highly susceptible and Tata Puri, C-72, C-73, C-302, 11-2010, 27-2010 were susceptible. All fungicides exhibited differential effects in inhibiting the mycelial growth of *C. capsici.* Among these fungicides Carbendazim at 0.3g/ml and 0.2 g/ml respectively was the most effective in inhibiting the mycelial growth after 4, 8 and 12 days followed by Acrobit, Dithan M-45 andAntracol. The least effective fungicide was Alliet. The results of this study will serve in finding the resistance source against *C. capsici*and to select the most effective means for the management of anthracnose disease.

**P08.003 Strategies to reduce primary *Phytophthora* infections in conventional and organic potato production**

*J. Nechwatal and M. Zellner*
*Bavarian State Research Centre for Agriculture, Lange Point 10, 85354 Freising, Germany*
*Email: michael.zellner@lfl.bayern.de*

Potato late blight epidemics, caused by *Phytophthora infestans*, will usually start from infested seed potatoes introduced into the fields. Recent PCR analyses have shown that on average 10% of the seed potatoes are latently infected with *P. infestans*. In the field, as soon as soils are sufficiently moist, primary stem infections will develop from these and serve as starting points for subsequent secondary infections after the pathogen has grown up the stem. Our tests have shown that such primary infections can only be effectively controlled by the application of systemic fungicides (such as Metalaxyl-M, Benalaxyl-M, Propamocarb) approximately one week ahead of the first stem blight symptoms, as these reach into the plant, inhibiting the growth of the pathogen. An alternative control measure is the use of seed dressings that can also considerably reduce primary infection rates in the field. In our tests, copper hydroxide and cymoxanil seed dressings had a significant effect against *P. infestans* primary stem infections when applied with planting in spring. In organic farming, systemic fungicides for the control of stem blight are not available. Therefore, seed dressings with $Cu(OH)_2$ and other registered preparations are also tested as a measure to reduce primary stem infections. Besides, alternative products for foliar applications in organic potato farming are tested as part of a management strategy to reduce the extent of secondary leaf infections, and to minimise the deposition of sporangial inoculum on the soil and on the crop. Several abscised potato leaf assays have revealed promising candidate substances.

**P08.004 Sensitivity of *Corynespora cassiicola* isolates from Brazil to fungicides**

*A. Teramoto[1], L.M. Santos[1], T.A. Machado[1], M.C. Meyer[2] and M.G. Cunha[1]*
*[1]Phytosanitary Section, School of Agronomy, Federal University of Goiás, P.O.Box 131, 74001-970, Goiânia, Goiás State, Brazil; [2]Embrapa Soybean, Santo Antônio de Goiás, GO*
*Email:adritera@terra.com.br*

The fungus *Corynespora cassiicola* is the causal agent of the soybean target spot and frequently occurs in the Brazilian Mid-West region. Under favorable climate conditions it can cause serious damage in this crop. In Brazil, the use of resistant cultivars and fungicides are recommended, although only few cultivars and foliar fungicides are available. This investigation was carried out to evaluate *in vitro* sensibility of thirty four isolates of *C. cassiicola* from several Brazilian states to the fungicides: boscalid, carbendazim, cyproconazole, fluopyram, fluxapyroxad, prothioconazole and thiofanate methyl, used in concentrations 0; 0,16; 0,8; 4; 20 and 100 $\mu g\ mL^{-1}$ of active ingredient, respectively. Fluxapyroxad and fluopyram showed higher levels of mycelial growth inhibition and lower values of $ED_{50}$. Thiophanate methyl and carbendazim showed no mycelial growth inhibition of most isolates of the fungus, while boscalid and cyproconazole presented an intermediate level of inhibition to most fungi isolates tested.

**P08.005 European legislation on pesticides: challenges for soilborne disease management**

*P. Colla[1], G. Gilardi[1], M.L. Gullino[1,2], E.C. Tjamos[3], M. Bradascio[4], C. Slusarski[5], J. Ciesielska[6] and A. Garibaldi[1]*
*[1]Centre for the Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Torino, 10095 Grugliasco, TO, Italy; [2]Department of Agricultural, Forest and Food Sciences and Technologies DISAFA, University of Torino, 10095 Grugliasco, TO, Italy; [3]Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens, Iera Odos 75, 118 55, Athens, Greece; [4]Dow AgroSciences Italia srl, Bologna, Italy; [5]Research Institute of Horticulture,*

*96-100 Skierniewice, Poland; [6]JWC Projects, Warsaw - Poland*
*Email: paola.colla@unito.it*

After the phasing out of methyl bromide, the containment of soilborne pathogens in horticultural sector has been enabled by the availability of alternatives practices, including the use of alternative fumigants such as 1,3-dichloropropene, methyl isothiocyanate generators (metam sodium/potassium and dazomet), chloropicrin, methyl iodide and dimethyl disulfide. However, stringent environmental regulations largely affect the continued availability of these products. In Europe the number of chemicals instruments available for plant protection did dramatically reduce after the re-evaluation of pesticides under the European Dir. 91/414/EEC on Plant Protection Products. With the completion of the review programme in 2009 it has become clear that soil fumigants have a very high risk profile given to their intrinsic characteristics such as toxicity and high application rates. The Dir. 91/414 was replaced in 2011 by the Reg 1107/2009 that introduced the evaluation of crop protection products through more stringent hazard-based cut-off criteria. To date only metam sodium/potassium and dazomet may be authorized limited to one application every third year on the same field. Chloropicrin will be withdrawn by June 2013 while 1,3-dichloropropene authorisation was withdrawn in 2009, although several Member States granted periods of 120-day authorizations for emergency uses. In the meanwhile the directive 2009/128/EC on sustainable use of pesticides requires the adoption of general principles of IPM by 2014. Within the LIFE+ Project "Sustainable Use of Fumigants" researchers, fumigant registrants and growers committed to finding practical solutions that simultaneously achieve efficacy to control soil pests and to comply with stringent regulations.

### P08.006 Correlation between triazole sensitivity and mutations in the CYP51 gene of *Mycosphaerella graminicola* in Flanders

*K. Curvers, B. Pycke, G. Haesaert and G. Gheysen*
*Department of Molecular Biotechnology, University of Ghent, Coupure Links 653, 9000 Ghent, Belgium*
*Email: katrien.curvers@ugent.be*

*Mycosphaerella graminicola* (anamorph *Septoria tritici*) is a fungal pathogen causing leaf or *Septoria tritici* blotch (STB) in wheat. Worldwide, STB is one of the most important wheat diseases, with yield losses up to 50%. No fully STB-resistant wheat cultivars are available and due to resistance to multiple fungicides, this economically important plant pathogen is difficult to control. Today, most of the fungicides still effective and used against *M. graminicola* belong to the group of the sterol 14á-demethylation inhibitors of which triazole derivatives are most commonly used. The use of azole fungicides is however jeopardized by the emergence of resistance, since a constant shift towards reduced sensitivity of *M. graminicola* has been observed in Europe. This shift is caused mainly by mutations (single nucleotide polymorphisms or SNPs) in the CYP51 gene encoding the demethylase target protein for these fungicides, whereby some specific mutations could be linked to the use of certain triazoles. Here, we present the CYP51 haplotype diversity of the *M. graminicola* population in Flanders, Belgium. Monitoring over a period of three years allowed us to get an idea of the spatio-temporal distribution of the *M. graminicola* haplotypes in this region. We also present the use of the High Resolution Melting (HRM) technology to identify the different CYP51 SNP mutants in field isolates, and show that HRM is a useful and promising technology for the screening and identification of mutations in single-spore isolates of plant pathogens.

### P08.007 Mechanism by which azoxystrobin protects sugar beet from *Rhizoctonia solani*

*M.F.R. Khan[1] and A. Noor[2]*
*[1]Department of Plant Pathology, North Dakota state University, Fargo, North Dakota, 58108, and University of Minnesota; [2]Department of Plant Pathology, North Dakota State University, Fargo, North Dakota, 58108, United States*
*Email: Mohamed.Khan@ndsu.edu*

Rhizoctonia crown and root rot caused by *Rhizoctonia solani* Kühn is the most common and serious root disease of sugar beet in the USA. Timely application of azoxystrobin provides effective disease control but the mechanism of control is unknown. The objective of this study was to determine how azoxystrobin provides control to sugar beet from *R. solani*. Research was conducted in the greenhouse at a temperature of 20 ± 2˚C. Sugar beet susceptible to *R. solani* were planted and maintained to the four leaf stage. Azoxystrobin was used at 167 g a.i. $ha^{-1}$ either in an 18-cm band or used as a root dip. Artificial inoculation was done using barley grain colonized with *R. solani* (2 infected grain / plant or pot). Treatments included the use of 4-leaf stage beet and dipping the roots in azoxystrobin followed by transplanting into fresh potted soil and inoculation; applying azoxystrobin in an 18-cm band to an inoculated soil into which was planted 4-leaf stage beet either 3 or 7 days later. Controls included inoculated and non-inoculated 4-leaf stage plants. Results showed that azoxystrobin provided control against *R. solani* when used as a root dip to cover the entire root and when band applied to the inoculated soil before planting. The research suggested that azoxystrobin provided control by probably killing the fungus or prevented germination, growth and infection by the fungus.

**P08.008 Controlling *Rhizoctonia solani* in sugar beet through fungicide application**

Y. Liu[1] and M.F.R. Khan[2]
[1]Department of Plant Pathology, North Dakota State University, Fargo, North Dakota, 58108, United States; [2]Department of Plant Pathology, North Dakota state University, Fargo, North Dakota, 58108, and University of Minnesota
Email: yangxi.liu@ndsu.edu

*Rhizoctonia solani* is a soilborne fungus that causes damping-off, crown and root rot of sugar beet (*Beta vulgaris* L.), and is a major limiting factor in sugar beet production affecting economic returns in 24% of the sugar beet producing acres in the United States. The objective of this research was to evaluate penthiopyrad for its efficacy at controlling *R. solani* under greenhouse conditions. Penthiopyrad, a systemic fungicide, employs the succinate dehydrogenase inhibition mode of action to suppress pathogens. Penthiopyrad was evaluated as an in-furrow application at planting (210, 280, 420 and 555 g a.i. $ha^{-1}$), followed by inoculation with *R. solani* AG 2-2 IIIB grown on barley. Penthiopyrad at all rates provided significantly better control than the inoculated check, especially the rates between 280 and 555 g a.i. $ha^{-1}$ were not significantly different than the non-inoculated check (P =0.95). Penthiopyrad was also evaluated as a soil drench where it was directly added to the soil-hypocotyl interface of four-leaf stage sugar beet plants at 210, 280, 420 and 555 g a.i. $ha^{-1}$, followed by inoculation with *R. solani*. All rates of penthiopyrad used as a drench provided effective control similar to the non-inoculated check. This research demonstrated that penthiopyrad provides effective control of *R. solani* when in direct contact with the pathogen before infections take place.

**P08.009 Effect of picoxystrobin and penthiopyrad on controlling *Rhizoctonia solani* in sugar beet**

Y. Liu[1] and M.F.R. Khan[2]
[1]Department of Plant Pathology, North Dakota State University, Fargo, North Dakota, 58108, United States; [2]Department of Plant Pathology, North Dakota state University, Fargo, North Dakota, 58108, and University of Minnesota
Email: yangxi.liu@ndsu.edu

*Rhizoctonia solani*, a soilborne fungus that causes damping-off, crown and root rot of sugar beet (*Beta vulgaris* L.), is the most serious production problem for growers in North Dakota and Minnesota who produce about 60% of the United States sugar beet. The objective of this research was to evaluate the efficacy of picoxystrobin and penthiopyrad alone and in mixtures to control *R. solani* on sugar beet in greenhouse conditions. Picoxystrobin is a systemic fungicide and a quinone outside inhibitor and penthiopyrad is a broad spectrum fungicide which belongs to the succinate dehydrogenase inhibitor group. Picoxystrobin was used alone at 565 g a.i. $ha^{-1}$ and penthiopyrad was used alone at 555 g a.i. $ha^{-1}$, and in picoxystrobin:penthiopyrad mixtures of 273:280; 419:410; 565:555 g a.i. $ha^{-1}$. Fungicides were applied in-furrow at planting, followed by inoculation with *R. solani* AG 2-2 IIIB grown on barley. In this trial, fungicides used alone and in mixtures provided effective control of *R. solani*, which had significantly more survivors than the inoculated check. This trial demonstrated that picoxystrobin and penthiopyrad have the potential to be used as an in-furrow application for providing control of *R. solani* of sugar beet.

**P08.010 Differential patterns of cross-resistance to SDHI fungicides in Japanese isolates of *Botrytis cinerea***

H. Ishii[1], H. Suzuki[2] and M. Kakishima[3]
[1]National Institute for Agro-Environmental Sciences; [2]Mie PrefectureAgricultural Research Institute; [3]University of Tsukuba
Email: hideo@affrc.go.jp

In Japan, succinate dehydrogenase inhibitor (SDHI) fungicides boscalid and penthiopyrad have been registered for grey mould disease control on strawberry. Soon after the introduction of boscalid, isolates of *Botrytis cinerea* resistant to this fungicide have been detected although field performance of fungicide seemed to be maintained (Suzuki et al., 2010). Although basic activity of penthiopyrad is superior to boscalid, cross-resistance between boscalid and penthiopyrad was confirmed in conidial germination tests carried out on agar plates as well as bioassays using potted cucumber cotyledons inoculated with the fungus. Interestingly, boscalid-resistant isolates carrying H272R mutation in *sdhB* gene encoding a subunit of succinate dehydrogenase (SDH) proteins remained sensitive to a novel SDHI fungicide fluopyram. This tendency was similar with our previous finding that high level of boscalid resistance caused by H278Y/R or H229Y mutations of *sdhB* gene in *Corynespora cassiicola* and *Podosphaera xanthii*, respectively, was counteracted with fluopyram (Ishii et al., 2011).

**P08.011 The optimization of the protection of winter wheat to Fusarium head blight in Poland**

J. Kaczmarek[1], D. Popiel[1], A. Dawidziuk[1], A. Brachaczek[2], G. Koczyk[1] and M. Jedryczka[1]
[1]Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland; [2]DuPont Poland Ltd., Postepu 17b, 02-676 Warsaw, Poland
Email: jkac@igr.poznan.pl

The yield of wheat is on average the half of its genetic potential, what is partially caused by plant pathogenic

fungi such as *Fusarium* spp. The aim of this work was to optimize fungicide protection of winter wheat against Fusarium head blight (FHB) based on the presence and quantity of *Fusarium* spores in air samples and on analysis of the potential risk of resistance of those fungi to different fungicides. The experiments were done using Polish winter wheat cultivar Bogatka, susceptible to FHB. From 1 May to 30 June 2011 and 2012 the concentration of *Fusarium* spp. spores in air samples was analysed. Field observations comprised incidence of FHB and the yield of grain. Seed quality was evaluated based on mass of 1000 kernels, the uniformity of kernels as well as density and percent of protein, wet gluten and starch content. Isolates obtained from each fungicide programme were tested to their resistance to different chemical groups of active substances fungistatic to *Fusarium* spp. It was found that the concentration of spores fluctuated in the season and was very high at wheat flowering time.The use of protective treatments significantly decreased FHB. The increase of seed yield after the fungicide treatment depended on the protective technology and experiment site. The use of fungicides positively affected the bulk densities of grains, as well as their uniformity. According to these data producers can find optimal technologies and treatment times to provide the sufficient quality and quantity of yield of winter wheat.

**P08.012 Effect of phosphite fertilizer on mycelial growth, sporangium formation and zoospore cyst germination of *Phytophthora palmivora***

*N. Miyake and K. Kageyama*
*United Graduate School of Agriculture Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
*Email:r6102009@edu.gifu-u.ac.jp*

A group of phosphorous acid compounds has been shown to suppress Phytophthora diseases in various crops. The objective of this study was to compare *in vitro* activity of phosphite fertilizer on mycelial growth, sporangium formation, and zoospore cyst germination of *Phytophthora palmivora*. Azoxystrobin, cyazofamid, cupric hydroxide, cupric sulfate basic, and 2,4,5,6-tetrachloroisophthalonitrile (TPN), which have been registered as fungicides to control of white powdery rot of fig caused by *P. palmivora* in Japan, were used as control chemicals. The 50% effective concentration ($EC_{50}$) values in inhibition of mycelial growth were low in phosphite fertilizer, 2.28-20.89 µg/ml smilar to cupric hydroxide, cupric sulfate basic, and TPN, ranging from 0.78 to 6.73 µg/ml. The $EC_{50}$ values in inhibition of sporangium formation were low, <12.47 µg/ml, in all chemicals. On the other hand, phosphate fertilizer was not effective on germination of encysted with the $EC_{50}$ values being 326.23-438.45 µg/ml. The results suggest that phosphite fertilizer will affect two stages, mycelial growth and sporulation of the life cycle of *P. palmivora* and will potentially provide disease control.

**P08.013 *In vitro* sensitivity tests of *Leptosphaeria maculans* field isolates from Canadian Prairies to Quinone outside inhibitors (QoI) and Demethylation inhibitors (DMI) fungicides**

*C. Liu, W.G. Dilantha Fernando, Y.T. Gan, G. Peng* and *H.R. Kutcher*
*Department of Plant Science, 222 Agriculture Building, 66 Dafoe Road, University of Manitoba, Winnipeg, MB R3T 2N2, Canada*
*Email: D_Fernando@umanitoba.ca*

Blackleg, caused by the fungal pathogen *Leptosphaeria maculans* is the most economically important disease of *Brassica* species in Western Canada. Quinone outside inhibitors (QoI) and Demethylation inhibitors (DMI) are two classes of fungicides registered to control blackleg disease on canola plants. Both of these chemical classes are site-specific and are thus susceptible to pathogen mutations at their functional loci. During 2011-2012, a total of 135 *L. maculans* isolates collected from fungicide treated canola fields in Western Canada were characterized based on mycelial growth to determine *in vitro* sensitivity to QoI fungicides azoxystrobin and pyraclostrobin, and to DMI fungicide propiconazole. The baseline $EC_{50}$ values of 79 *L. maculans* isolates for azoxystrobin ranged from 0.084 to 1.894µg/ml (mean – 0.525), and the $EC_{50}$ value for pyraclostrobin ranged from 0.046 to 0.463µg/ml (mean – 0.197). The $EC_{50}$ values for propiconazole ranged from 0.329 to 1.611 µg/ml (mean – 0.706). The mean $EC_{50}$ values of 56 *L. maculans* isolates collected from canola fields with two-year fungicide spray history were 0.695, 1.924, and 0.438µg/ml for azoxystrobin, pyraclostrobin, and propiconazole, respectively. Overall, there was no significant difference ($p$=0.05) in fungicide sensitivity to azoxystrobin and pyraclostrobin among isolates collected between 2011 and 2012. However, the $EC_{50}$ value for pyraclostrobin fungicide increased four to fivefold in 4 isolates collected in 2012. *L. maculans* fungicide sensitivity will be monitored during the next two years and molecular identification will be conducted on pyraclostrobin-resistant isolates to detect mutations in the cytochrome *b* gene which may explain the increased resistance observed in some isolates.

**P08.014 *CYP51* paralogues and azole sensitivity in *Rhynchosporium commune***

*N.J. Hawkins, H.J. Cools and B.A. Fraaije*
*Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom*
*Email: nichola.hawkins@rothamsted.ac.uk*

*Rhynchosporium commune* is an ascomycete pathogen causing barley leaf blotch or scald, resulting in yield

losses of up to 40%. Azole fungicides are an important component of control programmes, but sensitivity shifts have been reported, reducing the effectiveness of some older azoles. Azole sensitivity shifts reported in other fungal species are due to mutations or overexpression of the target-site-encoding gene *CYP51*, enhanced efflux of the fungicide or altered sterol metabolism. Genome sequencing has shown that some filamentous ascomycetes have a second *CYP51* paralogue, *CYP51A*, as well as the *CYP51B* paralogue present in all sequenced filamentous ascomycetes. In *Aspergillus fumigatus* and *Fusarium graminearum*, presence of *CYP51A* reduces intrinsic azole sensitivity, with mutations or over-expression of *CYP51A* causing further reductions in azole sensitivity in *A. fumigatus*. In *R. commune*, *CYP51A* is only present in some isolates, and a reduction in azole sensitivity is linked to an increase in the frequency of *CYP51A* in the *R. commune* population. The role of *CYP51A* in azole sensitivity in *R. commune* has been further investigated by gene expression analysis, heterologous expression, and detection of historical *CYP51A* levels in Rothamsted's Hoosfield long-term spring barley field experiment.

**P08.015 Evolution and spread of *Mycosphaerella graminicola* isolates overexpressing *CYP51* or carrying different CYP51 variants in response to azole fungicide applications**

*B.A. Fraaije, S. Atkins, J. Luo, P.D. de la Fuente, N.J. Hawkins and H.J. Cools*
*Department of Biological Chemistry and Crop Protection, Rothamsted Research, Harpenden, AL5 2JQ, UK*
*Email: bart.fraaije@rothamsted.ac.uk*

Azole fungicides have been used for three decades to control Septoria leaf blotch caused by the fungus *Mycosphaerella graminicola*. Recent research has shown that the efficacy of some azoles (e.g. tebuconazole) can be compromised by the evolution and spread of insensitive strains carrying combinations of up to six amino acid alterations (substitutions and a deletion) in the target protein sterol 14α-demethylase (CYP51). Since 2009, we have identified several novel CYP51 variants carrying up to eight CYP51 alterations and a novel resistance mechanism, a 120 bp insertion in the *CYP51* promoter which is linked with *CYP51* overexpression. *In vitro* testing shows significant shifts in azole efficacy for all compounds tested, including epoxiconazole and prothioconazole. Here we present the genotype-to-phenotype relationships for these strains and discuss the practical implications.

**P08.016 Advances in the use of fungicides to manage coffee leaf rust and coffee berry disease in Kenya**

*G. O. Alwora and E. K. Gichuru*
*Coffee Research Foundation P.O. Box 4 - 00232 Ruiru*
*Email:gedohal@yahoo.com; ekgichuru@yahoo.com*

Since sulphur was first used as a fungicide to control powdery mildew on grapes, chemical control of plant diseases, including coffee leaf rust (CLR) caused by *Hemileia vastatrix* and coffee berry disease (CBD) caused by *Colletotrichum kahawae*, has proved to be inevitable. Additionally, new races of these diseases have arisen thus increasing the need for rapid chemical control as genetic resistance are broken and cultural control rendered less effective. Recent climatic changes has also affected the effectiveness of spray programmes especially those of protective nature. There have been a lot of advancements in the chemical industry with manufacturers trying to survive in this controversial field. Major advances have been made in the type of molecules, concentrations, formulations, rates as well as spray intervals all being done with the concern of environmental pollution and consumer safety. Various categories of fungicides have therefore been developed for agricultural use in different generations. Copper based fungicide such as Bordeaux and Burgundy Mixtures were used to manage CLR by the last decade of the 19th century. The use of organic fungicides began in 1934 and has since played a major role in the world wide control of CBD and CLR. Systemic fungicides are the most recently developed fungicides as well as the most promising for the future. This review focuses on the major advances in the type of molecules, formulations, spray intervals, challenges and future perspectives in the control of Coffee berry Disease (CBD) and Coffee Leaf Rust (CLR) with special focus to the Kenyan situation.

**P08.017 Impact and chemical control of root rot in faba bean**

*K.F. Chang, R.L. Conner, S.F. Hwang, H.U. Ahmed, D.L. McLaren, B.D. Gosse, G.D. Turnbull and S.E. Strelkov*
*Alberta Agriculture and Rural Development, 17507 Fort Road NW, Canada*
*Email: kan.fa.chang@gov.ab.ca*

Root rot pathogens are a major impediment to cultivation of faba bean crops on the Canadian prairies. Surveys in Alberta and Manitoba revealed that *Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp. are the most prevalent pathogens associated with root rot of faba bean. Substantial yield losses (68%) were observed due to root rot of faba bean. In the most trials seedling emergence and seed yield declined, and root rot severity increased significantly with each increase in inoculum level of both *Fusarium avenaceum* and *Rhizoctonia solani*. Fungicidal seed treatments with Apron Maxx and Vitaflo 280 consistently improved emergence and seed yield in faba bean grown in soil inoculated with either *F. avenaceum* or *R. solani*. This study demon-

strates the losses incurred by root rot of faba bean, and shows that control of root rot through seed treatment is warranted.

### P08.018 Control of Sclerotinia stem rot in soybean by fungicides

*C.H. Sumida, M.G. Canteri, D.C. Peitl, I.P. Orsini, F. Tibolla, F.A. Araújo, N.S. Calvo, D.F. Chagas, G.D.O. Arieira and F.E. de Mello*
*Londrina State University, Rodovia Celso Garcia Cid. (PR 445), Km 380, CP 6001, Londrina, Paraná, CEP 86051-990, Brasil.*
*Email: cirosumida@hotmail.com*

Sclerotinia stem rot is widespread in most regions of soybean production in Brazil and causes yield losses of millions tons annually. The present paper evaluates the effect of fungicides on *S. sclerotiorum* inhibition and the damage caused by the fungus in soybean culture. Tests were developed about: 1) different concentrations of fungicides on mycelial growth inhibition of *S. sclerotiorum*, 2) carpogenic germination inhibition, 3) ascospores germination inhibition and 4) control of Sclerotinia stem rot in soybeans under field conditions. Treatment evaluated was fluazinam, procymidone, iprodione, thiophanate methyl, carbendazim, fluazinam + benzalkonium chloride and *Trichoderma harzianum* biological treatment. Fungicide fluazinam was the only one inhibit 100% the *S. sclerotiorum* mycelial growth and carpogenic germination inhibition. Procymidone and benzalkonium chloride inhibit ascospores germination by 13,5 and 13,9%, respectively. These same fungicides were most effective to reduce the production of ascospores per apothecia, reducing 65 and 82%. Fluazinam, procymidone and benzalkonium chloride + fluazinam were more efficient on control of Sclerotinia stem rot under field conditions, when treated twice during the flowering in interval of 15 days. These fungicides have reduced disease incidence by 71, 73 and 73% compared to control, in the 2009-10 crop. In 2010-11 crop, white mold incidence reduced by 77, 75 and 77%, respectively.

### P08.019 Wound protectants to prevent the stem canker (*Neofusiccoum parvum*) of blueberry

*B.A. Latorre, R. Torres and K. Elfar*
*Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Vicuña Mackenna 4860, Santiago, Chile*
*Email: blatorre@uc.cl*

Stem canker of blueberry (*Vaccinium* spp.), caused by *Neofusicoccum parvum* and other Botryosphaeriaceae spp., is an economically important disease in Chile. Symptoms include apical necrosis, reddish stem necrosis, internal vascular discoloration and partial or total death of the foliage. Infection occurs through injuries, being pruning wounds the main infection route. The objective of this study was to determine the effectiveness of fungicide pastes, applied as pruning wound-protectants, against *N. parvum*. Based on the results obtained 0.1% benomyl, 0.06% iprodione and 0.5% tebuconazole provided over 90% control efficacy and completely prevented the re-isolation of *N. parvum*. However, control efficacy below 20% was obtained with 0.1% pyraclostrobin, *Bacillus subtilis* (QST713), *Trichoderma* spp. 7.1% ascorbic acid (Citrus SL) and 5.0% boric acid. Benomyl, iprodione and tebuconazole were also highly effective against *N. parvum* mycelium in vitro with $EC_{50}$ values of 0.15-0.25, 0.26-0.33, and 0.52-0.68 $\mu gmL^{-1}$, respectively, while $EC_{50}$ values >2 $\mu gmL^{-1}$ were obtained with pyraclostrobin. Pastes and liquid formulations containing 0.1% benomyl, 0.06% iprodione or 0.5% tebuconazole significantly protected wounds against *N. parvum* under field conditions. Benomyl and tebuconazole provided 97% control efficacy against infection by *N. parvum* when the pruning wounds were inoculated 1 or 10 days after treatment. In conclusion, the results of this study demonstrate that the occurrence of *N. parvum*-caused stem canker on blueberry can be reduced by protecting pruning wounds with paste formulations containing benomyl, iprodione or tebuconazole. The tested biological control agents and natural products were relatively ineffective against *N. parvum* and 5% boric acid was phytotoxic.

### P08.020 Containment and eradication of *Phytophthora cinnamomi* from natural ecosystems

*W. Dunstan, M. Crone, T. Paap, C. Dunne and G.E. Hardy*
*Centre for Phytophthora Science and Management, School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, 6150*
*Email: g.hardy@murdoch.edu.au*

The exotic soil-borne plant pathogen is recognized as one of 15 'Key Threatening Processes' to Australia's biodiversity. In south west Western Australia an estimated 41% of the 5710 native described plant species are susceptible and over one million hectares of native vegetation is infested. Therefore, robust methods that can effectively contain and eradicate the pathogen are paramount for the protection of remaining uninfested areas. Based on observations that *P. cinnamomi* is a poor saprotroph and that it can survive in asymptomatic annual and herbaceous perennial species, we describe two methods that can effectively be used to eradicate and contain *P. cinnamomi* from spot infestations. Firstly, we applied herbicides to kill plants then fumigants Metham and Dazomet to depth. Secondly, we used herbicides to kill all plants and ensured that no recruitment from soil seedbanks occurred over a period of two years. With regular baiting of the soils we were able to show no recoveries of the pathogen up to 30 months after treatments. The methods can be applied to spot

infestations in a wide range of environments to ensure large areas do not become infested through autonomous or anthropogenic spread. A detailed description of the approaches together with the recommended containment, monitoring, sampling and hygiene procedures will be discussed.

**P08.021 Control of anthracnose of hot pepper by forecasting Information in Korea**

*K.H. Lee[1], K.S. Ahn[1], M.K. Song[1], Y.H. Lee[2], I.G. Song[1], H.T. Kim[3] and S.C. Yun[4]*
*[1]Environment-friendly Agriculture Division, Chungbuk ARES, Cheonwon, 363-883, Republic of Korea; [2]Crop and animal division, Extension Service Bureau, Rural Development Administration, Suwon, 441-707, Republic of Korea; [3]Department of plant medicine, Chungbuk National University, Cheongju, 361-763, Republic of Korea; [4]Department Biochemical Sciences, Sun Moon University, Asan, 336-708, Republic of Korea*
*Email: bjej@korea.kr*

Field experiment was conducted to development the control method of pepper anthracnose using the forecasting information provided by National Crop Pest Management System(NCPMS), a web GIS based online information system in Korea in 2012. NCPMS alert the infection of anthracnose when hourly summed infection risk of 2.5 by calculating cumulative infection risk every hour based on the leaf wetness and hourly averaged temperature is reached. Incidence of anthracnose was compared among treatments, periodic spray after disease development, forecasting spray when NCPMS alerts and no spray. Forecasting spray treatment was divided depending on a application of preventive spray and spray interval. There were total 22 times of anthracnose infection risk warning on the experimental field located in Cheongwon from June 1. The number of spraying times and diseased fruit rate in August 30 were 8 and 3.9% in forecasting spray with minimum 5-7 day interval, 4 and 63.1% in 10-day interval spray after disease development, 10 and 20.8% in preventive spray with protective fungicide before disease development and forecasting spray with minimum 10 day interval, 0 and 95.7% in no-spray, 6 and 32.7% in forecasting spray with minimum 10 day interval, 12 and 4.0% in preventive spray with protective fungicide before disease development and forecasting spray with minimum 5-7 day interval, respectively. In this study, it is thought that control effect of pepper anthracnose by chemicals was more affected by timing and interval of spray rather than application of preventive spray. For the control of pepper anthracnose, we recommended the forecasting spray maintaining minimum 5-7 days of interval when NCPMS alerts the infection of anthracnose.

**P08.022 Rapid throughput analysis of filamentous fungal growth using the Bioscreen C: screening anti-fungal compounds**

*A.Medina, R.J.W. Lambert and N. Magan*
*Applied Mycology Group, Cranfield Health, Cranfield University, Beds. MK43 0AL, U.K.*
*Email: n.magan@cranfield.ac.uk*

A rapid method for screening antifungal compounds and performing ecophysiological studies with filamentous fungi has been developed by the use of specific semi-solid media and spectrophotometric/turbidimetric measurements using the Bioscreen C with 2x100 microtitre well plates. The medium composition and separation, inoculum size, medium volumes, and incubation parameters for measuring initial germination and growth dynamics have been optimised. These have been applied to assess the effectiveness of 18 concentrations of propyl propane thiosulfinate (PTS) against Aspergillus flavus in YES medium under different environmental regimes. Minimum inhibitory concentrations (MIC), and non-inhibitory concentration (NIC) values, and a newly developed $MIC_{50}$, have been calculated for the efficacy of these concentrations of PTS under four different environmental conditions (0.995 and 0.95 water activity (aw); 20 and 25 _C) over 7 d periods with automated measurements every 20 min. Data were modelled using the LambertePearson Model, a mathematical modelling approach previously used for bacterial inhibition. Results have been compared with traditional growth rate data and Lethal Dose50 (LD50) values. This approach could have major implications for rapid screening assays for growth and secondary metabolite production by filamentous organisms and has major advantages in terms of time reduction, culture medium volumes required, measurement and the ability to integrate and model key parameters for comparing efficacy.

**P08.023 Baseline sensitivity of *Didymella bryoniae* to fluopyram**

*K.L. Stevenson, D.B. Langston*
*Department of Plant Pathology, University of Georgia, 2360 Rainwater Rd, Tifton, GA, USA*
*Email:ks@uga.edu*

Resistance of the cucurbit gummy stem blight pathogen, *Didymella bryoniae*, to succinate-dehydrogenase-inhibiting (SDHI) fungicides boscalid and penthiopyrad was recently reported in the southern U.S. However, resistance to the SDHI fungicide fluopyram has not yet been reported in this pathogen and isolates resistant to boscalid and penthiopyrad were confirmed as sensitive to fluopyram based on in vitro mycelial growth assays. In this study, 98 isolates of *D. bryoniae* with no previous exposure to SDHI fungicides were tested for sensitivity to fluopyram using an *in vitro* mycelial growth assay to establish a baseline for further resistance monitoring.

Colony diameters were measured after 4 days of incubation on YBA medium amended with fluopyram and compared to growth on non-amended medium. On YBA, $EC_{50}$ values for fluopyram ranged from 0.114 to 1.149 mg/L with a mean of 0.576 mg/L. Using 24 of the 98 isolates, a similar assay was conducted on fluopyram-amended PDA and results compared to those on YBA. For the 24 isolates tested on PDA amended with fluopyram, $EC_{50}$ values ranged from 0.045 to 0.194 mg/L with a mean of 0.102 mg/L, and were significantly lower than those obtained using a similar assay on fluopyram-amended YBA. Colony diameters were more irregular and diffuse on YBA compared to PDA, making measurement more difficult. Although YBA has been recommended for SDHI sensitivity assays, based on our results, the mycelial growth assay using fungicide-amended PDA was more reliable than YBA and produced more consistent results for determining sensitivity of this pathogen to fluopyram.

**P08.024 Lesion expansion as an epidemic component of wheat tan spot**

*C.A. Forcelini, C. Ranzi and R. Roherig*
*University of Passo Fundo, P.O. Box 611, Passo Fundo, RS, 99001-970, Brazil*
*Email: forcelini@upf.br*

Tan spot caused by *Pyrenophora tritici-repentis* is an important disease of wheat in Brazil. It can reduce grain yield by more than 40%, and most Brazilian cultivars are susceptible to this disease. Tan spot management is based on seed treatment, crop rotation and the use of fungicide. Over the years wheat growers have had to increase fungicide rates to achieve satisfactory control of tan spot, which might be due to difficulties in controlling lesion expansion, a toxin mediated process. In 2011, ten wheat cultivars were assessed regarding to lesion growth by tan spot in greenhouse. Lesion size measured four days after inoculation was very similar among cultivars (average of 0.63 $mm^2$) but it varied from 2.39 to 18.99 $mm^2$ 21 days later. The rate of lesion expansion ranged from 0.08 to 0.88 $mm^2$/day depending on wheat genotypes. The ones with higher rates in the greenhouse were also the more affected by tan spot in the field. In other greenhouse trials designed to compare disease severity after one to three sequential inoculations with the pathogen, lesion expansion from a previous infection accounted for 86% of the total disease, whereas new lesions only 14%. Curative sprays of strobilurin fungicides did not control lesion expansion and triazoles were efficient when sprayed within 10 days after inoculation. Lesion expansion is an important component of tan spot epidemics and it can be used to assess genotype susceptibility. Curative control of lesion expansion is limited to triazole applications within a few days after infection.

**P08.025 Fungicide resistance in *Cercospora beticola* the cause of sugar beet leaf spot**

*G.A. Secor, V.V. Rivera, M.D. Bolton and M.F.R. Khan*
*Department of Plant Pathology, North Dakota State University, NDSU Dept 7660, Box 6050, Fargo, ND 58108, USA*
*Email: gary.secor@ndsu.edu*

Cercospora leaf spot is a serious disease of sugar beet wherever sugar beets are grown throughout the world. The disease, caused by the fungus *Cercospora beticola,* is managed by crop rotation, resistant varieties and proper fungicide applications. Populations of *C. beticola* from the US have been monitored for changes in sensitivity to fungicides to four classes of fungicides (tin, benzimidazole, triazole, QoI) using bulk spore germination, growth reduction and spore germination for the past 10 years. The number of isolates with resistance to tin at 2 ppm has decreased from 65% to < 10% during this period. The percentage of isolates with resistance to thiophanate methyl ranged from 78-14%. Sensitivity of isolates to the triazole fungicides as measured by $EC_{50}$ values gradually increased over time, but increase dramatically in 2011. Reduced sensitivity correlates with increased disease losses. Triazole resistance is associated with overexpression of the Cyp51enzyme in *C. beticola*. There has been a 40 fold increase in $EC_{50}$ values of isolates recovered from ND and MN since the introduction of pyraclostrobin. Isolates of *C. beticola* collected from MI USA fields with high incidence of Cercospora leaf spot in 2011 had high $EC_{50}$ values to pyraclostrobin, and the G143A mutation was present. Bulk spore samples collected from 1412 fields in ND/MN USA in 2012 showed that 24 of the samples (1.7%) contained the G143A mutation. Limited testing in several European populations has shown high levels of resistance to triazole and QoI fungicides, and the presence of the G143A mutation.

**P08.026 Resistance of *Peronosclerospora maydis*, the causal agent of maize downy mildew, to metalaxyl and dimetomorf fungicides**

*A. Widiastuti, A. Wibowo, D. Yudistira and C. Sumardiyono*
*Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Jalan Flora Bulaksumur Yogyakarta 55281, Indonesia*
*Email: arif@faperta.ugm.ac.id*

The research had been conducted to detect any *Peronosclerospora maydis* which is resistant to metalaxyl and dimetomorf fungicides. Due to the emergence of resistant strains of *P. maydis*, it is suspected to cause variability of the fungal pathogen in endemic and non-endemic areas of maize downy mildew. Fungal spores were collected from infected maize plants in Klaten and Kalasan regions in Central Java. Examination

of the influence of metalaxyl and dimetomorf was performed *in vitro* using spore germination technique, while the greenhouse trial was conducted by inoculating diseased maize leaf towards 10 days old maize plants grown from seeds treated with metalaxyl and dimetomorf. The result showed that there had been a strain of *P. maydis* from Klaten region which was resistant to metalaxyl, but not resistant to dimetomorf. Klaten is maize downy mildew endemic region in Central Java, whereas in Kalasan is a non-endemic area with fewer occurence of disease incidence. Spores of *P. maydis* collected from Kalasan and Klaten had different sizes. These might be different strains of *P. maydis* arising from fungicide treatment with different frequency of application. To ensure the variability of these 2 strains, molecular research is needed in the future.

**P08.027 Scheduling of novel fungicides to manage location specific diseases in rice**

*M.K.P. Kumar, D.K.S. Gowda and S.V. Manjunath*
*Zonal Agricultural Research Station, V.C. Farm, Mandya, India*
*Email:babu_prasanna@rediffmail.com*

In India, Karnataka is important rice growing state with an area of 1.39 million hectares, cultivated in different ecosystems. The crop is commonly prone to attack of more than 10 diseases with blast disease causing serious damage followed by sheath blight, brown spot, and sheath rot diseases. At present disease management is mainly through use of chemicals due to lack of suitable and durable resistant varieties. The rice disease management is complicated, as different diseases occur at different growth stages of crop. As a result different chemicals are applied at different intervals, thereby increasing cost of plant protection. Hence the quest is to identify molecules effective against multiple pests. In this regard ten new generation broad spectrum fungicides were screened in the field with aim of controlling multiple diseases with limited sprays to know timing and effective rates so as to manage more than one disease with a spray. Kresoxim methyl 50% SC @ 500ml/ha, Azoxystrobin 23 SC @ 625 ml/ha and Trifloxstobin 50 WG @ 200 g/ha were effective as stand alone. However, the combination of Kresoxim methyl + Hexaconazole 48 WG @ 500 g/ha, Azoxystrobin +Hexaconazole 16.25 SC @ 900 ml/ha and trifloxystrobin + tebuconazole 75 WG @ 400 g/ha showed similar efficacy against leaf blast, sheath blight, sheath rot and neck blast diseases, when applied at tillering stage and 50% panicle emergence stage.

**P08.028 Potential use of fungicides to control Stemphylium blight of lentil**

*A.H.M.M. Haque[1], R. Podder[1], M.M. Kamal[1], M.G. Hossain[1], M.M.E. Rahman[1], M.A. Muktadir[1] and A. Sarker[2]*
*[1]Bangladesh Agricultural Research Institute, Joydebpur, Gazipur-1701, Bangladesh; [2]ICARDA, South Asia and China program, New Delhi, India*
*Email: mahfuzprc@gmail.com*

*Lens culinaris* Medik. ssp. *culinaris* is the only cultivated species under the genus *Lens* which has been accelerated susceptibility to different fungal diseases in Bangladesh. Stemphylium blight of lentil caused by *Stemphylium botryosum* Wallr the major one that limit seed yield and quality. Use of potential fungicides and method of application is an important component of this disease management. The experiment was conducted at Pulses Research Sub-Station, Gazipur, and Regional Agriculture Research Station, Jessore, Bangladesh to find out the effective fungicides and spray schedule for controlling Stemphylium blight during winter, 2011–2012. A total of five treatments (four fungicides viz. Rovral (Iprodion), Secure 600 WG(Fenamidione + Mancozeb), Companion(Mancozeb), Indofil M-45 (Mancozeb) and one control (water) were designed combined with three spray schedules (7, 10, 15 days interval) in this study. Fungicides with different spray schedule showed significant effect on controlling Stemphylium blight as well as yield of lentil. Among all combinations the lowest disease severity was recorded after Secure 600 WG application with 7 days interval spray schedule at Jessore and Gazipur followed by Indofil M-45 and Companion with 7 and 10 days interval spray schedule respectively at Jessore. The highest yield 1293 kg/ha and 1106 kg/ha were recorded from secure 600 WG at 7 days interval sprayed plots at Gazipur and Jessore respectively. The lowest yield was obtained from untreated control plot at both locations. A potential recommendation from this study can be drawn that would help the lentil growers to get optimum yield.

**P08.029 FGSG_03716, involved in the resistance of *Gibberella zeae* to JS399-19, a novel cyanoacrylate fungicide**

*J.F. Yu, Y.P. Hou, J.X. Wang, M.G. Zhou and C.J. Chen*
*College of plant protection, Nanjing Agricultural University, Nanjing, 210095, P. R. China*
*Email: changjun-chen@njau.edu.cn*

*Fusarium graminearum* (teleomorph: *Gibberella zeae*), the dominant pathogen of Fusarium head blight (FHB) on wheat in the southern of China, is a leading cause of economic loss. 2-cyano-3-amino-3-phenylancryic acetate, JS399-19 is a novel cyanoacrylate fungicide, which is developed by Jiangsu Pesticide Institute, China, and will be widely applied to control FHB in field in 2013, especially where resistance of *G. zeae* against carbendazim was severe. To reveal the JS399-19-resistant mechanism of *G. zeae*, we found that FGSG-03716, coding one member of the multidrug resistance family, was involved in the resistance. To confirm the conclusion,

FGSG -03716-deleted mutants and its complementary mutants was constructed through homologous double crossover *in situ* and validated by Southern blot. $EC_{50}$ values of FGSG-03716-deleted mutants against JS399-19 increased by 62~71μg/mL higher than its progenitor, highly JS399-19-resistant strain, Y2021A with the $EC_{50}$ value of 180.43μg/mL.

**P08.030 Exploring mechanism of resistance to isoprothiolane in rice blast fungus *Magnaporthe oryzae***

*M.J. Hu, Y. Lin and C.X. Luo*
*Department of Plant Protection, College of Plant Science and Technology and the Key Lab of Crop Disease Monitoring & Safety Control in Hubei Province, Huazhong Agricultural University, Wuhan 430070, P. R. China*
*Email: cxluo@mail.hzau.edu.cn*

The fungicide Isoprothiolane (IPT) has been widely used to control rice blast fungus *Magnaporthe oryzae* for decades. However, the molecular mechanism of resistance is still unexplained and was only believed to be associated with transmethylation in the biosynthesis of phosphatidylcholine. In this study the IPT sensitivity was tested for hundreds of *M. oryzae* isolates collected from Yunnan and Hubei provinces of China. And three IPT resistant mutants were generated by adapting mycelium to increasing concentrations of IPT subsequently. No fitness penalty was observed in these resistant mutants. In consideration of the role on transmethylation in the biosynthesis of phosphatidylcholine, three candidate genes, *PEAMT*, *CHO2* and *OPI3* were sequenced, and their expression profile was determined. There was no significant difference in the expression of those genes between resistant mutants and sensitive parental isolates. Additionally, Solexa sequencing was conducted to screen potential target gene. MGG_09793 was the mostly changed gene between resistant mutant H08-1a_mut and sensitive parental isolate H08-1a after IPT treatment. However, when this gene was knocked out in H08-1a_mut and H08-1a, the resistance status of the resulted transformants to IPT was not changed. This study indicates that expected *PEAMT*, *CHO2* and *OPI3* genes were not the targets of IPT. Interestingly, the DJ-1/PfpI family protein MGG_09793 was specifically and intensely induced by IPT treatment in the sensitive isolates, even this gene was also not the direct target of IPT.

**P08.031 *In vitro* binding characteristics of $\beta_2$-tubulins from *Fusarium graminearum* with carbendazim**

*J.Q. Xu[1,2] and M.G. Zhou[1 *]*
*[1]College of Plant Protection, Nanjing Agricultural University, Nanjing, 210095, P. R. China; [2] College of Forestry, Henan University of Science and Technology, Luoyang 471003, P. R. China*
*Email: mgzhou@njau.edu.cn*

Fusarium head blight caused by *Fusarium graminearum* is an important disease of wheat and barley and benzimidazole fungicides, including carbendazim and thiophanate, have been employed to control the disease nearly for forty years. However, the carbendazim resistance has developed in the population and the resistant mechanism has been revealed by genetic transformation and site-directed mutagenesis different from previously repots. The results showed that point mutation at $\beta_2$-tubulin different site could cause moderate or high resistance. There is no report of carbendazim resistance mechanism on the protein level up yet now. In this study, the genes of $\beta_2$-tubulins with different mutation sites, including F167Y, E198K, F200Y and E198L of *F. graminearum* have been cloned on the pET vectors respectively and expressed in *Escherichia coli*, and the *in vitro* binding characteristics between recombinant $\beta_2$-tubulins and carbendazim were studied by fluorescence quenching. Fluorescence of all recombinant protein was quenched following the addition of carbendazim, in which the $\beta_2$-tubulin of wild-type sensitive strain had the highest quenching rate and the $\beta_2$-tubulin with two mutation sites simultaneously had the lowest. The apparent assembly rates (Kon) of $\beta_2$-tubulin with different mutation sites was negatively correlated with the resistant level of starting strain to carbendazim. However, the disassembly rates (Koff) was positively correlated with the resistant level. The affinity constant (Ka, the value of Kon/Koff) of $\beta_2$-tubulin of sensitive strain was the largest, whereas those of resistant strains were smaller. These findings indicate that the resistance of *F. graminearum* to carbendazim was caused by the reduction of affinity power between the target protein and the fungicide.

**P08.032 Preliminary study on the action target of a novel antibiotic shenqinmycin**

*S. Xu, J.Y. Luo, X.Y. Pan, J. Wu, Y.P. Hou, C.J. Chen and M.G. Zhou*
*College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, P. R. China*
*Email: mgzhou@njau.edu.cn*

Shenqinmycin is a novel antibiotic firstly developed by Shanghai Jiao Tong University, and its main component is phenazine-1-carboxylic acid. It is a broad-spectrum antibiotic, and is extremely effective against *Xanthomonas oryzae* pv. *oryzae* (Xoo), with the mean $EC_{50}$ value of 0.110±0.006 μg/ml (61 Xoo strains). It has a better protective activity than curative activity, and the control efficacy *in vivo* can reach 78.91% when the concentration of shenqinmycin is 200 μg/ml. Proteomic analysis revealed that carbohydrate metabolism of Xoo was suppressed by shenqinmycin since one third of the differentially expressed proteins are involved in carbohydrate metabolism. In addition, shenqinmycin is able to inhibit spore germination of *Fusarium graminearum* at a

low concentration, while it is not able to inhibit the mycelial growth effectively, indicating that shenqinmycin inhibits energy metabolism. We found in the literatures that phenazines have redox activity and can maintain the balance of NADH /$NAD^+$ ratio in their producers. So we investigated the redox activity of shenqinmycin and found that it can react with NADH *in vitro*. Carbon source metabolism analysis further confirmed that carbon source metabolism ability of Xoo was suppressed by shenqinmycin, and ATP or NADH is required for the utilization of these suppressed carbon sources. Thus, we suspect that shenqinmycin may compete with NADH dehydrogenase, and this was further verified with gene knockout, functional complementation, prokaryotic expression and pharmaceutical combination of all the subunits of Xoo NADH dehydrogenase.

**P08.033 Molecular basis of tetraconazole resistance in the sugarbeet pathogen *Cercospora beticola***

*M.D. Bolton, G.A. Secor, V. Rivera-Varas and M.F.R. Khan*
*United States Department of Agriculture, 1605 Albrecht Blvd N, Fargo, ND 58102-2765, U.S.A.*
*Email: Melvin.Bolton@ars.usda.gov*

Cercospora leaf spot, caused by the fungus *Cercospora beticola*, is the most important foliar disease of sugarbeet. Control measures include the application of sterol demethylation inhibitors (DMIs). However, DMI-resistant isolates are becoming widespread, and therefore are a major threat to sugarbeet production. Understanding the molecular mechanism of fungicide resistance is critical for fungicide resistance management. The objective of our study was to determine the molecular basis of resistance to the DMI fungicide tetraconazole in order to develop tools for PCR-based detection of DMI resistance. To this end, we first cloned the *C. beticola Cyp51* gene, which encodes the DMI target Cyp51. We showed that resistance to tetraconazole in this fungus is related to over-expression of *Cyp51*. However, we believe that *Cyp51* over-expression only partly explains DMI resistance in this pathosystem. Therefore, we have utilized RNA-seq technology to identify genes differentially expressed in a resistant isolate upon exposure to tetraconazole. Additionally, we have identified genes that are inherently induced in a DMI-resistant isolate compared to a DMI-sensitive isolate. Genes of interest included those involved in transport, signal transduction, and sterol metabolism. These research findings will be discussed in detail.

**P08.034 Characterization of genotype and phenotypes of *B. cinerea* isolates to zoxamide and carbendazim**

*M. Cai, L. Chen and X.L. Liu*
*Department of Plant Pathology, China Agriculture University, Beijing Key laboratory of quarantine and prevention control on seed-borne diseases, Beijing, 100193, P. R. China*
*Email: cm122587@163.com*

Zoxamide and carbendazim are both β-tubulin inhibitors. Zoxamide is effective on oomycetes and some fungi such as *Botrytis cinerea*, while carbendazim is highly effective on fungi only. The aim of this study was to characterize resistant phenotype of *B.cinerea* isolates and to investigate the molecular basis of resistance. Monitoring of sensitivity in161 field isolates showed three phenotypes were present, including 26 $S^{zox}S^{car}$ (both sensitive to zoxamide and carbendazim), 88 $S^{zox}R^{car}$ (sensitive to zoxamide but resistant to carbendazim) and 47 $R^{zox}R^{car}$ (both resistant to zoxamide and carbendazim) isolates, but no one was $R^{zox}S^{car}$ (resistant to zoxamide but sensitive to carbendazim). Two $R^{zox}S^{car}$ phenotype isolates were obtained via laboratory selection on zoxamide-amended petri dishes. Analysis of amino acid (AA) sequence in β-tubulin gene showed the resistance was always associated with AA variations in resistant phenotypes. Two point mutations of E198A and V349I were found in all $S^{zox}R^{car}$ phenotype isolates; either point mutation of E198K and T351I or F200Y in $R^{zox}R^{car}$ phenotype isolates; and point mutation of M233I in $R^{zox}S^{car}$ phenotype isolates. No fitness penalty was affected in $S^{zox}R^{car}$ and $R^{zox}R^{car}$ phenotypes according to mycelial growth rate, temperature sensitivity, capability of sporulattion and sclerotia production, and virulent on detached tomato fruit, compared with sensitive isolates. However, the $R^{zox}S^{car}$ phenotype isolates lost ability to sporulate and produce sclerotia on PDA petri dish in darkness, but no significant differences with the other three phenotypes in virulent. The different resistant mechanism for these two β-tubulin inhibitors is important to design management strategies of resistance in *B. cinerea*.

**P08.035 Competitive fitness of pyrimorph-sensitive and -resistant isolates of *Phytophthora capsici***

*Z.L. Pang, J.P. Shao, L. Chen, J.R. Fan, J. Hu, Z.W. Wang and X.L. Liu*
*Department of Plant Pathology, College of Agriculture and Biotechnology, P. R. China Agricultural University, Beijing 100193, P.R. China*
*Email: pangzhilicau@hotmail.com; seedling@cau.edu.cn*

Pepper Phytophthora blight resulted by *Phytophthora capsici* causes significant yield losses to pepper production worldwide. Pyrimorph, a new Carboxylic Acid Amide fungicide, was registered to control this disease in China. To date, Q1077K site mutation in cellulose synthase 3 was reported referring to pyrimorph resistance of *P. capsici*. In this study, we aimed to develop a molecular assay to investigate the competitive fitness

of pyrimorph-resistant (R3-2, and R11-1, [$EC_{50}$] > 65 μg/ml) and -sensitive (Hd3, and Hd11, $EC_{50}$< 1.5 μg/ml) isolates of *P. capsici* with similar pathogenicity, with and without pyrimorph selection pressure. The mixtures of pyrimorph-resistant (R) and sensitive (S) isolates with ratios of 1R:9S, 3R:7S, 5R:5S, 7R:3S, and 9R:1S, were inoculated to the soil surface around in pepper plants (cultivar Tedaqiemen) with four euphylla, or made a stab inoculation in eggplant fruit treated with 1.5 μg/ml pyrimorph. After 7 days incubation, pathogens were recovered. The ratios of the resistant isolates were quantified by a real-time PCR method developed, combined with traditional tested assay. Ten successive transfers were performed. Results demonstrated that the efficiency of real-time PCR was consistent with traditional tested assay. Both assays showed that competitive ability of resistant isolates were similar or decreased in comparison with sensitive isolates, in the absence of selection pressure or in the presence of pyrimorph treatment. This real-time PCR assay will be used in high throughput monitoring high resistant isolates population in the field for pepper Phytophthora blight management.

### P08.036 Effect of different fungicides in the management of wheat blast

*A.A.B. Sussel, J.H. Chagas, A.C. Dianese and A.B. Zacaroni*
*Embrapa Cerrados, P.O.Box 08223, 73310-970, Planaltina, DF, Brazil*
*Email: angelo.sussel@embrapa.br*

The wheat blast (*Pyricularia grisea*) is responsible for large losses in quality and productivity of wheat grain, mainly in the Midwest region of Brazil. The aim of this study is evaluate the effect of fungicides tebuconazole+ trifloxystrobin (75+150 g/ha), prothioconazole+trifloxystrobin (87.5+75 g/ha), epoxiconazole+pyraclostrobin (60.8+98.8 g/ha), epoxiconazole+pyraclostrobin (37.5+ 99.8 g/ha), tebuconazole (150 g/ha), tebuconazole+ azoxystrobin (144+75 g/ha), tebuconazole+azoxystrobin (120+72 g/ha) in the incidence progress and severity of wheat blast and also in the wheat productivity. In March 2012 the wheat cultivar BRS208 was sown in the Federal District, Brazil. The fungicides were sprayed at begin of flowering plus two applications with ten-days intervals. The experimental design was a randomized block with 5 replicates and control treatment not sprayed. The wheat spikes were evaluated 6 times with five-day intervals for incidence. Upon reaching the stage 85 of Zadoks and colleagues scale, samples of 100 wheat spikes of each replicate were evaluated for severity. The productivity was evaluated with the harvesting, drying and weighing of wheat grains. Variance analysis and Tukey test at 5% probability were performed. There is no significant difference on the productivity and on the disease severity. The incidence progress curves fit better into the monomolecular model with the lowest mean square of error and the second highest determination coefficient. The rates of disease incidence progress among the fungicide treatments did not differ, however they differed from the control. It is concluded that treatment with fungicides reduced the disease progress in the field, however, without preventing losses in productivity.

### P08.037 Y123H mutation of *FvCYP51B* conferred to slow growth and resistant to prochloraz in *Fusarium verticillioides*

*J.R. Fan, F.P. Chen, Y.Z. Diao, H. Cools, S.L. Kelly and X. Liu*
*College of Agriculture and Biotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: 575933596@qq.com; chenfengping1207@126.com*

*Fusarium verticillioides* causes corn disease resulting in yield loss and fumonisin contamination harmful to humans and animals. Prochloraz as a potential fungicide showed a good inhibition against *F. verticillioides*. To explore resistant risk and mechanisms responsible for decreasing sensitivity of *F. verticillioides* to prochloraz, four resistant strains were generated by fungicide selection. The growth rates of mutants were varied from the resistant level. All the mutants are resistant to triazole fungicides tested including triadimefon, tebuconazole, and difenoconazole, however, there was no cross-resistance between DMIs and chlorothalonil or fludioxonil, the multiple site fungicides. The four resistant mutants were classified into two genotypes: PCZ-R1 with wild type *FvCYP51B* including two mutants 0113 and 0104, and PCZ-R2 carrying an Y123H substitution in *FvCYP51B* containing 0109 and 0110 mutants. Wild type *FvCYP51B* can complement the function of native *ScCYP51* in *Saccharomyces cerevisiae*, whereas *FvCYP51B* with Y123H mutation cannot. For the PCZ-R1 mutants, over-expression of *FvCYP51A* decreased the sensitivity to prochloraz, while for PCZ-R2 mutants, over-expression of *FvCYP51A* combination with Y123H mutation of *FvCYP51B* caused resistance to prochloraz. The Y123H mutation of *FvCYP51B* also caused slow growth in PCZ-R2 mutants.

### P08.038 Sensitivity of *Cercospora sojina* to demethylation inhibitor and methyl benzimidazole carbamate fungicides

*G.R. Zhang and C. A. Bradley*
*University of Illinois, Department of Crop Sciences, 1102 S. Goodwin Ave. Urbana, IL, 61801, U.S.A.*
*Email: grzhang@illinois.edu*

Frogeye leaf spot (FLS) is a disease of soybean caused by Cercospora sojina. Demethylation inhibitor (DMI) fungicides such as prothioconazole, tetraconazole, and

flutriafol, and the methyl benzimidazole carbamate (MBC) fungicide thiophanate-methyl can be applied to manage FLS. These two fungicide classes are recommended to manage FLS caused by *C. sojina* strains that are resistant to quinone outside inhibitor (QoI) fungicides. A total of 58 *C. sojina* baseline isolates collected from 9 states were tested with an in-vitro assay to determine their sensitivity to DMI and MBC fungicides. Baseline isolates had effective concentrations in which 50% mycelial growth inhibition ($EC_{50}$ ) values for prothioconazole, tetraconazole, flutriafol, and thiophanate-methyl ranged from: 1.70 to 10.92; 0.09 to 0.33; 0.15 to 0.84; and 0.45 to 0.95 ug/ml, respectively, with mean $EC_{50}$ values of 4.48: 0.19: 0.39: and 0.73 ug/ml, respectively. When QoI fungicide-resistant and sensitive *C. sojina* isolates were compared for their sensitivities to DMI and MBC fungicide, no significant differences were observed, which indicates that DMI and MBC fungicides should equally control FLS caused by QoI resistant or sensitive isolates. Sensitivities of *C. sojina* isolates to DMI and MBC fungicides will continue to be monitored to identify isolates that are resistant to these fungicide classes.

**P08.039 Influences of irrigation root time and concentration of cyazofamid on control effects against Chinese cabbage clubroot**

*G.X. Wu, Y.X. Wu, G.H. Ji, M.Z. Chao and Y.Q. He*
*College of Plant Protection, Yunnan Agricultural University, Kunming, 650201, P. R. China*
*Email: chonchon@ynau.edu.cn*

Clubroot caused by *Plasmodiophora brassicae* is the key factor affecting the yield and quality of Chinese cabbage in China. Some studies showed that cyazofamid were effective pesticides for control of clubroot. In this study, the influences of irrigation root time and concentration of cyazofamid on control effects against Chinese cabbage clubroot root were measured in the indoor potted efficacy trials. The result showed that the relative control effects were more than 80% with 50 mg/L cyazofamid irrigating at first 3 days after sowing, the control effects gradually decreased with 50 mg/L cyazofamid irrigating at more than 3 days after sowing, especially the control effects were less than 25% with 50 mg/L cyazofamid irrigating at more than 9 days after sowing. The higher the irrigating concentration of cyazofamid was, the better the relative control effect was. However, the difference of the relative control effects was small among 100 mg/L, 50 mg/L and 25 mg/L cyazofamid. Therefore, the recommended irrigating time of cyazofamid is the first 3 days after sowing, and irrigating concentration is 25-50 mg/L.

**P08.041 Fungicides and spraying technology for the management of Asian Soybean Rust**

*J.Z. Zhou*
*Shenyang Research Institute of Chemical Industry, No. 8 Shenyang Dong Road, Tiexi District, Shenyang, Liaoning, 110021, P. R. China*
*Email: zhoujizhong@simochem.com; zhoujizhong82@163.com*

Asian Soybean Rust (ASR), caused by the virulent pathogen *Phakopsora pachyrhizi*, is the most destructive disease for soybean in many soybean-producing areas of the world. The first symptom of ASR in the form of rust pustules is found on the lower leaves of canopy. The disease becomes severe as the upper leaves are re-infected by the spores produced by the pustules located on the abaxial surfaces of leaves. Significant yield losses resulted due to decreases in seed size and seeds per pod, and increased in pod abortion. It's reported that yield losses was as high as 60% in Paraguay in 2001, up to 63% in Brazil in 2003, and almost 100% in some fields of South Africa in 2001. Fungicides is the main tool to defend against ASR damage, due to the current unobtainable soybean cultivars with acceptable levels of resistance to ASR and the low efficiency of cultural practice to control of the disease. Protectant fungicides such as mancozeb, chlorothalonil, and strobilurin compounds and curative fungicides like triazoles were evaluated to be effective in Southern Africa and South America. The fungicides could reduce the severity of ASR and increase the yield. The timing and the number of fungicide applications for chemical control of ASR are critical factors to affect the results of ASR management, even the usage of the effective fungicides. Spraying technologies providing good penetration and canopy coverage were proved to be favor for ASR control.

**P08.042 A screening system for compounds to control of Asian Soybean Rust**

*J.Z. Zhou*
*Shenyang Research Institute of Chemical Industry, No. 8 Shenyang Dong Road, Tiexi District, Shenyang, Liaoning, 110021, P. R. China*
*Email: zhoujizhong@simochem.com; zhoujizhong82@163.com*

Asian Soybean Rust (ASR) causes huge damage to the world soybean-producing areas, especially in South Africa and South America. Fungicides are relied on to manage ASR in the field. New compounds are needed from the lab, even the effective triazole and strobilurin fungicides existed. Risk of fungicide resistance of the trazole and strobilurin is medium and high, separately, which means that the two classes of fungicides have to be used cautiously to management the issue of resistance. In the lab, a system was developed to screen new compounds to control of ASR. The system is consisted of spore germination, detached leaves and attached leaves assays. Several separated trials involved in the system, including the effect of temperature and time

on spore germination; parameters of leaves age, varieties, and the medium to keep the leaves on detached assays; parameters of temperature, humidity, spore concentrations on attached leaves in greenhouse. Efforts are made to ensure the system to screen new compounds efficiently and economically.

**P08.043 Chemical control of rape clubroot**

*J.C. Ma and G.J. Tan*
*Anhui agricultural university, Anhui province Hefei city, 230036, P.R.China*
*Emial:mjc6601@126.com*

Rape is one of the most important cash crops. During the last 20 years increasing intensity of vegetable production and the rapid growth in popularity of oilseed rape as a broadacre or arable break crop have increased the severity of clubroot and the area of land affected in both the vegetable and broadacre industries. Clubroot caused by *Plasmodiophora brassicae* affects the Brassicaceae family of plants. Resting spores of it have a great ability to survive in soil, so the disease is difficult to control. A number of control measures such as application of liming of the soil and use of resistant host genotypes are recommended for clubroot, but may not always be practical or effective. So we fixed our attention on the application of fungicides. We used 8 chemicals with different chemical treatments to protect rape clubroot in greenhouse and field, including 10% Cyazofamid suspending agent, 50% fluazinam SC, 75% of the Corning, 53% gold mine water dispersible granule, 70% Thiophanate-methyl, 45% enemy yellow sodium, 20% Zn thiazole suspending agent and 80% carbendazol wettable powder. The results indicated that the seedling stage in rape is the most important stage of protection; Chemical with seed has the best control effect; 50% fluazinam SC, 10% Cyazofamid suspending agent, 20% Zn thiazole suspending agent had good effect.

**P08.044 Two non-target recessive genes are involved with resistance to zoxamide in *Phytophthora capsici***

*L. Chen[1], Y. Bi[2], M. Cai[1], S.S. Zhu[3], Z.L. Pang[1] and X.L. Liu[1]*
*[1]Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China; [2]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, P. R. China; [3]Key Laboratory of Agro-Biodiversity and Pest Management of Education Ministry of China, Yunnan Agricultural University, Kunming, Yunnan, 650201, P. R. China*
*Email: seedling@cau.edu.cn*

*Phytophthora capsici* is a devastating oomycete plant pathogen which is distributed all over the world; it has been reported to cause severe epidemics on a wide range of hosts. Zoxamide is a sole benzamide fungicide used to control oomycete diseases. It disrupts microtubule by binding β-tubulin protein, whereas no mutation in α- and β-tubulin was found in zoxamide-resistant isolates, and the expression of α- and β-tubulin showed also no difference between zoxamide-resistant isolates and sensitive isolates. It suggested that *P. capsici* developed a non-target-site-based resistance to zoxamide. The segregations of zoxamide-resistance in sexual progeny of *P. capsici* were also analyzed. The progeny derived from the self-crossing of the sensitive isolate PCAS1 and PCAS2 showed a separation of sensitivity to zoxamide, indicating that the resistance to zoxamide is controlled by a recessive nuclear gene(s). Furthermore, the segregation of resistance in $F_1$, $F_2$, and $BC_1$ progeny accord with the $\chi^2$ test ($P$>0.05). These results suggested that the resistance to zoxamide was controlled by two recessive genes in *P. capsici*, and the isolate could exhibit resistance to zoxamide when at least one pair alleles were homozygous. It is implied that resistance risk could be low to moderate on the basis of genetic trait of zoxamide-resistance in *P. capsici*. We still should pay attention to the resistance developing especially if two compatible mating types co-exist in the same field.

**P08.045 Flower rusty spot disease of orchid and its control**

*P. Patanavipart and T. Tassakorn*
*Plant Pathology Research Group, Plant Protection Research and Development Office,Department of Agriculture, Chatuchak, Bangkok, 10900, Thailand*
*Email:peerawanp@gmail.com*

Flower rusty spot is one of the most important orchid diseases in Thailand. It can cause serious problem in many varieties of orchids especially *Dendrobium* sp. The flower lesions were collected from *Dendrobium* sp. *and Mokara* sp. and identified as *Curvularia eragrostidis.* Twenty fungicides were selected and tested for their effectiveness in inhibiting the growth of *C. eragrostidis* in culture media by poison food technique at four different concentrations. The results showed that ten fungicides could completely inhibit the mycelial growth of the fungus. The ten fungicides used for efficacy test on *Dendrobium* flowers were conducted under greenhouse conditions by randomized complete block design with 4 replicates. Only six fungicides had high effectiveness in controlling the *C. eragrostidis.* These fungicides were subsequently done for field efficacy test in a commercial orchid farm by spraying on *Dendrobium* sp. flowers for four times with 5 day-intervals. The experiment plots were designed by randomized complete block with 4 replicates. The symptom of flower rusty spot disease was evaluated before spraying fungicides and at 5, 10 days after the last spray. The disease incidence in treatments sprayed with four fungicides: mancozeb 80% WP, captan 50% WP, pyraclostrobin 25% W/V EC and iprodione 50% WP were 15.89%, 23.40%,

25.35% and 33.61%, respectively while the percentage of infection in non-treated with fungicide was 81.77.

**P08.046 Metabolites analysis of the fungicide SYP-Z048 in cucumber plant**

*P.F. Liu, X.W. Feng and X.L. Liu*
*College of Agriculture and Biotechnology, China Agricultural University, No. 2 Yuanmingyuan West Road, Beijing 100193, P. R. China*
*Email: pengfeiliu@cau.edu.cn*

Previous research has demonstrated that SYP-Z048 (3-[5-(4-chlorophenyl)-2,3-dimethyl-3-isoxazolidinyl] pyridine), a newly developed nitrogen heterocycle substituted isoxazoline compound, exhibited good protective and curative activities against a wide range of fungal diseases of fruits and vegetables caused by Ascomycetes, Basidiomycetes, and Deuteromycetes. In this study, the metabolic behavior of SYP-Z048 was investigated in cucumber plant. The fungicide degradation followed the first order kinetic equation in adult-plant leaf, seedling and fruit, with half lives of 2.67 d, 1.69 d and 2.04 d respectively. On the comparison of chromatogram and mass spectrometry of fungicide free control with treated samples, main metabolites of SYP-Z048 in host plant were identified by LCMS-IT-TOF. The metabolites structures and kinetics suggested that the bio-transformation of SYP-Z048 occurred via multiple reaction pathways that included hydrolysis reaction, demethylation and the cleavage of isoxazolidine ring.

**P08.047 Resistance risk and resistance mechanism of *Peronophythora litchii* to four Novel QoI fungicides**

*Y.X. Zhou, P.F. Liu, H.X. Duan and X.L. Liu*
*Department of Plant Pathology, College of Agriculture and Biotechnology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: pengfeiliu@cau.edu.cn*

Sensitivities of *Peronophythora litchii*, the causal agent of litchi downy blight, to four novel QoI fungicides (enestroburin, SYP-1620, SYP-2815, and ZJ0712) were tested *in vitro*. All four fungicides strongly inhibited sporangium and cystospore germination, mycelial growth, and sporangium production. For evaluation of the resistance risk and for determination of baseline sensitivities to the four fungicides, 68 field isolates of *P. litchii* were examined for fungicide sensitivity with a mycelial growth assay. Ranges and means of $EC_{50}$ values (in μg/mL) were 0.047–0.288 and 0.138 for SYP-2815, 0.056–0.992 and 0.148 for ZJ0712, 0.430–7.529 and 2.720 for SYP-1620, and 0.180–1.772 and 0.810 for enestroburin; the distributions were approximately unimodal and skewed. Eight mutants resistant to the three novel QoI fungicides and azoxystrobin were obtained by exposing field isolates to fungicide-amended agar and to ultraviolet irradiation. All eight mutants had a high and stable resistance, and most were as fit or more fit than sensitive isolates. The full length of the cytochrome b gene in *P. litchii* was cloned from both sensitive isolates and resistant mutants, and single-site mutations G142A, G142S, Y131C, or F128S were found in resistant mutants. Molecular docking results also indicate that *P. litchii* could develop resistance to the three novel QoI fungicides, and especially to SYP-1620. Resistance management should be considered for the three novel QoI fungicides.

**P08.048 Detection of benzimidazole resistance in New Zealand *Botrytis* spp. associated with onion neck rot**

*S.L.H. Viljanen-Rollinson, M.I. Khan, M.V. Marroni, S. Keenan, I.A.W. Scott, J. MacKay and S. Bulman*
*The New Zealand Institute for Plant & Food Research Limited, Private Bag 4704, Christchurch 8140, New Zealand*
*Email: suvi.viljanen@plantandfood.co.nz*

Neck rot is a serious disease affecting onion bulbs in most onion-growing areas of the world. It is usually latent during the growing season and harvest, becoming evident only after several weeks of storage. Although onions are affected by several *Botrytis* species, *B. aclada, B. allii* and *B. squamosa* have been indicated as the causal organisms of neck rot. The disease is controlled by the use of fungicides and by cultural methods. Growers in New Zealand reported control failures after using benzimidazole fungicides, and plate assays confirmed that resistant isolates of *Botrytis* spp. were present. We identified the *Botrytis* species in our collection using ITS, IGS and GAPDH sequence data and characterized point mutations in the β-tubulin gene associated with benzimidazole resistance. A new High Resolution Melt (HRM) assay was developed that allowed fast and simple identification of *B. aclada* versus other *Botrytis* in our collection. In addition, allele-specific PCR and HRM assays were developed to identify SNPs associated with the benzimidazole resistance. Resistance to benzimidazole fungicides was seen to segregate largely with the *Botrytis* species: almost all of the *B. aclada* isolates were resistant whereas the other *Botrytis* spp. were sensitive. The assays developed in this study allow rapid screening of *Botrytis* isolates causing onion neck rot, and facilitate improved management decisions, including new resistance management practices.

**P08.049 Detection on the sensitivity of *Botrytis squamosa* in Chinese Chives to four fungicides in Beijing**

*B. Hu[1], Z.Q. Huang[2], X.L. Liu[2], Y.L. Li[1], H. Sun[1], X.Q. Wang[1] and Y.C. Shi[1]*

[1]*Beijing Plant Protection Station, Beijing, 100029, P.R. China;* [2]*Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: hdashuai@126.com*

In 2011 and 2012, a total of 224 strains of *Botrytis squamosa* Walker were sampled from 8 suburban counties of Beijing, and 63 strains were chosen for determination of sensitivities to SYP-Z048, procymidone, difenoconazole and pyrimethanil *in vitro*. The results showed that the $EC_{50}$ values for SYP-Z048 ranged from 0.011 to 0.305 mg/L, the mean $EC_{50}$ value was (0.0914±0.0614) mg/L. The $EC_{50}$ values for procymidone varied from 0.044 to 3.879 mg/L, the most insensitive was 88.16 folds of the most sensitive, with a mean $EC_{50}$ value of (0.2511 ± 0.4820) mg/L. There was a highly resistant strain with 20 folds resistance factors to procymidone appeared in 63 experimental strains. The $EC_{50}$ values for difenoconazole varied from 0.015 to 1.590 mg/L, with a mean $EC_{50}$ value of (0.4351 ± 0.4383) mg/L. The $EC_{50}$ values for pyrimethanil ranged from 0.019 to 80.818 mg/L, and 9 highly resistant strains with more than 100 folds resistance factors appeared among these strains, which suggested appropriate precautions against resistance development should be taken.

### P08.050 Evaluation of fungicides Enestroburin and SYP1620 on their inhibitory activities to fungi and oomycetes and systemic translocation in plant

*P.F. Liu, H.Q. Wang, Y.X. Zhou, Q.X. Meng and X.L. Liu*
*Department of Plant Pathology, China Agricultural University, Beijing 100193, P.R. China*
*Email: seedling@cau.edu.cn*

To evaluate inhibitory activity of QoI fungicides enestroburin and SYP1620 *in vitro* and systemic translocation in planta, bioassays and high performance liquid chromatography (HPLC) analysis were conducted, using well-known azoxystrobin as a reference. All the three test fungicides inhibited the mycelial growth of *Sporisorium reiliana*, *Phytophthora infestans*, *Peronophythora litchi*, and *Magnaporthe grisea*, with effective concentration for 50% inhibition ($EC_{50}$) values ranging from 0.02 to 2.84 μg/ml. For inhibiting mycelial growth, SYP1620 was more effective than azoxystrobin and enestroburin on *Valsa mali*, *Gaeumannomyces graminis*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Alternaria solani*, and *Colletotrichun lagenatium*. The three fungicides were highly effective on biotrophic pathogens tested. Enestroburin and SYP1620 had penetration and spreading in wheat leaves, but the penetration and translocation levels were lower compared to azoxystrobin. HPLC results indicated that the three fungicides were rapidly taken up from solution by wheat roots and transported upwards, with greater fungicide concentrations in roots than in stems and leaves. Therefore, enestroburin and SYP1620 are systemic fungicides that inhibit a broad spectrum of fungi and oomycetes.

### P08.051 Characterization and sexual reproduction of field populations of *Phytophthora capsici* in China during 2006–2012

*Y. Bi, J. Hu, X.L. Cui, X.H. Lu and X.L. Liu*
*Department of Plant Pathology, China Agricultural University, Beijing 100193, P. R. China*
*Email: biyang0620@126.com; seedling@cau.edu.cn*

The isolates had been collected from 28 provinces in China from 2006 to 2012 and had no history of exposure to CAA fungicides. The sensitivities of 403 isolates of *Phytophthora capsici* to the fungicides metalaxyl, dimethomorph and flumorph were determined based on *in vitro* mycelial growth. Most isolates were sensitive to metalaxyl but a few from Hebei, Jiangsu, Gansu, Heilongjiang, and Tibet provinces had intermediate metalaxyl sensitivity. The distribution of the $EC_{50}$ values for dimethomorph and flumorph in *P. capsici* ranged from 0.126 to 0.339 μg/ml or 0.700 to 1.740 μg/ml, respectively. There was no evidence of geographical variation in the sensitivity of *P. capsici* to dimethomorph or flumorph. Both A1 and A2 mating types were detected on the same farms in four provinces (Gansu, Heilongjiang, Shandong, and Jilin) and in 1:1 ratio except in Jilin province. Single oospore progenies were obtained from the sexual hybridization and self-crossing *in vitro*. The 18 progeny derived from hybridization differed in sensitivities to metalaxyl, dimethomorph and flumorph, and also differed in fitness. Further research showed that the resistance of *P. capsici* against dimethomorph was controlled by two dominant genes by analyzing the segregation of the 337 progeny from self-crossing and sexual hybridization. To reduce the development and spread of fungicide resistance in *P. capsici*, we suggest that the possibility of sexual reproduction be reduced and that metalaxyl be alternated with dimethomorph or flumorph.

### P08.052 Identification of *Colletotrichum truncatum* from pepper and their sensitivity to 3 DMI fungicides in China

*Y.Z. Diao, C. Zhang, W.J. Mou, F.P. Chen and X.L. Liu*
*Department of Plant Pathology of China Agricultural University, Beijing Engineering Research Center of Seed and Plant Health, Beijing 100193, P.R. China*
*Email: diaoyongz@163.com;seedling@cau.edu.cn*

Anthracnose is an important disease on pepper in China, which caused by several species of *Colletotrichum*. The control of anthracnose mainly depends on chemical fungicides, especially 14α-demethylase enzymes inhibitors (DMIs), which exhibit high effect, broad spectrum,

systemic and long durable traits. The DMIs prochloraz, difenoconazole, and epxiconazole have been registered in China to control anthracnose in pepper and many other plants. In this study, we collected 260 diseased samples, and one isolate was single-spored from one sample. All the isolates were identified base on molecular, morphology, and multilocus molecular phylogenetic analysis of ITS, Tub2, Actin, GAPDH, CHS-1, and HIS3, which were reported previously as a method for identification of species of *Colletotrichum*. Furthermore, Koch's speculate was performed by inoculating the spore suspension of isolates on pepper fruits, causing similar symptoms as anthracnose in the field. The spores from inoculated peppers were isolated and confirmed as *C. truncatum.* The sensitivity of 73 *C. truncatum* isolates from 20 regions was determined to the three DMIs prochloraz, difenoconazole, and epxiconazole. The sensitivity frequency of all isolates to the three testing fungicides distributed as an unimodal curve, which indicated that there were no reduced sensitivity subgroup among these strains. The range of $EC_{50}$ and mean $EC_{50}$ value were 0.0037-0.1722 μg/ml and (0.0526±0.0295) μg/ml; 0.1916-3.7326 μg/ml and (1.0816±0.7237) μg/ml; 0.0914-5.4569 μg/ml and (1.6516±1.1322) μg/ml, respectively for prochloraz, difenconazole and epxiconazole. The results will be useful for monitoring resistance problem of *C. truncatum* to DMIs in field.

**P08.053 The sensitivity of *Colletotrichum* spp. and *C. acutatum* from pepper to 5 DMI fungicides**

*Y.Z. Diao, C. Zhang, F.P. Chen, J.R. Fan and X.L. Liu*
*Department of Plant Pathology of China Agricultural University, Beijing Engineering Research Center of Seed and Plant Health, Beijing 100193, P. R. China*
*Email: diaoyongz@163.com;seedling@cau.edu.cn*

Anthracnose is an important and heavy disease on pepper and leads to serious losses on pepper production in China, which can be caused by several *Colletotrichum* species. Previous research showed that the anthracnose can be well controlled by 14α-demethylase enzymes inhibitors (DMIs) fungicides. However, no information on the baseline sensitivity to different DMIs has been available. In this study, we aimed to determine the *in vitro* response profiles of different *Colletotrichum* species to 5 DMI fungicides including prochloraz, difenoconazole, tebuconazole, myclobutanil, and epoxiconazole, which were now widely used in China. During 2010 - 2013, a total of 727 strains of *Colletotrichum* species in pepper were collected from 29 provinces, including 373 *C. gloeosporioides* isolates and 354 *C. acutatum* isolates. 90 *C. gloeosporioides* isolates and 79 *C. acutatum* isolates were chosen for determination of sensitivity to five DMIs fungicide-amended PDA petri dishes by colony diameter assay. The results showed that the sensitivity frequency of *C. acutatum* and *C. gloeosporioides* to the five fungicides distributed as an unimodal curve, which indicated there was no resistant subgroup among these strains. Thus, it can be used as baseline sensitivity for field resistance monitoring. The mean $EC_{50}$ values of *C. gloeosporioides* isolates or *C. acutatum* isolates were (0.0370±0.0241) mg/L, (0.0480 ±0.0240) mg/L; (0.5198±0.3573) mg/L, (0.2458±0.1233) mg/L; (0.5268±0.3040) mg/L, (0.2530±0.0596) mg/L; (0.8262±0.3199) mg/L, (0.2687±0.0620) mg/L; (7.6856 ±3.2715) mg/L, (6.3653±2.0093) mg/L, respectively for prochloraz, epoxiconazole, difenconazole, tebuconazole, and myclobutanil. Our data showed that prochloraz was the best fungicide to inhibit mycelium growth of *C. gloeosporioides* and *C. acutatum in vitro;* while myclobutanil was the one with the lowest activity of inhibition.

**P08.054 Frequency distribution of sensitivity of *Ustilaginoidea virens* to four EBI fungicides, prochloraz, difenoconazole, propiconazole, and tebuconazole, and their efficacy in controlling rice false smut in Anhui Province of China**

*Y. Chen[1,2,3], W.X. Wang[1,2,3], Y. Zhang[1,2,3], X. Yang[1,2,3], A.F. Zhang[1,2,3,*] and T.C. Gao[1,2,3,*]*
*[1]Institute of Plant Protection and Aro-products Safety, Anhui Academy of Agricultural Sciences, Hefei 230031, P. R.China; [2]Laboratory of Quality & Safety Risk Assessment for Agro-Products (Hefei), Ministry of Agriculture, P. R. China; [3]Scientific Observing and Experimental Station of Crop Pests in Hefei, Ministry of Agriculture, P. R. China*
*Email: aifangz@163.com; gtczbs@yahoo.com.cn*

False smut, caused by *Ustilaginoidea virens* is an important emerging disease of rice (*Oryza sativa* L.) in China. Up to now, as most varieties with high yielding and good quality are susceptible or even highly susceptible to false smut in most rice growing ecological regions, especially in Anhui Province, chemical control with fungicides would be an important measure for the control of this disease. The ergosterol biosynthesis inhibitor (EBI) fungicides, such as prochloraz, difenoconazole, propiconazole and tebuconazole, are extensively used in China for the control of rice diseases, such as rice sheath blight and rice blast. In this study, a total of 102 *U. virens* isolates (from Anhui Province of China) were tested for their sensitivity to prochloraz, difenoconazole, propiconazole and tebuconazole during the stage of mycelial growth. The $EC_{50}$ ranges of values for prochloraz, difenoconazole, propiconazole and tebuconazole inhibiting mycelial growth of the 102 *U. virens* isolates were 0.04-0.75, 0.04-1.08, 0.04-0.38 and 0.03-0.57 μg/ml, with the average $EC_{50}$ values of 0.32 ± 0.08, 0.45 ± 0.08, 0.19 ± 0.03 and 0.21± 0.06 μg/ml, respectively. These values suggested that the tested *U. virens* isolates were very sensitive to these four EBI fungicides. Results of field trials showed that twice spraying of 3 of the fungicides exhibited higher control efficacy than a single spraying for the control of rice

false smut. Twice spraying of each was better than a single spray for the control of rice sheath blight. Twice spraying of 50% propiconazole EC at 300 g.a.i/ha showed the best control of rice false smut in both two sites during the two consecutive years, 2010 and 2011, with the control efficacy ranging from 71.5% to 74.3%. Sensitivity of the field *U. virens* isolates to EBI fungicides should be monitored. Mixtures, as well as alternation with other fungicides, with different modes of action, should be experimented.

**P08.055 Effect of different doses of fungicide (Mancozeb) against Alternaria leaf blight of tomato in Tunnel**

*A.S. Gondal[1], M. Ijaz[2], K. Riaz[1] and A.R. Khan[3]*
*[1]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan; [2]Barani Agricultural Research Institute, Chakwal, Pakistan; [3]Department of Plant Pathology, King Saud University, Saudi Arabia*
*Email: silentrose77@gmail.com*

Tomato (*Lycopersicon esculentum* Mill.) is an important commercial vegetable of the world. Tomato cultivars cultivated in Pakistan have low level of genetic resistance to *Alternaria* leaf blight disease. Farmers, in pursuance of high yield are inclined to cultivate some varieties which may be less resistant to the disease and rely on fungicide applications for the control of *Alternaria solani*, the casual organism of *Alternaria* blight of tomato. Five tomato varieties (Litah545, Litah514, Eurica, Ti-166 and Astra) were sown in five replications with one standard check in tunnel. Different doses of mancozeb (4 g/L, 8 g/L, 12 g/L and 16 g/L of water) were applied after 7 days intervals. Disease data was recorded after ten days interval from flowering stage to onward. Average yield of each variety was calculated after ten pickings. All fungicide doses reduce the disease severity as compared to untreated check. The highest reduction in the disease was achieved by applying mancozeb 12 g/L of water at an interval of 7, 14, 21 and 28 days. The yield of Litah545 and Litah514 give higher yield as compared to Eurica, Ti-166 and Astra. Overall results revealed that weekly sprays of mancozeb at 12 g/L of water were cost effective and eco-friendly for the management of Alternaria blight of tomato.

**P08.056 Impact of mutations in the SDH gene on the sensitivity to different SDHIs in various pathogens**

*G. Stammler[1], M. Semar[1], K. Klappach[1] and A. Wolf[2]*
*[1]BASF SE, Agricultural Center, Speyerer Strasse; [2]D-67177 Limburgerhof; [2]BASF SE, Specialty Chemicals Research, Carl-Bosch Strasse, D-67056 Ludwigshafen, Germany*
*Email: gerd.stammler@basf.com*

Succinate dehydrogenase (SDH) inhibitors block the fungal respiration by binding to the complex II of the respiratory chain. Several mutations conferring resistance to SDH inhibitors (SDHIs) in the target protein at different positions in three SDH subunits B, C and D were detected in field isolates of plant pathogens such as *Botrytis cinerea, Corynespora cassiicola, Alternaria alternata, Didymella bryoniae,* and *Sclerotinia sclerotiorum* and in laboratory mutants of *Mycosphaerella graminicola.* Even within a single species, different mutations were found at one location (*e.g.* B-P225L,F,T or B-H272Y,R,L in *B. cinerea*), and in different locations in different subunits (*e.g.* B-H277Y, C-H134R, D-H133R in *A. alternata*). Some mutations are part of the binding site with explainable effects on SDHI binding (e.g. in case of B-H272-exchanges) or outside of the binding area which excludes a direct influence on SDHI binding. The impact of the mutation on the resistance level is not correlated with its proximity to the binding site and exchanges at one position can cause different resistance factors (e.g. H272Y,R,L in *B. cinerea*). Pathogen populations in agricultural crops comprise already today a variety of the above mentioned target site mutations. Sensitivity monitoring programs are running for a number of pathogens based on *in vivo, in vitro* and mutation analysis. Diversity of mutations complicates the development of genetic assays and interpretation of sensitivity findings in an unprecedented manner. The high value of SDHIs for farmers worldwide and the complexity of SDHI resistance call for efforts to maintain efficacy of this mode of action.

**P08.057 The usefulness of reduced doses of fungicides in integrated pest management**

*M. Korbas, J. Danielewicz, E. Jajor and J. Horoszkiewicz-Janka*
*Institute of Plant Protection – National Research Institute, Poznan, ul. Wladyslawa Wegorka 20, Wielkopolska, 60-318, Poland*
*Email: j.danielewicz@iorpib.poznan.pl*

Integrated Pest Management (IPM) is an effective and environmentally sensitive approach to pest management that relies on a combination of common-sense practices. Fungi control can be assured successfully under IPM guidelines. Appropriate crop rotation, cultivation of resistance cultivars and harmonious fertilization allowed to reduce potential threats. If the non-chemical methods will not prove to be sufficiently effective, application of fungicides (which the IPM does not exclude) will be needed. The aim of the experiment was to determine the possibility of using lower doses (than those registered in Poland) of fungicides in wheat cultivation. It would give the opportunity to wheat producers to use reduced doses of chemicals (which is fulfill the idea of IPM very well). Winter wheat cultivar Tonacja was tested. The assessments of Fusarium root rot and Eyespot (*Tapesia yallundae*) were performed at full maturity of grains stage

(BBCH 75). The percentage of infected plants (in various degrees) was evaluated. Efficacy of applied fungicides was counted. Yield obtained from experimental plots was analyzed. Using of reduced doses of tested fungicides confirm the effectiveness of chosen active substances in disease control and reveal the usefulness of the lower doses in wheat protection.

**N08.001 Sensitivity of *Botrytis cinerea* to carbendazim from cucumber in Shanxi Province**

*H. Yin[1], J.B. Zhou[1], L. Ren[1, 2] and X.J. Zhao[1]*
*[1]Institute of Plant Protection, Shanxi Academy of Agricultural Sciences, Taiyuan 030032, Shanxi Province, China; [2]College of Agriculture, Shanxi Agricultural University, Taigu 030801, Shanxi, P. R. China*
*Email: zhoujianbo6067@163.com; zhaoxiaojun0218@yahoo.com.cn*

To understand the situation of resistance levels to carbendazim, 147 *Botrytis cinerea* isolates were collected from Shanxi province. The sensitivity was determined by the method of minimal inhibitory concentration. For the carbendazim, the $EC_{50}$ values widely are distributed in the range of 0.0130-6703.4679μg/mL. 57.14% isolates showed the $EC_{50}$ values of *B. cinerea* isolates to carbendazim ranged from 255.7112-1003.1263μg/mL (the $\log_{10}EC_{50}$ values to carbendazim ranged from 2.4-3.0). The average frequency of high resistant (HR) isolates and super high resistant (SR) isolates were 20.41% and 68.71% in Shanxi province. The average $EC_{50}$ values and resistant frequency and resistant level were 808.8028μg/mL and 93.20% in Shanxi province. The highest resistant frequency (100%) in Dadong, Changzhi, Jincheng, Shuozhou where more carbendazim had been used compared with other regions. This suggests that the higher resistant of *B. cinerea* to carbendazim from cucumber were generally developed in Shanxi province. Resistant isolates became dominant populations.

## Concurrent Session 9-Climate Change and Plant Diseases What Have We Learnt in 20 Years

### O09.001 Arable crop disease control, climate change and food security

*B.D.L. Fitt*[1], *N. Evans*[2], *P. Gladders*[3], *D.J. Hughes*[1] *and J.S. West*[4]
[1]*University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB, UK;* [2]*Weather INnovations Incorporated, 75 High Street North, Stewkley, LU7 0EZ, UK;* [3]*ADAS Boxworth, Battlegate Road, Boxworth, Cambridge CB23 4NN, UK;* [4]*Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK*
*Email:b.fitt@herts.ac.uk*

Global food security is threatened by crop diseases that account for average yield losses of 16%. Climate change is exacerbating threats to food security in much of the world, emphasising the need to increase food production in northern European countries such as the UK. However, to mitigate climate change, crops must be grown so as to minimise greenhouse gas emissions (GHG); results with UK oilseed rape demonstrate how disease control in arable crops can contribute to climate change mitigation. However, work examining impacts of climate change on UK epidemics of winter oilseed rape diseases illustrates unexpected, contrasting impacts of climate change on complex plant-disease interactions. In England, phoma stem canker is expected to become more severe whilst light leaf spot is expected to become less severe. Such work can provide guidance for government and industry planning for adaptation to impacts of climate change on crops to ensure future food security.

### O09.002 Realistic experimental approaches to climate change impact assessment and adaptation

*S. Chakraborty*
*CSIRO Plant Industry, Queensland Bioscience Precinct, 306 Carmody Road, St Lucia, Queensland 4067, Australia*
*Email: sukumar.chakraborty@csiro.au*

There has been a surge in interest on climate change impact on plant pathogens. But systemic issues have constrained the advancement of knowledge. For instance, research has ignored processes such as long distance dispersal of inoculum and host-pathogen adaptation at spatio-temporal scales relevant to climate change. Together with a lack of consideration of technological advances and human interventions, this has led to uncertainties in predictions of future impacts. This presentation argues that consistent findings from realistic empirical research are the foundations for accurate predictions. Only a handful of studies have used realistic field conditions, none has considered factorial combinations of 2 or more climate variables and all have used abrupt $CO_2$ increases known to overestimate community response. The fragmented and context dependent findings on pathogen biology have not been synthesised to improve understanding. Citing examples of realistic empirical studies, this presentation will offer guidelines to improve useful knowledge. Published and unpublished findings will be used to illustrate approaches and strategies to generate new and useful knowledge. The concept of fitness will be introduced to synthesise knowledge and to analyse pathogen strengths and vulnerabilities under climate change. Pathogens will follow migrating host communities to migrate and/or evolve as they adapt to climate change. Changed geographic distribution will potentially bring together diverse lineages that do not share a common ecological niche. A brief review of research will be presented on host-pathogen adaptation to summarise current understanding. Strategies and priorities for empirical research and important challenges and opportunities will be highlighted.

### O09.003 Climate change and virus diseases: vector interactions and epidemiological implications

*A. Fereres*
*Instituto de Ciencias Agrarias, ICA, CSIC, Spain*
*Email: a.fereres@csic.es*

Evidence for global warming includes observed increases in global average air and ocean temperatures that are being accelerated by human activities, especially those rising from greenhouse gas emissions, such as carbón dioxide ($CO_2$), methane and nitrous oxide. Plant viruses are one of the most yield-liming factors in agriculture leading to extensive crop losses worldwide. It is Increases in temperatures and concentration of $CO_2$ among other biotic factors have a strong impact not only in plant viruses themselves (replication, cell to cell movement, symptom expression, etc…) but also in their host plants, insect vectors and virus-vector interactions. More than 70% of plant viruses are transmitted by insects. Aphids, whiteflies and thrips have very short generation times and high fecundity (r-strategists) and are the most important vectors of plant viruses. All these vectors react very fast to any changes in temperature and other biotic factors, which may have clear implications under climate change scenarios. Changes in the abundance, phenology and distribution of certain insect vectors will have a clear impact in virus disease epidemics. For example, a slight increase in winter temperature will alter aphid peak densities and more importantly, aphid flights will occur earlier in the season increasing the risk of virus outbreaks. Changes in temperature will also affect the transmission efficiency by their insect vectors. For example, the transmission of pea enation mosaic virus in pea crops is very dependent of its efficiency of transmission by its vector, the pea aphid. This transmission was considerably greater at 20 and 30 ℃ than at 10 ℃. An increase in temperature will also increase the proportion of alate (winged) morphs. This has been shown for the lettuce

aphid Nasonovia ribisnigri that increased the proportion of winged morphs from 7% at 16℃ to over 40% at 20℃. Winged morphs have higher migratory ability and, and hence higher potential to move plant viruses over long distances. The impact that climate change may have on the incidence and spread of hemipteran-borne plant viruses, its potential effects on virus-vector-plant interactions, as well as other operating processes will be identified and discussed.

**O09.004 Climate change and plant biosecurity–implications for policy**

*J. Luck[1], I.D. Campbell[2], R. Magarey[3], S. Isard[4], J.P. Aurambout[5] and K. Finlay[1,6]*

*[1]Plant Biosecurity Cooperative Research Centre for National Plant Biosecurity, Innovation Centre, University of Canberra, Bruce, 2617, Australian Capital Territory, Australia; [2]Integrated Natural Resources Division, Agriculture and Agri-Food Canada, 1341 Baseline Rd., Ottawa, ON, Canada, K1A 0C; [3]North Carolina State University and Co-operator with USDA-APHIS-PPQ-CPHST PERAL 1730 Varsity Drive, Suite 300, Raleigh, NC, 27606; [4]Departments of Plant Pathology and Meteorology, Penn State University, 205 Buckhout Lab, University Park, PA 16802 USA; [5]Biosciences Research Division, Department of Primary Industries Victoria PMB 15 Ferntree Gully Delivery Centre 3156, Victoria, Australia; [6]Future Farming Systems Research, Department of Primary Industries Victoria, PO Box 4166, Parkville, 3052, Victoria, Australia*

*Email: j.luck@pbcrc.com.au*

Projected future climates, such as increasing temperature, increasing atmospheric $CO_2$, altered precipitation patterns and increases in the frequency of climatic extremes are likely to influence the entry, establishment and spread of invasive pests and pathogens. Climate change will require a revision of current biosecurity policies and practices, such as preparedness and prevention planning, containment techniques, surveillance response, incursion management and pest risk analyses. We provide a comprehensive review of the climate change events affecting the biology and distribution of species that represent a biosecurity threat to agricultural and forestry production. Using two case studies, the Asiatic Citrus Psyllid (*Diaphorina citri* Kuwayama) and Asian Soybean Rust (*Phakopsora pachyrhizi* Sydow and P. Sydow), we explore ways that climate change will affect invasive species and describe how biosecurity agencies can use this knowledge to minimise the spread of invasive pests and pathogens. Armed with the knowledge of the likely effects of climate change on the biology of organisms and their geographical distribution, we examine the implications to existing biosecurity policy. Gaps in research that will address the effects of climate change on plant biosecurity are also outlined, such as (i) the use of atmospheric transport models to predict the movement of species during major storm events; (ii) identification and prioritisation of new and existing pest threats; (iii) identification and documentation of pest status changes and potential new interactions that may occur with new cropping regimes; (iv) development of guidelines for incorporating climate change scenarios and their implications to pest risk analyses.

**O09.005 Impact of climate change variables on diseases of chickpea and pigeonpea in semi-arid tropics**

*M. Sharma and S. Pande*

*International Crops Research Institute for the Semi-Arid Tropics Patancheru, 502324, Andhra Pradesh*

*Email: mamta.sharma@cgiar.org*

Grain legumes primarily grown in rainfed systems will be most affected by increasing climate variability, particularly erratic rainfall patterns, high temperatures and elevated carbon dioxide. Chickpea and pigeonpea are the two major grain legumes that are largely grown in the rainfed environments in India. Analysis of long-term weather and disease data sets for last four decades in India indicated emergence of new diseases and shift in the geographical distribution of diseases/pathogens in chickpea and pigeonpea. A clear cut shift in soil borne diseases of chickpea was identified and dry root rot (DRR) caused by *Rhizoctonia bataticola* was found as a potentially emerging constraint to chickpea production than wilt. In pigeonpea, increasing frequency of Phytophthora blight (*Phytophthora drechsleri* f. sp. *cajani*) was recorded. Also the occurrence of minor diseases like Alternaria blight is being reported from places where it was not a problem earlier. Changed scenario of pathogen races has also been seen and the multirace scenario with in a region has been observed. This suggests that climate change is altering the spectrum of diseases in terms of pathogen distribution and virulence. The impact of high temperature and low moisture was investigated in detail on the dry root rot and Phytophthora blight diseases. Also the impact of elevated $CO_2$ levels (350, 550 and 750ppm) was studied on the Phytophthora blight of pigeonpea under the facilities established at ICRISAT, Patancheru (open top chamber and free air $CO_2$ enrichment). Detailed investigations to understand the impact of climate change on chickpea and pigeonpea diseases and devising methodologies to mitigate it with host plant resistance is underway.

**O09.006 Effects of experimental warming on the life cycle of economically important pathogens in oilseed rape**

*M. Siebold and A. von Tiedemann*

*Department of Crop Sciences, Division of Plant Pathology and Crop Protection, Georg-August-University of Göttingen, Grisebachstr. 6, D-37077 Göttingen, Germany*

*Email: msiebol@gwdg.de*

Rising temperatures due to global warming will influence both crop and fungal pathogen development in the future. Within the research framework KLIFF (Climate Change Research in Lower Saxony, Germany), potential effects of rising air and soil temperatures on soil- and plant debris-borne life cycle stages of the economically important oilseed rape pathogens *Phoma lingam*/*Leptosphaeria maculans, Sclerotinia sclerotiorum* and *Verticillium longisporum* were investigated experimentally utilizing climate chambers and a field soil warming facility. Treatments reflected warming scenarios for Lower Saxony, Germany, by 2050 (mid term) and 2100 (long term) as projected by regional climate models. Investigations included (1) development of Phoma crown canker in spring (field only), (2) apothecia production of *S. sclerotiorum* in spring and (3) colonization of winter oilseed rape by *V. longisporum*. Results of two climate chamber experiments and the two field growing seasons 2010/11 and 2011/12 showed that oilseed rape growth and development responded linearly to increasing temperatures with an average flowering advance of 7 days per 2 ℃ warming. Development of phoma crown canker in the field showed large variation in response to the warming treatments with no clear trend towards rising temperatures. Maximum germination of *S. sclerotiorum* sclerotia was 4 to 7 days earlier under a 2 ℃ temperature increase, potentially advancing the oilseed rape infection window in the future. *V. longisporum* colonization of the plants was advanced in warmer chambers and plots, which may lead to higher inoculum densities in the soil after harvest and an increased importance of this pathogen under future warming in Germany.

### O09.007 Effect of different atmospheric $CO_2$ levels on resistance of Arabidopsis against *Pseudomonas syringae*

*Y.L. Zhou, C.M.J. Pieterse and S.C.M. Van Wees*
*Plant-Microbe Interactions, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands*
*Email: Y.Zhou1@uu.nl*

The atmospheric $CO_2$ concentration has been rising at an accelerating pace since the Industrial Revolution. The influences of different atmospheric $CO_2$ levels on plant diseases vary significantly, depending on plant species, pathogens and research conditions. In general, stomata close in response to elevated $CO_2$ concentration, while they open under low $CO_2$ conditions. Using our $CO_2$-level-controlled climate chambers, we set out to study the effect of different atmospheric $CO_2$ levels on resistance of Arabidopsis to the foliar pathogen *Pseudomonas syringae pv tomato* DC3000 (*Pst*) that enters the plant through stomatal openings. Infection with *Pst* resulted in stomatal closure at 1 h after dip inoculation at all three $CO_2$ conditions tested (high (750ppm), ambient (450ppm), and low (150 ppm)). At 4 h, the stomata reopened by the action of coronatine (COR), a virulence factor produced by *Pst*, as was reported previously. Interestingly, this reopening occurred only in plants grown under ambient and high $CO_2$ conditions, but not when the plants grew under low $CO_2$ conditions. Different stomatal responses were observed when plants grew under ambient $CO_2$ condition first and were then transferred to low $CO_2$ at the same time as inoculation with *Pst*: stomata did reopen, which even occurred independently of COR. This indicates that different mechanisms of *Pst*-controlled stomatal regulation are engaged in guard cells when exposed to low $CO_2$ for different duration. Bacterial growth over a 4-day time interval was reduced in the low $CO_2$ treated plants compared to ambient-grown plants, which was independent of duration of low $CO_2$ exposure, suggesting that additional mechanisms besides stomatal responses contribute to the enhanced resistance level.

### O09.008 Spatial modelling of rice yield losses due to bacterial leaf blight and leaf blast in a changing climate

*A.H. Sparks , C. Duku, J. Aunario, M. Noel and S.J. Zwart*
*International Rice Research Institute (IRRI), DAPO Box 7777, Philippines*
*Email: a.sparks@irri.org*

To enhance Africa's rice production and reduce reliance on imports, rice yields per hectare need to be raised significantly, despite climate change. Major determinants of the current low yield levels per hectare (average of about 1 t ha-1 in upland ecologies, 1.5 to 2 t ha-1 in rainfed lowland ecologies and 3.0 to 4.0 t ha-1 in the irrigated ecologies) are biotic stresses, in particular two major rice diseases: blast and bacterial leaf blight, whose effect on rice growth and yield are very much affected by weather conditions, in particular air temperature and relative humidity. We linked two models, EPIRICE and RICEPEST, to generate spatial estimates of disease severity and yield losses for three countries in Africa, Rwanda, Tanzania and Uganda. The EPIRICE model models disease severity based upon weather data. The RICEPEST model simulates rice yield losses due to bacterial leaf blight and leaf blast (as well as other rice pests) under a range of specific production situations (e.g. crop establishment method, water and nutrient management). The objective of the study was to map and quantify the impact of climate change as forecasted by the Intergovernmental Panel on Climate Change (IPCC) on rice yield loss as result of bacterial leaf blight and rice leaf blast in Tanzania, Rwanda and Uganda. We found that bacterial leaf blight appears to be more of a biotic constraint in the future under three emission scenarios, A1B, A2 and B1, than leaf blast.

**P09.001 Increasing carbon dioxide and temperature effect on powdery and downy mildew of grapevine under controlled environment**

M. Pugliese[1,2], M.L. Gullino[1,2] and A. Garibaldi[1]
[1]Agroinnova – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy; [2]Disafa – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy
Email: massimo.pugliese@unito.it

The pathosystems grapevine (*Vitis vinifera)* – downy mildew (*Plasmopara viticola*) and powdery mildew (*Eysiphe necatrix*) were chosen as models to assess the potential impact of increased $CO_2$ and temperature on disease incidence and severity under controlled environment. Grapevine potted plants were grown in phytotrons under 4 different simulated climatic conditions: (1) standard temperature (ranging from 18 ° to 22 ° C) and standard $CO_2$ concentration (450 ppm); (2) standard temperature and elevated $CO_2$ concentration (800 ppm); (3) elevated temperature (ranging from 22 ° to 26 ° C, 4 ° C higher than standard) and standard $CO_2$ concentration; (4) elevated temperature and $CO_2$ concentration. Each plant was inoculated with a spore suspension containing $5x10^5$ cfu/ml. Disease index and physiological parameters (chlorophyll content, fluorescence, assimilation rate) were assessed. Results showed an increase of the chlorophyll content with higher temperatures and $CO_2$ concentration, to which consequently corresponded an higher fluorescence index. Disease incidence of downy mildew increased when both $CO_2$ and temperatures were higher, while an increase in $CO_2$ did not influenced powdery mildew incidence, probably due to the increased photosynthetic activity of plants under such conditions. Considering that the rising concentrations of $CO_2$ and other greenhouse gases will lead to an increase in global temperature and longer seasons, we can assume that this will allow more time for pathogens evolution and could increase pathogen survival, indirectly affecting downy and powdery mildews of grapevine.

**P09.002 Increasing carbon dioxide and temperature effect on black spot of basil and alternaria leaf spot of rocket salad under controlled environment**

M. Pugliese[1,2], E. Cogliati[1], G. Gilardi[1], M.L. Gullino[1,2] and A. Garibaldi[1]
[1]Agroinnova – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy; [2]Disafa – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy
Email: massimo.pugliese@unito.it

The pathosystems basil (*Ocimum basilicum*) - black spot (*Colletotrichum gloeosporioides*) and rocket (*Eruca vesicaria* subsp. *sativa*) – Alternaria leaf spot (*Alternaria japonica*) were chosen as models to assess the potential impact of increased $CO_2$ and temperature on disease incidence and severity under controlled environment. Potted plants were grown in phytotrons under 4 different simulated climatic conditions: (1) standard temperature (ranging from 18 ° to 22 ℃) and standard $CO_2$ concentration (400 ppm); (2) standard temperature and elevated $CO_2$ concentration (800 ppm); (3) elevated temperature (ranging from 22 ° to 26 ℃, 4 ℃ higher than standard) and standard $CO_2$ concentration; (4) elevated temperature and $CO_2$ concentration. Each plant was inoculated with a spore suspension containing $1x10^5$ cfu/ml of the pathogen. Disease incidence and severity were assessed 7, 14 and 21 days after inoculation. Basil plants grown at 800 ppm of $CO_2$ showed increased black spot symptoms compared to 400 ppm. Increasing $CO_2$ to 800 ppm showed a clear increment in the percentage of Alternaria leaf spot on rocket leaves compared to standard conditions. Disease incidence and severity were always influenced by the combination of rising $CO_2$ and increased temperature, compared to standard conditions (400 ppm of $CO_2$ – 22 ℃) for both pathosystems. Considering the rising concentrations of $CO_2$ and global temperature, we can assume that an increased severity of *Alternaria japonica* on rocket and *Colletotrichum gloeosporioides* on basil is expected in the future.

**P09.003 Effects of the four root rot pathogens on two pea (*Pisum sativum* L.) varieties in controlled conditions**

J. Bacanovic, A. Šišić, C. Bruns and M.R. Finckh
Department of Ecological Plant Protection, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen. Germany
Email: bacanovic@uni-kassel.de

Foot rot is an important limiting factor in pea production in Germany. The disease is caused by pathogens belonging to the Ascochyta complex (*Mycosphaerella pinodes*, *Phoma medicaginis* and *Ascochyta pisi*) and *Fusarium* spp. Climate change scenarios predict an increase in winter precipitation and temperatures. These conditions will favor soil borne pathogen survival rate and population build up. On the other hand, winter pea production will become more common. Little data are available about susceptibility of winter pea varieties to predominant soil borne pathogens. The present study was carried out to evaluate the susceptibility of the commonly used winter pea variety EFB33 to different isolates of *Fusarium avenaceum*, *F. solani* f. sp *pisi*, *M. pinodes* and *P. medicaginis* under controlled conditions in sterile sand compared to the spring pea variety Santana. Three weeks after sowing and inoculation disease symptoms were assessed and plant growth parameters measured. All of the tested pathogens resulted in disease. *F. avenaceum* was the most aggressive pathogen causing severe wilting symptoms on both varieties. Reductions in fresh weight per unit of external tissue damage on Santana were significantly different among pathogens with 12.5% for *F. avenaceum*, 8.8% for *P. medicaginis*, 8.4% for *M. pinodes* and 3.8% for *F. solani*,

respectively. Overall, EFB33 was less susceptible. Fresh weight reductions for *F. avenaceum* were 15.8% per unit tissue damage. There were no significant differences in aggressiveness between the three other pathogens.

**P09.004 Effects of compost application on pathogen presence and frequencies in the crop rotation winter pea-maize-winter wheat in organic agriculture**

*J. Bacanovic, C. Bruns, J.H. Schmidt and M.R. Finckh*
*Department of Ecological Plant Protection, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen. Germany*
*Email:bacanovic@uni-kassel.de*

The key for the success of the rotation winter peas for biomass - maize – winter wheat is the ability of peas to fix nitrogen which is highly dependent on pea health. However, little is known about the importance and specificity of pathogens affecting winter peas in the German climate. There are open questions about the role of peas as alternative host for mycotoxin producing Fusaria of cereals. Field experiments were carried out from 2009 to 2012 to assess the pathogens in the system and potential of composts to improve system performance. Winter peas were either untreated or inoculated with *Phoma medicaginis*, in the presences or absence of yard waste compost (YWC) at rate of 5 t dry matter $ha^{-1}$. A second application of YWC was made to the winter wheat. *Fusarium* ssp. were isolated and identified from the roots and stem base of all crops. In addition, the Ascochyta complex pathogens on peas were identified. Pathogen occurrence was highly variable across the experimental field and among years. More than 15 different Fusarium species were isolated from maize and wheat. Among them *F. avenaceum* was isolated also from pea. Overall frequencies of Fusarium spp. on peas were highest in 2012, while on maize and winter wheat it was in 2011. It appears that higher winter temperatures combined with lower rainfall favored *P. medicaginis* on peas over other pathogens in 2011 and 2012 in comparison to 2010. Application of composts overall stabilized crop performance but it did not lead to yield increases.

**P09.005 Diversity and global significance of irrigation pathogens under a changing climate**

*C. Hong*
*Virginia Polytechnic Institute and State University, Virginia Beach, VA 23455, USA*
*Email: chhong2@vt.edu*

Plant pathogens in irrigation water were recognized as a significant crop health issue in the early 20th century and this issue has since increased greatly in its scope and degree of impact. Today these irrigation pathogens affect not only plant biosecurity, but also water and environmental sustainability. For example, the potential risk of pathogen accumulation and redistribution is a significant deterrent to adoption of water recycling practices that capture nutrient-rich agricultural runoff in containment basins then reuse it for irrigation. These practices are crucial to reducing the environmental footprint of crop production and ensuring agricultural water security in light of global water scarcity. Their significance is further magnified by changing climate. This presentation aims to chart an integrated strategy to these highly interconnected challenges by focusing on irrigation pathogen mitigation. Specifically, the diversity of plant pathogens including bacteria, fungi, nematodes, oomycetes, and viruses found in water to date will be reviewed. Their importance to crop health, water supply and pollution prevention will be assessed using ornamental horticulture industry in the United States as an example. Available resources to help farmers, policy-makers, and the public understand the scope of impact and complexity of these issues also will be discussed. In addition, the areas of future studies that enable farmers to capture and reuse agricultural runoff water without recycling and spreading plant pathogens will be highlighted.

**P09.006 Climate change increases risk of fusarium ear blight on wheat in central China**

*X. Zhang[1,2,3,4], J. Halder[5], R.P. White[3], D.J. Hughes[1], Z. Ye[2], C. Wang[6], R. Xu[7], B. Gan[8] and B.D.L. Fitt [1,3]*
*[1]University of Hertfordshire, Hatfield, Hertfordshire, AL10 9AB, UK; [2]Elementary Education College, Chongqing Normal University, Chongqing, 400700, P. R. China; [3]Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK; [4]Southwest University, Chongqing, 400715, P. R. China; [5]School of Public Health, Imperial College, London, W2 1PG, UK; [6]Hadley Centre, Met Office, Meteorology Building, University of Reading, Reading, RG6 6BB, UK; [7]Hubei Plant Protection Station, Wuhan, Hubei, 430070, P. R. China; [8]Crop Research Institute, Anhui Academy of Agricultural Sciences, Hefei, Anhui, 230000, P. R. China*
*Email: b.fitt@herts.ac.uk*

In this work, a logistic weather-based regression model for estimating incidence of wheat fusarium ear blight in central China was developed, using up to 10 years of disease, anthesis date and weather data available for 10 locations in Anhui and Hubei provinces. In the model, the weather variables were defined with respect to the anthesis date for each location in each year. The model suggested that incidence of fusarium ear blight is related to number of days of rainfall in a 30-day period after anthesis and that high temperatures before anthesis increase the incidence of disease. Validation was done to test whether this relationship was satisfied for another

five locations in Anhui province with fusarium ear blight data but no nearby weather data, using weather data generated by the regional climate modelling system PRECIS. How climate change may affect wheat anthesis date and fusarium ear blight in central China was investigated for period 2020-2050 using wheat growth model Sirius and climate data generated by PRECIS. The projection suggested that wheat anthesis dates will generally be earlier and fusarium ear blight incidence will increase substantially for most locations.

**P09.007 Effect of climate change on the incidence and severity of blast and brown spot disease of rice in southwest Nigeria**

*D.B. Olufolaji*
*Dept. of Crop,Soil and Pest Management.TheFederal University of Technology, Akure, Nigeria*
*Email: tundefolaji022@gmail.com*

Rice (*Oryza sativa.* L) being a leading economic grain crop in Nigeria, suffered yield reduction due to diseases. Studies were carried out to ascertain the impact of climate change on the effect of two diseases on rice production in the southwest of Nigeria in order to plan for possible disease management. Incidence and severity of rice blast and brown spot diseases were studied on the farms in the ecological zone with special reference to variations in the now-a-days irregularities in climatic entities like, rainfall, humidity, wind speed, and temperatures patterns. It was observed in January 2011 when the rains are scanty and relative humidity low, (15.2mm and 64%) and the wind speed and the temperatures are low, (25kph and 21°C), low epidemics of both blast and brown spot diseases in terms of incidence and severity were 32.56%, 3.4 and 38.21%, 3.2 respectively. The yield then was also 2.4 and 2.1 t/ha respectively. When the rains were heavy and the humidity high as well as high temperatures – and high wind speed – in June, July August the two diseases picked up and caused significant ($P \leq 0.05$) damage to rice since the incidence and severity average for the three months were 58.63%, 4.5 and 60.12, 4.7 for blast and leaf spot diseases respectively. In the year 2012, when climate entities were altered the diseases incidence and severity also changed to align with high humidity, rainfall, temperature, wind speed, diseases increased and yield reduced considerably at un-expected period thus leading to slight reduction in rice availability in Nigeria. Importation thus increased and foreign reserves thus deplete as well.

## Concurrent Session 10-Disease Management in the Organic Farming System

### O10.001 Biological soil disinfestation: A practical and fundamental approach for the control of soil borne pests, using renewable resources

*A. Gamliel*
*Institute of Agricultural Engineering, Agricultural Research Organization (ARO) Volcani Center, Bet Dagan, 50250 Israel*
*Email: agamliel@agri.gov.il*

The use of available organic material, animal manures, composts, plant residues, and other as soil amendments can be an effective approach to managing soilborne pests. The combination of organic amendments with soil solarization can further improve pest control. The term "biofumigation" has often been used to describe the incorporation of organic amendments, reflecting the mode of action of active volatile compounds which are generated in the soil. This is not the case, however, since many different chemical and biological mechanisms are involved with organic amendments, beyond the active volatile compounds. These processes occur during the decomposition of organic material, and act directly and indirectly on pathogen survival. Moreover, organic amendments may result in the evolution of "soil suppressiveness", *i.e.* low disease incidence and severity, in the presence of a potent pathogen and a susceptible host. We demonstrated the evolution of soil suppressiveness against *Fusarium oxysporum* f. sp. *radicis-cucumerinum* following its amendment with dried leaves and stems of wild rocket (*Diplotaxis tenuifolia*). Soil suppressiveness is not pathogen-specific, as we demonstrated that amending soil with various crop residues, induced soil suppressiveness to the root knot nematode *Meloidogyne javanica* which was introduced into the soil after treatment, and reduced galling index in subsequently grown tomato, basil or snapdragon crops. The adoption of organic amendments as a disease managing approach has been extended to a broad spectrum of cropping systems. Improvements are continuously made by providing consistent and reproducible results, and by formulating organic material as commercial products (as with chemical pesticides) for maximal efficacy and consistency.

### O10.002 Bottlenecks and successes in fungal disease management of organic stone fruit production: epidemiology, forecasting and control

*I.J. Holb*
*Institute of Horticulture, University of Debrecen, H-4015 Debrecen, P. O. Box 36.Hungary*
*Email: holbimre@gmail.com*

Interest has turned from conventional to organic production as environmental considerations are becoming increasingly important. Management practices of organic production differ from those in conventional production. Only natural products are permitted in organic production according to IFOAM standards. Synthetic products are banned in organic production. As a result, disease control is less effective than in integrated or conventional production with the consequence that epidemics are likely to be more serious in such a system. This lecture will discuss bottlenecks and successes of disease management in organic stone fruit production and will provide current management options against key fungal diseases of stone fruit crops in the growing season. Then epidemics of fungal diseases and development of fungal disease management in stone fruit species will be demonstrated by focusing mainly on management practices against brown rot and cherry leaf spot. This will include e.g. epidemic features of brown rot and leaf spot in organic cherry and peach orchards, a risk of blossom and fruit brown rot epidemics in organic stone fruit orchards, possible control strategies in organic production systems, efficacy and phytotoxicity of approved fungicidal products and appropriateness of various sanitation practices in organic stone fruit production as well as the role of resistant *vs*. susceptible cultivars in disease epidemiology and management of organic stone fruit orchards. Based on above examples, a theoretical and practical DSS approach and future trends in fungal disease management will be provided for organic stone fruit orchards based on mechanical, agro-technical, biological and chemical control options.

### O10.003 Postharvest disease management in organic farming: integration of strategies

*D. Spadaro, A. Garibaldi, M.L. Gullino*
*AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*
*Email: davide.spadaro@unito.it*

Many strategies have been developed to control postharvest decays on various organic fruit and vegetables. They include good storage practice, such as low temperatures of storage, modification of relative humidity and atmosphere, and good hygienic practice. Biological control using antagonists has emerged as one of the most promising strategies. Several biocontrol agents have been widely investigated against different pathogens and fruit crops. Many biocontrol mechanisms have been suggested to operate on fruit including competition, biofilm formation, production of diffusible and volatile antibiotics, parasitism, induction of host resistance. Essential oils are gaining increasing interest due to their volatility, relatively safe status, wide acceptance by consumers, ecofriendly and biodegradable properties. Application of essential oils is an attractive method due to their bioactivity in the vapour phase and the limitation of aqueous sanitation for many commodities, make them useful as possible fumigants. Heat treatments have been

considered as a promising prestorage methods of postharvest control of decay. The beneficial effect of prestorage hot water immersion treatment to prevent rot development has been shown in numerous fruit. Thermotherapy has a number of advantages which include relative ease of use, short treatment time, reliable monitoring of fruit and water temperatures, and killing of skin-borne decay-causing agents. None of these methods used alone provided satisfactory levels of decay control, although some of them were useful when applied in combination, resulting in additive or even synergistic levels of decay control, in an integrated vision of disease management.

**O10.004 Crop management to minimize the risk of plant-associated enteric bacteria in organic production systems**

A.H.C. van Bruggen[1], G. Gu[2], J.M. Cevallos-Cevallos[3] and A.C.Wright [4]
[1]*Emerging Pathogens Institute and Department of Plant Pathology, University of Florida, Gainesville, FL 32611, United States;* [2]*Eastern Shore AREC, Virginia Tech University, Painter, Virginia, United States;* [3]*Centro Investigaciones Biotecnologicas del Ecuador, Guayaquil, Guayas, Ecuador; Food Science and Human Nutrition Dept., University of Florida, Gainesville, FL, United States*
*Email: ahcvanbruggen@ufl.edu; ggu@epi.ufl.edu; jmceva@espol.edu.ec; acw@ufl. edu*

Outbreaks of enteric pathogens have been traced back to contaminated vegetables. *E. coli O157:H7* and *Salmonella enterica* can enter into crop plants through natural openings or wounds in roots or leaves. Recently, we investigated effects of soil management and microbial diversity on ingress and survival of *S. enterica* Typhimurium in tomato plants. Tomato plants grown in conventional or organic soils were inoculated with GFP-labeled *S. enterica* Typhimurium suspension ($10^9$ CFU/ml) by leaf dipping or through guttation droplets. Endophytic bacterial communities were characterized by PCR-DGGE before and after inoculation. *Salmonella* entered tomato leaves through stomates and hydathodes and sometimes moved into the vascular system. More *Salmonella* survived in plants grown in conventional than in organic soil ($P<0.05$). This was related to higher bacterial diversity, Ca and Mg contents in organically managed plants. All contaminated fruits (1%) were from tomato plants grown in conventional soils. About 5% of seeds from infested conventional fruits were contaminated. We also investigated if irrigation ponds could be sources of *Salmonella*, and determined splash dispersal of GFP-labeled *Salmonella* from water onto tomato plants and into tomato leaves and fruits. *Salmonella* was isolated from each of 10 ponds, depending on oxygen content, eutrophication level and temperature. Splash dispersal was enhanced when soil was covered by plastic compared to bare soil or natural mulch. The risks of enteric pathogens in organic production systems can be minimized by enhancing soil health and plant microbial diversity, by using natural mulch, and by minimizing nutrient run-off and maximizing oxygenation in irrigation ponds.

**O10.005 Towards the production of healthy organic rooibos (*Aspalathus linearis*) seedlings**

S.C. Lamprecht[1], Y.T. Tewoldemedhin[1] and J.A. Brand[2]
[1]*ARC-PPRI, Private Bag X5017, Stellenbosch, 7599, South Africa;* [2]*Rooibos Ltd, PO Box 64, Clanwilliam, South Africa*
*Email: lamprechts@arc.agric.za*

Rooibos (*Aspalathus linearis*) is an indigenous legume crop grown commercially in the Western Cape province of South Africa primarily for the production of rooibos tea. Damping-off is the most important soilborne disease of organic rooibos (*Aspalathus linearis*) seedlings. The disease is caused by a complex of pathogens, including species of *Fusarium*, *Pythium* and *Rhizoctonia* and can cause losses of 50% or more in organic rooibos nurseries. In this study we evaluated the use of compost and rotation crops as part of an integrated management strategy against damping-off. Six composts at three soil incorporation rates (25, 50 and 75 % v/v) were evaluated under glasshouse conditions for their effect on seedling survival in naturally-infested soil. Four of the composts significantly improved survival and higher survival rates were recorded at higher compost application rates. Beginning in 2011, field trials have been conducted annually to determine the most effective compost, rotation crops (*Brassica juncea*, lupin, oat, rye triticale and vetch) and time of compost application prior to planting for control of diseases incited by soilborne pathogens in rooibos nurseries. In 2011 and 2012, application of compost significantly improved seedling survival compared to soil without compost. Rotation crops did not significantly affect seedling survival rates or growth in 2011, and in 2012 the most effective rotation crop (triticale) did not significantly improve survival compared to the control. Our findings indicated that compost amendment can be an important component of an integrated management strategy for control of damping-off in organic rooibos nurseries.

**O10.006 Evaluating biopesticides for managing diseases in vegetable and herb crops**

M.T. McGrath
*Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Long Island Horticultural Research & Extension Center, 3059 Sound Avenue, Riverhead, NY, 11901, USA.*
*Email: mtm3@cornell.edu*

Replicated experiments are being conducted routinely to

evaluate efficacy of products for diseases occurring naturally on field-grown crops in Riverhead, New York. Most experiments have been conducted in research fields dedicated to organic production. Diseases that have been investigated are powdery mildew and downy mildew of cucurbits, Septoria leaf spot and powdery mildew of tomato, downy mildew of basil, and Phytophthora blight of cucurbits. These are diseases that occur commonly and often are difficult to manage with cultural practices alone, leading to diminished yield and/or fruit quality. Products evaluated include Actinovate (*Streptomyces lydicus*), Kaligreen (potassium bicarbonate), Mildew Cure (cottonseed oil, corn oil, garlic extract), Milstop (potassium bicarbonate), Organocide (sesame oil), Oxidate (hydrogen dioxide), Regalia (extract of *Reynoutria sachalinensis*), Serenade (*Bacillus subtilis*), Serenade Soil, Sonata (*Bacillus pumilus*), Sporatec (rosemary, clove, and thyme oils), Trilogy (neem oil), and Timorex Gold (tea tree oil). They are being tested alone and in combination programs with other products, in particular copper. Products in development are also being tested. Almost all products evaluated were effective for powdery mildew diseases. Other diseases have proven more challenging to manage. Among the more effective treatments for Septoria leaf spot were Actinovate alternated with copper and low rate of Organocide combined with copper. Downy mildew in cucumber was suppressed best by Actinovate, Organocide plus copper, and Sonata. The products evaluated are defined by EPA as biopesticides because they are derived from natural materials. Most experiments are being funded by the IR-4 Biopesticide and Organic Support Research Program.

### O10.007 VineMan.org: innovative strategies for enhancing disease management in organic vineyards

S.E. Legler[1], T. Caffi[1], S. Poni[2], H.H. Kassemeyer[3], F.G. Rosner[4], H. Reisenzein[5], G. Strauss[5], M. Gorfer[6], P. Storchi[7], H.J. Schroers[8], J. Tardaguila[9] and V. Rossi[1]
[1]*Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, Italy;* [2]*Istituto di Frutti-Viticultura, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, Italy;* [3]*Dept. Biology , Staatliches Weinbauinstitut Freiburg, Merzhauser Str. 119, 79100 Freiburg im Breisgau, Germany;* [4]*Education and research centre for enology and pomology, Wiener Str. 74, 3400 Klosterneuburg, Austria;* [5]*Institute of Sustainable Plant Production, Austrian Agency for Health and Food Safety, Spargelfeldstr. 191, 1226 Wien, Austria;* [6]*Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Konrad-Lorenz-Str. 24, 3430 Tulln, Austria;* [7]*Consiglio per la Ricerca e la sperimentazione in Agricoltura Unità di Ricerca per la Viticoltura, Via Romea 53, 52100 Arezzo, Italy;* [8]*Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia;* [9]*Viticultural Research Group, University of La Rioja, Avda. de La Paz 93, 26006 Logroño, Spain*
*Email: saraelisabetta.legler@unicatt.it*

Organic viticulture has grown considerably in the last decade; nonetheless, organic farming still has a huge potential for innovation and improved solutions specifically for the control of pests and pathogens. The research project VineMan.org (www.vineman-org.eu), funded by European Union within the CORE Organic II ERA-Net (project no. 249667), aims at improving disease control, one of the most difficult tasks in organic viticulture, through innovative cropping systems based on integration of: i) enhanced plant resistance, ii) vineyard management practices, iii) use of biological control agents, and iv) targeted applications based on disease models. During the first year, the above mentioned components of an integrated system have been developed and tested. Natural compounds were tested with a leaf disk assay and some of them were able to activate plant innate immunity. Early leaf removal, resulting in modified microclimate and changed bunch compactness and berry skin structure, was effective in reducing grey mould incidence on grapes. The entomopathogenic fungus *Lecanicillium lecanii* was used against the leafhopper *Scaphoideus titanus*, the vector of the flavescence dorée phytoplasma, and proved to be virulent to its second instar nymphs. Disease models for downy and powdery mildews were implemented in a web-based decision support system for organic viticulture, and tested in several Italian vineyards; the number of copper treatments against downy mildew was reduced by 36% on average. Finally, innovative strategies based on these results were designed and evaluated for overall sustainability trough an *ex-ante* assessment. These strategies are under test in different viticultural areas of Europe.

### O10.008 Fungal disease management in organic French bean: Epidemiology and traditional organic management strategies

G.K.N. Chhetry and H.C. Mangang
*Department of Life Sciences, Manipur University Canchipur-795003, Imphal, India*
*Email: gknc2004@yahoo.co.in*

North Eastern region of India having sub-tropical humid climate is virtually organic by default and conducive for the cultivation of French bean F (*Phaseolus vulgaris* L.) in farmlands ranging from home garden to commercial farmland. However, occurrence of fungal diseases are common with significant variation in the epidemiology of diseases affecting leaf (*Alternaria alternata*, *Cercospora canescen* – leaf spot, *Uromyces appendiculatus* – rust, *Erysiphe polygoni* –powdery mildew etc), fruit (*Sclerotinia sclerotium* and anthracnose fruit rot etc.) and root (*Rhizoctonia solani* –root rot, *Fussarium solani* –wilt, *Sclerotium rolfsii* –white root rot etc.). Farmers

manage the fungal diseases using their traditional methods. Traditional amendments of soil with indigenous organic manures affect the epidemiology of French bean root, fruit and leaf diseases. Within the organic farming system, epidemiology of fungal diseases was more in commercial field as compared to home garden crops. However, epidemiology of fungal diseases of French bean in conventional farming system is more and faster as compared to organic farming system in all fungal diseases associated with French bean. Further, use of bio-pesticides (*Trichoderma* spp.), indigenous plants based disease control strategies and hygienic practices drastically reduce the fungal diseases in organic farming system. Observations made in the field and under control environment suggested that traditional approaches when blended with scientific methods makes the traditional approaches a practical decision making model for the management of fungal diseases in organic farming system. Details of organic practices for the management of fungal diseases have been reported and discussed in the paper with suggestions of further improvement.

**P10.001 Effect of compost tea on growth promotion of lettuce, soybean and sweet corn**

*C.K. Shim, M.J. Kim, Y.K. Kim, H.J. Jee, J.H. Park, S.J. Hong and E.J. Han*
*Organic agriculture Division, National Academic of Agricultural Science, Rural Development of Administration, Republic of Korea*
*Email: ckshim@korea.kr*

This study was aimed to enhance growth promotion of lettuce, soybean, and sweet corn treated with aerated compost tea. Application of compost tea made with vermicomposting to the root zone can increased the plant yield and root growth. The effect of four concentrations, 0.8%, 0.4%, 0.2%, and 0.1% of compost tea on growth of lettuce, soybean, and sweet corn was studied in greenhouse. The results pointed out that compost tea treatments significantly increased leaf number, plant height, and fresh weight of lettuce, soybean, and sweet corn. In lettuce, 0.1% of compost tea was the most effective on growth parameters in foliage part. However, 0.8% of compost tea was significantly promoted the growth of root and shoot in soybean and sweet corn. In soybean treated with higher concentration of compost tea was more effective on increasing of root nodule by 7.25 times than lower concentration of compost tea. In organic cultivation, water based extract of compost tea included active microorganisms are simple to make by soaking compost in water and aerated.

**P10.002 Culture Sensitivity Test of C*eratocystis fimbriata* associated with Mango sudden decline/death (MSD)**

*G.B. Poussio[1], I. Bally[2], L. Kumar[1], F.S. Fateh[3], M.R. Kazmi[3], M.M. Jiskani[4], M.Y. Channa[5] and A.J. Memon*
*[1]Plant pathology section ARI Tando jam; [2]Agriculture Sciences Queensland Department of Agriculture, Fisheries and Forestry; [3]National IPM Program, Institute of Plant & Environmental Protection, National Agricultural Research Centre, Islamabad; [4]Faculty of Crop Protection Department of Plant Pathology SAU Tando jam; [5]Agriculture Training Institute Sakrand*
*Email: gulbahar.2008@gmail.com*

The culture sensitivity tests of *Ceratocystis fimbriata* associated with MSD were studied at Plant Pathology Section, Agriculture Research Institute Tando jam during February and March, 2012. The target fungus was isolated from infected mango trees through standard methods by using PDA medium. Three fungicides viz. Topsin-M, Alliate and Nativo, as well as three plant extracts viz. Neem, Tobacco and Bitter apple were analyzed against the mycelial colony growth of *Ceratocystis fimbriata in vitro* under aseptic conditions. Initially, 25, 50, 75 and 100 ppm of Topsin-M, Alliate and Nativo fungicides, where as 3, 5, 6 and 8 ml of Neem, Tobacco and Bitter apple were mixed per 1000 ml PDA, while fresh PDA plates served as control. The 5 mm disc of fresh culture was inoculated. The experiment was designed as RBCD with 7 treatments and 4 repeats. The effect of tested fungicides and plant extracts on the mycelial colony growth of *Ceratocystis fimbriata* was examined after 10, 15 and 25 days of inoculation. The results showed highly significant difference at $P < 0.05$. The minimum colony growth of the fungus was recorded at the highest dose of fungicides and plant extracts, as compare to control. The growth rate was increased as the dose was decreased. The fungicides were most effective as compare to plant extracts. Topsin-M found to be the best followed by Nativo and Alliate, while Tobacco was less effective as compare to Neem followed by Bitter apple.

**P10.003 Fusarium wilt disease of shallot and response of 4 shallot cultivars against the disease in the field trial**

*A. Laila, A. Wibowo, E. Sulistyaningsih, P. Basunanda, S. Subandiyah and S. Harper*
*Department of Plant Protection Faculty of Agriculture, Universitas Gadjah Mada, Jalan Flora Bulaksumur Yogyakarta 55281, Indonesia*
*Email: awibugm@yahoo.co.id*

Fusarium wilt caused by *F .oxysporum* f.sp. *cepae* (Foc) is the most important constrain of shallot (*Allium cepa* group *aggregatum*) production in Indonesia. Bulb rot disease is another problem concerning for consumption and seed bulbs. Greenhouse and field trial was conducted to find out the response of some shallot cultivars against the disease and biofertilizer application for field disease

management. Before field planting, seed bulbs of 16 shallot cultivars were tested for the incidence of seed-borne diseases in the greenhouse with 20 plant replicates for each cultivar. The results suggested that bulb soft rot was the most abundance found with the incidence ranged from 5-40% on 12 cultivars and of 0% on the rest 4 cultivars. Twisted leaves followed by wilting as typical Foc infection was found 10% only on Kuning cultivar. Four cultivars with gradient incidence of seed borne diseases in the greenhouse trial, i.e., Crok (5%), Kuning (10%), Tiron (15%) and Trisula (40%) were selected for the response against Foc and biofertilizer application in the field with split plot design. Biofertilizer applications was done 4 times after planting. The result showed that Foc incidence were 6.94% on control soil, 6.60% on biofertilizer application, 43.75% on Foc inoculated, and 40.28% on Foc inoculated plus biofertilizer application. Among 4 cultivars there was no significantly different on Foc wilting incidence ranged from 21.53% to 26.04% respectively. The research was funded by ACIAR Australia No. HORT/2009/056.

**P10.004 A new formulation based compost used as a biofertilisant and biopesticide in organic farming**

*B. Mouria, A. Ouazzani Touhami and A. Douira*
*Unity of Botany, Biotechnology and plant pathology, faculty of Sciences, Ibn Tofaïl University, B.P:133, Kenitra, Morocco*
*Email: mouriab@gmail.com*

Morocco is one of the first African and Arab countries have introduced organic farming to improve quality of Moroccan agricultural products intended the international market. However, this production mode, prohibiting the use of synthetic products, has a problem of yield and disease control. Our results showed that the treatment of a greenhouse tomato crop with municipal solid waste compost improve growth and yield parameters up to 125% and inhibit gray mold, caused airborne fungal pathogen *Botrytis cinerea*, up to 73%. This unusual effect of compost on foliar pathogen is due to the induction of systemic resistance. A better performance of the compost was censured by systemic effect amplification, multiplying its antagonist potential. This formulation has resulted in a better growth of plants and total protection (100%) of tomato leaves against *B. cinerea*. This formulation is therefore a discovery that opens completely new prospects. Indeed, it could be used in organic farming under commercial conditions to ensure yield and plant protection while preserving the environment for the sake of sustainable agriculture.

**P10.005 Foliar fungal diseases of rice under upland rain fed organic farming system: Epidemiology and traditional ecological knowledge based management approaches**

*G.K.N. Chhetry and M. Khashimwoh*
*Department of Life Sciences, Manipur University, Canchipur-795003, Imphal, India*
*Email:gknc2004@yahoo.co.in*

Rice being the staple food of the people of northeast region of India, diversity of rice cultivars including scented and aromatic one are cultivated both in the law land and upland under various organic farming farmlands viz; rain fed terraced land, dry terrace land, upland plain dry land, slashed and burn dry land ,etc. Organic dry land rice farmers grow location specific indigenous rice cultivars but these local cultivars are prone to many foliar fungal diseases like brown leaf spot, leaf and neck blast of which brown leaf spot are the most devastating one and major constraint to yield. Eco-friendly traditional organic methods are the only methods adopted by the farmers for the management of diseases. Scientific evaluation of these traditional ecological knowledge based practices in the field and control environment showed encouraging results in the management of fungal diseases in organic dry land rice farming system. Details of range of traditional eco-friendly practices with procedures for target fungal disease in true organic spirit over a range of diverse rice cultivars, efficiency of methods in terms of cost benefit ratio in the context of organic dry land areas, extent of disease severity and incidence( under 5 points rating scale) with a variety of suggestions for further improvement in the future through blending the traditional ecological knowledge of the indigenous farmers with modern biotechnological approaches have been reported in the paper in order to make the farming system viable, affordable and sustainable in the region.

## Concurrent Session 11-Disease Modeling and Epidemiology

### O11.001 Practical applications of plant disease modeling in IPM

*V. Rossi and T. Caffi*
*Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, Italy*
*Email: vittorio.rossi@unicatt.it*

Integrated Pest Management is based on dynamic processes and requires decision-making at strategic, tactical, and operational levels. Relative to decision makers in conventional agricultural systems, decision makers in IPM systems require more knowledge and updated information, and must deal with greater complexity. It has become clear that IPM can be efficiently implemented only if the decision makers are adequately supported. Different tools have been developed for the support of decision-making in plant disease control, and these tools can be grouped in three categories: warning services, on-site devices, and decision support systems (DSSs). These tools work at different spatial and time scales, are provided to users by both public and private sources, focus on different communication modes, and can support multiple options for delivering information to farmers. Plant disease models are key components of any decision-support tool for disease control. Plant disease models produce predictions about the epidemic or single epidemic components that can be used as risk indicators. Characteristics, weaknesses, and strengths of the currently available decision tools are discussed and a new generation of model-based DSSs is shown, which is characterised by: i) a holistic treatment of crop management problems (pests, diseases, pesticide application timing and rates, etc.); ii) a conversion of complex decision processes into simple and easy-to-understand decision supports; iii) easy and rapid access through the Internet; and two-way communication between users and the provider that make it possible to consider context-specific information.

### O11.002 Integration of molecular biology tools into conventional epidemiology: the example of molecular tools to assess risk of disease and efficacy of management actions

*O. Carisse, M. Tremblay and H.V. der Heyden*
*Agriculture and Agri-Food Canada, 430 Gouin Blvd, St-Jean-sur-Richelieu, Quebec, Canada, J3B 3E6*
*Email: odile.carisse@agr.gc.ca*

Molecular plant disease epidemiology, defined as the integration of molecular biology into conventional epidemiology, has become of foremost importance in plant pathology. Molecular epidemiology could be used to understand the etiology, dispersal, and pathogenesis of plant pathogens. It also has practical applications for plant pathogens surveillance and disease management. Molecular tools were developed to quantify airborne plant pathogen populations such as *Botrytis* spp. or monitor genetic mutations associated with fungicide resistance. For instance, spatial statistics were used to characterize relationships between single nucleotide polymorphisms (SNP) related to fungicide resistance in *B. cinerea* populations. The results showed that three spatial relationships may arise when spatial point patterns representing the presence of SNPs are compared by pairs: exclusiveness (12%), co-occurrence (31%) and absence of relationship (56%). Despite the fact that the majority of the tested pairs of SNP showed no spatial relationship, the presence of spatial co-occurrence relationship support models of co-existence between sensitive and resistant strains, and suggest a higher degree of complexity in the resistant-sensitive interactions. Recently, we used molecular tools in surveillance networks to track airborne pathogens and to inform crop advisors and growers about risk of Botrytis-induced diseases and to evaluate efficacy of fungicide applications. Overall, the inclusion of molecular tools in the plant disease management toolbox has proven to be useful, resulting in a reduction of fungicide usage. However, it should be stressed that the information derived from molecular monitoring is sampling dependent and that representativeness is critical especially to time management actions.

### O11.003 Recent development in using molecular tools to monitor the regional epidemics of wheat stripe rust in China

*Y. Luo, X.F. Liu, J.H. Yan, J.M. Liang, Q. Wan and Z.H. Ma*
*Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: ygluo@ucanr.edu*

Monitoring development of initial inoculum and determining pathway of pathogen dispersal of wheat stripe rust in China were addressed in recent studies by using molecular approaches. Real-time PCR was developed to quantify latent infection level in fields. Multi-year and multi-location experiments demonstrated that the molecular disease index (MDX) was significantly correlated with field-observed disease index (DX). Spatial distribution analysis showed that MDX could be used to accurately and efficiently target latent infection loci of the fields and to timely estimate the initial inoclum level and potential risk of disease epidemics. The method was also useful to guide in chemical treatments on infection loci as very early stage. Amplified fragment length polymorphism (AFLP) was used to study the pathogen population genetic structures for western over-summering regions of China. Direct and indirect approaches for

molecular data analysis were applied to determine the possible pathway of pathogen dispersal among these regions. The results showed strong asymmetric dispersal and gene flows from Gansu and Sichuan to other regions including Shannxi, Ningxia and Qinghai. Various probabilities of asymmetric dispersal among 9 selected geographic subpopulations, representing above 5 provinces, were detected. Gansu and Xinjiang populations shared some genotypes, but gene flow was weak, indicating a certain level of isolation. Yunnan populations were clearly isolated from those of Gansu and were likely from clonal populations. Recombined or mixture of recombined with clonal populations were found in some locations in both Gansu and Ningxia. These studies demonstrated the molecular approaches as powerful tools in epidemiological studies.

**O11.004 Using satellite remote sensing in crop stress assessment in Iowa**

*X. Li and X.B. Yang*
*Department of Plant Pathology & Microbiology, Iowa State University, 351 Bessey Hall, Ames, Iowa, 50011, U.S.A.*
*Email: linuslee@iastate.edu*

Satellite remote sensing imageries from Landsate satellites and AQUA/TERRA satellite systems in 2009-2012 were used to analyze regional biotic and abiotic stresses in soybean and corn caused by foliar diseases, drought, and flood. Soybean foliar diseases (brown spot, sudden death syndrome, and white mold) and corn foliar diseases (gray leaf spot and northern blight) were used. In 2009 to 2012, Iowa experienced completely different extreme summer weather, from record cool in 2009 to regional record flood in 2010 and record drought in 2012. Two types of derived information, the normalized difference vegetation index (NDVI) and onset greenness were used to analyze seasonal changes of crop canopy and their associations with biotic and abiotic stresses. The remote sensing assessments were validated by limited disease field survey data. At 250 m resolution, NDVI was able to detect late season occurrence of brown spot, SDS, and gray leaf spot. Early planting has higher probability of foliar disease. We were able to detect the spatial differences of fields in stresses caused by different weather conditions. For example, the summer drought in 2012 occurred in areas where the disease pressure was much lower in the wet summer in 2010. The results can be useful in developing site-specific disease models for on-farm disease management.

**O11.005 Climate-disease integrated modeling of plant disease environment on sub-seasonal scale**

*Z. Pan, X.B. Yang, and X. Li*
*Department of Earth and Atmospheric Sciences, Saint Louis University, St. Louis, Missouri, 63108, USA*
*Email: panz@slu.edu*

The 42,600 ha in corns and soybeans in the Midwest represents 35% of all U.S. cultivated cropland and the Midwest accounts for a large portion of the $200 billion yearly U.S. agricultural production. Projecting meteorological environment on weekly-monthly scales during growing season is crucial to optimization of farming operation. We have integrated a regional climate mode (WRF), a dispersion model (HYSLPIT), and a disease model into an agricultural forecasting system. The integrated model has been used to forecast soybean rust and other plan diseases in past years over the U.S. main agricultural regions. This presentation reports the model skills in forecasting meteorological environment along with soybean rust forecast applications. Thirty-day forecast are generated each week, projecting the key parameters for disease occurrence, including precipitation amount and frequency, temperature, and moisture. We found that the model captures overall pattern of rainfall distribution although it has a overall positive bias compared with observations. Forecast of rainfall amount and rainy day matches well with observations throughout the growing season except for around May, the spring barrier when seasons are in transit. These forecasted meteorological variables are then used to predict movements of soybean rust, a potentially devastating disease. The model seems to have reasonably predicted rust spread from coastal states towards North Central Region. These forecasts have provided a useful guidance for the early detection of the disease for soybean producers.

**O11.006 A simulation model for multi-seasonal spread of Verticillium wilt of lettuce**

*B.M. Wu[1] and K.V. Subbarao[2]*
*[1]Department of Plant Pathology, China Agricultural University, Beijing, China 100193; [2]Department of Plant Pathology, University of California, Davis, c/o U. S. Agricultural Research Station, 1636 E. Alisal Street, Salinas, CA 93905, USA*

Because seedborne *Verticillium dahliae* is a concern to lettuce growers in California, it is desirable to establish an acceptable contamination threshold. As introduction of inoculums into lettuce fields for experiments is undesirable, we constructed a simulation model to study the spread of Verticillium wilt. The model consists of 4 components: one for simulating the infection of host plants, one for simulating the reproduction of microsclerotia on diseased plants, one for simulating the survival of microsclerotia, and one for simulating the dispersal of microsclerotia. The simulation results demonstrated that the ID-DI curve parameters and the dispersal gradients all affect disease spread in the field. While a steeper dispersal gradient facilitated the establishment of the disease in a new field with a low inoculum density, a

long-tail gradient promoted the disease spread in fields with a high inoculum density. The simulation results also revealed the surprising importance of avoiding successive lettuce crops to reduce the survival rate of microsclerotia between crops, and the importance of breeding *V. dahliae* resistant lettuce cultivars to lower the number of microsclerotia formed on each diseased plant. The simulation results, however, suggested that even with a low seed infestation rate, the pathogen would eventually become established if susceptible lettuce cultivars are grown consecutively in a field for many years. A threshold for seed infestation can be established only when two of the three drivers, low microsclerotia production per diseased plant, long tail dispersal gradient, and low microsclerotia survival between lettuce crops are present.

**O11.007 Global megatrends and plant diseases: a synthesis of shifting production situations and crop health syndromes in rice over 23 years in the lowlands of Asia**

*S. Savary, N.P. Castilla, A.H. Sparks, J. Bigornia, C. Torres, L. Willocquet, N. McRoberts and P.D. Esker*
*IRRI, DAPO Box 7777, Metro Manila, Philippines; Current addresses: S. Savary and L. Willocquet: Inra, UMR AGIR, Toulouse, France; N. McRoberts: Dpt. of Plant Pathology, UC Davis, USA; P.D. Esker: U. de Costa Rica, Costa Rica*
*Email: Serge.Savary@toulouse.inra.fr*

Production situations and crop health status were characterized at multiple sites surveyed in lowland rice across tropical and subtropical Asia between 1987 and 2010. A standardized characterization procedure was used over both time and space to generate approximately 50 variables pertaining to an individual surveyed rice field, defined here as the statistical unit. Production situation variables included amounts of inputs (e.g., mineral fertilizer, manure, pesticides), components of the cropping regimens (e.g., crop establishment date relative to neighboring fields at a site, rotation), crop management elements (e.g., crop establishment method, water management), and varieties. Crop health variables pertained to some 30 different diseases, animal pests, and weeds. Here we report a synthesis of analyses conducted over 1,000 surveyed fields. Similarly to previous reports, strong relationships are found between production situations and crop health, as well as a shift in crop health as production situations evolved. The analysis highlights the emergence of new crop health problems, especially diseases, as well as of entirely new crop health syndromes. Emergence of diseases or of entire health syndromes are analyzed in relation to shifts in production situations or their components. This extensive survey work also included direct actual yield measurements. A key result of this work is that the variation in yield was marginal over the considered period, despite considerable increases in inputs. This has heavy consequences for the future. While shifts in plant health may account for such a lack of progress, other hypotheses need consideration.

**O11.008 Global trade and its effects on the spread of plant diseases**

*M.J. Jeger, M. Pautasso, K. Dehnen-Schmutz and J.P. Stack*
*Division of Ecology and Evolution and Centre for Environmental Policy, Imperial College London, Silwood Park campus, Ascot SL5 7PY, United Kingdom*
*Email: m.jeger@imperial.ac.uk*

The emergence of non-endemic plant pathogens is a function of their: (i) introductions through dispersal or evolution within an endemic population; (ii) establishment through suitability or adaptation to host plants, habitat and climate; and (iii) further spread through natural or human-mediated mechanisms. Such an invasive process often leads to the long-term persistence of plant pathogens and the damaging impact of disease epidemics in crops and other economically-important or socially-valued ecosystems. Climate and global trade are major drivers of the dispersal and invasion of plant pathogens affecting agricultural and horticultural crops, plantation and woodland trees, and plants in natural and semi-natural environments. Patterns of weather, notably large-scale air movements, have been implicated in the long-range dispersal of pathogens between and within continents. In some cases intercontinental dispersal has led to the catching-up with crop plants that accompanied human population movements over centuries and millennia. However the movement of humans, seeds and ornamental plants, and their associated pathogens, has intensified markedly over recent decades with increasing globalisation of the world economy. This has led to sudden host switching, emergence of hybrid pathogens, and depending on climatic suitability major disease epidemics, which often were not predicted. The situation is then exacerbated by climate change, either in terms of an enlarged range of plants traded globally or through direct effects on plant pathogens. In this talk we make a distinction between dispersal mediated by natural means and that by human activity, but show that these processes interact and that such interactions are important in analysing, understanding and predicting epidemics. For this, new modelling methodologies based on network approaches are required to supplement those used previously in plant disease epidemiology.

**O11.009 A strategy to identify and quantify partial resistance interacting with other plant attributes: the case study of rice sheath blight**

*L. Willocquet, Srinivasachary and S. Savary*
*IRRI, DAPO Box 7777, Metro Manila, Philippines;*

*Current addresses: S. Savary and L. Willocquet: Inra, UMR AGIR, Toulouse, France. Srinivasachary: Dupont Pioneer Oversees Corporation, India Branch, 3rd Floor, 6-3-1099/1100, Babukhan's Millenium Centre, Rajabjavan Road, Somajiguda, Hyderabad-500 082, Andra Pradesh, India.*
*Email: Laetitia.Willocquet@toulouse.inra.fr*

Host plant resistance in many pathosystems does not depend solely on resistance genes or QTLs, but also on other genetic attributes, which enable a crop to escape disease. Identifying and quantifying components of partial resistance in such pathosystems is therefore both difficult and critical for breeding because of the confounding effects of escape and resistance processes. Rice sheath blight (*Rhizoctonia solani*) is an economically important disease in most rice producing countries. Two main groups of mechanisms for partial resistance are hypothesized for this disease: disease escape, linked with morphological traits, and physiological resistance, i.e., induced or constitutive plant processes that decrease the infection and reproduction of the pathogen. Methods were developed to measure (1) physiological resistance, using detached tiller tests yielding lesion densities, sizes, and expansion, and (2) overall partial resistances using microfield experiments where morphological characteristics, disease intensification and disease spread were measured. Both types of experiments were conducted on a range of rice genotypes, and several multivariate analyses were performed. The results indicate that disease escape represents an important component of partial resistance, affecting both disease intensification and disease spread, and is associated with morphological traits. The number of lesions on detached tillers varied amongst genotypes, and was significantly associated to disease intensity in microfields at low infection efficiency. The experimental characterization of 163 cultivated rice accessions allowed identifying 27 genotypes with low level of disease intensity and suitable agronomic traits. These genotypes may be used as sources of resistance to ShB in breeding programs.

### O11.010 Clonal population foci of *Monilinia fructicola* during epidemics within peach tree canopies

*S.E. Everhart and H. Scherm*
*Department of Plant Pathology, University of Georgia, Athens, GA 30602 (USA)*
*Email: scherm@uga.edu*

We determined the fine-scale genetic structure of populations of the brown rot pathogen, *Monilinia fructicola*, in individual peach tree canopies to better understand fine-scale pathogen diversity and to complement previous work on spatio-temporal disease development at the canopy level. Across 3 years, six trees were monitored for disease development season-long, tagging each individual symptom (blossom blight, green fruit rot, pre-harvest fruit rot, and twig canker) and mapping it in three dimensions using a magnetic digitizer. Trees had between 244 and 861 fruit total, with a final fruit rot incidence of 11.1 to 58.2%. The pathogen was isolated from each of the mapped symptoms, and all 694 single-spored isolates were evaluated with 13 polymorphic SSR markers. Each canopy population had 65 to 173 isolates per tree, and these populations showed high genetic (average *uh* = 0.529) and genotypic diversity (average *D* = 0.928). The percentage of unique multilocus haplotypes within trees was greater for blossom blight isolates (average 78.2%) than for fruit rot isolates (average 51.3%), indicating a greater contribution of clonal reproduction during the pre-harvest epidemic. Spatial genetic structure was observed among fruit rot isolates, with all six populations showing positive and significant autocorrelation up to ~1.1 m, which was positively correlated with the size of the clonal groups within those trees ($r$ = 0.890, $P$ = 0.018). Despite high levels of within-tree pathogen diversity, the relative contribution of locally available inoculum combined with short-distance dispersal is likely the main factor in generating the observed fine-scale spatial patterns within trees.

### O11.011 Modelling Aflatoxin contamination in maize: the AFLA-maize experience

*M.C. Leggieri, P. Giorni, V. Rossi and P. Battilani*
*Institute of Entomology and Plant Pathology, UCSC, Piacenza, Italy*
*Email: marco.camardoleggieri@unicatt.it*

Maize is the third most important cereal crop after wheat and rice and it is grown on about 120 million hectares worldwide. This crop is a major source of food and feed but, unfortunately, it is also a well-known host for toxigenic fungi as *Aspergillus flavus* able to contaminate the ripening kernels with aflatoxin $B_1$($AFB_1$) which is reported as carcinogenic for humans and animals. EU legislation fixed $AFB_1$ thresholds for raw maize destined to humans, dairy animals and other animal species (Commission Regulation 1181/2006 and Directive 100/2003). Modelling of the interactions between host, plant and environment during the season can enable prediction of pre-harvest $AFB_1$ risk and its potential management. In this work the relational diagram of *A. flavus* infection cycle was developed; state variables, rates and driving variables were decided and organized in a coherent structure. Quantitative data for crucial steps of the cycle were collected from literature and equations were elaborated to connect driving variables to rates; an algorithm was then developed to finalize the model. The model predicts the risk of maize contamination by $AFB_1$ above the legal limit of 5 µg/kg. The model was validated with a six year data set and around 70% of maize samples were correctly classified, below or above the threshold, by AFLA-maize. Therefore, AFLA-maize, giving a pre-

diction on a daily base, allows following the risk dynamic along the season and it is a useful support to alert farmers and technicians. Apart real time predictions, historical and predicted data can be used as input to draw risk maps in poorly studied areas or in climate change scenarios.

### O11.012 Disease warning models in greenhouses: *Botrytis cinerea* in rose and *Didymella bryoniae* in cucumber

*A.J. Dik*[1], *M. Hubers and M. Kockelkoren*
*Aleid Dik Consultancy, Wolvenplein 5, 3512 CJ Utrecht, The Netherlands*
*Email: contact@aleiddik.nl*

Most disease warning models have been developed for field crops and fruit trees and are used for timing of fungicide applications. In heated glasshouses, disease warning models can in addition be used to prevent disease by changing the climate settings. Two models were developed in The Netherlands, one for *Botrytis cinerea* in roses in collaboration with Syngenta Crop Protection B.V., and one for *Didymella bryoniae* in cucumber, both in glasshouses. *Botrytis cinerea* (grey mould) is a major problem in the post-harvest phase of greenhouse-grown roses. A model was developed that calculates survival of the spores on the flowers during cropping and subsequent post-harvest disease. The model is based on two experiments followed by validation and improvement by testing in four commercial greenhouses during three years. For fine-tuning, in two greenhouses a grid of nine plots was used which showed that large horizontal differences in climate and disease occurred. For *Didymella bryoniae* (*Mycosphaerella*, gummy stem blight), the approach was similar. In 2012, data on temperature, RH and diseased fruits were collected in six to ten plots per grower, in three commercial glasshouses and in total in seven crops. The data were analysed to establish the driving factors in fruit infection. In 2013, more growers are collecting data so that the model can be validated and improved. These models are both based on regression analysis. This method of model development is relatively cheap and fast with results that are of good use to growers. The models are marketed as modules for the climate computer.

### O11.013 Mathematical models based on different thermal and moisture regimes for development of Ascochyta blight of chickpeas

*A. Riaz, F. Naz and A.R. Chaudhary*
*Plant Pathology Department, University of Arid Agriculture, Rawalpindi, Pakistan*
*Email: abidriaz@uaar.edu.pk*

Two separate models have been developed to describe the mathematical relationship of temperature and leaf wetness durations with blight development on two chickpea cultivars under controlled conditions. Plants of Balkasar-2000 (moderately resistant cultivar) and AUG-424( highly susceptible cultivar) of our field trials were artificially inoculated with *Ascochyta rabiei* isolate ID-1 prepared @5x $10^5$ conidia ml $^{-1}$ and subjected to various controlled environments to determine the impact of temperature and leaf wetness durations on disease establishment. Disease severity (%) was significantly affected by temperature, wetness durations and their interactions and depended a lot on the level of resistance of the cultivar. It increased with increasing wetness duration (6-96h) at all the temperatures tested (10-25 ℃). At least 18h of leaf wetness were required for significant disease establishment (50%) at the optimum temperature of 20 ℃ in AUG-424 and for Balkasar-2000, this value was 96h. Quadratic trend in disease severity was found in relation to temperature and linear trend was recorded with regard to leaf wetness periods in both the cultivars. This study gives a systematic evaluation of host-pathogen interactions in controlled conditions. This approach of finding out quantitative relationship of disease with these most important variables may serve as a criterion for selection of cultivar for a specific area.

### O11.014 Predicting the occurrence of a major outbreak of disease using compartmental epidemiological models

*R.N. Thompson, C.A. Gilligan and N.J. Cunniffe*
*Department of Plant Sciences, University of Cambridge, Cambridge, CB2 3EA, UK*
*Email: rnt22@cam.ac.uk*

A key question in epidemiology is whether or not, given the arrival of a pathogen in a system, a major outbreak of disease is going to occur. We consider stochastic versions of the well-studied SIS and SIR models, showing analytically that traditional measures of the probability of a major outbreak rely on various assumptions – including a "large" population and a basic reproduction number much larger than unity. We will then consider the exact definition of a major outbreak. In particular, we define a major outbreak to be hitting a threshold number of infected individuals, and consider the probability of a major outbreak occurring given early disease spread data. We find an interesting phenomenon whereby the probability of a major outbreak is commonly overestimated, and provide a method for amending standard estimates. Our results have clear implications in many areas of epidemiology, with many important diseases modelled using compartmental models. One example is *Huanglongbing* (citrus greening). A control strategy that is used is roguing (the removal of infected plants, replanting with new susceptible plants). Since legislation in Brazil states that, if the number of infected individuals reaches twenty-eight percent of the grove, then the all plants in the grove must be removed, our methods provide a framework that can be used to

predict whether grove removal is going to be necessary.

### O11.015 Evaluation of forecasting models for *Alternaria* brown spot of mandarins with receiver operating characteristic curve analysis

*D.D.M. Bassimba[1], J.L. Mira[2] and A. Vicent[2]*
*[1]Laboratorio de Fitopatologia. Instituto de Investigação Agronómica (IIA). Chianga 2104, Huambo, Angola; [2]Centro de Protección Vegetaly Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Apdo. Oficial, Moncada 46113, Valencia, Spain*
*Email: avicent@ivia.es*

Alternaria brown spot (ABS) caused by the tangerine pathotype of *Alternaria alternata* (Fr.) Keissl. is a serious disease of some mandarin cultivars. In general, the efficiency of current fungicide schedules in Spain is not satisfactory, mainly due to the asynchrony between spray timing and the distribution of infection periods. Several forecasting models for ABS were developed and evaluated under semiarid conditions in Valencia, Spain. The infection efficiency of the pathogen was studied by inoculating nursery plants of the highly susceptible cultivar 'Fortune' and the moderately susceptible 'Nova'. Inoculated plants were maintained under controlled conditions with different combinations of temperature (8-35 ℃) and wetness durations (8-48 h). Disease incidence and severity in inoculated plants were used as response variables, and temperature and wetness duration were considered as explanatory variables. Data were fitted by logistic regression to a polynomial model with parameters related to temperature, wetness, and their interaction. A simple generic infection model was also fitted by nonlinear regression, which included five parameters: minimum (Tmin), optimum (Topt) and maximum (Tmax) temperature, and minimum (Wmin) and maximum (Wmax) wetness duration for infection. The two models were evaluated using independent infection data from trap plants of 'Fortune' and 'Nova' exposed weekly in the field during 2011 and 2012. The sensitivity and specificity of each model were calculated and evaluated with Receiver Operating Characteristic Curve analysis (ROC). A decision action threshold was established for each cultivar, integrating the economic impact of incorrect predictions, both false negatives and false positives.

### O11.016 Artificial neural networks for forecasting development of Septoria leaf blotch of wheat

*T.Z. Ibragimov and I.T. Ibragimova*
*All-Russia Scientific Research Institute of Phytopathology, Bolshie Vyazemy, Moscow region, 143050, Russia*
*Bit Logic, Moscow, Russia*
*Email: tibragimov@vniif.ru*

Modeling and subsequently based on this forecast the development, distribution and damage Septoria leaf blotch of wheat complicated nonlinear dependencies and large-scale factors influence the process. One of the possible ways to overcome these and other difficulties in the construction of forecasting systems is the use of artificial neural networks. Prediction problem can be divided into two main classes: classification and regression. Regression problems can be solved using the following types of networks: multilayer perceptron, radial basis function, generalized regression network and linear network. As parameters in our study were used:
- The observed degree of development of *Septoria* at the time of the forecast;
- The average temperature;
- The number of days with precipitation at;
- Phenological stage of development wheat at the time of the forecast;
- Average moisture content of the atmosphere;
- Average annual rainfall.

Projected output parameter - the degree of development of Septoria leaf blotch of wheat to phase 75. With the use of software packages Neuroph and KNIME built predictive neural networks of the following types: linear network, radial basis function and multi-layer perceptron. Obtained results show that for such a simple set of input parameters, there is a tendency to receive neural networks satisfactory prediction of the disease. So with the use of neural network - multilayer perceptrons managed to accurately predict Septoria leaf blotch epidemic on wheat on the territory of Russia in 1997 and 1999.

### P11.001 A selective medium to isolate airborne spores of *Microdochium nivale*, causing winter wheat scab

*Y. Hayashi, T. Kozawa, S. Akino, N. Kondo, D. Aiuchi, M. Tani and M. Koike*
*Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, 080-8555, Japan*
*Email: s19195_hayashi@yahoo.co.jp*

Winter wheat scab in Hokkaido, Japan is caused predominantly by *Gibberella zeae* and *Microdochium nivale* and can result in significant yield losses. A selective medium for isolation of *G. zeae* was previously developed, but not for *M. nivale*. The purpose of this study therefore was to develop a selective medium for isolation of airborne spores of *M. nivale*. Based on the basic composition of Komada's Fusarium-selective medium, carbon and nitrogen sources and the most suitable vitamin B component for the basal composition were examined. Hyphal growth of *M. nivale* was promoted when galactose was replaced with lactose and combined with L-asparagine, while aerial hyphal formation increased with thiamine hydrochloride as the vitamin B source. In antimicrobial composition, colony formation

of other filamentous fungi was greatly inhibited by spiroxamine. Thiophanate methyl, to which *M. nivale* shows resistance, selectively inhibited the growth of *Fusarium* spp. only. LATTS (initials of components) medium developed in this study could distinguish by colony color between the varieties within *M. nivale*. *M. nivale* var. *majus* formed characteristic deep pinkish colonies for seven days incubation, whereas *M. nivale* var. *nivale* remained forming white colonies. From the 12 days on, *M. nivale* var. *nivale* formed either light pinkish colonies or white colonies. Spore trapping using LATTS medium was subsequently performed in a wheat field. *M. nivale* formed characteristic pinkish colonies in the case of contamination with other filamentous fungi, making differentiation easy. Overall, the findings show that LATTS medium is effective for isolation of airborne spores of *M. nivale*.

**P11.002 Epidemiological assessments and husk spot control in macadamia**

*O.A. Akinsanmi and A. Drenth*
*The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Centre of Plant Science, Ecosciences Precinct, GPO Box 267 QLD 4001, Australia*
*Email: uqoakins@uq.edu.au*

Husk spot, caused by *Pseudocercospora macadamiae*, is a major disease of macadamia in Australia. The disease occurs on the husk (pericarp) of fruit of the macadamia nut and gives rise to premature nut drop. Infection of the fruit takes place early followed by a long quiescence period. The timing of infection of the husk and the number of infections at early stages of nut development play a major role in disease intensity. Husk spot development is influenced by the incidence of latent infections and certain climatic conditions. Our regression models revealed that number of rainy days between fruit set and nut physiological maturity stage influenced disease incidence, whereas, accumulated precipitation from anthesis to nut maturity stage influenced disease severity. Fungicides are used for disease control but the timing and frequency of application are crucial for effective disease control. We developed an initial decision support system for fungicide spray applications utilising a checklist of risk factors that affect infection and husk spot development: history of crop loss due to husk spot, short-term weather forecast, previous disease levels, susceptibility of cultivars, inoculum load in the tree canopy and flowering pattern. In this paper, we describe how the forecasting method was developed and compared to deliver different decision support tools for effective fungicide spray applications against husk spot.

**P11.003 Spatial distribution of single nucleotide polymorphisms related to fungicide resistance and implications for sampling**

*H.V. der Heyden, P. Dutilleul, L. Brodeur and O. Carisse*
*Agriculture and Agri-Food Canada, 430 Gouin Blvd, St-Jean-sur-Richelieu, Quebec, Canada, J3B 3E6 Email: odile.carisse@agr.gc.ca*

Spatial distribution of single nucleotide polymorphisms (SNPs) related to fungicide resistance was studied for *Botrytis cinerea* (vineyards) and for *Botrytis squamosa* (onion fields). Heterogeneity in these distributions was characterized through the fitting of discrete probability distributions. Two SNPs related to boscalid resistance (H272R and H272Y), and one SNP related to dicarboximide resistance (I86S) were studied for *B. cinerea* in grape. For *B. squamosa*, one SNP responsible for dicarboximide resistance (I86S) was studied. One onion field was sampled in 2009 and another one was sampled in 2010 for *B. squamosa*, and two vineyards were sampled in 2011 for *B. cinerea*, for a total of four sampled sites. Cluster sampling was carried on a 10x10 grid, each of the 100 nodes being the center of a 10mx10m quadrat. In each quadrat, 10 samples were collected and analyzed by RFLP-PCR or PIRA-PCR. Mean SNP incidence varied from 15 to 68%, with an overall mean incidence of 43%. For all eight data sets, the beta-binomial distribution was found to fit the data better than the binomial distribution. This indicates local aggregation of fungicide resistance among sampling units, as supported by estimates of the parameter θ of the beta-binomial distribution ranging from 0.09 to 0.23 with an overall median value of 0.20. On the basis of the spatial distribution patterns of SNP incidence, sampling curves were computed for different levels of reliability, emphasising the importance of sample size, especially for detection of mutation incidence below the at risk threshold for control failure.

**P11.004 Rootstocks effects on Entomosporium leaf spot in 'Abate Fetel' pear**

*M.J. Gonçalves, A. Bogo, G.C. Weber, B. de Bem and L. Rufato*
*Crop Production Program. Santa Catarina State University - UDESC, Avenida Luiz Camões, 2090, Lages/SC, CEP. 88.520-000, Brazil*
*Email: amauribogo@udesc.br*

The Entomosporium Leaf Spot (ELS) is caused by the fungus *Fabreae maculata* (anamorph: *Entomosporium mespili)* and affects most of the pear cultivars and quince rootstocks in Brazil. The aim of this study was to characterize the behavior of Abate Fetel pear cultivars under quince rootstocks (EMA, EMC and Adams) to ELS in southern Brazil, during the crop season of 2009/2010 and 2010/2011. The incidence and severity were quantified weekly from the first symptoms appearance in 100 randomly leaves distributed in four medium-height branches per plant on each eight replications per

treatment. Curves of ELS progress were constructed and the epidemics compared according to: a) the beginning of symptoms appearance (BSA); b) the time to reach the maximum disease incidence and severity (TRMDI and TRMDS); c) area under the incidence and severity disease progress curve (AUIDPC and AUSDPC). The incidence and severity data were analyzed by linear regression and adjusted for three empirical models: Logistic, Monomolecular and Gompertz. All combinations of Abate Fetel cultivar and rootstocks were susceptible to *E. mespili*. However, there were significant differences in ELS intensity among rootstocks evaluated. The highest ELS intensity was observed in combination with EMA rootstock and the lowest with EMC rootstock. Abate Fetel grafted on EMC rootstock showed BAS, TAMID and TAMSD significantly smaller, when compared with EMA and Adams rootstocks. The Logistic epidemiological models were the most appropriates to describe the ELS progress of Abate Fetel cultivar in the edafoclimatic conditions of southern Brazil.

**P11.005 Can nitrogen nutrition of the host plant influence the aggressiveness of secondary inoculum? The intriguing case of *Botrytis cinerea* on tomato**

*M.A. Abro, F. Lecompte, F. Bryone and P.C. Nicot*
*INRA, UR407 Pathologie végétale, Domaine Saint Maurice, CS 60094, F-84143 Montfavet cedex, France*
*Email: philippe.nicot@avignon.inra.fr*

The influence of nitrogen (N) fertilization on a plant's susceptibility to pathogens is fairly well documented. However, little is known about possible effects on spore production by fungal pathogens on diseased tissue and on the aggressiveness of this resulting secondary inoculum. To address this question, sporulation by two strains of *Botrytis cinerea* was quantified on tomato plants produced in hydroponic conditions under different N irrigation regimes with inputs of nitrate from 0.5 to 45 mmol per liter (mM). Sporulation decreased significantly ($P < 0.05$) with increasing N fertilization up to 15 to 30 mM nitrate. The spores were collected and used to inoculate tomato plants produced under a standard fertilization regime. The aggressiveness of this secondary inoculum was significantly influenced by the nutritional status of its production substrate. Disease severity was highest with spores produced on plants with very low or very high N fertilization (0.5 or 30 mM nitrate). It was lowest for inoculum from plants with moderate levels of N fertilization. The results will be discussed in terms of possible mechanisms involved and in terms of potential consequences for disease control.

**P11.006 Pathogenicity of *leptosphaeria maculans* isolates obtained from *brassica napus* (oilseed rape) cultivars with the *rlm7* resistance gene**

*G.K. Mitrousia, Y.J. Huang, A.M. Hall and B.D.L. Fitt*
*School of Life and Medical Sciences, University of Hertfordshire, Herts, AL10 9AB*
*Email: g.mitrousia@herts.ac.uk*

Isolates obtained from winter oilseed rape cultivars with the resistance gene *Rlm7* were examined for their pathogenicity by inoculation onto cotyledons and true leaves of the susceptible cultivar Drakkar (no *R* gene) and cultivars with the *Rlm7* gene (Excel, Roxet, Hearty and line 01-23-2-1). After assessment of lesions, cotyledons and true leaves were detached 17 and 21 dpi, respectively, and incubated in darkness under high humidity to assess pycnidial development and conidial production. All the isolates tested produced typical large/grey lesions (susceptible phenotype) on both cotyledons and true leaves of Drakkar; large numbers of pycnidia with conidial masses were produced in and outside the lesions on cotyledons after 5 days of incubation and on true leaves after 3 days of incubation. Small lesions surrounded by dark margins (resistant phenotype), with no difference between isolates in lesion area, were produced on cotyledons of the four *Rlm7* cultivars. There was no difference in lesion area between the isolates tested on true leaves of Excel, Hearty and 01-23-2-1 but there was difference between isolates on Roxet. Most of the isolates produced small numbers of immature pycnidia on the lesions on cotyledons after 5 days of incubation and on true leaves after 3 days of incubation of the four cultivars with the *Rlm7* gene. However, these pycnidia were not able to produce conidia. With increased incubation period and the senescence of cotyledons and true leaves, mature pyncidia developed and produced conidia outside the lesions.

**P11.007 Potato yield gaps in SE Asia and China and the role of plant pathogens**

*D. Crook and G.A. Forbes*
*CIP China Center for Asia and Pacific; International Potato Center, Room 709, Pan Pacific PlazaA12 Zhongguancun Nandajie, 100081, Beijing, P. R. China*
*Email: g.forbes@cgiar.org*

Yield gap can be defined in many ways but frequently is seen as the difference between actual yields on farmers' fields and the amount attainable by implementing best practices (attainable yield), or the theoretical yield. Theoretical yields were estimated from published values for net primary production (NPP) in 5 countries: China, Vietnam, Myanmar, Indonesia and the Philippines. Actual and attainable yields were estimated based on productivity statistics (e.g., FAO) and published studies. Data for the later were sketchy but demonstrated that potential yields averaged 79 T/ha, attainable yields averaged 28 T/ha and actual yields averaged 14 T/ha. Identification of the role of plant pathogens in these yield gaps is difficult due to the scarcity of published assessments under actual farm conditions. Expert opinion indicates

that a large part of the gap is probably due to seed degeneration caused in large part by viruses, and in some locations also to late blight. The results indicate that host plant resistance to these pathogens together with on-farm disease management could have immediate positive impacts on potato productivity in the region.

**P11.008 Ants and millipedes as vectors of *Phaeomoniella chlamydospora* to grapevine pruning wounds**

*P. Moyo[1], F. Roets[2], L. Mostert[1]* and *F. Halleen[1, 3]*
*[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa; [2]Department of Conservation Ecology and Entomology, University of Stellenbosch, Private Bag X1, Matieland (Stellenbosch) 7602, South Africa; [3]Plant Protection Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa*
*Email: halleenf@arc.agric.za*

*Phaeomoniella chlamydospora* is one of the causal organisms of Petri disease and esca, important grapevine trunk diseases globally. Although extensive research has been carried out to understand the epidemiology of this pathogen, several questions remain uncertain with regards to dissemination in vineyards. In this study, we investigated the potential of Portuguese millipedes (*Ommattoiulus moreleti*) and cocktail ants (*Crematogaster peringueyi*) to vector this fungus to fresh pruning wounds. We also determined whether sap formed after pruning of shoots is a potential food source for these taxa, *in vitro*. Millipedes were offered a choice between grapevine sap and water in Petri dishes and monitored for ingestion of sap. Laboratory-kept colonies of ants were presented with a choice of grapevine sap, water and tuna and monitored to identify feeding preferences. Both arthropod species preferred sap over the other items presented to them, implying that they would visit fresh pruning wounds for sap. Subsequently, it was determined whether both arthropod taxa can effectively transmit a DsRed transformed *Phaeomoniella chlamydospora* isolate to fresh wounds and cause infection *in vitro*. Arthropods were exposed to the fungus for 24 hours and transferred to the base of pruned healthy potted plants and were removed after three days. Isolations from pruning wounds, one month later, confirmed that both arthropod species were able to vector the fungus to pruning wounds and cause infection. These results provide the first compelling evidence that arthropods play a role in the dissemination of trunk disease pathogens significantly contributing towards understanding the epidemiology of grapevine trunk disease pathogens.

**P11.009 A web-based decision support system for sustainable vineyard management**

*T. Caffi[1], F. Salinari[2], S. Giosuè[2], S.E. Legler[1] and V. Rossi[1]*
*[1]Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, Italy; [2]Horta s.r.l., Via E. Gorra 55, 29122 Piacenza, Italy*
*Email: tito.caffi@unicatt.it*

The implementation of Integrated Pest Management requested by the European Directive on the sustainable use of pesticide (2009/128/CE) requires complex decision-making to growers, who need real time information about the vineyard environment and the disease risk, as well as increased knowledge on plant protection options, limitations and fulfilments. A web-based Decision Support System (DSS) was developed for helping viticulturists in decision-making about tactical management of the vineyard. The DSS, named Vite.net®, is provided by Horta srl (www.horta-srl.com), is available for registered users via the internet and is composed by: i) a network of weather sensors; ii) a server repository that stores weather data and forecasts; iii) an user-friendly interface that makes it possible to insert vineyard-specific information (e.g., cultivar, trellis system) and monitoring data (e.g., date of disease onset) and show up-to-date graphical supports for informed decision-making; iv) mathematical models that use weather data, vineyard-specific information and monitoring data to predict the development of the main grapevine pests and diseases, the plant growth and development, and the risk of abiotic stresses, such as low temperature and drought. The DSS was developed within the MoDeM-IVM project, funded by the European Union's FP7 (grant-agreement n°262059), and was validated in commercial vineyards in Italy, Spain and Portugal. In these vine yards, plots managed according to the DSS were compared with the grower's practice and an untreated control. Results confirmed the advantages rising from the use of the DSS, with a 40-50% reduction of the number of fungicide treatments. In organic vine yards, the DSS made it possible a 37% reduction of the copper used to control downy mildew.

**P11.010 Effect of light, temperature and leaf wetness on the infection efficiency of *Plasmopara viticola*, the causal agent of grapevine downy mildew**

*S.E. Legler, T. Caffi, and V. Rossi*
*Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via E. Parmense 84, 29122 Piacenza, Italy*
*Email: saraelisabetta.legler@unicatt.it*

Although the *Plasmopara viticola* pathogen's life cycle and the disease epidemiology have been studied intensively, quantitative dependency of some infection processes on environmental variables is still unclear. The combined effects of light, temperature, and wetness duration on the release of zoospores from zoosporangia and the infection efficiency of these zoospores were

investigated trough environmental controlled and semi-field experiments. Zoospores were released after 6h of incubation between 10 and 25 ℃, after 9h at 5 ℃, and at lower percentages at 30 ℃. When these zoospores were inoculated on *Vitis vinifera* leaf disks, their infection efficiency were negatively related to the time elapsed between release and inoculation. It seems that swimming in water progressively reduced the infection efficiency of zoospores. Irrespective of the light regime, sporangia inoculated on *V.vinifera* leaves caused infection after only 1h of wetness when incubated between 10 and 25 ℃, nonetheless longer wetness duration was needed to reach the maximum infection efficiency: 3h at 20/25 ℃ and 6h at 10/15 ℃. No infection occurred at 5 and 30 ℃. Combination of temperature/wetness duration also influenced disease severity. These results were fit to a nonlinear model to predict infection severity for each combination of temperature/wetness duration; this model can be used in infection prediction instead of the simpler criterion of Blaeser and Weltzien (1979) as occurs for *V.labrusca* (Lalancette et al., 1988). Incubation and latency periods were mainly influenced by temperature; when incubated at 10/15 ℃ the pathogen required 9days for the appearance of symptoms, and only 4days at 20/25 ℃. Below 15 ℃, *P.viticola* caused infection but did not produce sporangia.

**P11.011 Diagnosis of pathogens responsible of pre- and post-harvest rice diseases in Madagascar**

*M.L. Razanakoto[1], C. De Clerck[1], O. Parisi[1], F. Zalmine[2], C. Rabemanantsoa[2], M. El Jaziri[3] and M.H. Jijakli[1]*
*[1]Laboratory of Phytopathology, Gembloux Agro-bio tech, University of Liège, Passage des Déportés 2, Gembloux 5030, Belgium; [2]Department of Biology, Laboratory of Education and Resaerch in Medicinal Biologiy, Faculty of Medicine, University of Antananarivo, Antananarivo Madagascar; [3]Laboratory of Plant Biotechnology, University of Brussels, Belgium*
*Email: MH.Jijakli@ulg.ac.be*

Rice (*Oryza sativa*) is the most cultivated crop and the staple food in Madagascar. However, the country has never achieved food self-sufficiency and continues to import rice abroad. Rice diseases are one of the factors limiting rice production and causing significant yield reductions in the country. Our study aims to identify the rice pathogens present in Madagascar as a first step in the development of new biological method to control them. Rice samples were collected from farmers in several regions of Madagascar and were plated in standards media (Potato Dextrose Agar for fungi and Nutrient Agar for bacteria). A survey questionnaire was also conducted with the farmers in order to collect information about the context in which the diseases appeared. Isolated strains were identified by morphological characterization for fungi, gram stain and anaerobic growth test for bacteria. Two sets of oligonucleotides were used for amplification of hypervariable regions of the nuclear ribosomal DNA: ITS1 and ITS4 for fungi, F357 and R538 for bacteria. Amplicons were sequenced and analyzed with BLAST. Almost all the fields visited had rice presenting disease symptoms. We observed also that one kind of symptom was specific of one region. Predominant pathogens were thus different in the regions of survey. In Analamanga and Vakinankaratra, *Alternaria sp*. and *Xanthomonas oryzae* pv. *oryzae* were pre-dominant. In the Atsinanana region, however, *Curvularia lunata* and *Pseudomonas fuscovaginae* were the most present.

**P11.012 Effect of inoculum concentration on the development of anthracnose fruit rot on flowers and fruit of different strawberry cultivars**

*B.B. Forcelini[1], F.P. Gonçalves[2] and N.A. Peres[1]*
*[1]Gulf Coast Reserach and Education Center – Wimauma, Florida, USA; [2]Escola Superior de Agricultura Luiz de Queiroz / USP, Piracicaba, Brazil*
*Email: bforcelini@ufl.edu*

Anthracnose Fruit Rot (AFR), caused by *Colletotrichum acutatum*, is a major strawberry disease in the southeastern United States and can cause up to 80% yield loss. Pathogen-free plants, fungicides and resistant cultivars are important tools to control the disease and reduce spread. The effect of inoculum concentration on AFR development in cultivars with different susceptibility levels was determined in field and detached fruit experiments. In the field study, the AFR incidence on three cultivars ('Strawberry Festival' 'Treasure' and 'Camarosa') was compared using five inoculum concentrations ($10^3$ to $10^6$ conidia/ml) on flowers and green fruit for 16 days. On detached fruit, AFR incidence was assessed on green fruit of 'Strawberry Festival' and 'Camarosa' using six inoculum concentrations ($10^2$ to $10^6$ conidia/ml) for 9 days. There was a significant interaction between inoculum concentration, cultivar, and plant organ. Disease incidence was lower on 'Strawberry Festival' than on 'Treasure' or 'Camarosa' on green fruit and flowers independent of the inoculum concentration. For all cultivars and inoculum concentrations, green fruit was more susceptible than flowers. Moreover, minimum inoculum concentration for symptom development for 'Camarosa' was $10^3$ in the field and $10^4$ on detached fruit and for 'Strawberry Festival' was $10^4$ in the field and $10^5$ in detached fruit. Cultivars less susceptible to AFR, such as Strawberry Festival should be used in areas where weather conditions are extremely favorable for AFR and *C. acutatum* is present.

**P11.013 Applying quantitative real-time PCR (qPCR) and spore trapping techniques for the development of a rice blast monitoring and forecasting model**

*C.M. Huang, D.J. Liao and C.L. Chung*

*Department of Plant Pathology and Microbiology, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei, 10617 Taiwan, China*
*Email: r00633009@ntu.edu.tw*

Rice blast is one of the most devastating diseases of rice worldwide. In Taiwan, despite the development of a rice blast forecasting model in 1970s, nationwide disease monitoring has long been relying on periodic surveys by trained plant protection personnel. The conventional naked-eye examination approach, however, has its limitations. The objective of this study is to first develop an approach which allows the collection and quantification of *Magnaporthe oryzae* conidia in the field. A modified rice blast forecasting model, using amount of conidia along with weather factors as parameters, will then be established. We have successfully developed a cyclone-based spore trap and a standard sample processing protocol for extracting DNA from collected airspores. Using qPCR technology and a specific primer pair designed based on *Magnaporthe* infection structure specific protein (*mif23*) gene, the amount of *M. oryzae* conidia can be easily quantified. While detection limit for the qPCR method can be as low as 4 copy numbers of *M. oryzae* gDNA, the limit for reliable and accurate quantification is 10 copy numbers. Aiming to build a forecasting model, airspore samples, weather data, and disease severity have been periodically collected from a few selected monitoring stations located in rice fields. Our preliminary data showed that the new technique was able to detect *M. oryzae* conidia before the appearance of blast symptoms, and the amount of conidia and disease severity were positively correlated. We are working to set up a regression model that better explains the relationships among all the variables.

**P11.014 VIPS – a technology platform for integrated pest and disease management (IPM) in agriculture**

*B. Nordskog, J. Netland, H. Eikemo, H. Hole, T.E. Skog, A.F. Schjøll and G. Brodal*
*Bioforsk – Norwegian Institute for Agricultural and Environmental Research, Hogskoleveien 7, N-1432 Aas, Norway*
*Email: berit.nordskog@bioforsk.no*

VIPS is a web-based forecasting and information service developed for integrated management of pests and diseases in cereals, vegetables and fruit crops. It also includes decision support for management of weeds in cereals based on the Danish Plant Protection Online. VIPS is a collaborative project between Bioforsk and the Norwegian Agricultural Extension Service (NAES) under a government-funded action plan for reduced risk in use of pesticides. Forecasting models that predict the likelihood of pest or disease outbreak can support growers in determining if or when pesticides are needed. Input in these models are historical weather data from a network of weather stations located in crop production areas, in combination with weather prognosis from the Norwegian Meteorological Institute and biological/field observations collected by NAES. A general interface is used for all models, allowing new models to be implemented into the existing system. The web site is open and free of charge (www.vips-landbruk.no), including an application for smart phones. Future perspectives include implementation of data from virtual weather stations based on farm specific weather prognosis and radar measured rainfall. Although VIPS is purpose-made to serve the needs of Norwegian farmers, Bioforsk is currently involved in several international projects where our experience with VIPS is integrated in the development and objectives of the projects.

**P11.015 Distribution and incidence of apple powdery mildew in a mixed cultavar orchards and relationship to disease severity**

*A. Singh, K.P. Singh and G.S. Rajwar*
*22, GaneshDham, Newada, Sunderpur, Varanasi – 221005, India*
*Email: amitabhs777@gmail.com*

Apple powdery mildew epidemics, caused by *Podosphaera leucotricha* (Ell. and Ev.) Salm., can be readily described in terms of the disease triangle. The role of different environmental factors, viz., temperature, relative humidity, leaf wetness, sunshine and rainfall were studied in relation to disease development. The present experiment was conducted during the season 2003 to 2005 to determine a simplified assessment procedure by which apple powdery mildew severity/index could be predicted from incidence data and develop incidence-severity relationship in apple cultivars under Uttaranchal hilly conditions. The development of powdery mildew on ten popular cultivars of apple, viz., Mollies Delicious, Red Chief, Braeburn, Bakingham, Early Shanbery, Jona Mac, Red Free, Red Fuzi, Golden Spur, and Chaubatia Anupam were studied to determine incidence-severity relationship. The use of percentage scales and keys of visual disease severity, remote sensing, and some indirect methods like spore counts and disease incidence are considered valid approaches for disease assessment. The relationship between increase in incidence of powdery mildew in relation to severity can be established either by making sequential records in one tree during the progress of an epidemic or by assessing many trees with different amounts of disease at one point of time. Thus, from the above analysis, it is evident that a combination of several factors like the presence of susceptible host, virulent pathogen, and congenial environment for disease development during receptive phenological stage of apple tree, was responsible for the incidence of the powdery mildew on apple in Uttaranchal during 2003 - 2005 orchard seasons.

**P11.016 Effect of low temperature and host on survival of *Puccinia striiformis* f. sp. *tritici***

*J.X. Qiao, L.J. Ma, X.Y. Kong and X.P. Hu*
*State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest Agriculture and Forestry University, Tai Cheng Road 3, Yangling 712100, P. R. China*
*Email: xphu@nwsuaf.edu.cn*

Wheat stripe rust (yellow rust), caused by *Puccinia striiformis* Westend f. sp. *tritici* Eriks (PST), is one of the most destructive diseases in many wheat-growing regions worldwide. Overwintering survival of PST is one of the most important factors determining the epidemic of wheat stripe rust. In the natural condition, PST begins to overwinter in hypha (mycelia) in host plant when the average temperature in January (monthly average of daily air temperatures) is 1~2°C. When temperature declines to -6~-7°C, or as low as -10°C when wheat is under snow cover, PST might not survive according to the previous research results. Based on those results, the overwintering boundary of PST in China was confined as Huangling (Shanxi), Jiexiu (Shanxi), Shijiazhuang (Hebei), and Dezhou (Shandong). Recent results showed that the overwintering boundary of PST in China was Daxin (Beijing), Xushui (Hebei), Yangquan (Shanxi), Yanchang (Shaanxi), Qinyang (Gansu), Pingliang (Gansu), Gangu (Gansu), Lixian (Gansu), Songpan (Sichuan), and Maerkang (Sichuan) using the probability 70-85% of monthly average temperature more than -7°C in December or January as standard. We studied that PST survival varies with different low temperature and time duration using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) method. The results showed that PST could not survive after 48h at -10°C, 12h at -15°C, and 24h at -20°C in wheat leaves. Cultivar also affected the survival of PST. This study can provide theoretical guidance for disease field investigation and prediction.

**P11.017 The impact of ecology factors for rape clubroot**

*J.C. Ma, Z.M. Bao, H. Li, C.H. Fang and G.J. Tan*
*Anhui Agricultural University, Anhui province Hefei city; Huangshan Agricultural Extension Service Center, Huangshan city, P.R.China*
*Email: tgj63@163.com*

As one of the most important cash crops, rape has played a significant role in the national economy. Rape clubroot disease caused by *Plasmodiophora brassicae*, poses a threat to the rape production industry. *P. brassicae* is a soil-borne obligate Parasite. Resting spores of it have a great ability to survive in soil. It is good for rape clubroot in the south of Anhui Province where is much of acid soil. The objective of this study was to identify The characteristic of rape clubroot in Anhui. We investigated four villages in Huangshan city. For example Xiuning,Huizhou,Tunxi and Shexian,a total of 102 parcel of rape fields.Difference in cultivars, soil types , plant arrangements, previous crops and areas were researched to make sure the occurrence characteristics and effectively control the damage of it. The results indicated that significant difference in rape clubroot was led to by different ecological factors. Acid soil, highly susceptible cultivars, direct seeding can trigger the serious disease.

**P11.018 Survival quantity and loci of *Puccinia striiformis* f. sp. *tritici* on overwinter wheat**

*L.J. Ma, X.Y. Kong, J.X. Qiao, J.J. Wang and X.P. Hu*
*State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest Agriculture and Forestry University, Tai Cheng Road 3, Yangling 712100, P. R. China*
*Email: malijie1016@163.com*

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (PST), is an important airborne epidemic disease on wheat, seriously threatening food production. Spring epidemic is the most destructive period of wheat stripe rust, and the disease severity is mainly determined by the level and distribution of overwinter pathogen and the climate conditions during the spring (Li and Zeng, 2002). Spring field investigation of wheat stripe rust is conventionally conducted through visual observations, which could not detect the pathogen inside plant tissue, leading to forecasting inaccurately for wheat stripe rust. To better understand the quantity level and distribution of overwinter pathogen on wheat, the qRT-PCR technique had been used for determining PST survival quantity in different loci on wheat, especially on leaf sheaths. At the same time, overwinter wheat plants infected by PST at last autumn were separately cultured to clear and definite that whether the overwinter pathogen had infected activity in the next spring. In 2011, 68 samples were collected from Gansu province, the survival pathogen were detected and quantified by qRT-PCR, from which Zhuanglang county was supposed to the hardest areas for PST overwinter compared with Qinzhou and Li county. According to the separate experiment, 6 sporulation plants were observed from 25 wheat plants in Li county, 2 sporulation plants were observed from 10 plants in Qinzhou county, and there were none sporulation observed in Zhuanglang county. The loci of overwinter PST on wheat were preliminary studied.

**P11.019 PODYAM: a modeling framework for simulating population dynamics in agricultural mosaics**

*M. Gosme[1], F. Vinatier[1,2], L. Hossard[1], M. Valantin-Morison[1] and M.H. Jeuffroy[1]*
*[1]INRA, UMR211 Agronomie, F-78850 Thiverval-Grignon, France; [2]INRA, UMR1221 LISAH, F- 34060 Montpellier,*

*France*
*Email: marie.gosme@grignon.inra.fr*

Integrated Pest Management (IPM) requires that several control methods be combined in order to control simultaneously several pests (weeds, diseases, animal pests). Many models have been developed to predict disease epidemics and insect pests' population dynamics for a single pest on a single crop. But it is often difficult to design IPM strategies based on these models because of the lack of common formalisms between the different models. Here, we propose a modeling framework in which any pest or disease could be simulated in order to predict the effect of the configuration of different control strategies in agricultural landscapes. The framework is spatially explicit and strives to represent the population dynamics of all organisms in a common framework. The organisms' life cycles, as well as cultural practices, are described with generic processes acting on the abundance matrices (organism's stages x cells of the grid lattice representing the landscape). Different pests can be simulated by using specific functions and parameters for each process. In order to demonstrate the genericness of the approach, three existing single-pest models working at the landscape scale were adapted within the framework: a model of brown rust of wheat, a model of blackleg of oilseed rape and a model of pollen beetle of oilseed rape. The exercise proved to be more or less difficult depending on the complexity of the initial model and simplifications were sometimes necessary to comply with the constraints of the approach.

**P11.020 Dispersal behavior of *Diaphorina citri Kuwayama* (Homotera: Psyllidae) under laboratory condition**

*C.Q. Ruan[1], B. Liu[1], Z.Q. Wu[2], T. Li[2], H.Q. Hu[1], G.C. Fan, Y.P. Duan[3]and D.G. Hall[3]*
*[1]Fujian Academy of Agricultural Sciences, Fuzhou, Fujian 350003, P.R. China; [2]Fujian Agriculture and Forestry University,Fuzhou,Fujian 350002, P. R. China; [3]U.S. Horticultural Research Laboratory (USDA/ARS), Fort Pierce, FL 34945, USA*
*Email: fzliubo@163.com*

Abstract: Asian citrus psyllid (ACP), *Diaphorina citri Kuwayama*, is the vector of Huanglongbing (HLB), the most devastating disease of citrus worldwide. Knowledge of ACP behavior to search host plants is helpful for understanding HLB spreading within or between citrus trees. In present research in laboratory, the behaviors of ACP adults to evaluate host plants were observed. ACP adults could disperse to seedlings of *Rhododendron simsii* (non host plant for ACP), *Murraya panciculata* L. and "Lugan" *Citrus reticulata Banco* when the plants were put together. The mean number of adults per plant on *R. simsii* was significantly lower than those on citrus and murraya at 18 h and 42 h after ACP were released, respectively. The numbers of adults on citrus and murraya became significantly different 90 h after treatment. The numbers of ACP adults per plant were not significantly different among the 3 types of murraya seedlings classified by development degrees of flushing shoots. Within a tree, the ratio of ACP adults on the new flushing shoots did not significantly increased in comparison to other parts of the tree during 1-4 d after the psyllids were released. Even on 7th day after ACP release, about 30 % adults habituated on the parts of trees other than flushing shoots. The above indicated that ACP adults spent less time to differentiate between host and non-host plants in comparison to the differentiation between different host plants species or different parts of a host plant.

**P11.021 Disease occurrence in 'Fuji' apples in major apple growing regions of Korea during 2002~2012**

*Y.S. Do[1], D.H. Lee[2], S.Y. Lee[1], K.H. Choi[1] and M.J. Kim [1]*
*[1]Apple Research Station, National Institute of Horticultural & Herbal Science, Gunwi 716-812, Republic of Korea; [2]Technology Cooperation Bureau, Rural Development Administration, Suwon 441-857, Republic of Korea*
*Email: nudge@korea.kr*

Disease occurrence in Fuji apple cultivars on 11~22 apple orchards from late March to late October 2002~2012 was investigated at intervals of 30 days. Major diseases infecting apple orchards including Bitter rot (*Glomerella cingulata*), White rot (*Botryosphaeria dothidea*), Marssonia blotch (*Marssonia mali*), Alternaria blotch (*Alternaria alternata*), Gray mold (*Botrytis cinerea*), Sooty blotch (*Gloeodes pomigena*), Fly speck (*Schizothyrium pomi*) and Valsa canker (*Valsa mali*) was surveyed. The medial disease incidence of infected orchards by white rot, bitter rot, Alternaria blotch, Gray mold, Valsa canker, soothy blotch and fly speck, and marssonia blotch was recorded as 0.39, 60.1, 4.95, 0.14, 2.45, 0.10, and 28.2%, respectively. The medial percentage of infected orchards by those diseases was showed as 57.1, 91.4, 35.4, 39.9, 17.7, and 89.5%, respectively. Incidence of white rot in 'Fuji' apple fruits sharply decreased compared to those of the 1990s. The reason why diseases were reduced since 2000s seemed to be the development of effective fungicidal spray schedules. Marssonina blotch was the most severe disease in the 'Fuji' apple in all year of the survey. Consequently, it becomes the most important disease in apple growing regions in Korea.

**P11.022 Effect of UV-B radiation on pathogen germination and epidemic components of wheat yellow rust**

*P. Cheng, H.G. Wang and Z.H. Ma*
*Department of Plant Pathology, China Agricultural*

*University, No.2 Yuanmingyuan West Road, Haidian District, Beijing, 100193, P. R. China*
*Email: wanghaiguang@cau.edu.cn*

Global climate has changed since pre-industrial times and is increasingly recognized as one of main influencing factors of plant diseases. Particularly, the effects of increased solar ultraviolet-B (UV-B: 280-315 nm) irradiance at the ground level, resulting from stratospheric ozone depletion, have stimulated considerable studies in recent decades. The effect of UV-B radiation on pathogen germination and epidemic components of wheat yellow rust (caused by *Puccinia striiformis* f. sp. *tritici*) was evaluated in this study. Germination experiments showed that the germination ability of the urediniospores reduced as the UV-B radiation enhanced and the radiation time extended. The assessment of epidemic components of the disease showed that the enhanced UV-B radiation could cause the decline of sporulation quantity, lesion expansion rate, number of infection sites and disease index, and could prolong incubation period. According to the research results of other scientists, global atmospheric concentration of ozone in the next 60 years could reduce 2-10% and commensurable UV-B radiation could increase 4-20%. Therefore, our results are of great significance for the prediction of wheat yellow rust with enhanced UV-B radiation in the future. This study was supported by the National Natural Science Foundation of China (31101393) and the National Key Basic Research Program of China (2013CB127700).

### P11.023 Quantification of airborne inoculum of *Puccinia striiformis* and *Blumeria graminis* using duplex real-time qPCR

*Y. Li, Y. Luo and Z.H. Ma*
*Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: mazh@cau.edu.cn*

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), and powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), are two major diseases of wheat in China. For both diseases, airborne inocula are major sources causing primary infections and disease development. Quantification of airborne inoculum requires efficient spore trapping, as well as accurate and fast sample processing and analysis methods. In this study, Burkard multi-vial cyclone samplers were used to collect air samples into 1.5 mL centrifuge tubes followed by DNA extraction. A duplex TaqMan quantitative real-time PCR assay was developed using primer pairs and probes each specific to *Pst* or to *Bgt*. Sensitivity tests demonstrated that this assay can detect DNA extracted from less than 10 urediniospores of *Pst* or conidial spores of *Bgt*. The experimental result also demonstrated that co-existence of the two species in a sample had no negative effect on respective quantification for each species without interference each other in quantification. Additionally, environmental dust, tiny insects, plant tissues and others in the samples showed no negative effects on DNA quality from extraction and on quantification of each species comparing with using pure samples. Thus, quantification of spore density in the air for each species using this duplex real-time qPCR is reliable. The standard curves for quantifying *Pst* and *Bgt* were y = -0.321x + 11.75 and y = -0.279x + 10.65, respectively (y: $\log_{10}$ spore number, x: Ct value). This method provided an efficient way to quantify airborne pathogens to estimate potential inoculum of disease epidemics.

### N11.001 Preliminary study on mating system of *Villosiclava virens*

*J.J. Yu, X.L. Yin, M.N. Yu* and *Y.F. Liu*
*Institute of Plant Protection, Jiangsu Academy of Agricultural Science, No.50, Zhongling Street, Xuanwu District, Nanjing, 210014, P.R. China*
*Email: liuyf@jaas.ac.cn*

*Villosiclava virens*, the causal agent of rice false smut, produces ascospores in sexual propagation, which could be one of the primary infection sources of the disease. To better understand its sexual life cycle, the mating-type genes of *V. viren* were initially studied to reveal its sexual propagation. The 185 base pair (bp) conserved coding sequence of housekeeping α-domain of mat1-1-1 mating-type protein was cloned, and this sequence had a similarity of 61% , 63% and 68% to that of *Cordyceps militaris*, *Cor. bassiana* and *Claviceps purpurea*, respectively. Two pairs of specific primers were designed to detect *mat1-1-1* and *mat1-2-1* mating-type genes in *V. viren* strains, and 138 bp and 220 bp length DNA fragments could be amplified by PCR, respectively. Two hundred and forty single-ascospore strains, which were isolated from three stromata belonging to different sclerotium, and 50 randomly selected field isolates of *V. viren* were employed in the mating-type gene detection. And it was found that *mat1-1-1* and *mat1-2-1* mating-type genes existed in the alternative type of strains. The subsequent inoculation assay further proved that *V. viren* was a heterothallic fungus, since almost all the sclerotia and fertile perithecia could be observed upon pair-inoculated strains of “mat1-1-1” and “mat1-2-1” type, and barely observed upon inoculated strains of “mat1-1-1” or “mat1-2-1” type.

## Concurrent Session 12-Diseases of Ornamentals and Turfgrass

### O12.001 Newly important powdery mildews and rusts on herbaceous and woody ornamentals

*M.L. Daughtrey and L. Kiss*
*Cornell University, Long Island Horticultural Research & Extension Center, Riverhead, NY 11901 USA*
*Email: mld9@cornell.edu*

A number of rust and powdery mildew diseases of ornamentals have extended their geographic ranges and become important in their new locations since the turn of the century. North American examples include the pear trellis rust caused by *Gymnosporangium sabinae* on ornamental pear; the Japanese apple rust, *G. yamadae* on crabapple; daylily rust caused by *Puccinia hemerocallidis*; rusts of chrysanthemum, caused by *P. horiana* and possibly *P. chrysanthemi*; as well as powdery mildews such as *Podosphaera xanthii* on petunia and verbena, and other species infecting *Calibrachoa* x *hybrida*, *Torenia fournieri*, *Catharanthus roseus*, and *Acer palmatum*. A large number of rust and powdery mildew fungi have become problems on ornamentals in other parts of the world: for example, *Erysiphe pulchra* on *Cornus florida*, previously known from Japan and the U.S. has recently been reported from Italy; *E. alphitoides* was identified on wisteria in the U.K.; *Leveillula taurica* was newly found on lisianthus, nasturtium, calla lily, *Impatiens balsamina* and balloon plant in Brazil, and *Gymnosporangium* spp. infecting *Crataegus* spp. have been recorded in new places in Europe. DNA sequence data, especially ITS sequences, have been widely used in the identification of both powdery mildew and rust fungi newly found in different parts of the world; however, their use can sometimes be misleading, as one to a very few nucleotide differences may be correlated with important differences in host range and, in addition, some powdery mildew and rust taxa exhibit variability in their ITS regions.

### O12.002 Bacterial diseases of flowering plants, foliage plants and shrubs and their control

*D.J. Norman and J. Chen*
*University of Florida, Mid-Florida Research and Education Center, Apopka, FL, 32703, USA*
*Email: djn@ufl.edu*

Bacterial diseases cause significant economic losses in the floricultural and ornamental plant industries. In today's competitive marketplace, plants are not saleable with disease symptoms such as spotted leaves and flowers, wilted stems, or retarded growth. Standard bactericides containing copper and / or mancozeb are frequently used; but they leave unacceptable residues on plant surfaces. To produce disease free plant materials, modern facilities rely on strict sanitation and cultural controls to prevent bacterial outbreaks. However, even with strict sanitation protocols in place, bacteria can still enter production facilities via contaminated seeds, propagative materials, aerosols or human contacts. In many cases the initial mode of introduction of the pathogen into the production facility is never identified. Once introduced, bacteria become established and spread rapidly through crop contacts or irrigation, causing significant crop loss. In this presentation we will emphasize new effective *Bacillus*- containing biologicals and systemic acquired resistance (SAR) compounds such as benzothiadiazole, ascorbic acid, alpha-keto acids, humic acid, fish oil, *Reynoutria sachalinens* extract and phosphonate for disease control. The effectiveness of these compounds depends on the crop, specific bacterial pathogens targeted, as well as application timing and repeat applications before pathogen challenge. Additionally, titanium dioxide ($TiO_2$) and the nanoparticles of titanium and copper are showing promise as new bactericides.

### O12.003 Diversity and ecophysiology of cryophilic fungal pathogens on turfgrass

*T. Hoshino and N. Matsumoto*
*Biomass Refinery Research Center (BRRC), National Institute of Advanced Industrial Science and Technology (AIST),3-11-32, Kagamiyama, Higashi-Hiroshima, 739-0046, Japan*
*Email: Tamotsu.hoshino@aist.go.jp*

Snow molds attack dormant plants such as forage crops, winter cereals, and conifer seedlings at low temperatures under snow cover. These fungi belong to various taxa (oomycetes, ascomycetes, and basidiomycetes). Oomycetes, *Pythium* spp. were reported to be less tolerant to chilling and freezing temperatures than other fungal taxa. Free mycelia and hyphal swellings, structures for survival, of *P. iwayamai* lost viability after cycles of freezing-thawing. However mycelia in host plants survived the treatment. Basidiomycetous snow molds produce extracellular antifreeze proteins which attach to the surface of ice crystal to inhibit ice crystal growth. An ascomycete, *Sclerotinia borealis* prevails where soil freezing is severe. Mycelial growth rate on frozen plates at -1℃ was faster than that on unfrozen medium at the optimal growth temperature of 4-10℃. *S. borealis* can grow at low water potential on media, and an increase in intracellular osmosis enhances mycelial growth at low temperature. Though, as a whole, snow mold fungi can tolerate low temperatures to prevail under snow, strategies to adapt cryoshpere differ from fungus to fungus, according to the adaptability in their particular habitats.

### O12.004 Biological control of turfgrass diseases

*G.Y. Yuen*

*Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722, USA*
*Email:gyuen1@unl.edu*

Integrated management of turfgrass diseases requires greater use of nonchemical strategies such as biological control. The availability of commercial biocontrol agents and their use in controlling turfgrass diseases, however, is limited. This may be due largely to biocontrol agents exhibiting lower disease control effectiveness than chemical fungicides under variable environmental conditions. In this presentation, over 15 years of research into the control of fungal turfgrass diseases using the bacterium *Lysobacter enzymogenes* strain C3 will be summarized. Investigations into microenvironmental effects on foliar population dynamics of strain C3 revealed a number of factors, such as ultraviolet light and carbon substrate availability, which limit foliar colonization by C3. At the same time, strategies to overcome such limiting factors, including the application of protective and nutritional substrates and the establishment of endophytic bacterial populations, have been identified. Strain C3 was found to express many antifungal mechanisms, and several were demonstrated to have important roles in biocontrol of fungal pathogens in turfgrass. These mechanisms varied as to the plant parts on which they were active and the environmental conditions under which they were expressed. Research using strain C3 has shown that biological control activity of C3 can be improved through understanding the nature of the microenvironment of the turfgrass canopy and the manner in which populations of the bacterium respond to changes in environmental conditions. This knowledge can serve as a model useful for developing other microbial agents into effective biological tools for managing turfgrass diseases.

### O12.005 Differences in the timing and mechanisms of the infection processes of *Microdochium nivale* and *M. majus* on wheat (*Triticum aestivum*) and Kentucky bluegrass (*Poa pratensis*)

*L.E. Jewell and T. Hsiang*
*School of Environmental Sciences, University of Guelph, Guelph, Ontario, N1G 2W1, Canada*
*Email: ljewell@uoguelph.ca*

*Microdochium nivale* and *M. majus* are fungal plant pathogens causing pink snow mold on cereals and grasses. Both species are among the causal agents of Fusarium Head Blight, and *M. nivale* is also responsible for Fusarium patch, a common disease on turfgrasses. Although these species are closely related, *M. majus* has not been observed growing on turfgrass in the field, whereas *M. nivale* is known to infect both cereals and grasses. A histological study was undertaken to investigate the timing and the mechanism of the infection processes of both species on detached leaves of Kentucky bluegrass (*Poa pratensis*) and wheat (*Triticum aestivum*). Detached leaves were inoculated with either conidia or hyphae from single isolates, and were incubated for up to seven days at room temperature. For *M. nivale*, isolates originally collected from both turfgrasses and wheat were included in these experiments, whereas only wheat-derived isolates of *M. majus* were available. Inoculated leaves were collected every 24 hours, stained, and observed under magnification. Hyphal inoculum derived from both species caused infection of both types of plant tissue within four days of inoculation. Conidial inoculum failed to cause infection within this time period. In all cases where infection was observed, hyphae were observed growing directly into stomata, without the formation of appressoria. Isolates of *M. nivale* from both wheat and from grass caused infection on both plant species. These observations suggest that individual isolates of *M. nivale* and *M. majus* can infect multiple host species at least under artificial inoculation conditions.

### O12.006 The infection process and specific detection of the pathogen *Cylindrocladium buxicola* using probes developed from comparative genomic analyses

*S.E. Healy* and *T. Hsiang*
*School of Environmental Sciences, University of Guelph, Guelph, ON. N1G 2W1, Canada*
*Email: shealy@uoguelph.ca*

Box blight causes a serious foliar disease on *Buxus* spp. and the causal pathogen *Cylindrocladium buxicola*, has been very recently been found in Ontario creating concerns for nursery growers. The infection process of *C. buxicola* was investigated on detached leaves of 'Green Gem' using a spore suspension as inoculum. Under optimal conditions, 3 hrs after inoculation, germination was observed, within 24 hrs penetration was seen, and by 72 hrs sporulation was visible. Specific primers for the ribosomal DNA internal spacer region (ITS) specific primers were generated for detection of *C. buxicola.* The complete genome sequence of *C. buxicola* was obtained using Illumina next generation sequencing technology and used in comparative genomic analyses to identify genes which are unique to this fungus but absent in other sequenced fungi to develop primers for these unique genes. These unique primers were compared to the ITS specific primers to assess sensitivity and specificity as well as their ability to detect fungal DNA in infected plant tissue.

### O12.007 Pathogenicity and control of *Armillaria* mellea and *A. gallica* in the UK

*E.J. Beal and B. Henricot*
*The Royal Horticultural Society, Wisley, Woking, Surrey, GU23 6QB, UK*
*Email: lizbeal@rhs.org.uk*

*Armillaria* spp. (honey fungus) are root pathogens and the primary cause of death and decline of woody plants in UK gardens. A 4-year survey found *A. mellea* and *A. gallica* to be the two most common species. Experiments have been designed to further our understanding of their pathogenicity. Rhizomorphs are produced by *Armillaria* to travel from plant to plant but their role in spreading the disease once severed is not known. In order to test this, rhizomorphs of *A. mellea* and *A. gallica* were severed and their growth and viability were examined. Results collected over 3 trials showed that severed rhizomorphs of both species did not grow, could survive up to 6 months but their survival was low (less than 9%). *A. gallica* survived longer than *A. mellea*. This suggests that in the absence of woody material the risk from severed rhizomorphs is not very significant. Preliminary trials were designed to look at host susceptibility to *A. mellea*. Six plant genera including *Betula*, *Fragaria*, *Ligustrum*, *Prunus*, *Rosa* and *Sorbus* growing in pots, standing outside and in a controlled environment, were inoculated with *A. mellea*. Results on infection level in both environments will be presented. Allicin is a stabilised garlic product known for its antibacterial and fungicidal properties but little data is available on its effect on *Armillaria* growth. *In vitro* experiments showed that Allicin was more effective in inhibiting mycelium growth of *A. gallica* (mean EC50=7.9 ppm at 14 days) compared to *A. mellea* (mean EC50=26.4 ppm at 14 days). However, Allicin at a concentration of 20-30 ppm also stimulated the growth of rhizomorphs of both *Armillaria* species limiting the use of this product to control the disease.

**O12.008 Relationship of inoculum level to the development of gray bulb rots on tulips and iris**

*G.A. Chastagner, K. Coats and A. DeBauw*
*Washington State University Research and Extension Center, Puyallup, WA 98371, USA*
*Email: chastag@wsu.edu*

Gray bulb rot, caused by *Rhizoctonia tuliparum*, can cause significant losses in the production of tulips and bulbous iris. Two field trials were conducted to examine the relationship between the levels of *R. tuliparum* sclerotia in soil and the development of gray bulb rot on tulips and iris. At the time of planting, inoculum was added to the planting cells at various rates. The inoculum consisted of sclerotia that were grown on sterilized rice grains. Zero to 33.3 grams of the inoculum per 30.5 cm of row was added to the soil covering the bulbs at the time of planting. No disease developed on any of the iris or tulips when the soil was not infested with inoculum. The number of iris and tulips that emerged varied with inoculum level. Data analysis showed that there was a highly significant correlation between the number of emerged and/or healthy iris and tulips plants. The highest inoculum levels resulted in a three-fold reduction in the number of tulips that emerged and reduced the number of healthy iris plants by one half. A similar trend was observed with respect to the yield of iris bulbs. A qPCR-based assay is being developed to quantify inoculum levels of this pathogen in soils.

**P12.001 Identification and distribution of fungi associated with root and crown rot of hopbush (*dodonaea viscosa* ) in Khuzestan province, South west, Iran**

*H. Alvani por and R.Farrokhi Nejad*
*Department of Plant protection, Shahid chamran University of Ahvaz, Iran*
*Email: Alvani_2006@yahoo.com*

Hopbush (*Dodonaea viscosa*) is a member of *Sapindaceae* family that is an ornamental hedge plant and has valuable medicinal properties. Its cultivation in khuzestan province is faced with some difficulties including root and crown rot disease caused by different pathogens. In order to identify fungi associated with *Dodonaea viscosa* crown and root rot and their distribution, during 2011 samples were collected from Ahvaz, Sousangerd and Hamidie regions in Khuzestan province. Samples were sterilized with 1% and 5% sodium hypochlorite or ethanol and finally were transfered on PDA, CMA and Nash & Snyder media. The isolates were purified using single spore and hyphal tip methods. Sixty-five fungal isolates were obtained including 3 isolates of *Lasiodiplodia hormozganensis*, 60 isolates of the genus *Fusarium* and 2 isolates of *Pythium* sp. Based on their morphological characters, *Fusarium* isolates were identified as *F.solani* and *F.equiseti*. The frequency of isolates of *L. hormozganensis*, *F. solani*, *F. equiseti* and *Pythium* sp. were 4.6, 84.6, 7.6 and 3.0 percent, respectively. Pthogenicity tests were conducted in petri plates revealing that the *L. hormozganensis*, *F.solani*, *Pythium* sp. isolates were able to cause seed rot, root rot and damping off. Pathogenicity *invivo* tests (on pot ) that conducted with virulence two *F. solani* from Ahvaz and Sousangerd showed that 2-3 days after inoculation, fungi causes root and crown rot and damping off. This is the first report of *L. hormozganensis* on *Dodonaea viscose* in the world. Different species of *Lasiodiplodia* cause diseases on plants as well as humans.

**P12.002 Powdery mildews recently detected on ornamental plants in Italy**

*D. Bertetti[1], P. Pensa[1], M.L. Gullino [1,2] and A. Garibaldi[1]*
*[1]Center of Competence for the Innovation in the Agro-environmental Sector (AGROINNOVA), University of Torino, Via Leonardo da Vinci, 44, 10095 Grugliasco (TO), Italy; [2]DISAFA, University of Torino*
*Email: domenico.bertetti@unito.it*

In the last five years several powdery mildews were observed, for the first time in Italy, on ornamental plants

growing in farms, in nurseries, in public and private gardens located in Piemonte and Liguria regions. Symptoms were described. Features of conidia and conidiophores observed on the microscope were reported. If the perfect stage was observed, chasmothecia, asci and ascospores were described. The ITS analysis was carried out to confirm the morphological identification of the fungal causal agents of the diseases. Symptoms were reproduced inoculating artificially healthy plants. In farms and nurseries located in Liguria the following pathogens were detected: *Podosphaera* sp. on *Euphorbia susannae*, *E. inermis*, *E. perdorfiana* and *E. aggregata*, *Golovinomyces biocellatus* on spearmint (*Mentha spicata*) and *G. cichoracearum* on Paris daisy (*Argyranthemum frutescens*). The last pathogen was reported also on moth mullein (*Verbascum blattaria*) cultivated in a greenhouse located in the campus of the University of Torino, while *Erysiphe cruciferarum*, *G. cichoracearum* and *E. heraclei* were detected respectively on spider flower (*Cleome hassleriana*), *Rudbeckia fulgida* and English ivy (*Hedera helix*) growing in public gardens of Piedmont and Liguria. Finally, in private gardens of Piedmont, we identified *Golovinomyces cichoracearum* on gerbera (*Gerbera jamesonii*), *Erysiphe pulchra* on *Cornus florida*, *Podosphaera* sp. on common phlox (*Phlox drummondii*), *Golovinomyces orontii* on creeping bellflower (*Campanula rapunculoides*) and *Golovinomyces cichoracearum* on michaelmas daisy (*Aster novi-belgii*).

### P12.003 Ornamental plants infected by soil-borne pathogens for the first time in Italy

*D. Bertetti[1], G. Gilardi[1], M.L. Gullino[1,2] and A. Garibaldi[1]*
*[1]Center of Competence for the Innovation in the Agro-environmental Sector (AGROINNOVA), University of Torino, Via Leonardo da Vinci, 44, 10095 Grugliasco (TO), Italy; [2]DISAFA, University of Torino*
*Email: domenico.bertetti@unito.it*

In the last five years, several soil-borne pathogens were detected on ornamental plants, for the first time in Italy. New hosts were cultivated in nurseries, in farms, in private and public gardens located in Piemonte and Liguria regions. Symptoms were described. The causal agents were isolated and identified observing their morphological characteristics appeared on colonies grown *in vitro*. The ITS analysis confirmed the identifications. Koch's postulates were fulfilled reproducing the symptoms of the diseases on healthy plants, from which were reisolated the same pathogens artificially inoculated. *Fusarium oxysporum* was isolated from *Papaver nudicaule* cultivated for cut flowers and from two succulent plants: *Cereus peruvianus monstruosus* and jade plant (*Crassula ovata*). *F. oxysporum* isolated from jade plant was ascribed to the new forma specialis *crassulae*. Another new forma specialis is evaluating for *F. oxysporum* isolated from *P. nudicaule*. *Verticillium dahliae* was reported on *Lampranthus* sp. cultivated in Liguria, on *Rudbeckia fulgida* and *Coleus verschaffeltii*, both growing in a public garden of Torino. *Pythium aphanidermatum* caused collar and stem rot on *Lampranthus* sp., *Phytophthora nicotianae* was the causal agent of collar and root rot on *Daphne odora* and *Edgeworthia papyrifera*, while *P. cinnamomi* was isolated from affected roots of *Kalmia latifolia*. *Rhizoctonia solani* belonging to anastomosis group AG-4 was detected on *Hosta fortunei*, *Lupinus poliphillus* and *Aquilegia flabellata*, while *R. solani* AG-1 was isolated from *Digitalis purpurea*; both the groups AG-1 and AG-4 were found on *Salvia nemorosa*. Finally, *Sclerotinia sclerotiorum* was reported on *Aquilegia flabellata*, *Borago officinalis* and *Petunia × hybrida*.

### P12.004 *Pythium* as a cause of foliar blight of mature woody plants

*G. Denton[1], S. Roy[2], J. Denton[1] and S. Archer[2]*
*[1]The Royal Horticultural Society, Wisley, Woking, Surrey. GU23 6QB UK; [2]Imperial College, London UK*
*Email: geoffdenton@rhs.org.uk*

Foliar blights have been observed on evergreen mature shrubs in the UK during 2006 – 2009 via the Royal Horticultural Advisory (RHS) Service. *Rhododendron, Ilex* and *Osmanthus* plants had dark spotting, often causing a V-shape lesion, similar to those typical for Phytophthora blights. Following identification of organisms associated with the lesions through sequencing of the ITS region, over 50% could be ascribed to three species of *Pythium* , namely *Pythium intermedium*, *Pythium attrantheridium* and a third probably undescribed species closely related to the other two. Detached *Rhododendron, Ilex* and *Osmanthus* leaves were used in infection assays with all three species, the results indicating varying susceptibility of the host plants and differing aggressiveness of the *Pythium* species. The *Pythium* species were re-isolated from the lesions and identification confirmed by morphology or sequencing of the ITS region. We conclude that some *Pythium* species can cause foliar blights that might previously have been ascribed to *Phytophthora*.

### P12.005 Exploring alternative possibilities of non-chemical methods in managing stem rot (*Rhizoctonia solani* Kühn) of carnation under protective cultivation

*S. Chandel and S. Sharma*
*Department of Plant Pathology, Dr.Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P)-173230, India*
*Email: schandelmpp@yahoo.co.in; schandelmpp@rediffmail.com*

Carnation (*Dianthus caryophyllus* L.), a native of Mediterranean region belonging to family caryophyllacea is

one of the major cut flowers of the world and ranks 7th in world flower market. Having realizing the importance of asthetic value in recent years, the demand of this flower has increased manifolds in National and International markets including India. The agro-climatic conditions of Himachal Pradesh are highly suited for seasonal and off-seasonal growing. The flower very often is subjected to attack by number of plant pathogens causing stem rot, wilt, leaf spots, bud rot and rust like diseases. However the devastating nature of stem rot causes high mortality rate upto 37 to 42 percent. Hence looking into the ill effects of the chemicals a suitable ecofriendly technology in integrated form involving the use of soil amendments, biological control agents, bio-fumigants and botanicals was devised and applied under protected cultivation on disease reduction and enhancement of growth parameters. Soil amendment in form of seed powder of *Melia azadarach* was found most effective when applied @2% (w/w basis). The incidence of stem rot was reduced to 17.33% from 40.33% in case of native strains of *Trichoderma viride* followed by *T. harzianum* (20.00%) showing increase in plant height, stem length, flowers/plants, flower size and reduction in number of days to first flowering. Integration of soil amendment and biocontrol agents resulted into minimum incidence (6.00%) in *T. viride+ Melia azadarach, T. harziamum +Melia azadarach* and *T. harzianum* + neem cake treatments followed by *Bacillus subtilis+ Melia azadarach*. Residues of cruciferous crops possessing properties of fumigants due to releasing of volatile substances showed significant superiority over untreated carnation plants. Cauliflower and cabbage among other crop residues were most effective in lowering disease incidence to 22.28 and 24.25% from 48.34% by registering lowest inoculum load of $22.82x10^3$ and $26.43x10^3$ c.f.u.$g^{-1}$ soil, respectively compared to control. Plant extracts of *Melia azadarach* and *Adhatoda vasica* out of various botanicals and two commercially available neem formulations, Neemazil and Neemgold showed maximum inhibitory action against *Rhizoctonia solani* at higher concentration of 40 per cent.

**P12.006 Volutella leaf and stem blight of boxwood in Canada**

*F. Shi and T. Hsiang*
*School of Environmental Sciences, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada*
*Email: fshi@uoguelph.ca*

In Canada, an outbreak of a disease of *Buxus* species (boxwood) was observed in 2008, although appears of the disease was first noticed over 10 years before that in Canada. To determine the causal agent of this boxwood disease, over 300 fungal isolates were obtained from diseased samples from 2008 to 2010. Eight major fungal morphotypes associated with blighted tissues were identified using morphological and molecular techniques. Among them, only *Pseudonectria buxi (DC.)* Seifert, Gräfenhan & Schroers showed pathogenicity to boxwood, and the symptoms and signs were typical of Volutella leaf and stem blight caused by *P. buxi*. In detached tissue tests, one-month-old leaves were found to be more susceptible to Volutella blight than one-year-old leaves. Among commonly grown *Buxus* cultivars in Ontario, the cultivar 'Green Gem' was the most susceptible compared to 'Green Velvet', 'Green Mound', 'Green Mountain' or 'Pincushion'. Wounds may be the major infection points for *P. buxi* since non-wounded inoculated tissues did not become visibly diseased. The genome of this fungus was sequenced, and a *MAT1-2* gene was detected. By PCR amplification of the genes around the mating type gene locus, the *MAT1-1* idiomorph was found in other isolates, and the ratio of the two idiomorphs approximates 1:1 implying a sexually reproducing population in Ontario, Canada.

**P12.007 Investigations on the occurrence and control of crabapple canker at the Summer Palace**

*S. Wang[1], Y. Li[2] and Q. Wang[2]*
*[1]Administrative office of the Summer Palace, Beijing Municipal Administration Center of Parks, Beijing, 100091, P. R.China; [2]Department of Plant Pathology, China Agricultural University, Beijing 100193, China*
*Email: kingnery05@hotmail.com*

Confronting the extreme climate and the following secondary disaster，the landscaping management should have the corresponding operating efficiency and coping capacity. This paper investigated and summarized the pathogenesis regularity, havoc symptom, inducement and control for crabapple canker after heavy snow falls and freezing weather in the Summer Palace. Freezing injury was the important factor to cause the spread of this disease in 2010. The results of field test showed that 3 kinds of disinfectant had obvious treating effect on infected trees. The control efficiency of amobam, benziothiazolinone and berberine was 86.7%, 80% and 73.3% respectively. A calendar had been established which can be easily used to guide the operation for preventing crabapple canker. We hope to provide scientific basis for management system of the secondary disaster, boosting the sustained and sound development of greening industry.

## Concurrent Session 13-Endophytes

### O13.001 Endophytic fungi are involved in multiple balanced antagonisms

*B. Schulz[1],C. Junker[1], S. Haas[1], M. Schobert[1], C. Citron[2] and J. Dickschat[2]*
*[1]Institute of Microbiology, Technical University of Braunschweig, Spielmannstr. 7, 38106 Braunschweig, Germany; [2]Institute of Organic Chemistry, Technical University of Braunschweig, 38106 Braunschweig, Germany*
*Email: b.schulz@tu-bs.de*

In order to infect, grow and survive within their plant hosts, fungal endophytes must deal not only with the plant's physical barriers and defense responses, but also with bacterial and fungal competitors. Secondary metabolites of both host and endophyte are involved in these interactions. Whereas endophytic colonization of the host often activates host defense reactions, endophytic fungi may secrete metabolites toxic to the host. In fact, endophytes produce a higher proportion of herbicidal metabolites than even phytopathogens do. But endophytes also secrete metabolites active against competitors. Co-culture of fungal endophytes with bacterial or fungal competitors resulted in altered secondary metabolite profiles, with each of the partners secreting metabolites toxic to the other. This is the case when the root endophytes *Pseudomonas aeruginosa* and *Fusarium* sp. are grown in co-culture, with the fungus secreting metabolites that inhibit *P. aeruginosa* that are not synthesized in monoculture. In another example, in co-culture, endophytes of *Fraxinus excelsior* secreted metabolites that inhibited growth of *Hymenoscyphus pseudoalbidus*, the causal agent of ash dieback. However, *H. pseudoalbidus* secreted metabolites that inhibited growth of the endophytes. This co-culture also resulted in reduced synthesis by *H. pseudoalbidus* of its phytotoxic metabolite, viridiol. In conclusion, we hypothesize that in order to grow asymptomatically within their hosts, endophytic fungi secrete metabolites toxic to the host to counter plant defense reactions, as well as those that inhibit growth of competitors. Thus, in order to grow asymptomatically, they are involved in multiple balanced antagonisms, responding with phenotypic plasticity to the respective situation.

### O13.002 Fungal Endophytes: Better players of biodiversity, host protection, antimicrobial production and nanotechnology

*R.N. Kharwar and S.K. Gond*
*Mycopathology and Microbial Technology Labaratory, Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi-221005, India*
*Email: rnkharwar@yahoo.com*

Endophytic fungi, a group of microbes that reside inside plant tissues have been the important source of bioactive compounds in addition to their biodiversity. Only about 5-8% of fungal species are yet known, and rests are awaiting their exploration from different niches and one of them may be unexplored endophytes. Since endophytes live in unique biotope that may lead to produce novel bioactive compounds. Presently, the study on endophytic fungi is confined only to some geographical regions and viewing India's plant biodiversity, the idea to isolate the endophytic fungi from Indian medicinal plants and to screening their abilities to produce bioactive compounds may be of great interest. Endophytic fungi found promising in producing natural bioactive having new mechanism of action within the cellular metabolism. Thus my group explored fungal endophytes of *Azadirachta indica*, *A. marmelos, N. arbor-tristis, Eucalyptus, Tinospora cordifolia, Adenocalymma alleaceum* and *Maduca indica* and addition to huge diversity, found some interesting bioactive compounds such as javanicin, 1-Iodo, naphthalene and flavoglucinol with impressive results. Biosynthesis of metal and semiconductor nanoparticles using microorganisms has emerged as a more eco-friendly, simpler and reproducible alternative to the chemical synthesis, allowing the generation of rare forms such as nanotriangles and prisms. *Aspergillus clavatus*, *A. terreus*, *Phoma herbarum*, were used for biosynthesis of silver and gold nanoprticles using aqueous solution of silver nitrate ($AgNO_3$), and tetra auro chlorate ($HAuCl_4$) respectively. Transmission electron microscopy (TEM), Atomic force microscopy (AFM), UV-Vis spectroscopy, and X-ray diffraction (XRD) were used to decide the sizes and shapes of Nps.

### O13.003 Xylariaceous endophytes: production of bioactive compounds, phylogeny, ecology and anamorph-teleomorph relationships

*M. Stadler*
*Dept. Microbial Drugs, Helmholtz Centre for Infection Research, Inhoffenstr. 7, 38124 Braunschweig, Germany*
*Email: marc.stadler@helmholtz-hzi.de*

The Xylariaceae is one of the largest families of Ascomycota and exhibit a great biological diversity, above all in the tropics. They are traditionally known for their ability to form conspicuous stromata on dead wood, but are among the most frequently encountered endophytes of seed plants. Some species are closely associated with insect vectors, which mediate the dispersal of their propagules. Perhaps owing to their interesting ecology, the Xylariaceae are prolific sources for unique secondary metabolites. The antiparasitic agents, PF-1022A and nodulisporic acid are among the very few endophyte-derived compounds that have entered into drug development. We have used the Xylariaceae as a model family to establish correlations between biodiversity and chemical diversity, in an international network of mycologists

and analytical chemists. More than a decade of intensive field work and monographic studies, using classical morphological methods, secondary metabolite profiling by HPLC/DAD-MS, and molecular phylogenies have resulted in a data matrix. This now serves well to link he endophytic states of these fungi to their wood-inhabiting teleomorphs. In the process of this work, numerous new and interesting species that also produce novel, unique secondary metabolites have been discovered. In addition, we were able to clarify the life cycle of certain xylariaceous endophytes and found various novel and/or interesting secondary metabolites with chemotaxonomic, ecological, or even phylogenetic significance. Aside from a review of our past work, some recently published and unpublished data on the above topics will be presented.

**O13.004 Arbuscular mycorrhizal fungi: in vivo imaging of their interaction with host plants and *Trichoderma***

*P. Bonfante, A. Genre, B. Lace, S. Woo and M. Lorito*
*Department of Life Sciences and Systems Biology, University of Torino, Italy*
*Email: paola.bonfante@unito.it*

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs which colonise the roots of most land plants, gaining plant-assimilated carbon, and provide benefits to their hosts, mostly based on an improved mineral nutrition. Thus far, genetics and molecular-based approaches have mainly illustrated plant responses, although genomics and transcriptomics on AMF are starting to give light on the biology of these widespread endophytes. In vivo confocal microscopy observations on *Medicago truncatula* root organ cultures expressing GFP tags, allowed us to describe early plant responses to AMF molecules as well AMF contact (oscillation of calcium spiking; cytoplasmic reorganization). We also describe how the introduction of *Trichoderma atroviride* PKI1, a biocontrol agent of fungal pathogens, leads to important changes in the tripartite interaction. Results indicated that PKI1 is capable to parasitize *Gigaspora gigantea* hyphae, through localized cell wall degradation, and is a strong root colonizer, causing localized cell death. Different from the AMF, PKI1 did not activate a cytoplasmic aggregation and its exudates were ineffective in triggering nuclear calcium oscillations. These in vivo imaging results show that the symbiosis between an AMF and its host may be strongly perturbed by the introduction of another endophyte, like PKI1. Under these conditions *T. atroviride* likely exploits its enzymatic arsenal against both the AMF and the plant host, while in nature the complexity and competitiveness of the rhizosphere environment may mitigate Trichoderma aggressiveness, resulting in a beneficial outcome of the fungus plant interaction.

**O13.005 Fungal endophyte-assisted soil phytoremediation using willow: an integrated metabolomics approach**

*K.A. Aliferis and S. Jabaji*
*McGill University, Department of Plant Science, 21111 Lakeshore Rd., Sainte-Anne-de-Bellevue, QC H9X 3V9, Canada*
*Email: konstantinos.aliferis@mcgill.ca; suha.jabaji@mcgill.ca*

Soil decontamination is challenging and costly task, and currently, 'dig-and-dump', encapsulation, immobilization, soil washing, and extraction are the most common practices. However, none of these methods meets the criteria for fast, cost-effective *in situ* remediation without the disturbance of soil features and microbial diversity. Phytoremediation has emerged as an alternative of high potential and lower cost compared to the above-mentioned practices. Willows have high phytoremediation potential, and their inoculation with symbiotic endophytes is possible to further improve their efficacy. The main objectives of the present research are (i) the study of the willow's (*Salix purpurea* cv Fish Creek) metabolome perturbation in response to mycorrhization by the arbuscular-mycorrhizal fungus (AMF) *Glommus irregulare* and exposure to soil contaminants, and (ii) the detection of metabolite-biomarkers performing GC/MS, LTQ-Orbitrap-MS, and $^{1}$HNMR metabolomics. Metabolomics/bioinformatics analyses revealed the impact of AMF and soil contamination on the plant's metabolome. Higher contents of metabolites involved in the phenylpropanoid, terpenoid, and flavonoid biosynthetic pathways in leaves of mycorrhized compared to non-mycorrhized trees grown in contaminated or not soils, were observed. These pathways are well known for their critical role during plant defense responses to biotic and abiotic stimuli, including exposure to xenobiotics. A closer look at these pathways revealed elevated levels of primary and secondary metabolites belonging to carboxylic acids, terpenoids, and flavonoids. Results suggest that the presence of *G. irregulare* in willow roots directly and indirectly enhances its phytoremediation capability, which in turn, is advantageous when used as components of phytoremediation strategies with the corresponding beneficial effects.

**O13.006 Molecular characterization of novel leaf endophytes as potential bioresource to control mango anthracnose**

*S.T. Basha, N.P.E. Reddy and M.N. Devamma*
*Microbiology Division, Department of Virology, Sri Venkateswara University, Tirupati-517502, A.P., India; College of Food Science and Technology, ANGRAU, Pulivendula-516390, A.P. , India; Department of Botany, Sri Venkateswara University, Tirupati-517502, A.P., India*
*Email: thahirbashas@yahoo.com*

Indian agriculture is facing several challenges in increasing the productivity from its limited resources to combat aggressive plant pathogens. However, in order to give the reader a sense of the excitement of discovery that comes with research on potential leaf endophytes that paved the way that meets farmer's expectation are herewith presented. Mango the "king of all fruits", shares around 56 per cent of total global production by India, is severely affected with anthracnose caused by *Colletotrichum gloeosporioides*, the most important biological constraint to mango production resulting substantial yield loss (39.4%) in Andhra Pradesh, India. Among the leaf endophytes screened, the potential endophytes EB9 (100%) and EB35 (100%) completely suppressed the pathogen were cloned and identified as *Brevundimonas bullata* (EB09) and *Bacillus thuringiensis* (EB35) based on 16S rRNA analysis. The potential bacterial leaf endophyte *Bacillus thuringiensis* (EB35) along with its compatible fungicide thiophanate methyl proved to be the best combination in combating the anthracnose disease both *in vitro* and in field trials and further delayed in ripening of the fruits up to 21 days. The talc based formulations of these endophytes evaluated up to 90 days were viable and potent against the aggressive pathogenic isolate Cg23. The RAPD banding profiles with random primers generated reproducible and scorable polymorphic bands reflected the high level genetic diversity among the bacterial leaf endophytes with formation of two main clusters and the novel endophyte EB35 amplified with OPC-12 primer yielded unique band can be used as SCAR marker. These novel leaf endophytes which reside inside the tissues of most plant species usually have associated with novel secondary bioactive metabolites were identified by spectroscopy techniques. The formulations developed for specific geographical region and their enormous bioresource for commercialization will be discussed.

### O13.007 Endophytic *Muscodor* (Xylariaceae, Ascomycota) Species in China: Basic Biology, Diversity and Functions

*Z.L. Yuan [1], L.B. Rao [1], Y.C. Chen [1], C.L. Zhang [2] and F.C. Lin [2]*
*[1]Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang, Zhejiang Province, 311400, China; [2]Key Laboratory of Molecular Biology of Crop Pathogens and Insects of Ministry of Agriculture, Institute of Biotechnology, Zhejiang University, Hangzhou 310029, P. R. China*
*Email: zlyuanmycology@gmail.com*

Xylariaceae fungi have been well known for their efficient production of a wide range of bioactive natural product. Of them, the newly erected genus *Muscodor*, attracts an increasing attention due to its unique biological functions. Many *Muscodor* species, are recently recorded as obligatory plant endophytes in China. Molecular phylogeny supports the description of a novel species-*M. fengyangensis*, and also it indicates that most Chinese *Muscodor* species are positioned as the basal branch in phylogenetic trees, suggesting the remaining ones may evolve from these putative ancestors. *Muscodor* spp. are able to produce an array of volatile small molecules that could inhibit or even kill most pathogens, which shows great promise towards commercialization of *Muscodor* products in control of fruit postharvest diseases and soil-borne pathogen management.

### O13.008 Phylogenetic and alkaloid profile diversity of endophytes in drunken horse grass (*Achnatherum inebrians*)

*L. Chen[1,2], C.J. Li[2], Y.R. Wang[2], C.A. Young[3] and C.L. Schardl[1]*
*[1]University of Kentucky, Lexington, KY, U.S.A.; [2]Lanzhou University, Lanzhou, P. R. China; [3]The Samuel Roberts Noble Foundation, Ardmore, OK, U.S.A.*
*Email: Schardl@uky.edu; li.chen1@uky.edu*

Fungal endophytes, mainly *Epichloë* and *Neotyphodium* species are important bioprotective symbionts of major forage-turf grasses and wild rangeland grasses, such as *Festuca*, *Lolium*, *Elymus*, *Agrostis*, *Poa*, *Achnatherum*, *Agropyron* and *Melica* species, etc. Endophytes of grasses play an important role in both competitiveness and toxicity, which is attributable to their production of alkaloids. The grass-endophyte symbionts have been found that produce four classes of bioprotective alkaloids (peramine, lolines, indole-diterpenes and ergot alkaloids). Drunken horse grass (*Achnatherum inebrians* (Hence) Keng) is a dominant perennial intoxicating grass in the degraded grasslands of north and northwest China. Two different endophyte taxa are reported in *A. inebrians*: *Neotyphodium gansuense* Li et Nan (*Ng*) and *N. gansuense* var. *inebrians* C. D. Moon et Schardl (*Ngi*). In this study, we sequenced the genomes of an *Ng* isolate (E7080) from Northwest Gansu Province and two *Ngi* isolates from Southeast Gansu Province (E7478) and Xinjiang Province (E818), China. To clarify the population structures of endophytes in *A. inebrians*, we also screened 45 samples of seeds from Xiahe and Sunan locations in Gansu Province, and from Murengaole Sumu, Alxa, Inner Mongolia, China. We report dramatic differences in alkaloid gene contents and alkaloid profiles of the *Ng* versus *Ngi* isolates, and confirm the taxa of the *A. inebrians* endophyte by using housekeeping gene phylogenetic analysis.

### P13.001 Microbial endophytes: an alternative source for phytochemicals mimetic to their host plants

*V.C. Verma*
*Department of Microbiology and Infectious Diseases, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221 005 UP, India*

*Email: chandravcv@gmail.com; vijay.verma@daad-alumni.de*

Microbial endophytes have the ability to produce a huge chemical diversity that includes alkaloids, peptides, steroids, terpenoids, isocoumarins, quinones, phenylpropanoids, lignans, phenols, phenolic acids, aliphatic compounds, lactones, and many others. Some time they are capable to produce the bioactive compounds which are originally reported from their host medicinal plants. This concept is established with the isolation of a very potent anti-breast cancer agent 'Taxol' from an endophytic fungus *Taxomyces andreanae* isolated from *Taxus brevifolia* plant (Science; Stierle *et al.* 1993), followed by some other bioactive compounds such as hypericin, camptothecin, vincristine, podophyllotoxin etc. There are a number of reports available that claims the phyto-mimetic chemicals from the endophytic microbes of the host plants. All such reports are based on the selection of some well known medicinal plants with a known key phytochemical like *Catheranthus roseus-vincristin, Camptotheca foetida-camptothecin, Hypericum perforatum-hypericine, Podophyllum peltatum-podophyllotoxin* etc. Host-endophytes symbiotic relationship thus offers opportunity to harvest phyto-mimetic bioactive constituents from medicinal plants, and we know that a microbial source of a high value product may be easier and more economical to produce effectively, thereby reducing its market price. We are particularly interested in validating this hypothesis and successfully reported two most important phyto-molecules from their corresponding fungal endophytes and these molecules are Azadirachtin and Piperine. We have screen fungal endophytes from *Azadirachta indica-Eupenicillium pervum* and *Piper longum- Periconia* spp. that have potential of producing Azadirachtin and Piperine in significantly detectable amounts. But it requires further insight research about the biosynthetic pathway dissection of these two molecules in host as well as in microbes. This achievement however provides an exciting platform for further scientific exploration within both the ecological and biochemical contexts.

### P13.002 Diversity and distribution of latent pathogenic Botryosphaeriaceae on native *Acacia* in South Africa

*F. Jami[1], B. Slippers[2], M.J. Wingfield[1] and M. Gryzenhout[3]*
*[1]Department of Microbiology and Plant Pathology, [2]Department of Genetics,[1,2]Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; [3]Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa*
*Email: Fahimeh.jami@fabi.up.ac.za*

The fungal communities on native trees are typically less well studied than on their commercial counterparts, especially in the Southern Hemisphere. Recent studies have highlighted the Botryosphaeriaceae as common and diverse members of native woody ecosystems that often have broad host ranges and infect trees of commercial importance. The aim of this study was to consider the mycogeography of the Botryosphaeriaceae associated with *Acacia karroo*, as important and widespread part of the South Africa landscape. Samples were collected form healthy trees from 23 sites across the distribution of this tree in South Africa, with intense, systematic and hierarchical sampling in the Tshwane area over a number of years. Isolates were grouped based on PCR-RFLPs of the ITS region as well as morphology of cultures, and representatives were identified based on comparisons of sequence data for the ITS, TEF-1α, β-tubulin and LSU gene regions. In total, 16 Botryosphaeriaceae species were identified, including five previously underscribed taxa. Of these, 11 were found distributed across South Africa, while 13 species found in the more intensively sampled sites in Tshwane. The results illustrate a rich diversity of Botryosphaeriaceae that can exist on a native host, even in the absence of obvious disease. There was a clear pattern of geographic isolation, with some species occurring only in some parts of the country. There was also a clear temporal pattern, with isolation frequencies of the different species varying at sites where sampling continued for a number of years. This study provides rich information to enhance an understanding of the patterns and processes that shape the diversity of Botryosphaeriaceae across native landscapes.

### P13.003 Host specificity of Botryosphaeriaceae occurring on *Acacia karroo* and surrounding native trees in South Africa

*F. Jami[1], B. Slippers[2], M.J. Wingfield[1] and M. Gryzenhout[3]*
*[1]Department of Microbiology and Plant Pathology, [2]Department of Genetics, Forestry & Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; [3]Department of Plant Sciences, University of the Free State, Bloemfontein, South Africa*
*Email: Fahimeh.jami@fabi.up.ac.za*

The Botryosphaeriaceae is an important and diverse family of latent fungal pathogens of woody plants. While some species appear to have wide host ranges, others are found only a limited number of Hosts. It is, however, not clear, if the latter character reflects specificity or is an artifact of sampling. In this study, we addressed this question by sampling native South African trees from four different families, including *Acacia karroo* (Fabaceae), *Celtis africana* (Celtidaceae), *Searsia lancea* (Anacardiaceae) and *Gymnosporia buxifolia* (Celastraceae). Based on DNA sequence data of the ITS, TEF-1α, β-tubulin and LSU gene regions, two new species of the Botryosphaeriaceae were found on *C. africana* and *S. lancea* and these were described as *Tiarosporella africana* sp. nov. and *Aplosporella javeedii* sp.

nov. In addition, five known species were identified including *Neofusicoccum parvum, N. kwambonambiense, Spencermartinsia viticola, Diplodia pseudoseriata* and *Botryosphaeria dothidea*. The diversity of these species was different on the various hosts sampled. *S. viticola* occurred on all the hosts except *S. lancea.* Some species such as *N. parvum, N. kwambonambiense* were not found on *A. karroo*, but have been found on this host in previous studies. Some species never occurred on *A. karroo*, despite the extensive sampling (267 isolates) of this host over a three year period. This suggests that some level of host specificity occurs in some of these fungi. It is curious, however, that species such as *B. dothidea*, known to have broad host ranges, was found only on one of the tree species sampled. This could be due to a sampling effect, but it may also reflect the fact that some intrinsic host factors, possibly combined with local environmental conditions, affect the distribution of Botryosphaeriaceae. This would be in contrast to intrinsic characters of the Botryosphaeriaceae alone determining their distribution. It could possibly explain why some Botryosphaeriaceae species appear to infect different suites of hosts in different areas of the world, possibly reflecting hosts with which they have co-evolved or not.

**P13.004 Development of Endophytic bacterial quorum quenching activity as a potential biocontrol strategy against quorum sensing controlled virulence using *Pectobacterium carotovorum* model**

*R.V. Rai and P.S. Rajesh*
*Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore 570 006, Katnataka, India*
*Email: raivittal@gmail.com*

Quorum sensing (QS) is a mode of communication in many species of bacteria to coordinate gene expression according to local density of their population. QS signalling molecules produced by the bacteria are specific for each species or strains. Most of Gram negative bacteria such as *Pectobacterium carotovorum* produce *N*-acyl homoserine lactones (AHLs) as intercellular messengers, which play a major role in virulence as well as QS regulated mechanisms. A number of organisms possess the capacity to block the quorum sensing systems either by hydrolyzing the AHLs or inhibiting the receptors. In our study, the endophytic bacteria were isolated from bark and leaves of *Pterocarpus santalinus* and screened for inactivation of QS-controlled virulence in *P. carotovorum*. Quorum quenching (QQ) activity of endophytic bacteria was confirmed by inhibition of violacein production in mutant biosensor strain *Chromobacterim violaceum* CV026. Among 94 isolates, two endophytic bacteria were capable of degrading AHLs under tested conditions. The cell-free lysates of selected endophytic bacteria suppressed the virulence of *P. carotovorum.* This result was further substantiated by the reduced pathogenicity of *P. carotovorum* in potato tubers. This study demonstrates the targeting of QS-controlled virulence as an important biocontrol strategy.

**P13.005 Volatile fungicidal compounds and antifungal chaetoglobosin production by endophytic fungi isolated from *Nothapodytes foetida* and *Hypericum mysorense***

*R.V. Rai and P.V. Samaga*
*Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore–570006 Karnataka, India*
*Email: raivittal@gmail.com*

Microorganisms, which spend the whole or part of their life cycle colonising inside the healthy tissues of the host plants, typically causing no apparent symptoms of diseases are called as endophytes. They have become an attractive source of novel bioactive molecules because of their diversity, possible horizontal metabolic as well as genetic transactions with the hosts and capacity to inhabit unique niches. The plants with medicinal values or those growing in biodiversity hotspots were hypothesized to harbour hyper-diverse endophytic biota with useful bioactive compounds. In this light, the foliar endophytic fungi were isolated from two medicinal plants growing in Western Ghats of India – the one among ten important biodiversity hotspots of world. The foliar endophytic fungal isolates *Bionectria ochroleuca* NOTL33 and *Chaetomium globosum* HYML55 from *Nothapodytes foetida* and *Hypericum mysorense* respectively exhibited significant antifungal activity and also volatile antifungal activity. The phylogenetic distinction of these endophytes from their pathogenic and free living counterparts was studied by large subunit ribosomal RNA secondary structure analysis. The volatile antifungal compounds in the ethyl acetate extract of *B. ochroleuca* culture broth were characterized by GC-MS analysis. A yellow amorphous antimicrobial compound was purified from the culture broths of *C. globosum* HYML55 isolate. The compound was identified based on $^{1}H$ and $^{13}C$ NMR spectral analysis as a cytochalasan alkaloid similar to chaetoglobosin F. The active compound production by the isolate in different liquid media was quantified by reverse phase high performance liquid chromatography.

**P13.006 Cross-fertility of endophytic and pathogenic *Fusarium sacchari* isolates from banana and sugarcane**

*S.A. McFarlane, A. Viljoen, S. Athman, L.A. Martin and R.S. Rutherford*
*South African Sugarcane Research Institute, Private Bag X02, Mount Edgecombe, 4300, KwaZulu-Natal, South Africa*
*Email: sharon.mcfarlane@sugar.org.za*

*Fusarium sacchari* is a common inhabitant of sugarcane and has been isolated from stalks showing pokkah boeng disease symptoms, from reddened tissue associated with the borings of the insect pest *Eldana saccharina* Walker as well as from asymptomatic stalks. It has also been found as an endophyte in banana rhizomes, and did not cause any disease symptoms upon inoculation of banana plantlets. In this study, the relationship between a population of *F. sacchari* from sugarcane stalks and a collection of endophytic *F. sacchari* isolates from the rhizomes of banana plants was determined to assess the potential of cross-infection in a mixed cropping system. Phylogenetic analysis of a region of the translation elongation factor 1α gene showed that the isolates from banana formed a closely related but distinct group from the sugarcane isolates. After mating type idiomorphs (*MAT-1* and *MAT-2*) were identified using a PCR assay, crosses between the banana and sugarcane isolates were made on carrot agar, with each isolate used as both male and female parents. Crosses were also made with known representative tester strains within the *Gibberella* species complex. Certain pathogenic and endophytic *F. sacchari* isolates from sugarcane produced fertile crosses with endophytic isolates from banana, generating abundant oozing perithecia and viable ascospores. This confirmed that isolates of *F. sacchari* from banana and sugarcane belong to the same biological species, with the possibility of pathogenic isolates from sugarcane surviving in banana in a mixed cropping system.

**P13.007 The effect of endophyte presence on biotic and abiotic stress resistance of perennial ryegrass (*Lolium perenne* L.) plants**

*B. Wiewióra and G. Żurek*
*Plant Breeding and Acclimatization Institute– National Research Institute, Radzików, 05–870 Błonie, Poland*
*Email:b.wiewiora@ihar.edu.pl*

The aim of our studies was to discover the possibility of increasing plants resistance for biotic and abiotic stress by means of presence of endophytes. Plants of 12 perennial ryegrass ecotypes with (E+) and without (E-) endophyte were selected for our study. The experiment was carried out in three combinations: control, abiotic stress (drought – water withheld for 3 weeks) and biotic stress (inoculation of *Drechslera siccans*, causing brown blight). No significant effect of the endophyte presence on the changes of morphological parameters (number of shoots, plant dry matter yield) examined under the influence of drought were observed. The presence of the fungal mycelium in plants of perennial ryegrass subjected to drought stress increased the chlorophyll fluorescence parameters such as initial fluorescence (Fo), maximum (Fm) and variable (Fv) and the time to maximal fluorescence level (Tfm). The increase of the latter (46.1% in E- plants as compared to E+) is the evidence of slower transport of high energy electrons from the reaction center to plastochinons in E- plants as compared to E+ plants. The results of the biotic stress on plants indicate a significantly higher incidence of disease symptoms on E- plants (91.7% ecotypes) than on E+ plants (58.3% ecotypes). Average attendance of infected plants for E- ecotypes was 39.5%, and was statistically significantly different from the average for the E+ plants (6.5%). The results indicate the possibility of increasing the resistance to certain biotic and abiotic stress factors on the basis of presence of endophyte mycelium in plants.

**P13.008 Fusaria and Acremonium-like fungi as hyperparasites of the loose smuts of wheat and barley**

*E. Koch, J. Wunderle, P. Zink and M. Gossmann*
*Humboldt-University of Berlin, Division Phytomedicine, Berlin, Germany; JKI, Institute for Biological Control, Darmstadt, Germany*
*Email: eckhard.koch@jki.bund.de*

*Ustilago nuda* and *U. tritici*, the causal agents of the loose smuts of barley and wheat, respectively, grow endophytically during most of their life time. Following floret infection by smut spores, both fungi invade the scutellum where they remain in a state of dormancy until the seed starts to germinate. Within a few days after the onset of germination the loose smut fungi colonize the apical meristem and leaf initials. The plants remain symptomless until emergence of the smutted ears. Using an enzyme-linked immunosorbent assay (ELISA) and light microscopy, *U. nuda* was detected in nodes and leaves of growing spring barley plants. On field- and greenhouse-grown plants, fusaria and *Acremonium*-like fungi where observed growing on smutted ears. In most cases these fungi were only present on parts of the smutted ears, but occasionally the latter were fully covered with mycelium, indicating a hyperparasitic relationship. The origin of the infections is not clear. Contamination by air-borne inoculum cannot be totally ruled out. However, the fact that in some cases the smutted ears appeared already substantially colonised when they emerged from the boot strongly indicated that the infections occurred within the plant. It appears worthwhile to explore if these internal infections can be utilised for controlling loose smut fungi or other systemically growing plant pathogens.

**P13.009 Root-endophyte diversity in banana and it potential use for biological control of Fusarium wilt**

*N. Chaves, A. Schilly, M. Guzmán, Jorge Sandoval, C. Staver and M.A. Dita*
*Bioversity International, Turrialba, 30501, Cartago, Costa Rica*
*Email: m.dita@cgiar.org*

Plants in natural ecosystems host microbial communities of endophytes which may play essential roles in fitness. Target endophytes have been studied as biological control agents in banana, but little is known about their diversity. In this work, root-associated endophytes (bacteria and fungi) from 20 banana genotypes with different genome compositions and ploidy levels present in the CORBANA germplasm bank in Guapiles, Costa Rica, were isolated. Identification was performed based on analyses of specific genome regions (16S rDNA for bacteria and *Tefa-1α* or Internal Transcribed Spacer for fungi). Twenty-one bacteria and 13 fungi genus were identified. Among bacteria *Klebsiella, Enterobacter, Bacillus, Acinetobacter and Burkholderia* in this order were more frequent, while *Trichoderma* spp. *and Fusarium oxysporum* predominated among fungi. Bacterial and fungal isolates with not significant similarities in the database analyzed were also found and will probably constitute new descriptions. Some endophytes were more frequent or uniquely found on certain banana genotypes, but a correlation on endophyte-host specificity need to be further verified. *In vitro* tests against *Fusarium oxysporum* f. sp *cubense* (Foc), revealed that some endophytes such *Bacillus* spp., *Burkloderia* spp. and *Trichoderma asperellum* produce antibiotic compounds that significant reduces Foc growth. Greenhouse tests showed that Fusarium wilt severity is reduced in banana plants previously inoculated with two *Bacillus* spp., four *T. asperellum* isolates, or their combinations. Field tests to further verify the effect of these isolates against Foc are on-going.

### P13.010 Endophytic fungi isolated from surface-sterilized seeds of *indica* hybrid rice cultivars in China

*D. Xue and B.D. Gao*
*Hunan University Key Laboratory for Control and Utilization of Plant Diseases, Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, College of Bio-Safety Science & Technology, Hunan Agricultural University, Changsha 410128, P. R. China*
*Email: bdgao@yahoo.com.cn*

In order to clarify endophytic fungi in rice seeds, we tried isolation of fungi from seeds of 150 commercial *indica* hybrid rice cultivars, and then microscope observation of and molecular identification of isolated fungi. The results showed 2-100% fungus contamination rate. Blast of sequence of PCR products of rDNA-ITS of different isolates indicated presence of nine pathogenic fungi i.e. *Magnaporthe oryzae, Sarocladium oryzae, Cochliobolus miyabeanus, Curvularia lunata, Curvularia geniculata, Trichoconis padwickii, Nigrospora oryzae Penicillium citrinum* and *Aspergillus niger* on rice seeds. On the other hand, several endophytic fungi were also detected i.e. *Coprinus radians, Cladosporium cladosporioides, Arthrobotrys sp., Chaetomium brasiliense, Chaetomium globosum, Arthrobotrys cladodes,* and *Gelasinospora udagawae.*

### P13.011 Diversity and biocontrol potential of the culturable endophytic fungi from oilseed rape in China

*Q.H. Zhang, J. Zhang, L. Yang, D.H. Jiang* and *G.Q. Li*
*The State Key Laboratory of Agricultural Microbiology and the Key Laboratory of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan 430070, P. R. China*
*Email: guoqingli@mail.hzau.edu.cn*

A study was conducted to evaluate diversity and biocontrol potential of endophytic fungi in oilseed rape (*Brassica napus*). We isolated 97 endophytic fungal strains from healthy leaves (13 strains), stems (49 strains) and roots (35 strains). These fungi belong to 27 genera and 39 species with Shannon diversity index being 3.277 and Simpson diversity index being 0.955. Thirty-three species (84.6%) belong to Ascomycota, whereas 5 species belong to Basidiomycota (12.8%) and only one species (2.6%) belong to Zygomycota. The dominated genera are *Alternaria, Fusarium, Chaetomium* and *Gliocladium.* Twenty-four strains showed antifungal activity against *Sclerotinia sclerotiorum* in dual cultures on potato dextrose agar (PDA) and 7 strains formed inhibition zones with width >10 mm. The filtrates of *Gliocladium roseum* strain CanS-43 in potato dextrose broth cultures showed effective suppression of *S. sclerotiorum* infection on *B. napus* leaves. Meanwhile, 5 strains were detected to be able to produce volatile organic compounds (VOCs) inhibitory to *S. sclerotiorum* growth. The VOCs from strain CanR-46 of *Fusarium oxysporum* were effective for suppression of gray mold caused by *Botrytis cinerea* on post-harvest tomato fruits under air tight conditions. This study suggests that *B. napus* harbors diversified endophytic fungi, from which biocontrol agents against *S. sclerotiorum* or *B. cinerea* can be screened.

### P13.012 Biocontrol and elicitor pre-harvest treatments effect on the fungal ecosystems and the Ochratoxin A contamination of grapes

*H. Ahmed [1, 2], F. Hilaire [1], A. Stemmelen [1], N. Salah[1], G. Bastard [1], C. Strub [1] and S. Schorr-Galindo [1]*
*[1]UMR n°95 QUALISUD (CIRAD – UM2 – UM1 – SupAgro), C.C. 023, Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France; [2]Agricultural Botany Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt*
*Email: hodahusen@hotmail.com*

The grape and wine industry is affected by the presence of Ochratoxin A (OTA) because of grapes contamination

by *Aspergillus* section *Nigri* strains. The European Commission has set the maximum limit for OTA in wine and grape juice to 2 mg/Kg. The objective of this work is to provide non-chemical alternative methods to control OTA contamination in grapes and wine, in respect with environment and stakeholder health (producers and consumers). Different treatments were compared in experimental vineyard on near parcels after artificial contamination by *A. carbonarius*: a chemical fungicide; *Saccharomyces cerevisiae* and *Trichoderma atroviride* as antagonists; and a plant extract as elicitor (Stifénia). Two untreated parcels served as controls, one was artificially contaminated. Q-PCR using universal and specific primers for *A. carbonarius* had estimated the effect of the different treatments on the presence of *A. carbonarius*. The lowest occurrence of black aspergilli strains was obtained for treatment used elicitation. While, the DGGE gave an overview on their effect on the fungal ecosystem, that showed higher similarity between the non-contaminated and elicitor treatment (76%) followed by yeast one and the lowest treatment was the contaminated one. These results were confirmed with the results obtained from traditional methods of isolation that showed the elicitor treatment had a higher proportion of fungal species not isolated in the other treatments. Some *Penicillium* spp. isolated from grapes treated by elicitation showed interesting antagonist effect in vitro both on growth and toxigenesis of *A. carbonarius*. That highlights the possibility of replacing fungicides by the tested elicitor.

**P13.013 Bread wheat root colonization by mycorrhiza (*Glomus intraradices*) in Jalisco, Mexico**

*J. Ireta, N.Y. Zácamo, J.F. Pérez and H.E. Flores*
*INIFAP, Campo Experimental Centro-Altos de Jalisco, Tepatitlán, Jalisco, México 47600*
*Email: ireta.javier@inifap.gob.mx*

The overuse of nitrogen fertilization generates an excessive expense, as well as high soil contamination by nitrogen decomposition, since recent studies indicate that only 30% of the nitrogen applied is utilized by plants. Nitrogen is of special interest since it is the most limited nutrient element for plant growth; in addition, the nitrites and nitrates produced can move quickly and contaminate the under and superficial water. The increase efficiency on nitrogen use has an impact on plant production and it is a fundamental factor for reducing contamination of the environment; therefore, the development of technologies for slow nitrogen release could decrease contamination and improve crop yield. The so called "biofertilizers" such as mycorrhizae, which are natural fungal soil inhabitants that live symbiotically with plants in combination with some minerals, represent an alternative for reducing nitrogen consumption in the irrigated wheat areas of Jalisco, Mexico. The objective of this study was to determine the level of bread wheat root colonization by *Glomus intraradices* which was isolated from the soil by the moist sieving technique. Roots were stained with trypan blue and the presence of arbuscules, spores, and colonization were quantified. Treatments evaluated were: wheat seed treated zeolite + urea (25+75), zeolite+urea (25+75) + *G. intraradices*, urea, urea + *G. intraradices* the absolute untreated check, and the absolute untreated check + *G. intraradices*. The percentage of colonization was determined for each treatment. The best treatment was urea + *G. intraradices* which showed 35%, in contrast zeolite+urea (25+75) showed only 10.8%.

**P13.014 Diversities of endosymbiotic 16/18S rDNAs and endophytic bacteria in *Dendrobium officinale***

*J. Yu, X.F. Zhou, S.J. Yang, W.H. Liu and X.F. Hu*
*College of Life Science, Zhejiang Sci-Tech University, Xiasha, Hangzhou, Zhengjiang, 310018, P. R. China*
*Email: huxiuf@zstu.edu.cn*

Prokaryotes play a critical role in plants as the origin of evolution and as an extra genome. Diversities of 16/18S rDNAs from endosymbiotic and endophytic prokaryotes were investigated in the traditional Chinese herb *Dendrobium officinale.* Using the universal bacterial primer pair 27f/1492r targeting 16S rDNA, diversities of endosymbiotic 16/18S rDNAs were elucidated in *Dendrobium officinale*. They shared 99-100% similarity separately with the chloroplast 16S rDNA of *Dendrobium sp.* and with mitochondrion 18S rDNA from *Asparagus officinalis*, and both shared high homologies with bacterial 16S rDNAs. A novel 16S rDNA-targeted primer pair fM1 (5'-CCGCGTGNRBGAHGAAGGY YYT-3') and rC5 (5'- TAATCCTGTTTGC TCCCCAC -3') was designed, which showed good specificity compared to the plant 16/18S rDNAs, and perfect universality within bacteria from different species and genera. This primer pair selectively amplified a 400 bp amplicon from total genomic DNA of potted plants with endophytic bacteria other than from that of the tissue culture seedlings, demonstrating its specificity for bacteria and excluding the endosymbiotic 16/18S rDNAs. The novel primer pair was subjected to nested-PCR-DGGE to analyze the diversity of endophytic bacteria in *Dendrobium officinale* from 3 different sources. The results showed diversities in all roots and stems of the plants from all 3 locations. Altogether, 29 bands were identified, with the dominant group being *Proteobacteria* and the dominant genus being *Burkholderia*, which commonly has the function of nitrogen fixation and thus may play potential roles in *Dendrobium officinale*. The results shed light on a new role for endophytic bacteria in *Dendrobium officinale* as well as fungi.

**P13.015 Identification of a strain endophytic fungus *Acremonium implicatum* from root gall of tomato and**

**investigation of its bioactivities against *Meloidogyne incognita***

*X.L. Tian, Y.R. Yao, G.H, Chen, Z.C. Mao and B.Y. Xie*
*[1]College of Resources and Environment, Henan Institute of Science and Technology, Xinxiang 453003; [2]Institute of Vegetables and Flowers, CAAS, No.12 Nandajie Zhongguancun, Haidian District, Beijing, 100081, P. R. China*
*Email: tianxueliang@sohu.com*

A strain of endophytic fungus was isolated from root gall of tomato infected by M. incognita. The fungus was identified as *Acremonium implicatum* using morphological characteristic and 18S rDNA sequences. Biocontrol and growth-promoting potentials of this fungus was test under laboratory and greenhouse condition. The fungus not only exhibited nematicidal activity by killing the second stage larvae, but also suppressed egg hatching of M. incognita. Greenhouse test showed that the fungus effectively inhibited forming of root galls and nematodes populations in soil and also promoted growth of tomato. As a whole, this fungus had broader prospect for biocontrol of *M.incognita*. The result will give a new insight into endophytic microorganism in diseased plant and also provide a novel pathway to find biocontrol microorganism against target pathogens.

**P13.016 Biocontrol Potential of an endophytic *Bacillus pumilus* JK-SX001 against Poplar Canker**

*J.H. Ren, H. Li and J.R. Ye*
*Institute of Forest Protection, College of Forest Resources and Environment, Nanjing Forestry University, Nanjing, 210037, P. R. China; Jiangsu Key Laboratory for Prevention and Management of Invasive Species, Nanjing, 210037, P. R. China*
*Email: jrye_njfu@yahoo.com.cn*

Several endophytes play a vital role in plant protection and plant growth promotion. In China, poplar canker disease caused by three pathogens (*Cytospora chrysosperma, Phomopsis macrospora* and *Fusicoccum aesculi*) is difficult to control by use of chemicals; therefore, use of biocontrol agents is desirable. The aim of this study was to examine the effects of strain JK-SX001 on plant growth and poplar canker caused by three pathogens. It is clearly demonstrated that strain JK-SX001 is belonging to *Bacillus pumilus* by the Biolog identification system combined with morphological, physiological, biochemical tests and phylogenetic (16S rDNA) studies. Strain JK-SX001 produced lytic enzymes such as cellulases and protease. In vitro antagonistic assay showed that secondary metabolites of JK-SX001 extracted using methylbenzene could suppress the growth of three pathogens effectively. Derivative of this strain, labeled with *gfp*, was also used to study the colonization of its poplar hosts. This strain was verified as an endophytic bacterium of poplar by confocal laser scanning microscopy. The population of the GFP-labeled JK-SX001 inoculant was larger and more stable in roots and stems than that in leaves. The evaluation of the antagonistic strains against poplar canker indicated that JK-SX001 effectively reduced disease incidence. In greenhouse studies, poplar seedlings inoculated with this strain showed high increase in biomass production, shoot length, stem diameter and photosynthetic activity. Strain JK-SX001 colonized poplar roots, stems and leaves endophytically, promoting plant growth and suppressing pathogenic activities on seedling of poplar. Our results demonstrate JK-SX001 as a promising candidate for biocontrol of poplar canker.

**P13.017 Exploration of *ketosynthase* gene on the endophytic bacterial root of *Vetiveria zizanioides* L.**

*A. Fitriani, A. Aryani and H. Yusuf*
*Program Study on Biology, Indonesia University of Education, Dr. Setiabudhi 229 Bandung 40154 West Java, Indonesia*
*Email: anyfitriani@upi.edu*

Exploration of *ketosynthase* gene has been conducted on the 17 isolate of endophytic bacterial root of *Vetiveria zizanioides* L. *Ketosynthase* gene was detected by two pairs of *degenerate oligonucleotide* primers, that is DKF-DKR and HGLF-HGLR. Partial sequence analysis was conducted on *16S rRNA* gene that amplified by 63F and 1387R primers. Partial sequence of *ketosynthase* gene was obtained through *sequencing* result of the 400 and 700 bp amplicon in size. The result of this gene amplification showed that only five species of endophytic bacteria detected to have ketosynthase gene. Through the analysis of *16S rRNA* gene, the species information of that detected bacteria was shown, that is *Lysinibacillus sphaericus* (Isolate A), *Pantoea* sp (Isolate H), *Bacillus* sp (Isolate K), *Acinetobacter* sp (Isolate M) and *Pseudomonas aeruginosa* (Isolate O). Through bioinformatics and filogenetic study, it showed that isolate H, M, and O are included in a group of *proteobacteria* which has a gene of type I *ketosynthase*, while isolate A (*Lysinibacillus sphaericus)* and isolate K (*Bacillus* sp.*)* belong to a group of *Firmicutes* bacteria which has a gene of type II *ketosynthase*. The separation of the branch on bacterial family tree showed the evolution of ketosynthase genes in the bacteria itself.

**P13.018 Metagenome analysis of polyketide synthase ketosynthase domain in endophyte bacteria of *Ageratum conyzoides* L.**

*A. Fitriani and S.N. Saputra*
*Program Study on Biology, Indonesia University of Education, Dr. Setiabudi 229 Bandung 40154 West Java, Indonesia*
*Email: anyfitriani@upi.edu*

Metagenome analysis of polyketide synthase ketosynthase domain in endophyte bacteria of *Ageratum conyzoides* L. has been conducted. DNA isolated from root directly, which was cleaned and sterilzed before. Gene of ketosynthase amplified employing primer DKF/DKR and HGLF/HGLR. The size of amplicon is 700 bp for DKF/DKR ang 400 bp for HGLF/HGLR, approximately. Amplicon cloned to pGEM-T Easy vector and transformed to *Escherichia coli* DH 5a. Selection of transformans has been done by white-blue selection. DNA sequences showed that DKF/DKR amplified ketosynthase domain type I from many groups of bacteria such as Cyanobacteria, Proteobacteria, and many bacteria which has no identified. Meanwhile HGLF/HGLR amplified diversity of ketosynthase domain type I and II.

**P13.019 Integrated regulation of pyrrolnitrin production in the endophytic *Serratia plymuthica* G3**

*Y. Wu[1], S. Guo[1], X.L. Yu[1], S. Atkinson[2], S. Heeb[2], M. Cámara[2] and X.G. Liu[1,2]*
[1]*Institute of Life Sciences, Jiangsu University, Zhenjiang 212013, Jiangsu, P. R. China;* [2]*School of Molecular Medical Sciences, Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD,UK*
*Email: xgliu66@yahoo.com*

The antibiotic pyrrolnitrin [(3-chloro-4-(2'-nitro-3'-chloro-phenyl) pyrrole, PRN] is a secondary metabolite derived from tryptophan with broad-spectrum antimicrobial activity. Natural derivative products of PRN have been used as synthetic lead compounds for the production of novel agricultural fungicides (fludioxonil and fenpiclonil) as an alternative to traditional pesticides. The PRN *prnABCD* biosynthetic gene cluster is highly conserved amongst the Gram-negative PRN-producing bacteria *Burkholderia*, *Pseudomonas* and *Serratia*. However, there is still much less known regarding the molecular mechanisms controlling PRN biosynthesis in these organisms, compared to those regulating well characterised antibiotics such as phenazines or 2, 4-diacetylphloroglucinol (DAPG). We have recently found that PRN produced by *S. plymuthica* G3 is controlled by a complex regulatory network involving multiple regulators at different levels. Both LysR and TyrR family of transcriptional regulators were shown to be involved in the positive control of PRN biosynthesis, as well as the small RNA RsmB and the AHL-mediated quorum sensing (QS) network. We have further explored the molecular mechanisms underlying the integration of RsmA/B, the RNA chaperon Hfq and QS in the control of pyrrolnitrin production in the endophytic strain G3 of *S. plymuthica*. We acknowledge support from NSFC (31240046), the EU project PROAGROBAC (297882) and the Talent Summit Project of Jiangsu province.

**P13.020 Role of the GGDEF/EAL domain protein PigX in *Serratia plymuthica***

*Y. Wu[1], K.X. Gao[2], Y.F. Duan[1], M. Cámara[3] and X.G. Liu[1,3]*
[1]*Institute of Life Sciences, Jiangsu University, Zhenjiang 212013, P. R. China;* [2]*Department of Plant Pathology, Shandong Agricultural University, Taian 271018, P. R. China;* [3]*School of Molecular Medical Sciences, Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD,UK*
*Email: xgliu66@yahoo.com*

Cyclic dimeric guanosine monophosphate (c-di-GMP) is a common, nucleotide-based second messenger that regulates diverse cellular processes in bacteria. Diguanylate cyclases (DGCs) proteins containing the GGDEF domain produce c-di-GMP, while phosphodiesterases (PDEs) proteins bearing the EAL or HD-GYP domains degrade it. Many microbes have a large number of DGCs and PDEs to control c-di-GMP homeostasis in the cell implying its importance. Here we cloned and identified a GGDEF/EAL domain protein PigX from an endophytic strain G3 of *Serratia plymuthica*. Mutational analysis showed that the *pigX* gene is involved in positive control of swimming motility, proteolytic activity and antifungal activities. In contrast, the biofilm formation was up-regulated in a mutant of *pigX* compared to the wild type G3. More importantly, PigX is required for biosynthesis of the antibiotic pyrrolnitrin (PRN), which is a key biocontrol determinant responsible for suppression of phytopathogens. Furthermore, *lacZ*-based bioreporter fusion assays demonstrated that both post-transcriptional regulators Hfq and CsrB family small RNAs have impact on the expression of *pigX* gene. These findings suggest that the PigX protein in *S. plymuthica* G3 functions as a pleiotropic regulator to affect a variety of biocontrol-related phenotypes, although the signaling mechanisms and signal transduction pathways remain to be explored. We acknowledge support from NSFC (31240046), the EU project PROAGROBAC (297882) and the Talent Summit Project of Jiangsu province.

## Concurrent Session 14-Fastidious and Wall-less Bacterial Plant Pathogens

### O14.001 The Liberibacters, an overview: from *Candidatus* Liberibacter asiaticus and *Ca.* L. africanus to *Liberibacter crescens*

*J.M. Bové*[1] *and W.R. Nelson*[2]
[1]*Université de Bordeaux & INRA, Biologie du Fruit et Pathologie, 33140 Villenave d'Ornon, France;* [2]*The New Zealand Institute for Plant & Food Research Ltd, Private Bag 4704, Christchurch 8140, New Zealand*
*Email: joseph.bove@wanadoo.fr*

In 1970, sieve tube-restricted bacteria were discovered in citrus trees affected by Huanglongbing (HLB). First thought to be mycoplasma-like, they were soon recognized as walled gram negative bacteria and demonstrated in 1994, to be unculturable species of a new genus, *Candidatus* Liberibacter, in the alpha *Proteobacteria*. Two species were recognized: the heat-tolerant *Candidatus* Liberibacter asiaticus (Las) associated with the disease in Asia, and the heat-sensitive *Ca.* L. africanus (Laf) with the disease in Africa. Subspecies 'capensis' of Laf was detected in South Africa in 1998 in a Rutaceous tree, *Calodendrum capense*, but not in citrus. In 2004, when HLB was initially observed in South America (São Paulo State, Brazil), a new, heat-sensitive species was discovered: *Ca.* L. americanus (Lam), but the heat-tolerant Las was also present. Today, Las is widespread in Asia, and North, Central, and South America, and has entered Africa via Ethiopia in 2010. Lam has been identified in Texas in 2013. The first non-citrus liberibacter, *Ca.* L. solanacearum (Lso), was reported in 2008. It is associated with zebra chip of potatoes in New Zealand and North and Central America as well as with diseases of carrot and celery in Scandinavia and Spain, including Canarias Islands. Laf, Lam, Las and Lso are strictly sieve tube-restricted and transmitted by various psyllid vectors. The fifth species, *Ca.* L. europaeus (Leu), was discovered in Italy in 2011, within a psyllid, *Cacopsylla pyri*, the vector of the pear-decline phytoplasma. Leu was transmitted by *C. pyri* to Pear trees, in whose sieve-tubes the liberibacter reached high titers, but induced no symptoms. However, in New Zealand, Leu was reported in 2013 in Scotch Broom (*Cytisus scoparius*) where it induced severe symptoms, as well as in the broom psyllid, *Arytainilla spartiophila*. Finally, a sixth species, *Liberibacter crescens* (Lcr), was reported in 2012 from a hybrid mountain papaya in Puerto Rico. Unlike the *Candidatus* liberibacter species, Lcr has been cultured. Recently, a Pangaean origin of Lcr and the five *Candidatus* Liberibacter species has been proposed.

### O14.002 "*Candidatus* Liberibacter solanacearum": its geographic distribution, vectors, and global threat to annual crops

*J.E. Munyaneza*
*USDA-ARS, Yakima Agricultural Research Laboratory, Wapato, WA 98951, USA*
*Email: Joseph.Munyaneza@ars.usda.gov*

"*Candidatus* Liberbacter solanacearum" (Lso) is a phloem-limited, Gram-negative, unculturable bacterium that belongs to the *Alphaproteobacteria* group. The bacterium was first identified in 2008 and shown to be associated with zebra chip disease of potato in North and Central America and New Zealand. Lso also severely affects other solanaceous crops. In addition, the bacterium was recently reported on carrot and celery crops in Northern Europe and the Mediterranean region. Lso is transmitted to solanaceous species by the psyllid *Bactericera cockerelli* and to carrot by the psyllids *Trioza apicalis* and *Bactericera trigonica.* Four geographic haplotypes of Lso have so far been described; two haplotypes (LsoA and LsoB) are associated with diseases caused by this bacterium in potato and other solanaceous species, whereas the other two (LsoC and LsoD) are associated with diseased carrots and their insect vectors. The complex Lso/psyllid vectors has caused serious damage to the potato and tomato industries in the Americas and New Zealand, occasionally leading to abandonment of entire fields. In Europe, damage to carrots by Lso-infected carrot psyllids can cause up to 100% crop loss. An overview of Lso geographic distribution, biology, epidemiology, economic impact, and management will be discussed.

### O14.003 Tomato as a latent carrier of '*Candidatus* Liberibacter solanacearum', the causal agent of potato zebra chip disease

*X. Li, J. Nie, H. Arsenault, D.L. Hammill, H. Xu and S. H. De Boer*
*Canadian Food Inspection Agency, Charlottetown Laboratory, 93 Mt Edward Road, Charlottetown, PE, C1A 5T1, Canada*
*Email: sean.li@inspection.gc.ca*

*Candidatus* Liberibacter solanacearum' (CLs) was recently described as the causal agent of potato zebra chip disease. The disease is known occur in North America from Guatemala to the northern US, New Zealand, and northern Europe on various crops, and may spread to other potato growing regions. Observation of both zebra chip-infected tomato and potato plants propagated in growth rooms during the last four years indicated that tomato (varieties Moneymaker and Roma) can be a latent carrier of CLs. Tomato plants graft-inoculated with scions from latently infected tomato plants remained symptomless, but tested positive in a zebra chip-specific PCR assay. '*Can.* L. solanacearum' was consistently detected in the top, medium and bottom portions of symptomless tomato plants, including stem, petiole, midrib, vein, flowers and fruits. In tomato fruits, CLs

was evenly distributed in the tissues at the peduncle and style ends, and was present in the pericarp, columella and placenta tissues. In contrast, potato plants (cultivars Jemseg, Atlantic, Shepody, Frontier Russet, Russet Burbank, Red Pontiac, and Russet Norkotah) grafted with scions from the same latently infected tomato plants developed typical symptoms of purple top, leaf scorch, and other disease symptoms, four weeks after being grafted. Tubers harvested from the graft-inoculated potato plants also showed typical symptoms of brown discoloration in the vascular ring and medullary rays. While CLs could not be detected in some tissues such as aerial tubers of graft-inoculated greenhouse-grown plants, it was readily detectable in the stems and progeny tubers of the same plants.

**O14.004 Detection and Characterization of Miniature Inverted-repeat Transposable Elements in *'Candidatus* Liberibacter asiaticus'**

*X.F. Wang[1], J. Tan[1] , Z.Q. Bai[1], H.N. Su[1], X. Deng[2], Z. Li[1], C.Y. Zhou[1] and J. Chen[3]*
*[1]Citrus Research Institute, Southwest University, Chongqing, 400712, P. R. China; [2]Citrus Huanglongbing Research Center, South China Agricultural University, Guangzhou, 510642, P. R. China; [3]San Joaquin Valley Agricultural Sciences Center, USDA-ARS, Parlier, CA 93648, U.S.A.*
*Email: wangxuefeng@cric.cn*

Miniature inverted-repeat transposable element (MITE) is a non-autonomous transposons (devoid a transposase gene, *tps*) involving insertion/deletion of genomic DNA in bacterial genomes. No transposon has yet been reported in '*Candidatus* Liberibacter asiaticus', an alpha-proteobacterium associated with citrus Huanglongbing, which is highly destructive to the world citrus production. In this study, MITEs in '*Ca*. L. asiaticus' were detected and characterized through a pan-genomic study. A total of 326 isolates collected in China and Florida were examined for variations in a genomic locus, flanked by primer set LapPF1-f/LapPF1-r. Three PCR amplicons, B-SC1 (~720bp), B-SC2 (~640 bp) and B-CH1 (~300 bp), were observed. B-SC2 was found to be identical to B-CH1 but a DNA insertion, characterized as a MITE, MCLas-A, namely, for the presence of terminal inverted repeats (TIRs), small size (<500 bp), a non-coding central region (CR) and evidence of mobility. Sequence analyses showed B-SC1 contained a different MITE, MCLas-B. Both MITEs used phages / prophages as hosts and had variants found in both China and Florida. The representative variant, MCLas-A1, had a pair of 54-bp TIRs, a 217 bp CR and a pair of 6 bp direct repeats (DRs). Upstream 230 bp on its host was a putative *tps*. MITE mobility was evidenced by the presence of "full" (with MITE) / "empty" (without MITE) isolates in the bacterial pan-genome. All "empty" isolates had TIR remnants at the excision sites with significant variations according to geographical origins. This is the first observation and characterization of MITEs or transposons in '*Ca*. L. asiaticus'.

**O14.005 Single chain antibodies for proteins encoded by '*Ca*. Liberibacter asiaticus' and *Xylella fastidiosa***

*Q. Yuan, F. Ding, L. Lashley, O. Minenkova, R. Brlansky, R.L. Jordan and J.S. Hartung*
*USDA ARS Molecular Plant Pathology Laboratory, 10300 Baltimore Avenue, Beltsville, MD, USA 20705*
*Email: John.Hartung@ars.usda.gov*

We have developed and applied immunization and affinity screening methods to develop primary libraries of recombinant single chain variable fragment (scFv) antibodies against '*Ca*. Liberibacter asiaticus (CLas) and the citrus strain of *Xylella fastidiosa*. The primary libraries each encode more than $10^7$ unique antibodies. We have selected scFv against several proteins found on the surface of CLas, including the major outer membrane protein, OmpA; the polysaccharide capsule expressing protein KpsF; protein components of the type IV pilus (CapB and CapF); and flagellar proteins (FlhA and FlgI). We have also selected scFv that bind to proteins involved in virulence, including TolC. The scFvs expressed in phage particles have been used in ELISA and dot blot assays against CLas infected plant and psyllid extracts. We have also recloned many of these scFvs into a plasmid expression vector and used them in tissue print assays of infected and healthy plant parts. We have also isolated scFv from the *Xylella fastidiosa* library that bind isolates from citrus (citrus variegated chlorosis) but not from grapevine (Pierce's disease). We have demonstrated a technology to produce antibodies at will and against any protein target encoded by '*Ca*. Liberibacter asiaticus' and the psyllid vector, and to differentiate strains of *Xylella fastidiosa* that cause citrus variegated chlorosis from those that cause Pierce's disease. Future applications will include advanced diagnostic methods for huanglongbing and the development of immune labeling of CLas in infected plants and psyllids. Similar applications can be made to the *Xylella fastidiosa* and citrus variegated chlorosis pathosystem.

**O14.006 Host switching in a vector-borne plant pathogen, *Xylella fastidiosa***

*F. Labroussaa, S.E. Lindow and R.P.P. Almeida*
*Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720, USA*
*Email: flabroussaa@berkeley.edu*

*Xylella fastidiosa* is a xylem-limited bacterium that causes disease in several crops of economic importance such as grape, almond, citrus and coffee. Transmitted by xylem-sap sucking leafhoppers, *X. fastidiosa* must

respond appropriately to multiple environmental cues in order to be able to colonize its two hosts. In addition, switching between hosts is likely a complex process that requires the establishment of an infection in its novel partner while colonizing its current host; otherwise the transition may not be successful. Recent findings showed that traits involved in *X. fastidiosa* plant colonization are incompatible with insect colonization, and vice-versa; thereby transitioning between hosts represents an abrupt and absolute environmental change. Such mechanisms are dependent on the interplay of environmental cues and a cell-density signal (i.e. quorum sensing); while the later appears to also modulate phenotypic changes required for host switching. Interestingly, signal-deficient mutants do not lose their capacity to colonize plants but are not capable to colonize insects, indicating that cell-cell signaling suppresses movement and plant host colonization, but is required for plant-to-plant dispersal. However, accumulation of the cell-density signal is not sufficient for the transition from plant to insect, as structural polysaccharides, such as pectin or chitin, are a requisite for a state change required for efficient vector colonization. Understanding how *X. fastidiosa* switches from one life stage to another will lead to insights on how horizontally transmitted symbionts recognize, respond to, and switch between different environments as well as giving a more complete view of microbe-host interactions.

### O14.007 Spiroplasma-insect host interactions: shedding light on the black box

*N. Arricau-Bouvery[1], L. Béven[2], S. Duret[1], B. Batailler[3], Marie-Pierre Dubrana[1], C. Saillard[2], J. Renaudin[1]*
*INRA, UMR Biologie du Fruit et Pathologie, INRA-Univ Bordeaux, VILLENAVE d'ORNON, France ; Université de Bordeaux, UMR 1332 Biologie du Fruit et Pathologie, Villenave d'Ornon, France ; Université de Bordeaux, UMS 3420 Bordeaux Imaging Center, Bordeaux, France*
*Email: renaudin@bordeaux.inra.fr*

*Spiroplasma citri* is a phloem-limited plant pathogenic bacterium, which is transmitted by the leafhopper vector *Circulifer haematoceps*. Successful transmission requires invasion of insect cells and thus tight interactions between the spiroplasma surface proteins and the insect cells. Experimental transmission of *S. citri* mutants as well as data from protein overlay assays have led to the conclusion that several spiroplasma proteins, including spiralin, phosphogycerate kinase and plasmid-encoded proteins were involved in these interactions. In particular we have shown that the *S. citri* mutant G/6 lacking plasmid-encoded adhesins ScARPs was poorly transmitted. Through infection of the Ciha-1 cell line (issued from *C. haematoceps*) we also showed that the ScARP-less mutant G/6 was affected in its ability to adhere and invade the insect cells. By using latex beads cytadherence assays we showed that the N-terminal repeat domain (Rep) of ScARPs promoted binding to insect cells and that internalization of the beads was actin-dependent. These data suggested that ScARPs, via their repeat domain, were implicated in adhesion of *S. citri* to cells of the leafhopper vector. In agreement with, inhibition tests performed with anti-Rep polyclonal antibodies and competitive binding assays with recombinant Rep, both hindered invasion of the insect cells by the spiroplasmas. In addition, internalization of the spiroplasmas was found to be dependent on de novo actin polymerization, as cell treatment with cytochalasin D reduced the spiroplasma entry. As a whole these results indicate that ScARPs play a role in insect-transmission through their implication in cell invasion. However other functions cannot be excluded.

### O14.008 Recent progress on phytoplasma-host interactions and future of phytoplasma genomics

*S. Kakizawa and Y. Kamagata*
*National Institute of Advanced Industrial Science and Technology (AIST), Bioproduction Research Institute AIST Tsukuba Central 6-10, 1-1-1 Higashi, Tsukuba, Ibaraki, 305-8566, Japan*
*Email: s.kakizawa@aist.go.jp*

Many symbiotic and pathogenic bacteria were thought to be under the reductive evolution during adaptation to nutrient-rich environments. It causes extreme shrinkage of their genomes, and these bacteria lack a lot of genes for indispensable metabolic pathways because they can relay many metabolites on their hosts. Thus some of them could not be cultured outside their hosts any more. It is a big problem because knockout or over-expression of their genes are difficult therefore understanding of these gene functions and these bacterial biology must be also difficult. Phytoplasmas are plant pathogenic bacteria that can infect several hundreds of plant species and cause devastating yield losses in diverse crops worldwide. Although phytoplasma are unculturable, four complete phytoplasma genomes and several partial genomes have been determined so far. These phytoplasma genomes lack most metabolic pathways, including those for ATP, amino acid and nucleotide syntheses, most likely because many metabolites are available within the host cell environment, leading to a reduced selective constraint on genes for biosynthetic capabilities. To analyze functions of phytoplasma genes, *in planta* expression of phytoplasma genes has been widely used because they are unculturable. To this end, we are using the "genome engineering" techniques that was recently developed at J. Craig Venter Institute (JCVI). In this presentation, I would like to talk about the overview of phytoplasma-host interactions and our on-going studies.

**O14.009 Improved phylogenetics for the Weligama coconut wilt phytoplasma found in Sri Lanka**

*S. Abeysinghe[1], P. Abeysinghe[1] and M. Dickinson[2]*
*[1]Dept. of Botany, University of Ruhuna, Matara, Sri Lanka; [2]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK*
*Email: saman@bot.ruh.ac.lk*

Phytoplasmas are responsible for a number of diseases in coconut worldwide. Phytoplasmas have also been found associated with the wilt diseases of coconut in Sri Lanka (Weligama wilt) and India (Kerala wilt). However, the titre of these organisms in wilted coconuts appears to be very low when compared to the lethal-type diseases, making them difficult to detect, which in turn has made it difficult to confirm whether the phytoplasmas are the causal agent of the diseases or simply opportunist organisms that are sometimes found associated with the symptoms. In previous work, we have designed universal nested PCR primers based on the *secA* gene for amplification of DNA from phytoplasmas in most 16Sr groups, and have shown the value of the sequences generated for phylogenetic analyses. Recently, we have designed more specific sets of primers based on 16Sr gene and provided preliminary evidence that suggests the Weligama wilt phytoplasma has a 16S rDNA sequence most closely related to the 16SrXI sugarcane type phytoplasmas in Sri Lanka. However, we will be providing evidence that according to *secA* gene Weligama wilt phytoplasma is more similar to the16SrXIV Bermudagrass type, suggesting the possibility that the Weligama wilt phytoplasma may be a recombinant between two different phytoplasmas. Further work on more samples is required to resolve these findings. The universal real-time PCR detects the phytoplasmas in rice and sugarcane in Sri Lanka with low Ct values, it also detects the Weligama wilt phytoplasma in coconut samples, but the Ct values are much higher confirming the fact that when the phytoplasma is present it appears to be at very low levels.

**O14.010 Phytoplasmal diseases in southwest China and the classification of phytoplasmas associated with these diseases**

*H. Cai and H.R. Chen*
*Deparment of Plant Protection, Yunnan Agricultural University, Kunming, 650201, P. R. China*
*Email: caihong0623@gmail.com*

Phytoplasmas are phloem-inhabiting, cell wall-less bacteria of the class Mollicutes. Numerous plant diseases worldwide are caused by or associated with infection by phytoplasmas. Plants infected by phytoplasmas exhibit an array of symptoms such as yellowing, stunting, stem fasciation, big bud, little leaf, virescence, phyllody, shoot proliferation, and witches'-broom growth. Southwestern China is one of the global hotspots of biodiversity. A three-year survey was conducted from 2010 to identify phytoplasmal diseases and phytoplasmas present in Southwestern China. Samples were collected from various plants showing phytoplasmal disease-like symptoms. Nested PCR assays were performed to amplify genomic segments containing 16S rRNA and other conservative genes with phytoplasma-universal primers. Amplicons were cloned and sequenced to confirm the presence of phytoplasmas. So far, plants belonging to at least 24 species were found to have phytoplasma infection. The identified phytoplasmas belong to six distinct 16Sr groups (16SrI-B, 16SrII-A, 16SrII-C, 16SrIII-B, 16SrV-B, 16SrXII and 16SrXXXII) and are affiliated with six '*Candidatus* Phytoplasma' species, namely, '*Ca.* Phytoplasma asteris', '*Ca.* Phytoplasma aurantifolia', '*Ca.* Phytoplasma pruni', '*Ca.* Phytoplsma ziziphi', '*Ca.* Phytoplasma australiense' and '*Ca.* Phytoplasma malaysianum'. The impact of the phytoplasmas and the phytoplasmal diseases on the ecosystems in the region is currently being evaluated.

**O14.011 Classification trees based on the gene expression information from selected genes for the prediction of the BN disease status of grapevine**

*A. Rotter, P. Nikolić, M. Ravnikar, K. Gruden and M. Dermastia*
*National Institute of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia*
*Email: marina.dermastia@nib.si*

Phytoplasmas are bacteria without cell walls from the class Mollicutes that colonize plant phloem and are transmitted by phloem-feeding insect vectors. In grapevine, phytoplasmas cause grapevine yellows (GY) diseases, which were identified in the majority of grapevine growing countries worldwide. Several molecularly distinct phytoplasma groups which cause the GY were identified; however, a phytoplasma from the stolbur group/16SrXII-A, associated with the GY disease Bois noir (BNp), is very common. In our previous study we have shown. Based on the results of a comparison of the global transcriptome profile of healthy and BNp infected grapevine (*Vitis vinifera*) cv. Chardonnay, in this study a set of 22 genes was chosen. The selected genes were differentially expressed in various pathways and were confirmed to be suitable for a reliable classification of infected plants and for the characterization of susceptibility features in the field conditions. After their analysis in the period of five growing seasons classification trees were used in order to reveal possible marker genes that may be used in new diagnostic assays. Out of the 22 genes tested over seasons, three of them were shown to be useful for disease status determination; i.e. genes encoding flavanone 3-hydroxylase (*VvF3h*), large subunit of ADP-glucose pyrophosphorylase (*VvAgpL*) and histidine-containing phosphotransfer protein (*VvHP*).

Calculating the expression of those genes is enough for over a 60 %-accuracy for disease status determination.

**O14.012 Transmission of chestnut yellow crinkle phytoplasma by leafhopper *Parabolopona ishihari* Webb (Homoptera: Cicadellidae)**

*C.L. Lin[1], G.Z. Tian[1], X.Q. Zhu[2], Z.P. Li[2], C.S. Song[1], Z.G. Ren[1], Y. Li[1] and C.G. Piao[1]*
*[1]Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry/Key Laboratory of Forest Protection of State Forestry Administration, Beijing 100091, P. R. China; [2]Huairou Forest Pest and Disease Control and Quarantine Station, Beijing, 101400, P. R. China*
*Email: lincl@caf.ac.cn*

Chinese chestnut (*Castanea mollissima* BL.), a deciduous tree native to China, belongs to the family *Fagaceae* and is widely cultivated in eastern Asia. Recently Chinese chestnut trees planted in a suburb of Beijing, China developed symptoms including yellowing, leaf crinkling, little leaf, shortened internodes, and empty burrs. Transmission electron microscopy revealed the presence of phytoplasma cells in phloem sieve elements of the symptomatic chestnut trees. Molecular cloning and sequence analysis of PCR-amplified near-full length 16S rRNA gene indicated that the phytoplasma associated with the Chinese chestnut yellow crinkle (CnYC) disease is closely related to Japanese chestnut witches' -broom phytoplasma. Eleven insect species with piercing-sucking mouthparts were collected in chestnut plantation with symptomatic trees. CnYC phytoplasma was detected in *Parabolopona ishihari* Webb by PCR amplification of 16S rRNA gene using CnYC phytoplasma specific primer pairs. Nymphs and adults of *P. ishihari* Webb collected from chestnut trees infected with phytoplasmas were feed with healthy periwinkle (*Catharanthus roseus*) growing in green house. The symptoms of yellowing, leaf crinkling, little leaf and shorten internodes were observed and the CnYC phytoplasma was also detected by PCR amplification in periwinkles at 30 days post inoculation. These results suggested that CnYC phytoplasma could be transmitted from Chinese chestnut to other plant by *P. ishihari* Webb.

**P14.001 Effects of environmental temperature on the corn stunt spiroplasma disease**

*E.O. Sabato, S.R. Galvão and E.C. Landau*
*Embrapa, Rod. MG 424, Km 65 C.P. 151 35701970 Sete Lagoas, MG, Brazil*
*Email: elizabeth.o.sabato@embrapa.br*

The corn stunt spiroplasma (CSS) is caused by *Spiroplasma kunkelii*, transmitted by the leafhopper *Dalbulus maidis*. The objective of this study was to verify the spiroplasma transmission by *D. maidis*, and the development of CSS symptoms in maize, under acclimated chambers and screen-house conditions. The maximum and minimum temperatures ($^0$C) and amplitude, respectively, were: chamber I (27.35; 23.58; 3.76), II (27.22; 18.53; 8.69), III (24.8; 22.33; 2.48), IV (28.4; 23.29; 5.1), screen-house (32.29; 18.33; 12.96). In each condition, 24 spiroplasma-infective, and 6 healthy leafhoppers were confined on maize seedlings, for 6 days (one per seedling). After that, half of these seedlings were cultivated inside the chambers, and the other half was cultivated in the screen-house. More than 80% of the plants submitted to spiroplasma inoculation, and cultivated in the screen-house, showed CSS symptoms, indicating no effect of that temperatures on this pathogen transmission. Some plants without CSS symptoms could be due the death of the infective leafhopper, before spiroplasma transmission. For the maize seedlings submitted to spiroplasma inoculation, and cultivated under the five temperature conditions, only in the chamber II none plant presented CSS symptoms, until 60 days age. These asymptomatic plants were transferred to the screen-house where, in few days, the CSS symptoms appeared, indicating that bland temperature condition can stop the spiroplasma growth in maize.

**P14.002 Effect of the corn stunt spiroplasma disease on maize production**

*E.O. Sabato, E.C. Landau and A.M. Coelho*
*Embrapa, Rod. MG 424, Km 65 C.P. 151 35701970 Sete Lagoas, MG, Brazil*
*Email: elizabeth.o.sabato@embrapa.br*

The effect of corn stunt spiroplasma (CSS) on maize development and production was evaluated in the screen-house, with spiroplasma inoculation, and in the field, using one maize cultivar and one popcorn cultivar. In the screen-house 20 seedlings of each cultivar were submitted or not to spiroplasma inoculation, using one spiroplasma-infective or healthy leafhoppers *Dalbulus maidis* confined for six days in each eight-days-seedling. The CSS symptoms were detected on 40% and 60% of the maize and the popcorn cultivar plants that had the development drastically reduced by this disease, in relation to the healthy plants. Each cultivar was sowed in 10 lines (10m each one) in three different areas at Embrapa experiment station, Sete Lagoas, MG, Brazil, and, in each area, 10 plants with and 10 plants without CSS symptoms were marked for grain production evaluation. The averages of the corn stunting diseases symptoms incidence were 21.9%; 24.6%; 15.7% and 65.6%; 77.5%; 74.2% for the maize and the popcorn cultivar, respectively, with CSS symptoms predominance. The periodic insect sampling showed *D. maidis* leafhoppers presence since 15 days after sowing. The averages of CSS reductions on the maize and the popcorn cultivars grain production were, respectively: 84.07%; 75.40%; 76.80%; and 63.17%; 72.62%; 60.66%. These results indicate

that damage by CSS on the field maize crop can be severe.

**P14.003 Identification of sesame phyllody phytoplasmas and incidence of sesame phyllody disease in Antalya, Turkey**

*Z. Özdemir, H. Topuz, and M. İ. Çağırgan*
*Department of Plant Protection, Adnan Menderes University, Aydın 09100 Turkey*
*Email:zahide_ozdemir@yahoo.com*

Sesame phyllody is one of the major diseases of sesame (*Sesamum indicum*) reducing the yield significantly, in Antalya, Turkey. In 2011 and 2012, surveys conducted to sesame fields and incidence was recorded in three to four different times per growing season. In 2011, through the end of growing season, disease was found in all of the sesame fields and incidence was ranged from 0.7 to 11 % in 112 da survey area. In 2012, at the end of the season in 73 percent of the fields surveyed, disease was present and incidence ranged from 0.2 to 11% in 99 da survey area. Phytoplasmas detected from genomic DNA of diseased plants collected from surveys by the amplification of 16SrDNA using nested PCR with primer pairs P1/P7 and R16F2n/R16R2. An amplification product of 1.24 kb band was detected by nested PCR. Amplified 1.24 kb product of 16SrDNA were cloned and sequenced from seven phytoplasma isolates from different survey locations. According to BLAST search, six of the isolates were identified as Peanut Witches Broom (16SrII) and one isolate was identified as Clover Proliferation (16SrVI) phytoplasma group. Previously sesame phyllody phytoplasmas reported from Eastern Mediterranean region of Turkey were grouped in Clover Proliferation (16SrVI) phytoplasma group. Identification of more of the phytoplasma isolates from different locations is being carried out.

**P14.004 An update on Awka wilt disease of coconut caused by Phytoplasma In Nigeria**

*I.J. Osagie, E.E. Ojomo and F. Pilet*
*CIRAD, UMR RPB (CIRAD/IRD/UM2), F-34398 Montpellier, France;NIFOR, PMB 1030, Benin City, Nigeria*
*Email: fabian.pilet@cirad.fr*

Lethal yellowing disease (LYD) caused by phytoplasma is the most damaging threat to coconut in Africa. Nigeria is the first African country where the disease (Awka wilt) was reported in 1917. The symptoms of the disease are similar to those reported in other West and East African countries. In West Africa, LYD is caused by a phytoplasma belonging to the 16SrXXII group, and the disease is still very active in Nigeria and Ghana. In early 1990s, sequencing of the 16SrDNA of one Ghanaian and one Nigerian isolate confirmed the implication of a very similar but not identical phytoplasma strains in the two countries. In 2012, a new survey for coconut LYD covering most of the coastal Nigerian States was done. Symptomatic coconut trees were observed in all the States surveyed. Stem samples were collected from symptomatic trees from three Eastern States around Awka. From each sample, DNA was extracted and the 16SrDNA was amplified by PCR using P1/P7 primers. The PCR products were sequenced and compared with each other and with the LDN sequence published 20 years ago. A homology of 100% was observed between the 16SrDNA sequence previously published and the sequences of our samples. Based on the known conserved 16SrDNA sequences, this result suggests a relative stability of the LYD phytoplasma populations in Eastern Region of Nigeria and absence of introduction of the "Ghanaian strain". A similar investigation for symptomatic coconut from the Western Region and by using more variable genes is necessary.

**P14.005 Dissecting the role of *NPR1* in Defense against periwinkle leaf yellowing phytoplasma in *Catharanthus roseus* using virus-induced gene silencing**

*Y.C. Sung, C.P. Lin and J.C. Chen*
*Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan, China; Institution of Biotechnology, National Taiwan University, Taipei, Taiwan, China*
*Email: R00633002@ntu.edu.tw*

Phytoplasmas are prokaryotic plant pathogens causing considerable loss in many economical crops globally. Previously, we found that periwinkles (*Catharanthus roseus*) infected with periwinkle leaf yellowing (PLY) phytoplasma contained both symptomatic and non-symptomatic shoots. The expressions of *CrPR1* genes were up-regulated in both symptomatic and non-symptomatic shoots, indicating a systemic resistant machinery might be activated after PLY phytoplasma infection. Therefore, we aimed to investigate on *NPR1* gene, a critical gene in SAR activation, to realize the effects of SAR on phytoplasma pathogenesis. Because an analysis for gene functions was lacking for periwinkle, we aimed first to develop an effective virus-induced gene silencing system in periwinkle. *Tobacco rattle virus* (TRV)-based VIGS system was tested in periwinkle, and several potentially influential factors (temperature, plant age, and leaf age) were analyzed to optimize the VIGS system. The results show that high temperature had negative effects on VIGS efficacy and virus accumulation. Plant age showed no significant differences, while newborn leaves had better VIGS efficacy and virus accumulation compared to other leaves. Moreover, silencing effects and phytoplasma symptoms were able to coexist, indicating that the VIGS system we established can be used for functional analysis. Using this system, I generated

*CrNPR1* and *CrNPR3* silencing plants, and preliminary inoculation test of PLY phytoplasma showed that disease symptoms seemed to develop faster in *CrNPR1*-silenced plants than that of plants treated with vector only, suggesting that the importance of *NPR1* in defense against phytoplasma infection.

**P14.006 Large scale management of Coconut Lethal Yellowing Disease in Mozambique**

*N. Munguambe[1], R. Timbrine[2], M. Freire[1], S. Gadaga[3], P. Dias[3], B. do Rosario[2], J. Pudivitr[2] and F. Pilet[4]*
*[1]Instituto de Investigacao Agraria de Mocambique (IIAM), Quelimane, Mozambique; [2]Farmer Income Support Project, Millenium Challenge Account - Mozambique, Quelimane, Mozambique; [3]Farmer Income Support Project, ACDIVOCA, Quelimane, Mozambique; [4]CIRAD, UMR RPB (CIRAD/IRD/UM2), F-34398 Montpellier, France*
*Email: fabian.pilet@cirad.fr*

Mozambique was, at the beginning of the 1900's, one of the biggest world copra producers. Since the 1990s the coconut crop became seriously threatened by Coconut Lethal Yellowing Disease (CLYD), an insect transmitted disease caused by phytoplasma, which killed, up to 2008, at least 7,000,000 coconut palms. To support the smallholder coconut sector the MCA-FISP project started in 2009 was designed to reduce the impact of the disease through the use of containment measures consisting on culling and elimination of symptomatic disease trees to reduce its spread. In the early 1980s it was estimated that Nampula and Zambezia Provinces had about 122,000 ha of coconut trees. By 2009, 40,000 ha were destroyed by the disease, with an additional 25,000 ha under heavy disease pressure. Since 2010, the FISP project hired a contractor that culled with chainsaws about 600,000 coconut trees from an area of 50,000 ha belonging to smallholder farmers. The felled palms were selected based on the visual observation CLYD symptoms. The efficiency of the selection system was further confirmed through molecular analysis showing that the level of selection mistakes was within acceptable levels. This activity effectively reduced the average disease incidence from 5% to below 2% based on annual field surveys. Because the FISP project will end by September 2013, manual tree culling by the smallholders was recently started as a strategy for sustainability which is recognized to be a reasonable solution to maintain the disease at acceptable economic levels.

**P14.007 New findings on spartium withches' broom disease**

*R.E. Spallino, S. Rizza, V.D'Urso, C. Marzachì and M. Tessitori*
*Department of Agricultural and Food Science, Sect. Phytopathology and Plant genetics, University of Catania, Via S. Sofia 100 Catania, I-95123 Italy*
*Email: mtessitori@unict.it*

*Spartium junceum* is an important pioneer species of Mediterranean Basin. Spartium witches' broom (SWB) disease occurs in Italy and Spain. Besides the characteristic symptom, the plants show fasciation, yellowing of twigs, flower malformation and death of the whole plant. Both '*Candidatus* Phytoplasma spartii' (16SrX-D) and a phytoplasma member of the group 16SrV-C have been associated to SWB. Since 2010 increasing reports of this syndrome were registered in Sicily region (South Italy) and particularly in the Etna area, a valuable viticultural territory. The rapid spread of the disease suggested the presence of an active vector of the phytoplasma/s. Detection and molecular characterization of phytoplasmas was carried out through analysis of the 16S ribosomal RNA gene amplified by nested-PCR using universal primer pairs on samples of spartium and insects collected. Obtained sequences allowed to classify the detected phytoplasmas, on the basis of virtual RFLP analysis performed by *i*PhyClassifier, in 16rRNA V-C and X-D groups and subgroups. Further phylogenetic analysis of *sec-y* gene of 16SrV-C isolates evidenced a homogeneous cluster distinguishable from other reference strains of group 16Sr-V including the flavescence dorée associated phytoplasmas. Among all the insects species collected only one monophagous psyllid of *S. junceum* (*Livilla spectabilis*) resulted positive to both phytoplasmas. The epidemic of SWB in Sicily and the finding of a potential vector pose interest on the disease especially in consideration of a potential risk of occasional trasmission of 16SrV-C to grapevine in a region free of flavescence dorée.

**P14.008 Research on *Ca. Phytoplasma asteris* causing Oilseed Rape Phyllody disease in Poland**

*A. Zwolińska, T. Klejdysz, K. Krawczyk, H. Pospieszny and J. Nawrot*
*Virology and Bacteriology Department, Plant Protection Institute - National Research Institute, Węgorka 20, 60-318 Poznań, Poland*
*Email: a.zwolinska@iorpib.poznan.pl*

Phytoplasmas are single-celled microorganism that infect plants worldwide and are transmitted by phloem feeding leafhoppers. In a particular ecosystem phytoplasmas have specific insect vector which is responsible for spreading the disease between plant hosts. Usually there are a few plant species involved in life cycle of phytoplasma, serving as its reservoir. Both insect vectors and alternative hosts are factors determining the maintenance of phytoplasma in environment. The objective of the study was an identification of these factors in order to examine a natural life cycle of '*Ca*. Phytoplasma asteris' (Aster Yellows phytoplasma) causing oilseed rape phyllody in Poland. Phytoplasma belonging to the

Aster Yellows group is present on winter oilseed rape plants (*Brassica napus*) in Poland, for over a decade. The diseased plants exhibit phyllody of flowers which lead to siliques malformations and losses in seed production. We tested two potential *Auchenorrhyncha* vector species, which are numerous in agricultural environment: *Macrosteles laevis* and *Psammotettix alienus*. We found that *Macrosteles laevis* leaf hoppers transmit phytoplasma to the test plants: periwinkle (*Catharanthus roseus*) and broad bean (*Vicia faba*). We also found some weed species which tested positive for phytoplasma presence.

## Concurrent Session 15-Forensic Plant Pathology

### O15.001 Molecular diagnostic challenges for the detection of new pathogen introductions: A case study involving the detection of *Pseudomonas syringae* pv. *actinidia*e on kiwifruit in New Zealand

*R.K. Taylor, J.R. Chapman, L.I. Ward, L.W. Liefting, B.S.M. Lebas, and B.J.R. Alexander*
*Plant Health and Environment Laboratory, Ministry for Primary Industries, PO Box 2095, Auckland 1140, New Zealand*
*Email: robert.taylor@mpi.govt.nz*

In November 2010, *Pseudomonas syringae* pv. *actinidiae* (Psa) was detected for the first time in New Zealand on kiwifruit. The response to this finding triggered one of the largest surveillance and diagnostic programmes seen in New Zealand's horticultural industry. During this response the New Zealand Ministry for Primary Industries' Plant Health and Environment Laboratory tested samples from 912 properties and screened over 14,500 samples for the presence of Psa. The initial objectives of the response were to confirm the aetiology and disease prevalence, identify possible mechanisms of spread, and identify introduction pathways, with the aim of informing management options to contain the outbreak. Molecular characterisation of the Psa strains isolated during the response was conducted using a range of molecular techniques that included rep-PCR fingerprinting, multilocus sequence analysis, and sequence enrichment for targeted next generation sequencing. The usefulness and challenges of using molecular techniques for Psa detection and characterisation during the response will be presented.

### O15.002 Forensic techniques for toxins produced by plant pathogens

*A. Gamliel, P. Karlovsky and H.W. Dehne*
*ARO Volcani Center, Bet Dagan, 50250 Israel; University of Goettingen, Dept. of Crop Science, Griesebachstrasse 6, D-37077, Goettingen, Germany and University of Bonn, INRES-Phytomedicine, Nussallee 9, D-53115 Bonn, Germany*
*Email: Heinz-W.Dehne@t-online.de; agamliel@agri.gov.il*

Plant pathogens affect worldwide plant yields to a certain degree depending on the regions and climatic conditions of plant production. Besides quantitative losses plant pathogens also influence the quality of harvests and the worth giving and essential constituents of plant products. Of high importance for the quality of food and feed provided by plant production is the lowest contamination with mycotoxins produced by plant pathogens. Mycotoxins are highly active substances impairing the physiology of mammals – but in part they are also important for the virulence of these pathogens at plants as it could be demonstrated for *Fusarium* species on various host plants.
The forensic analytics of especially the *Fusarium*-mycotoxins are difficult – trace analysis has to be applied to identify and quantify the toxins. This is also necessary for rating in different host plant material and host debris as well. When certain fungi like *Aspergillus*-species produce toxins within 2 classes, the toxins formed by *Fusaria* cover more than 10 different chemical groups. This implicates that those compounds can only be identified and quantified that they are expected and appropriate methods are applied for this. The identification from plant material and organic debris is difficult but efficient techniques have been developed recently. Mycotoxins are not only found in harvested material but also in vegetative plant material and plant residues.
Besides immunological tests for single toxins the technique of LC-MS-MS can provide reliable and differential results for various mycotoxins. Most important for following the introduction of different mycotoxins in the environment methods following this can be assisted by the identification of the pathogen and the genetic characteristics of the particular isolate of the pathogen. All information together will lead to successful identification of pathogens responsible for mycotoxin contamination of plant material and allow to identify the source of introduction or of the primary inoculum of particular pathogens. The relevant techniques and results will be presented and discussed.

### O15.003 Forensic approach to the investigation of a *Fusarium proliferatum* disease outbreak in Israel

*J. Fletcher, I. Moncrief, A. Gamliel and J. Stack*
*National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Department of Entomology & Plant Pathology, 127 NRC, Oklahoma State University, Stillwater, OK. 74078, USA*
*Email: jacqueline.fletcher@okstate.edu*

Forensic plant pathology is an emerging, multidisciplinary approach, for the application of scientific tools and principles to the investigation of plant diseases that could have been criminally incited. Forensic technologies developed for plant disease assessment, pathogen detection and strain discrimination, sample collection, and other critical forensic methods must be validated in realistic settings. In 2008, *Fusarium proliferatum* (Matsushima) Nirenberg (*Fp)*, a mycotoxigenic fungus, was isolated in southern Israel from onion bulbs having disease symptoms of salmon-colored blotches on their outer scales and has since become widespread on southern Israeli onion farms. Although no criminal activity is suspected, the fact that the source of the fungus is not known provided an opportunity for the first complete forensic field and laboratory investigation of a plant disease. Methods employed include a decision tool for assessing criminal intent, a validated real time PCR *Fp*

detection assay, and ISSRs and SSRs (microsatellite or repetitive genomic region based methods) to characterize *Fp* populations and discriminate among isolates sampled from different locations and vegetation in and near the affected onion fields and irrigation water, as well as in natural vegetation distal to the fields. Sampling extended also to fields, in northern Israel where onion seeds and sets were produced for planting in the south, and in imported seed lots and other key sites. In addition to providing a useful characterization of *Fp* populations in the Israeli onion production system the work will form the basis for enhancement of forensic investigation applications in crop systems.

**O15.004 Deciphering the genome of Shiga-toxin-producing *Escherichia coli* O104:H4**

*Q. Feng, K.Y. Cai, H.J. Jia and J. Wang*
*Beijing Genomics Institute-Shenzhen, Central Building of Beishan Industrial Road, Yantian District, Shenzhen, 518083, P.R.China*
*Email: wangj@genomics.org.cn*

An outbreak caused by Shiga-toxin-producing *Escherichia coli* O104:H4 occurred in Germany in May and June of 2011, with more than 3000 persons infected. The pathogen analysis work concerned an isolate from a 16-year-old female member (TY2482) of an infected family. The studies included rapid and high-throughput DNA sequencing technologies, open-source data releases and prompt crowd-sourced analyses. First, we sequenced the sample using an Ion Torrent Personal Genome Machine (PGM) and generated 79Mb for a draft genome. Then, we released PGM sequencing data and the curiosity-driven analyses began. Meanwhile, high-throughput sequencing of this isolate on an Illumina HiSeq2000 platform was continued, and we obtained 2G reads later for a fine map. Analyses of the draft genome were carried out within 3 days by bioinformaticians on four continents facilitating the completion of the study in less than one week. Finally, we found the outbreak strain had a very close relationship to *E. coli* strain 55989, with an average nucleotide identity of 99.8%. From a detailed comparison of the chromosomes of TY2482 and *E. coli* strain 55989 plus a detailed examination on plasmids in TY2482, we revealed the outbreak strain belonged to an enteroaggregative *E. coli* lineage that had acquired prophage-encoded Shiga toxin 2 and plasmid-encoded antibiotic resistance gene CTX-M-15 ESBL, both essential for the strain's virulence.

**O15.005 Advanced diagnostic and discriminatory technologies for forensic investigation**

*D.G. Luster*
*USDA-ARS, Foreign Disease - Weed Science Research Unit, 1301 Ditto Ave.,Ft. Detrick, MD 21702*
*Email: doug.luster@ars.usda.gov*

Forensic investigations require rapid decision-making tools to streamline the identification of suspect agents, in order to make appropriate decisions on downstream procedures. Unless suspect organisms can be confirmed and or identified on site in a rapid manner, resources may be wasted in transportation and analysis costs. Inexpensive immunological detection systems such as immuno-strips can provide rapid identification of suspect agents saving investigator's time and money in subsequent procedures. While immunological detection reagents are available for many viruses and a few bacterial plant pathogens, very few reagents for eukaryotic pathogens have been developed, due to the difficulty in identifying and purifying potential immunogens with the appropriate level of specificity. Recent advances in RNA sequencing and protein identification by mass spectrometry have revolutionized the search for extracellular proteins and hold promise for development of sensitive and specific monoclonal antibodies. We will discuss methods and techniques used in our lab for the generation of monoclonal antibodies specific for fungal plant pathogens, and present new technologies used in deploying immunological reagents for rapid detection and identification in forensic investigations.

## Concurrent Session 16-Fruit Trees Diseases

### O16.001 Grapevine canker diseases: advances and findings over the past decade

*J. R. Úrbez-Torres*
*Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, 4200 Highway 97, Box 5000, Summerland, British Columbia, V0H 1Z0, Canada*
*Email: joseramon.urbez-torres@agr.gc.ca*

Dieback and consequent death of grapevines (*Vitis vinifera* L. and *Vitis* spp.) caused by perennial cankers is known to occur in vineyards since the early 1900s and nowadays is still one of the major concerns to the grapevine industry worldwide. Research conducted first in Australia and later in California during the 1970's decade revealed the diatrypaceous fungus *Eutypa lata* as the causal agent of grapevine dieback, and the disease was named Eutypa dieback. Since then and for over 30 years, *E. lata* was considered the sole fungus causing grapevine cankers and consequent dieback and so most research on cankers focused on only this fungus. However, morphological, phylogenetic, and pathogenicity studies conducted over the past decade have demonstrated that grapevine cankers and consequent dieback is a much more complex plant pathosystem, which involves not only *E. lata* but over 40 other different fungal species in both the *Diatrypaceae* and *Botryosphaeriaceae* families as well in the *Phomopsis*/*Diaporthe* genera. These findings have been of great significance to scientists who over the past decade have focused their efforts in trying to elucidate the role that such a broad number of taxonomically unrelated fungi play on this disease complex. This presentation summarizes the most relevant advances and findings that scientists around the world have achieved over the past decade on better understanding the biology and epidemiology of the different fungal pathogens associated with grapevine cankers and the subsequent development and implementation of effective chemical, biological and cultural control management strategies in vineyards worldwide.

### O16.002 Current status on bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* of kiwifruit in Italy

*M. Scortichini*
*C.R.A.- Centro di ricerca per la Frutticoltura, Via di Fioranello, 52, I-00134 Roma, Italy*
*Email: marco.scortichini@entecra.it*

A sudden, destructive epidemic of bacterial canker of kiwifruit was recorded during spring-autumn 2008 to *Actinidia chinensis* cvs. Hort 16A, JinTao, Soreli, the yellow-fleshed kiwifruit, and their pollinators in central Italy. *Pseudomonas syringae* pv. *actinidiae* (Psa) was consistently isolated from leaf spots, wilted twigs, cankers along leader and main trunk, lenticels as well as from whitish to reddish exudates oozing out the tree. The current Psa population most probably originated in China and it is genetically different from another which, during 1980-90, such population is spread in all major areas of kiwifruit cultivation of the world (i.e., China, Italy, New Zealand, and Chile) and it is retained as pandemic. Genome comparisons have revealed that Psa can gain and lose the phaseolotoxin gene cluster, as well as mobile genetic elements, such as plasmids and putative prophages, and that it can modify the repertoire of the effector gene arrays. In addition, the strains currently causing worldwide severe economic losses display an extensive set of genes related to the ecological fitness of the bacterium *in planta*, such as copper and antibiotic resistance genes and very effective siderophores. The rapid and reliable detection of propagative material of kiwifruit circulating worldwide is a fundamental step toward reducing the further risk of spread of the pathogen within and between countries. Field control benefits of the knowledge obtained from epidemiological studies and critical periods to effectively reduce the multiplication and dispersal of the pathogen have been pointed out. For a more effective success, the strategies for controlling Psa should be taken at area level.

### O16.003 New recognition on the infection of *Valsa mali* on apple tree

*K.Q. Cao, S.T. Wang, T.L. Hu and Y. Zhang*
*College of Plant Protection, Agricultural University of Hebei, Baoding, Hebei 071001, P.R. China*
*Email: ckq@hebau.edu.cn*

Apple canker caused by *Valsa mali* is a destructive disease that causes serious economic losses in China. According to the survey in Hebei, Shandong and Shanxi provinces, we found that the infection occurred generally on the trunk and the branches with a wound created by pruning, spreading, twisting, kicking, sun scald, freezing injury, insect damage and so on. However, 80% of disease occurred in pruning areas. New wounds were more likely to be infested than the old wounds. The conidia of *Valsa mali* had strong adaptability to the environment and could be released in winter at foggy or snowing weather when the bark was wet. At the temperature of 0℃, the germination rate of conidiospore could reach 64% after treated for 18 days. Pruning in winter has been proved to be the main process for disease infection and the pruning tools played an important role for disease spreading. Ten genera of fungi were isolated from and xylem of the apple tree, which was infected and turned black. *Valsa mali* had the highest frequency (44% of incidence). Anatomical observation and measurement of the 45 samples with canker were made in lab. The results showed that the average length of scab was 21.1 cm in xylem, which was 4.4 cm longer than in phloem. Much difference was found on trees when the

canker is getting older. It explained why the disease is hard to control at the late stage of the canker. Renewed disease control measures were designed based on what we have found.

**O16.004 Epidemiology and strategies for integrated control of *Neonectria galligena* (European canker of apple)**

*M. Wenneker, P.F de Jong and P.A.H. van der Steeg*
*Applied Plant Research, Business Unit Flower Bulbs, Tree Stock & Fruit, Wageningen UR, The Netherlands*
*Email: marcel.wenneker@wur.nl*

European canker, caused by *Neonectria galligena*, is an important disease of apple trees worldwide. *N. galligena* is a wound parasite which infects by either conidia or ascospores through leaf scars in autumn. When cankers are formed on side shoots or minor branches the damage can be relatively insignificant. However, if cankers are formed on the main stem or a major branch, the whole tree will be lost. Rain is an important vector of the pathogen, both by aerial rain splash from tree to tree, and rain splash and runoff within infected trees. Due to the biology of the fungus – the infection may be symptomless for a long period, for a few months or for the summer following the infection – it is difficult to control this disease. Control measures such as spraying of fungicides, covering wounds with a paint, cutting out diseased wood, and sanitation practices do not prevent the occurrence of epidemics. Strategies for integrated control of *N. galligena* are currently developed in the Netherlands to help the planning of efficient pathogen surveillance, eradication, or disease control measures. Research is focusing on the sources of infection of apple trees (including tree nurseries), susceptibility of pruning cuts, development of detection methods, warning models and alternative control methods.

**O16.005 Sooty blotch and flyspeck of apple: Ecology, diversity, and evolution**

*M.L. Gleason, J.C. Batzer, G.Y. Sun and R. Zhang*
*Department of Plant Pathology and Microbiology, 351 Bessey Hall, Iowa State University, Ames, IA 5001, USA*
*Email: mgleason@iastate.edu*

Fungi in the sooty blotch and flyspeck (SBFS) complex blemish apples worldwide in regions with moist summer weather and also colonize the waxy epicuticle of many other crop plant species, resulting in economic losses for growers. Research progress was slowed for 160 years by inability to reliably identify component species based on morphology alone. Recently, adding phylogenetic analysis of DNA sequence data revealed that the SBFS complex is comprised at least 80 species in contrast to four species that had been described previously. Collaborative studies in many countries found that some SBFS species were cosmopolitan whereas others had restricted geographic ranges. New insights into pathogen ecology and pathosystem phenology are helping to optimize regional weather-based warning systems for timing of fungicide sprays. Current and planned studies seek to reveal the evolutionary origins of SBFS fungi as well as the functional genomics of adaptation to their surface-dwelling niche.

**O16.006 Genetical engineering fruit plants to reduce fungicide input in orchards**

*C. Gessler and G.A.L. Broggini*
*Plant Pathology, IBZ, Universitätstrasse 2, CH-8092 ETH-Zürich, Switzerland*
*Email: cesare.gessler@usys.ethz.ch*

Apple production in temperate climate with often wet springs needs a relevant input of pesticides to control apple scab, powdery mildew and fire blight. Classical breeding has produced many scab resistant cultivars overwhelmingly based on the resistance from *M. floribunda* 821 (Vf) and some mildew resistant cv and efforts to breed also fire blight resistant cultivars are currently undertaken. The first resistance gene that has been isolated and cloned into a susceptible apple cultivar is the gene *HcrVf2*, responsible for the Vf scab resistance. A second has been recently identified and introduced into Gala, both incite full scab resistance. Similarly, after identification we introduced into Gala the candidate fire blight resistance gene from *Malus robusta* 5 and showed that it incited full fire blight resistance. However the final goal is the creation of a product, e.g. an ameliorated apple cultivar by the addition of resistance to scab and fire blight, with advantages to the environment, producer and consumer, raising as less concern as possible. As the presence of selectable marker genes and any other foreign gene is highly questioned, we opted for the cisgenic approach. We introduced by Agrobacterium transformation the target genes with their own regulatory sequences into the highly susceptible apple cultivar Gala, and eliminated post transformation all marker genes. The results demonstrate that introduction of apple own genes with their own regulatory sequences without the presence of foreign genes in the end product (cisgenic) nor any overexpression of apple genes is possible and a popular cultivar can be ameliorated through addition of genes by genetic engineering. The used methodology allows the sequential addition of genes; e.g a second transformation.

**O16.007 Limitations and prospects in control of bacterial diseases of fruit trees**

*E. Montesinos, I. Llorente, A. Bonaterra* and *C. Moragrega*
*Institute of Food and Agricultural Technology-CIDSAV, University of Girona, 17071 Girona, Spain*
*Email: emilio.montesinos@udg.edu*

Fruit tree production is one of the main agricultural activities in the Mediterranean climate countries. Hundreds of diseases affect fruit trees, but bacterial diseases have threaten orchards since the very old times , limiting their productivity and the quality of fruit, often devastating extense areas by killing high value trees. Classical bacterial diseases like fireblight of pomefruits (*Erwinia amylovora*), bacterial spot of stone fruits (*Xanthomonas arboricola* pv. *pruni*), citrus canker ( *Xanthomonas citri*), and citrus variegated chlorosis and Pierce disease (*Xylella fastidiosa*), are still threatening and expanding in new areas. However, emerging bacterial diseases, such as bacterial blight of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* or huanlongbing caused by Candidatus *Liberibacter*, have overtaken in importance the classical diseases in certain areas. In spite of the advances in knowledge of these bacterial plant pathogens, mainly in the genetics and mechanisms of interaction with their hosts, and the availability of molecular tools for its detection, the improvement in disease control have progressed slowly. Chemical control still rely on old compounds like copper derivatives or antibiotics, current biological control agents suffer in general of moderate to low efficacy levels and inconsistency, and plant strengtheners available did not cover in general expectations. Therefore, there is a need of more efficient bactericides and microbial biopesticides to provide tools for control of bacterial diseases. The constrains and prospects of new potential compounds like antimicrobial peptides and novel biocontrol agents like lactic acid bacteria, will be discussed.

**O16.008 *Guignardia bidwellii*, the agent of black rot of grapevine, is spreading in European vineyards**

*P.A. Rinaldi[1], G.A.L. Broggini[2], C. Gessler[2], D. Molitor[3], J. Sofia[4] and L. Mugnai[1]*

*[1]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DiSPAA)- Sez. Patologia vegetale ed Entomologia, Università degli Studi di Firenze, P.le delle Cascine 28, 50144 Firenze, Italy; [2]Plant Pathology Integrative Biology Zurich (IBZ) ETH Zurich, LFW - C16 Universitaetstrasse 2 8092 Zurich – Switzerland; [3]Centre de Recherche Public Gabriel Lippmann, Department Environment and Agro-Biotechnologies, 4422 Belvaux, Luxembourg; [4]Direcção Regional de Agricultura e Pescas do Centro, Estação de Avisos do Dão, 3504-504, Viseu, Portugal*

*Email: laura.mugnai@unifi.it*

Black rot of grapevine, caused by *Guignardia bidwellii*, has recently shown an increased incidence in the last ten years in Germany, Austria, Switzerland and Luxembourg. Since 2010, the disease has, in addition, become more common in warmer countries, such as Italy and Portugal, often leading to a complete crop loss. Several causes might be hypothesized for this sudden increase in the areas where it was previously almost unknown: changes in the application timing, in the types of chemicals used against downy and powdery mildew, increasing existence of abandoned vineyards. The climatic changes (warmer and more humid spring) were supposed to widely contribute to the disease spread too. In the Mediterranean countries, severe early symptoms on shoots and rachises may also contribute to an unexpected heavy damage build-up of inoculum. In addition, in these warmer climates, atypical symptoms, such as shriveling of the berries without the typical diagnostic fruiting structures, may have led to the misinterpretation of the symptoms which were often identified as cluster shriveling caused by *Plasmopara viticola*. The incorrect diagnosis and subsequent lack of specific control measures may have led to the inoculum increase. A molecular approach was used to confirm this hypothesis: clusters with shriveled berries without fruiting bodies were tested using one microsatellite of *Plasmopara viticola* and one of *G. bidwellii* which were amplified by PCR to detect the two pathogens. Studies on the genetic variability of the population of *G. bidwellii* present in European countries, as well as their sensitivity against different fungicide classes, are on-going. Further, a model simulating the epidemics of the disease is presently been validated under different climatic conditions.

**O16.009 A novel cultural approach to managing Armillaria root rot disease**

*G. Schnabel*

*School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson SC 29634, USA*

*Email: schnabe@clemson.edu*

Armillaria root rot (ARR) is caused by many *Armillaria* species, but in the southeastern United States it is primarily caused by *A. tabescens*. It is the number one cause of premature peach tree decline in South Carolina and Georgia. This species does not produce rhizomorphs or grow into above ground tree parts. Many chemical and biological approaches have been tried with little success to control this disease and commercially viable resistant rootstocks are currently unavailable. In a prototype study we investigated a new cultural method for ARR control where trees were planted about 25 cm higher than normal in open-bottom smart pots. After one year of establishment, the root crown was excavated. Five years after planting, tree mortality was reduced in trees left in pots by about 50% and in excavated trees by up to 100%. Larger scale field trials were established on several sites in South Carolina heavily infested with ARR to test the procedure's commercial viability. Mounds were made with disk plows to a height of about 20 cm and root collars were excavated after 2 growing seasons using an airspade powered by an air compressor. Above ground root collar excavation may be one of the most effective methods to control ARR caused by *A. tabescens* on replant sites with heavy ARR pressure.

**O16.010 Distribution of *Xylella fastidiosa* in blueberry stem and root sections in relation to disease severity**

*R.M. Holland and H. Scherm*
*Department of Plant Pathology, University of Georgia, Athens, GA 30602 (USA)*
*Email: scherm@uga.edu*

*Xylella fastidiosa* causes bacterial leaf scorch (BLS), a lethal disease of southern highbush blueberry in the southeastern U.S. To assess the prospects for mitigating BLS through pruning or hedging of affected plants, we determined the localization of the pathogen in naturally infected blueberry plants with varying levels of BLS severity. Stem segments from the top to the base of the plant, as well as root segments, were sampled in three affected plantings. Xylem sap was extracted from each segment and population densities of *X. fastidiosa* determined using real-time PCR. Detection frequencies were lowest in xylem sap from asymptomatic plants and highest in plants with severe symptoms. In asymptomatic plants, detection was least frequent (0 to 20.0%) in top and root sections and highest (4.6 to 55.6%) in middle and base stem sections. As disease severity increased, detection frequencies in roots increased to >80% in two plantings and to 60% in the third planting. Overall, detection frequencies were highest (>80%) in middle and base stem sections of plants from moderate and severe disease classes. The lowest bacterial titers (0 to $2.1\times10^1$ CFU/50 µl of sap) were observed in top and root sections of asymptomatic plants, whereas the highest titers (between $10^4$ and $10^5$ CFU/50 µl of sap) were obtained from middle, base, and root sections of plants from moderate and severe classes. Since the pathogen accumulates in the roots at moderate and high disease levels, management strategies such as pruning and mowing will not be effective in curing plants from BLS.

**O16.011 Etiology and management of diseases caused by Botryosphaeriaceae fungi on almond, pistachio, and walnut in California**

*T.J. Michailides, S.F. Chen, D.P. Morgan,* and *R. Puckett*
*University of California-Davis, Kearney Agricultural Research and Extension Center, Parlier, California 93648, USA*
*Email: tjmichailides@ucanr.edu*

Identification of the Botryosphaeriaceae species involved in causing canker and blight diseases of almond, pistachio, and walnut trees in California revealed that there are several species that occur in all three crops. These include *Botryosphaeria dothidea*, *Diplodia seriata*, *Lasiodiplodia theobromae*, *Neofusicoccum mediterraneum* and *Neof. parvum*. In addition, *Neof. nonquaesitum* was isolated from almond and walnut, *Dothiorella sarmentorum* from almond; while *Dip. mutila*, *Dot. iberica*, *L. citricola* and *Neoscytalidium dimitiatum* (*Nattrassia mangiferae*) only from walnut. Furthermore, *Macrophomina phaseolina* was isolated from both almond and pistachio, and *Neof. vitifusiforme* from both almond and walnut. Most of these species caused cankers when shoots of almond, pistachio, and walnut were inoculated. All these fungi overwinter in the nut crop orchards as pycnidia in cankered and/or blighted tissues. Only the anamorph stage of Botryosphaeriaceae species was found in pistachio, while both the anamorph and the teleomorph were detected in cankers of almond and walnut. Pathogenicity tests indicated that there are major differences in the virulence of these fungi with *L. theobromae*, *Neof. nonquaesitum*, *B. dothidea*, *L. citricola*, *Neof. mediterraneum*, and *Neof. parvum*, being more aggressive in causing cankers than *Diplodia* spp., *Dot. sarmentorum*, and *Neof. vitifusiforme*. Furthermore, some species caused disease on shoots without infecting fruit (*L. theobromae* and *Dip. seriata*) while others (*B. dothidea* and *Neof. mediterraneum*) infected both shoots and fruit. Effective management of these diseases depends on sanitation (removal of infected parts by pruning - pistachio, walnut), cultural practices (irrigation management - almond, pistachio, walnut) and multiple protective fungicide sprays (pistachio).

**O16.012 Histological analysis of warts on apple shoots induced by *Botryosphaeria dothidea***

*Y.P. Du and L.Y. Guo*
*Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: ppguo@cau.edu.cn; ppguoly@126.com*

The causal pathogen of apple ring rot, *B. dothidea*, can infect apple branches and induce the wart formation, a typical symptom of apple ring rot in China. Previous study has showed that isolates of *B. dothidea* from apple or pear induced significantly larger warts on apple shoots than those isolates from other hosts. In order to investigate the mechanism involved in the wart formation, the structure of the warts was studied using cryosection with a Leica cryostat microtome and the fluorescence microscopy technique. With a chitin-specific stain, the fluorescein-conjugated wheat germ agglutinin, the mycelia of *B. dothidea* were observed in big and small warts. Two types of mycelium were observed in the warts: the thick hyphae (ca. 12µm) served as the infection hyphae, and the thin hyphae (ca. 3µm) with multiple branches extended to nearby cells. Around each infection hypha several layers of cells with suberin deposition were evident with autofluorescence, which indicated the formation of suberization layer by plant in corresponding to the infection of pathogen. Significantly more mycelia were observed in large warts than small warts. The infection of pathogen in the small warts was limited to the periderm by the suberization layers formed by plants, but the mycelia of pathogen in large warts expended to the phloem of plant and breaking through the suberization layer by the infection hyphae

was observed. Therefore, the formation of large wart suggested a more severe damage to the host plant.

**O16.013 Morphological variation, genetic diversity and pathogenicity of *Colletotrichum* species causing citrus anthracnose in Portugal**

*A.P. Ramos, P. Talhinhas, S. Sreenivasaprasad and H. Oliveira*
*Centro de Investigação das Ferrugens do Cafeeiro, Instituto de Investigação Científica Tropical. Quinta do Marquês. 2780-505 Oeiras, Portugal*
*Email: ptalhinhas@iict.pt*

In the last two decades the importance of citrus production in Portugal has increased but significant losses related to anthracnose symptoms have been registered. These anthracnose symptoms were attributed to *Colletotrichum gloeosporioides*, but preliminary data led to evidence of diversity within the populations of the causal agent, raising the hypothesis of other species of *Colletotrichum* being involved in the disease. In the present work a field survey of the main citrus growing areas in Portugal was conducted and the pathogenicity of a group of *Colletotrichum* spp. isolates was studied along with the characterisation of morphological and genetic variability. DNA profiles based on ISSR-PCR using eight primers and analysis of DNA sequence data of rDNA-ITS and β-*tubulin* 2 gene regions distinguished between *C. boninense* and *C. gloeosporioides*. Sequence analysis from the ITS region differed from the results of the ISSR-PCR in that polymorphisms were revealed among *C. gloeosporioides* isolates, indicating the presence of genetic variation. *Colletotrichum boninense* isolates displayed a distinct polymorphism regarding cultural and morphological characters as well as different ISSR-PCR profiles and a sequence polymorphism in the ITS region. Results confirmed that anthracnose symptoms observed on leaves, branches, flowers and fruits of several citrus cultivars from different areas are caused by *C. boninense s. lat.* and *C. gloeosporioides*.

**O16.014 Sustainable control of post harvest apple rots - from orchard to store**

*R.J. Saville, K. Lower and A.M. Berrie*
*Robert Saville, East Malling Research, New Road, East Malling, Kent, ME19 6BJ, UK*
*Email: robert.saville@emr.ac.uk*

Fungal rots can result in significant losses of apple in store, particularly in fruit stored beyond January. Successful control of storage rots depends on a clear understanding of the rots to be controlled. Over the past two decades we have carried out surveys to determine the rots present in commercial apple stores in the UK. The survey involves weekly visits to Pack houses from January-March. At each visit at least 100 rotted fruit were removed from the rot bin or collected from the grader of fruit that was being graded at the time of the visit. Rots were identified visually and numbers recorded. The resulting data set allows us to observe trends in the rot profile over time. Results from the rot survey have helped inform practical advice for growers through the rot risk assessment concept. Rot risk assessment is based on assessment of various factors pre-harvest from which a decision can be made on the likely rotting in store and therefore the need for treatment and/or early marketing. Field trials were undertaken in 2009 and 2010 to evaluate components of the rot risk assessment concept including treatments, treatment timing (late bloom and/or pre harvest) and cultural control strategies such as selective picking. The rot survey data and field trial results will be drawn upon to present our current understanding of post-harvest apple rots, how this can be used practically by growers and future areas of research.

**O16.015 Etiology of moldy core and core rot of apple and temporal dynamics of the pathogen complex**

*G.Y. Sun[1], L.L. Gao[1], Q. Zhang[1], R. Zhang[1] and A.R. Biggs[2]*
*[1]State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, 712100, P. R. China; [2]Kearneysville Tree Fruit Research and Education Center, West Virginia University, Kearneysville 25430*
*Email: sgy@nwsuaf.edu.cn*

Fuji apples were collected and 186 fungal isolates were obtained from the fruit core region. Fungi were isolated from fruit with symptomless core regions, as well as from the core regions of fruit showing browning, typical moldy core, or core rot. All fungi were identified to species based on phylogenetic and morphological analyses. Pathogenicity was determined by cutting apples into halves and daubing spore suspensions containing $1\times10^4$ up to $1\times 0^8$ spores/ml on the carpel in the core region. Pathogenicity varied significantly among genera, with *Alternaria* spp. and *Cladosporium* spp. causing core browning at lower inoculum concentrations and moldy core at higher inoculum concentrations. Combinations of pathogens initiated more browning and moldy core than the pathogens applied alone. Core browning was introduced as a new type of core symptom, along with moldy core, dry core rot and wet core rot. Isolation at different development stages of flower and fruit showed that the main pathogens, *Alternaria* spp. and *Cladosporium* spp. were present in dormant flower buds from the previous November. The main pathogen causing core rot, *Trichothecium roseum*, was only found in young fruit, from about one month after last petal fall. Based on the variable infection stages of pathogens, to control moldy core and core rot effectively we suggest fungicide prophylaxis using multi-site fungicides or fungicide combinations beginning just prior to bloom and continuing through to 4 to 6 weeks after petal fall.

**O16.016 Heat treatment of Huanglongbing –affected citrus trees in field for reduction of "*Candidatus* Liberibacter asiaticus"**

*X.L. Deng, L. Guan, M. Liang, M. Xu and Y. Xia*
*Laboratory of Citrus Huanglongbing Research, Department of Plant Pathology, South China Agricultural University, Guangzhou, P. R. China, 510642*
*Email: xldeng@scau.edu.cn*

Huanglongbing (HLB, yellow shoot disease) is a devastating citrus disease worldwide. Research conducted by Lin Kung-Hisang et al. in 1960s China suggested that heat treatments were effective at eliminating HLB pathogen in scions. We tested effect of high temperatures on the reduction of "*Candidatus* Liberibacter asiaticus" (CLas) titers in HLB-affected citrus trees in Guangdong, China. Heat treatments were delivered via covering a tree with a temporary enclosed tent of plastic sheeting, which used natural sunlight to raise ambient temperature. Twenty-four sweet orange trees with HLB symptoms in an orchard were selected and divided into six blocks with three blocks of four trees, each receiving heat treatments, and the other three blocks used as controls. Heat treatments were performed three times in August with temperature exceeding 38 C for three hours each time. Leaf samples were collected in the following months and real-time PCRs with primer set HLBasf/HLBasr and TaqMan probe HLBp were used to monitor CLas titer changes. For three consecutive months (October, November, and December), average reduction rates (%) from heat treated trees were 85.0, 83.3, and 85.2. In contrast, average reduction rates (%) for untreated control trees were 19.0, 12.7, and 25.0 for the three months. Further evaluation is underway to confirm the efficacy of plastic sheeting heat treatment for controlling HLB.

**P16.001 Isolation and identification of *Brenneria nigrifluens* the causal agent of bark canker disease on walnut in Iraq**

*E.M. Al-Maaroof and P.S. Amin*
*Faculty of Agricultural Sciences, Sulaimani University, Sulaimania, Iraq*
*Email: ealmaaroof@yahoo.com*

Walnut is an old traditionally important tree in kurdistan mountains, Iraq. Bark canker disease recently found in Sulaimania. Disease incidence reached to 17.2% in Tawella. Many bacterial isolates were isolated and identified on the basis of standard morphological, biochemical characterization and API 20E system. 51.5% of the isolates identified as *Brenneria nigrifluens* and 36.3% as *Pantoea spp*. *B. nigrifluens* isolates formed single circular colonies with entire margins and creamy color on NA, while appeared as single colonies, circular with entire margin, and dark purple with green metallic sheen on EMB. Biochemical tests classified *B. nigrifluens* isolates into seven groups. Viteck GN system was further used to confirm the identification. High differences detected between the isolates in producing necrotic lesion on artificial inoculated walnut branches, while no symptoms appeared on detached leaves. Isolate number 22, 28 and 31 explored typical symptoms on two year old seedlings. All *B. nigrifluens* isolates showed absolute resistance to Erythromycin and Cephalexin, highly resistance to Ampicillin (94.1%), Vancomycin (76.4%), Rifampin (70.5%) and Amikacin (70.5%); Moderate resistance to Penicillin (58.8%); Moderate susceptibility to Streptomycin (41%), Gentamicin (35%). Chloramphenicol, Tobramycin, and Tetracycline showed high efficiency in bacterial growth inhibition. Minimum Inhibitory and Minimum Bactericidal Concentration activities of five chemicals against 17 bacterial isolates showed high efficiency of Kocide in killing 94.1% and inhibition of 100% of the isolates at (1/8field dose) *In vitro*, followed by Nordox which killed 70.6% and inhibited 76.4% of the isolates at (1/2 field dose) and (1/4 field dose) respectively. *In vivo* studies confirmed the high efficacy of Kocide in disease control and restriction of vertical and horizontal expansion of the cankers followed by Nordox. No significant differences detected between Champion, Courey and Melody.

**P16.002 Phomopsis cane and leaf spot disease of grape in Egypt**

*E.Z. Khalifa, M.A. Awad and M.E. Leila*
*Agricultural Botany Department, Faculty of Agriculture, Minufiya University, Shebin El-Kom 32516, Egypt*
*Email: khalifasz@yahoo.com*

Phomopsis cane and leaf spot caused by *Phomopsis viticola* is widely distributed throughout the grape-growing regions of Egypt. Crop losses due to this disease reached 30% in some regions of the world, but losses were not determined in Egypt. Five isolates of the causal organism were isolated from different localities of Egypt and proved to be pathogenic to different grape varieties. The observed symptoms were spots on shoots and leaves. It was also observed small, black, spots on the first three to four internodes of the developing shoots. The spots developed into elliptical lesions and grew together to form irregular, black, crusty areas. Field survey revealed that the disease is existed in different regions in Egypt. The highest disease percentages and severity was recorded in Behaira governorate, while the lowest ones were recorded in Sharkiya governorate. Evaluation of different grape varieties revealed that all the tested varieties were susceptible. Superior was the highest susceptible variety while kermson and flame were less susceptible. Macronutrients in the ratio 1N : 2P : 2K decreased the infection to some extent. From the antioxidants which were used to control the disease; ascorbic acid gave the best result in reducing the disease severity. Four calcium salts were used as spray to control the

disease; calcium phosphate gave the best results. Some fungicides were also used for the control; filint and topas were more effective than the other tested fungicides.

**P16.003 Fungal diseases of date palm in Qatar**

E.H. Al-Turaihi
*Agricultural Affairs Department, Ministry of Environment, P.O. Box 1966 Doha-Qatar*
*Email: emadhussain30@yahoo.com*

Date palm (*Phoenix dactylifera*) is an extremely important fruit tree in Qatar which is subject to attack by several fungal diseases causing damage on palm trees and resulted direct loss in production of dates. The major fungal diseases which normally attack date palm trees in Qatar are Black Scorch (*Thielaviopsis paradoxa*), False Smut (*Graphiola phoenicis*), Leaf Spots (*Alternaria, Drechslera, Cladosporium*) and Fruits Rot (*Aspergillus, Penicillium, Alternaria*). The description of symptoms, field diagnosis and environmental factors affecting the development of the diseases were shown in this study. The study also revealed that these fungi occur on date palm trees wherever are cultivated under stress conditions. All date palm cultivars are considered to be potential hosts of the fungi. Moreover, the high level of salinity in water, tended to increase the infection of date palm trees by the fungi which can be efficiently spread by air, tools, insects and irrigation water.

**P16.004 *Pestalotiopsis* spp., the causing agent of guava and wax apple diseases in Taiwan**

*B.Y. Fang, C.F. Hong, T.C. Lin and* W.H. Chung
*Department of Plant Pathology, National Chung Hsing University,No. 250, KuoKuang Rd., Taichung City, Taiwan, China*
*Email: wenchung@nchu.edu.tw*

*Pestalotiopsis* spp. are important pathogens that cause canker on guava and fruit rot on wax-apple in production areas and affect fruit quality during storage period. Previous studies indicated that four species of *Pestalotiopsis* could cause guava canker in Hawaii and two species caused fruit rot on wax-apple in China. In Taiwan, *P. psidii* reportedly caused canker on guava whereas *P. eugeniae* caused fruit rot on wax-apple. However, two types of symptom, purple- and brown-rot,exhibited on guava and wax-apple in fields. The objectives of the study are to re-classify the pathogens of *Pestalotiopsis* species and carry out its pathogenicity on guava and wax-apple. Ninety isolates from guava (13 isolates from purple-rot symptom) and 49 isolates from wax-apple (43 isolates from purple-rot symptom) were collected and analyzed for their biological characters. Morphological analysis showed that the conidia of all isolates from guava and wax-apple were similar. All isolates could cause symptoms on guava and wax-apple by wound inoculation with mycelialdisc. However, the isolates from purple-rot of guava and wax-apple caused severe symptoms at 25-30℃ whereas isolates from brown-rot caused severe symptom at 20-25℃. For phylogenetic analyses, isolates of *Pestalotiopsis* could be separated into four molecular groups based on ITS sequences. However, all isolates were scattered on phylogenetic analysis based on β-tubulin gene sequences. Based on the results, at least four *Pestalotiopsis* species were suspected as pathogens on guava and wax-apples in Taiwan.

**P16.005 Etiological characteristics of *Collectotrichum gloeosporioides* isolated from each progress and static symptoms on apple**

W. Cheon, D.Y. Kim, S.G. Lee and Y.H. Jeon
*Department of Bioresource Sciences, Andong National University, Andong 760-749, Republic of Korea*
*Email: applesident@naver.com*

*Collectotrichum gloeosporioides* is the fungal pathogen which causes the anthracnose disease in many crops including apple. This fungus is responsible for yield loss and reduces the commercial value of the fruit. Recently, static symptoms (SS) were observed with typically progress symptoms (PS) on apples. In the SS, small lesion is magnified in the beginning of growth period. Spot doesn't expand further and remains at state condition until harvesting season. We found that the shape and size were different between these two types of anthracnose conidia. The conidia of PS-fungi were straight, cylindrical, with an obtuse apex and a truncated base, and measured 13.43~20.14 x 4.19~6.15μm. However, the conidia shape and size of the SS-fungi were smaller than the PS fungi. It was smaller, cylindrical and spore size was 11.98~15.73 x 4.44~7.15. Pathogenicity test of these two types were done on immature apple cv. Fuji by inoculating with a conidial suspensions (105 conidia/ml). In a PS fungus inoculated apple, anthracnose symptoms were progressed and also soft and sunken. But, in the SS fungal inoculation, there was not expansion in size of static spot was observed. However, The nucleotide sequences of ITS region revealed 100% consistent. More research about two type symptoms of anthracnose should be conducted.

**P16.006 Branch dieback caused by *Neofusicoccum parvum* on walnut in Korea**

W. Cheon, Y.S. Kim, S.G. Lee and Y.H. Jeon
*Department of Bioresource Sciences, Andong National University, Andong 760-749, Republic of Korea*
*Email: applesident@naver.com*

Walnut (*Juglans sinensis* Dode) is an economically important tree in the world for its wood and its fruit. In

May, 2012, lethal dieback disease of walnut tree was detected in two orchards in Andong, Kyoungsangbuk-do, Korea. Disease symptoms included blight and dieback of the stems, flowing resin, dark decay inside the bark of dead twigs and defoliation. To analyze the morphology, dead twigs were detached from diseased plants in Andong orchards. They removed the bark and sliced thinly using a razor blade, and water-mounted, without staining, on a slide for microscopic observation of the fungi. The conidia embedded within the bark of dead twigs were mostly characterized by fusoid, hyaline, smooth, thin-walled, unicellular and 16.25~21.25 μm long and 4.37~6.87 μm wide. These characteristics are consistent with those reported previously for *Neofusicoccum parvum (Pennycook & Samuels*). To confirm the identities of the isolates, the sequence of internal transcribed spacer (ITS) region was analyzed. The sequences were compared with other DNA sequences in the GenBank database, using a BLAST search. BLAST analysis of the polymerase chain reaction product showed that the sequence had 99% identity with the nucleotide sequences for *Neofusicoccum parvum* (accession nos. JQ411396.1 and GU997688.1). Thus, both morphological and molecular characters confirmed this species as *Neofusicoccum parvum*. To our knowledge, this study is the first report of *N. parvum* as a pathogen of *Juglans sinensis* in Korea.

**P16.007 Banana wilt-a new emerging threat to banana cultivation of Sindh, Pakistan**

*A.M. Lodhi, M.A. Khaskheli, M.M. Jiskani and R.N. Syed*
*Department of Plant Pathology, Sindh Agriculture University Tandojam, Pakistan-70060*
*Email: mubeenlodhi@gmail.com*

Banana (*Musa* sp.) is an important cash fruit crop of Pakistan. It is cultivated on 30,000 ha, with an annual production of 141,000 tonnes. Of which, Sindh province contributes about 90%, where the 'Dwarf Cawendish' type of banana is mostly grown. Recently, banana crops grown in district Thatta (costal belt of Sindh) have been found severely affected with wilting. The affected plants showed profound yellowing, stunted growth and dying of the whole plant. The farmers have observed this type of abnormality in their banana crops for the first time. Preliminary investigations revealed that affected plants contained variety of typical symptoms including radish brown to dark brown discolouration of the vascular tissues of the pseudostem, the appearance of brown streaks in the central cylinder of the corm and dark brown discolouration of the cortex region of the rhizome. Isolation from affected plant parts revealed the abundant presence of *Fusarium oxysporum* f. sp. *cubense*, identified on the basis of the morphology of micro and macro conidia, and chlamydospores. Different species of plant parasitic nematodes including *Tylenchorhynchus, Helicotylenchus, Hoplolaimus* and *Meloidogyne* spp. were also found to be associated with the affected plants. Being a Fusarium wilt disease, it has the ability to become a devastating threat in banana plantations of Sindh province, and may cause loss levels similar to the epidemic of banana bunchy top disease which until it was controlled, virtually eradicated banana cultivation from the Sindh in 1980s. Results showing symptomatology, isolation and pathogenecity of the recently recorded banana wilt disease are presented herein.

**P16.008 *Alternaria* species associated with a new apple disease in North Italy**

*L. Languasco[1], K. Marschall[2], M. Collina[3], E. Ortalda[4], E. Pattori[1], F. Rotondo[3] and V. Rossi[1]*
*[1]Instituto di Entomologia e Patologia Vegetale, Università Cattolica del S. Cuore, Via E. Parmense 84, 29122 Piacenza, Italy; [2]Research Centre for Agriculture and Forestry Laimburg, Vadena, Laimburg 6, 39040 Ora, Italy; [3]Department of Agricultural Sciences, Università degli Studi di Bologna, Viale G. Fanin 46, 40127 Bologna, Italy; [4]Settore Fitosanitario Regionale – Regione Piemonte, c/o Environment Park palazzina A2, Via Livorno 60, 10144 Torino, Italy*
*Email: vittorio.rossi@unicatt.it*

About 10 years ago, a new apple disease spread in North Italy, starting from Alto Adige, then in Trentino, Veneto, Piedmont and Emilia-Romagna. The disease affects leaves and fruits. Leaves show small circular brown spots, sometimes with concentric ring and purple-red halo, while fruits show rounded brown-black spots, often centered on lenticels, sometimes with a reddish halo. These symptoms look like the "Alternaria blotch" (caused by *Alternaria mali*) but preliminary studies showed that the strains of *Alternaria* isolated in Italy don't belong to *A. mali*. Within the research project PRIN, 204 strains of *Alternaria* spp. were isolated from either leaves (40%) or fruits (60%) sampled in the most important apple-growing areas of northern Italy, and tested for pathogenicity. Different inoculation protocols were used, with either conidia or cultural filtrate (autoclaved or not), different inoculation methods on wounded or unwounded fruits or leaves, and incubation periods. Inoculation of drops of cultural filtrates on wounded leaves provided consistent results; 84 and 27% of the strains isolated from fruits and leaves were pathogenic, respectively. Highest frequency of pathogenic strains was observed in Piedmont (81% of strains) and the lowest in Emilia-Romagna (17%). Based on a morphological characterization, 17 out of 37 pathogenic stains belonged to the Tenuissima species-group, 12 to Gaisen, 4 to Alternata, 3 to Arborescens, and 1 to Armoraciae. This study made it possible to develop an efficient bioassay for pathogenicity of *Alternaria* strains and increased knowledge about etiology of this new apple disease which is spreading in North Italy.

**P16.009 Host specificity of selected species of genus *Coniothyrium* causing leaf spot of fruit tree leaves**

N. bitsadze
*Agricultural University of Georgia, 13 km. David Aghmashenebeli Alley, 131, Tbilisi, Georgia*
*Email: n.bitsadze@agruni.edu.ge*

Some of the representatives of genus *Coniothyrium* (*C. persicae, C. Cydoniae, C. armeniaceae, C. cerasi, C. piricolum*) were used for cross inoculation different fruit tree leaves (*Malus orientalis, Cydonia oblonda, Cerasus vulgaris, Prunus armeniaca, Persica mill*). It was discovered that besides host plants the above mentioned fungi have ability to inoculate other fruit tree leaves as well with various degree under controlled conditions. Length of Incubation period, production of fruiting bodies, sizes of the pycnidia and conidia of the same Coniothyrium species were varied depending on host plant. It gives us the base to suppose that *Coniothyrium* species have large pathogenic character and in natural environment each fungus species may cause the diseases in the representatives of other fruit trees.

**P16.010 Apple white rot control strategies in Southern Brazil**

R.M.V. Sanhueza, J.M.C. Fernandes and W. Pavan
*Proterra Engenharia Agronômica, CPPro. BR116, 7320, Cep 95200-000, Vacaria, RS, Brazil*
*Email: rosamaria@cipnet.com.br*

In hot and humid summers white rot causes can losses up to 30% of the apple production in orchards where the disease is present. This work reports the results of a warning system for disease outbreaks and fungicides efficacy in controlling white rot. The first epidemiological warnings were deployed in the 2002 to 2003 season. It consisted of emitting warnings when inoculum was available and a requirement of one or two consecutive rainy days was met. The fungicides were evaluated by spraying cv Fuji apples trees in the pre and post-harvest and with inoculation after or before the use of products. The warning system with the rain requirement managed the disease equally well to the preventive treatment. A more efficient control was achieved with fungicides belonging to the benzimidazol group sprayed at pre-harvest stage. In the post-harvest trials, the most effective products were thiophanate methyl, mancozeb and calcium chloride 0.3%. The healing action of the treatments was higher in treatments with potassium phosphite.

**P16.011 Variation in susceptibility of spring shoots of Nova mandarin (*Citrus reticulata* Blanco) to the "tangerine pathotype" of *Alternaria alternata* (Fr:Fr) Keissl**

E. Pérez[1], L. Burdyn[2], O. Blanco[1], P. Alves[1], A. Otero[1] and S. Garrán[2]
[1]*Instituto Nacional de Investigación Agropecuaria (INIA), Camino al Terrible s/n Salto Grande, Salto CP 50000. Uruguay;* [2]*Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Yuquerí Concordia_Entre Ríos, R. Argentina*
*Email: elenaperez@inia.org.uy*

Plant resistance to fungi infections is frequently associated with maturity of plant tissues. Young tissues used to be considered more sensitive to infections and pathogen colonization than older ones. Aim of this work was to study the relationship between phenological development of spring shoots of Nova mandarin and their susceptibility to *Alternaria* infections. An arbitrary scale considering 6 categories was used to discriminate among different phenological stages of shoot tissues. According to the degree of shoot development, categories of the scale were named as follows: B1 (initial shoot growth), B2 (shoot lengthening), B3 (end of shoot lengthening, initial leaf expansion), B34 (leaf expansion), B4 (end of leaf expansion, leaf thickening) and B5 (leaf seasoning). Shoots samples obtained from healthy plants were classified into the six different categories; they were artificially inoculated with a strain of *Alternaria citri* "mandarin-pathotype" and then incubated for 96 hours under optimal conditions for symptom expression. 24 hours after inoculation, type B2 shoots were significantly more sensitive than the rest of the categories assayed. However, when evaluations were made 96 hours after inoculation, no differences were observed between stages B2 and B3 neither among stages B3, B34 and B4.These results would be indicating that the pathogen can infect shoots throughout all their growth period, being latency greater when infection occurs in more seasoned shoots. In humid regions, where weather conditions are very conducive to this disease, it should be essential to apply protective treatments early in spring and they should be reinforced before shoots reaching final size.

**P16.012 Mango wilt disease: A serious threat to mango industries worldwide**

A.O. Al Adawi[1], I. Barnes[2], M.L. Deadman[3], B.D. Wingfield[2] and M.J. Wingfield[4]
[1]*Ghadafan Agriculture Research Station, Ministry of Agriculture and Fisheries, P.O. Box 204, Sohar, 311, Sultanate of Oman;* [2]*Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa;* [3]*Department of Crop Sciences, P.O. Box 34, Sultan Qaboos University, Al Khod 123, Sultanate of Oman;* [4]*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*
*Email: aliadawi74@gmail.com*

In recent years, a serious wilt disease has killed thousands of mango trees in Oman and Pakistan. In an attempt to better understand the etiology of the disease, several intensive surveys of the problem have been undertaken in these countries and research has been conducted to better understand the causal agent. Our investigations have shown that the fungus, Ceratocystis manginecans is the primary cause of the disease in Oman and Pakistan and that it is highly pathogenic. We have further demonstrated that the pathogen has an association with the bark beetle Hypocryphalus mangiferae in both countries. The beetle vectors the pathogen from diseased to healthy trees, thus contributing to the spread of the disease epidemic. Mango wilt disease management currently includes chemical treatments that are expensive and effective for only short periods of time. The selection of resistant cultivars is an attractive option for the long-term effective control of this disease. Recent inoculation trials with *C.* manginecans in Oman have confirmed that local mango cultivars and the cultivar 'Pairi', are highly susceptible. In contrast, the cultivars 'Hindi Besennara', 'Sherokerzam', 'Mulgoa', 'Baneshan', 'Rose', and 'Alumpur Baneshan' had only small lesions after inoculation and are considered relatively resistant to *C.* manginecans. The inoculation trial results correlate with the incidence of wilt in these cultivars under field conditions. Studies undertaken during last twelve years have provided substantial knowledge concerning mango wilt disease etiology, epidemiology and possible management options to reduce the problem.

**P16.013 Use of alternative fungicides for apple scab control**

*V. Philion*
*IRDA, Saint-Bruno-de-Montarville, Québec, Canada*
*Email: vincent.philion@irda.qc.ca*

Apple scab caused by *Venturia inaequalis* is an important disease in wet climates where this fruit is grown and intensive cropping systems favour this disease. Because scabbed fruits are unmarketable, intensive fungicide programs are used to manage this disease. Consequently, fungal populations are becoming increasingly more tolerant to a wide array of fungicides. Because of public concerns over the use of fungicides and resistance, alternative fungicides including products acceptable in organic agriculture are being investigated. Liquid lime sulphur (LLS) has been used extensively against apple scab but the rates in use can cause phytotoxicity. Potassium bicarbonate can be effective against apple scab, but the application timing is critical. We found that a fungicide program consisting of a very low rate of LLS timed during ascospore germination and potassium bicarbonate timed in post infection for main infection events can result in similar scab levels than conventional fungicides with few extra sprays. Furthermore, the overall cost of fungicide applications was lowered.

**P16.014 Pathogenic and Genetic characterization of strains of *Ceratocystis* sp. affecting mangoes in Pakistan**

*A. Rashid*[1], *S. Iram*[1], *I. Ahmad*[1], *G.B. Poussio*[2] *and F.S. Fateh*[1]
[1]*PARC Institute of Advanced Studies in Agriculture, National Agricultural Research Center, Islamabad, Pakistan;* [2]*Plant Pathology Section, Agriculture Research Institute Tandojam, Sindh; Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi*
*Email: asma_friend@yahoo.com*

Mango (*Magnifera indica* L.) is one of Pakistan's primary fruit crops. Major mango diseases include mango sudden death (MSD), mango malformation, powdery mildew, die-back and anthracnose. The most serious mango disease in Pakistan is mango sudden death caused by *Ceratocyctis manginecans* which results in the reduction of whole production unit (tree) at once. A survey by the Pakistan Agricultural Research Council (PARC) in 2008 recorded an incidence of 43% of mango sudden death in the nurseries of the Punjab. Therefore the present study was planned to assess the pathogenic and genetic characterization of different *Ceratocystis* strains affecting mangoes in Pakistan. A total of n=45 different isolates was isolated from different areas of Sindh (Hyderabad (4), Mirpur khas (4), Tando jam (4), Tando Allah Yar (4), Matiyari (3), Sanghar (6) and from the Punjab (Multan (7), Shujabad (4), Rahim Yar Khan (5), Muzafargargh (2) and Khanewal (2) during 2012, by the direct isolation method using malt extract agar media. Morphological studies showed that the colony color was initially mouse grey turning to brown. Spores were hyaline, cylindrical and truncated and ranged from 4.96-6.20X 32.59-51.21X µm. Perithecia were brown to black with globose base with long neck, and their surface area ranged from 160.89-239.09X µm whereas ascospore dimensions ranged from 2.48-3.0 X 3.72-4.65 µm. Various procedures (detached leaves method, detached twig, potato tuber method and on whole plants) of pathogenicity testing were performed to analyze the aggressive and non aggressive behavior of isolates. Detached leaves showed no symptoms, whereas other methods showed significant results. Disease symptoms including gummosis, bark splitting, leaf dropping/drying, canker development, mortality of the plant were observed and rated using 0-5 severity scales. Total DNA was extracted and ITS1 and ITS4 used for the comparison of different sequences through PAUP.

**P16.015 Hemibiotrophic infection of the Colletotrichum fungi causing anthracnose in olives**

*V. Sergeeva*
*School of Natural Sciences University of Western Sydney, Locked bag 1797 Penrith South DC, NSW 2751, Australia*
*Email: sergeeva@tpg.com.au*

Phytopathogenic fungi of the genus Colletotrichum cause economically significant diseases on many crops, including olives. *Colletotrichum gloeosporioides* and *C. acutatum* can affect all parts of the olive tree growing above the ground, causing disease symptoms typically known as anthracnose. This condition is characterised by brown necrotic tissue with orange masses of spores and has the capacity to also produce asymptomatic latent (quiescent) infections on various olive tree tissues, including flower buds, flowers, leaves, twigs, suckers and unripe fruits. Latent infection on pedicels after flowering can then move into and infect the fruit. Fungal spores and mycelium permit survival of the anthracnose pathogen during hot and dry summers or after fungicide applications, making it difficult to eradicate. Hemibiotrophic infection of the Colletotrichum pathogens incorporate aspects of both biotrophic and necrotrophic infection strategies. It shows initial symptomless intracellular growth, where the colonized host cells remain viable (biotrophy), and then switches to necrotrophic growth, killing the colonized olive plant tissues. During the necrotrophic phase the Colletotrichum pathogens actively kill plant tissue as they colonize and feed on the contents of dead or dying cells. This activity contrasts with that of biotrophic pathogens which derive nutrients from living cells and therefore maintain host viability. Explanation of life style of this phenomenon and current research approaches in future developments leading to the disease management strategies are suggested.

**P16.016 Transmission of causal fungi of mango sudden death disease in mango orchards of Pakistan**

*S. Saeed[1], I. Ahmadd[2], A. Masood[1], N. Iqbal[1], M.R. Kazmi[3] and C. Achem[3]*
*[1]College of Agriculture, BZU, Bahadur Sub- Campus, Layyah, Pakistan; [2]Pakistan Agricultural Research Council, Islamabad, Pakistan; [3]Australian High Commission, Mango ASLP Project, Islamabad*
*Email: shafqatsaeed@bzu.edu.pk*

The Mango Sudden Death (MSD) Disease has become serious threat for mango production all around the world. It has been shown that MSD is caused by a consortium of five fungi in Pakistan. The present work investigated the means of transmission of MSD from diseased to healthy trees in mango orchards. The role of bark beetle, *Hypocryphalus mangiferae,* drip, flood & basin irrigation techniques and wounds was evaluated. *Lasiodiplodia theobromae*, *Ceratocystis fimbriata*, *Fusarium* sp., *Alternaria* sp. and *Aspergillus* sp. were the most frequently isolated fungi from mango trees, soil, water and bark beetles. Flood irrigation was a major source of spread of MSD fungi from diseased trees in the orchards. Damage to collar or roots by implements during cultural practices has maximum chance of disease spread during flood irrigation in orchards. Basin formation around the trunk and drip irrigation may reduce the chances of disease dissemination in orchard. Bark beetle was attracted towards diseased/stressed trees.

**P16.017 Study on phylloplane mycoflora of peach and plum trees in Iran**

*M.R. Ahmadi and K. Rahnama*
*Department of plant protection, Faculty of crop production, Gorgan University of Agricultural sciences and Natural Sciences, Golestan province, Iran*
*Email: kamran_ra@yahoo.com*

In Golestan Province that is located in northeastern of Iran, peach and plum are two of the most important fruit trees. Twenty leaf samples of peach and plum trees were collected from a garden which is located in the campus of agricultural sciences & natural resources university of Gorgan in November and December 2012. It should be noted that the above mentioned trees have not been sprayed with fungicides and pesticides for few years, therefore it is expected that fungal flora on these plants has high variation. By the applying standard methods including petri dish moist chamber incubation and then embedded grown fungal samples on potato dextrose agar (PDA), More than 50 isolates were obtained. The main aim of the present study was to identify and enumerate the fungal species on phylloplane mycoflora of these trees. Microscopic observation revealed that major of the isolates belonged to the genus *Alternaria* (15 isolates), *Fusarium* (12 isolates), *Aspergillus* (10 isolates), *Acremonium* (6) and *Trichoderma* (5). Also six species were identified which are new to the fungal flora of Iran. These species were *Clonostachys macrospora, C. compactiuscula, Cylindrocarpon macrodidymum, Memnoniella leprosa, Periconia epiphylla* and *Stachybotrys suthepensis*.

**P16.018 A new disease caused by *Acremonium spp.* on bagged apple fruits**

*B.H. Li, X.L. Dong, Z.F. Zhang and C.C.Wang*
*College of Crop Protection and Agronomy, Qingdao Agricultural University; Key Lab of Integrated Crop Pest Management of Shandong Province; Qingdao, 266109,Shandong province, P. R. China*
*Email: baohuali@qau.edu.cn*

Fruit bagging became a main technique on apple production in China. The technique effectively prevented the infections by pathogens and damages by insects, such as *Botryosphaeria dothidea*, *Glomerella cingulata* and *Carposina niponensis*. However, a new disease, called black spot, became the main disease of bagging fruit. The disease caused about 5% productions loses on normal years and about 20% on serious years. Black spot wasn't a simple disease and cause by several fungus pathogen and nutrition deficiency. The main pathogen isolated from the black spots was *Acremonium spp.*

The internal transcribed spacer (ITS) region was amplified with primers ITS1/ITS4 from DNA extracted from six isolates of the pathogen. The results of BLASTn showed that six isolates had more than 99% similarity to records for *A. alternatum.* Inoculation experiment results showed that the pathogen infection only from wounds and cause circular and sunken lesions with diameter from 2 mm to 2 cm. The lesion's color was red brown with dark edge. So, the disease was named Acremonium Brown Spot (ABS). The fruit with Acremonium Brown Spots occupied about 45% of all disease fruit with black spots sampled from Shandong province in 2012.

**P16.019 Rough bark and botryosphaeria cankers, the two symptoms caused by *Botryosphaeria dothidea* on apple branches**

*B.H. Li, C.X.Wang, X.L. Dong, W.F. Leng and G.L. Zhang*
*College of Crop Protection and Agronomy, Qingdao Agricultural University; Key Lab of Integrated Crop Pest Management of Shandong Province; Qingdao, 266109,Shandong province, P. R. China*
*Email: baohuali@qau.edu.cn*

Rough bark and botryosphaeria canker were ever thought to be two different diseases on apple branches, caused by *B. berengeriana* and *B. berengeriana s. sp. piricola*, the different name was *Physalospora piricola*, respectively. Our experiment results showed that the rough bark and botryosphaeria canker were two kinds of symptom cased by the same pathogen, *B. dothidea.* On the normal growth branches, the pathogen infection motivated the division and suberization of parenchyma cells under lenticels and stomata and formed raised tumors. In the later stage, a cork layer was formed around the tumor. The diseased tissues were separated from the normal bark tissues by cork layers, and died off. When a large number of growing and died tumors collected together on branches, the symptom of rough bark was shown. When the branches stressed by water or were weak, the pathogen grew in tumor tissues broke through host defense, extended to living bark tissues, killed all the reached living cells and finally formed canker lesion. The canker lesions produced a large amount of conidia and asco-spore and played very important role on epidemics of the disease. On younger apple fruit, the infection active division and suberization of fruit cells as well.

**P16.021 Defense related biochemical changes by elicitor of *Fusarium mangifarae* in mango (*Mangifera indica* L.)**

*G. Chand, C.S. Azad, A. Kumar, S.P. Yadava and D.K. Chakrabarti*
*Department of Plant Pathology, Bihar Agricultural University, Sabour -813210, Bhagalpur (Bihar), India; Department of Plant Pathology, N. D. University of Agriculture and Technology, Faizabad-224229 (U.P.), India*
*Email: gireesh_76@rediffmail.com*

Investigation of defense responses at the initial stage of infection in a compatible host-pathogen interaction after treatment with biotic (*F. mangiferae*) and abiotic (Salicylic Acid) elicitors as well as inoculation of the host tissues with the pathogenic strain. Besides the effect of SA in controlling vegetative malformation in mango under field conditions was also investigated. In response to the application of both the elicitors and inoculation with the fungus, amount of PR-protein, that causes lyses of fungal hyphae increased by many folds. But the active oxygen species, hydrogen peroxide that induces hypersensitive death of host cells was reduced to the minimum level after treatment with biotic elicitor. $H_2O_2$ in the inoculated buds also reduced substantially. In malformed shoots and panicles no hypersensitive cell death takes place. The amount of SA increased marginally with SA treatment and in the inoculated buds. But in biotic elicitor treated buds, the quantity of SA was reduced. SA, an important signal molecule enhances $H_2O_2$ production by suppressing the $H_2O_2$ - degrading activity of catalase. The reduction in the amount of SA in biotic elicitor treated buds resulted into reduction in quantity of $H_2O_2$ in elicitor treated buds. In all the treatments a reduction in catalase activity over the control was noticed but the reduction was not enough to arrest the degradation of $H_2O_2$. Magiferin, quantity as compared with control was more in all the treatments; the maximum was recorded with biotic elicitor. A PR-protein of 20 KDa which resists the symptoms development was absent in control buds, although it appeared in all the treatments. But the spots for the PR-protein were very light. The maximum dark spot for the PR-protein was observed with fungus inoculated buds followed by SA treatment while it was the minimum in the biotic elicitor treated buds.

**P16.022 Occurrence and integrated control of Alternaria blotch on Cripps Pink apple fruits in Israel**

*L. Gur[1, 2], M. Reuveni[1], R. Stern[3] and Y. Cohen[2]*
*[1]Golan Research Institute, University of Haifa, P.O. Box 97 Katzrin 12900, Israel; [2]Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900, Israel; [3]MIGAL, Galilee Technology Center, P.O. Box 831, Kiryat-Shmona 11016, Israel*
*Email: mreuveni@research.haifa.ac.il*

Alternaria blotch, caused by *Alternaria alternata* apple pathotype, (*A. mali*) is an economically important disease of apple. An increase in disease severity on leaves and mainly on fruits of the prestigious cv. Cripps Pink (Pink Lady) was recently observed in Israel. The fungus caused external decay on the fruit body or on cracks around the fruit calyx, and damaged up to 80% of the

fruits in some orchards. Such a heavy infection on fruits barely reported in other regions of the world. Severe damage was especially observed in fruits facing the sun, or fruits on the top of the tree. As an integrated control strategy we used a fungicide, a growth regulator (to reduce calyx cracks) and shade nets. The results showed that the fungicide Ortiva-Top (Azoxystrobin + difenoconazole) was most effective against conidial germination, mycelial growth and decay development in detached fruits. Six applications of the fungicide Ortiva-Top at a concentration of 0.05% starting from mid of August (two weeks before cracks appearance) and three sprays of 0.2% of the growth regulator Superlon (GA4+7, 6-Benzyladenine) at 60, 75 and 90 days after full bloom provided the best disease control in orchards. Integration of a fungicide and a growth regulator, together with shade net (20%), in a commercial orchard, highly improved disease control. The combination of a fungicide and a growth regulator together with a shade net, seems to provide an adequate integrated disease control strategy.

**P16.023 Identification of gummosis of mango trees in Guangxi, south China**

*Q.L. Li, T.X. Guo, S.P. Huang and J.Y. Mo*
*Institute of Plant Protection, Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, 530007, P. R. China*
*Email: liqili@gxaas.net*

Mango (*Mangifera indica* L.) is an economically important fruit in southern provinces of China including Hainan, Guangdong, and Guangxi. Gummosis on trunks and branches is one of important disease in Guangxi, China. In order to identify the gummosis-causing agents, diseased trunks and branches were collected from 15 mango orchards in Guangxi province. Fungal isolates were obtained from these samples, including three species: *Botryosphaeria rhodina* (anamorph *Lasiodiplodia theobromae*), *Botryosphaeria dothidea* (anamorph *Fusicoccum aesculi*), and *Neofusicoccum parvum*. They were identified based on conidial morphology and cultural characteristics, as well as analyses of nucleotide sequences of three genomic regions: the internal transcribed spacer region, a partial sequence of the β-tubulin gene, and the translation elongation factor 1-α gene. *Lasiodiplodia theobromae* was found in all mango orchards but *Botryosphaeria dothidea* was found only in Wuming County in the Xixiangtang region and *Neofusicoccum parvum* only in Tiandong County in the Baise region. These three species were all found to be pathogenic via artificial inoculation using mycelia plugs on wounded twigs or branches, causing dark lesions on the twigs and branches and, sometimes, gum exudation from diseased parts. Isolates of *L. theobromae* were the most virulent and caused the largest lesions and most copious gummosis. This report represents the first description of *Botryosphaeria dothidea* and *Neofusicoccum parvum* as causal agents of gummosis on mango in China.

**P16.024 *Valsa mali,* an important fungus pathogen causes cherry gummosis**

*X.L. Dong[1], B.H. Li[1], L.M. Chen[1] and F.X. Zhang[2]*
*[1]College of Crop Protection and Agronomy, Qingdao Agricultural University; Key Lab of Integrated Crop Pest Management of Shandong Province; Qingdao, 266109, Shandong province, P. R. China; [2]Fruit Research Institute, Yantai Academy of Agricultural Sciences; Yantai, 265500, Shandong province, P. R. China*
*Email: dxl01234@yahoo.com.cn*

Cherry gummosis is one of the important disease affect cherry industry. Several isolates of *Valsa spp.* were isolated from section of cherry xylem producing gum and single spore isolates of the pathogen was also obtained. The single spore isolates were identified as *Valsa mali* according to morphological, ITS sequence and pathogenicity on apple branches. One of isolates was inoculated to young stems of cherry tree with mycelia. The infection and gumming processed were examined by observing frozen section under fluorescence microscope. The result showed that: One day after inoculation, hyphae were examined in phloem, but the morphology of phloem cells and cambial cells did not show obvious change. 3 days after, the cambial cells became swelling, the intercellular space expanded, and some cells were observed lyses. 5 days after, the duct cavities were formed. 7 days after, the duct cavities were expanded, the cells around the duct cavities were subErized. 14 days after, a large amount of gum duct cavities were observed in the cambial zone, and fulfilled with gum. The gum duct was extended vertically and was observed at 20cm high above the infected site. Gum overflowed out of the bark through the disease area. One month later, the gum duct was embedded in xylem.

**P16.025 Characterization and pathogenicity of fungal pathogens associated with grapevine Botryosphaeria dieback in China**

*J.Y. Yan, W. Zhang and X.H. Li*
*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, P.R. China*
*Email: lxh1962@yahoo.com.cn*

Grapevine Botryosphaeria dieback (Botryosphaeria canker) is becoming a serious disease problem for the production of table and wine grape production worldwide. The important disease has resulted in higher control costs for growers and has reduced productivity of the vine in China. Botryosphaeria dieback occurs in 19 out of 20 grape-growing provinces in China. Morphological

studies combined with multi-gene phylogenetical analyses confirmed that *Botryosphaeria dothidea*, *B. obtusa* (*Diplodia seriata*), *B. rhodina* (*Lasiodiplodia theobromae*) and *B. parva* (*Neofusicoccum parvum*) were associated with different symptoms of the disease, three other suspect species were still on identification. The geographical distribution of *Botryosphaeriaceae* species was significant, and analysis of more than 100 isolates showed that there was genetic differentiation among species. Koch's postulates were satisfied for all species, and pathogenicity tests showed that no cultivar among the 25 main cultivars growing in China were resistant to above four taxa. (The study was supported by CARS-30).

**P16.026 Occurrence of grapevine leafroll-associated virus complex in China**

*M.H. Liu[1], M.J. Li[1], H.H. Qi[2], R. Guo, X.M. Liu[1] and Y.Q. Cheng[1]*

*[1]Department of Pomology/Lab of Stress Physiology and Molecular Biology for Tree Fruits, A Key Lab of Beijing Municipality, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, P. R. China; [2]Plant Science and Technology College, Beijing University of Agriculture, Beijing 102206, P. R. China*

*Email: chengyuqin@cau.edu.cn*

A total of 249 grapevine (*Vitis* spp.) samples (86 popular varieties and a rootstock) from 19 provinces and regions in China were tested for *Grapevine leafroll-associated virus 1* (GLRaV-1), GLRaV-2, GLRaV-3, GLRaV-4 and GLRaV-5 by SYBR Green real-time RT-PCR and RT-PCR-based testing and sequencing methods to identify which species is the major causal agent of grapevine leafroll disease. We then characterized the genetic variability of Chinese GLRaV-3 isolates based on the entire coat protein (CP) gene. GLRaV-3 was the predominant virus, found in 100% of the samples. GLRaV-1, GLRaV-2 and GLRaV-4 were observed in 24.9% (62/249), 15.3% (38/249) and 0.80% (2/249) of the samples, respectively. Single infections with GLRaV-3 were found in 66.3% (165/249) of the samples, and the remaining samples were mixed infections of GLRaV-3 with one or two other GLRaVs, those with GLRaV-1 being the most common (18.5%, 46/249). A total 153 full-length CP gene sequences (94 sequences newly generated) of Chinese GLRaV-3 isolates from different grape-growing regions showed 89.3% to 100.0% and 92.7% to 100.0% identity at the nucleotide and aa levels, respectively. The average nucleotide diversity for the population of Chinese GLRaV-3 isolates was estimated at 0.037 (SE 0.0032). GLRaV-3 isolates from China were segregated into five distinct phylogenetic groups and two novel recombination events were found in the viral population. This is the first and most extensive report of the prevalent types of GLRaVs in China, which also provides an assessment of genetic variability of GLRaV-3 Chinese isolates.

**P16.027 The molecular and biological characterization of a novel double-stranded RNA mycovirus from *Botryosphaeria dothidea***

*Z. Ding, B. Zhan, J.B. Chen, Y.F. Zhao, X.M. Wu and L.Y. Guo*

*Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*

*Email: ppguo@cau.edu.cn; ppguoly@126.com*

A new double-stranded RNA virus named *Botryosphaeria dothidea mycovirus* 1 (BdMV1) was isolated from G1, a virulence-defect isolate of *Botryosphaeria dothidea*, the pathogen of apple ring rot. This virus formed rigid spherical particles approximately 45 nm in diameter and contained four dsRNAs (2.4 to 2.9 kbp). The results of SDS-PAGE and protein BLAST showed that this virus had two kinds of coat protein (CP) encoded by two different ORFs, which suggested that the virus particles had a double layer CP structure. BLAST analysis showed that the proteins encoded by virus dsRNA1 possess motifs that are conserved in RNA-dependent RNA polymerases, and the protein encoded by dsRNA2 resembles the hypothetical protein encoded by dsRNA4 of *Magnaporthe oryzae chrysovirus* 1. No significant sequence similarities were evident between known proteins and BdMV1 structural proteins shown to be encoded by dsRNA3 or dsRNA4. Repeated hyphal-tip isolation was applied on isolates G1 and five virus-cured isolates were obtained and all of them showed significant increase in virulence to apple shoots. These results suggest that BdMV1 is a new latent virus that possibly belongs to the family of *Chrysoviridae*.

**P16.028 Molecular characterization of genetically distinct groups of *Colletotrichum acutatum* from blueberry and comparing with isolates from other fruit crop hosts**

*J. Volkova[1] and A. Baženova[2]*

*[1]University of Latvia, Kronvalda Bv. 4, Riga, LV- 1586, Latvia; [2]Latvian Plant Protection research centre, Struktoru Str. 14a, Riga, LV-1039, Latvia*

*Email: julija.volkova@laapc.lv*

*Colletotrichum acutatum* (Simmonds) is important fungal plant pathogen worldwide, but in Latvia before it was detected only on rhododendrons, not on the fruit crops. In different studies before it has been discovered, that *C. acutatum* belongs to eight A1-A8 distinct molecular groups that are related to the geographical distribution of host plants. During this study samples of *C. acutatum* from different fruit crops (blueberry, strawberry, apple, cherry, and strawberry and raspberry) were collected and analyzed. For analysis rDNA ITS1 – 5.8S – ITS2 region was sequenced, using ITSF1 and ITS4 primers, sequencing were performed by Macrogen Europe Inc. (Netherlands). DNA sequence data were analyzed using MEGA 5.1. (Tajima, Nei 1984). All isolates of *Colleto-*

*trichum* sp. from Latvian fruit crops were identified as *C. acutatum*, and during phylogenetical analysis of sequences it was found, that isolates belongs to different molecular groups, related to origin of planting material. Isolates from blueberries belonged to molecular groups A3, and A4. Isolates from strawberries belonged to group A4 and A2, and group A2 is more specific for South part of Europe, and in this case origin of planting material was Italy.

**P16.029 Molecular diagnostics, characterization and management of major virus / virus-like pathogens infecting citrus in India**

*D.K. Ghosh*
*National Research Centre for Citrus (ICAR), Nagpur - 440010, Maharashtra, India*
*Email: ghoshdk@hotmail.com*

Diseases caused by virus and virus-like pathogens are considered as an important limiting factor to sustainable citrus industry in India where mandarin (*Citrus reticulata* Blanco), sweet orange (*Citrus sinensis* Osbeck) and acid lime (*Citrus aurantifolia* Swingle) are grown as commercial crop. About sixteen such pathogens are reported to infect citrus plant among which citrus tristeza virus (CTV), Indian citrus ringspot virus (ICRSV), citrus yellow mosaic badna virus (CiMV), citrus exocortis viroid (CEVd) and citrus greening bacterium (HLB), transmitted either by insect vectors or mainly by infected budwoods are of major concern. All three commercial citrus cultivars are infected by these pathogens singly or as mixed infection resulting into gradual decline of citrus orchards. Conventional bio-diagnosis, though routinely being used, has its own limitation. Serodiagnosis using pathogen specific polyclonal/monoclonal antibodies and nucleic acid based diagnosis by PCR/RT-PCR techniques are more reliable, rapid and less costly. A multitude of PCR based techniques viz. PCR, RT-PCR, IC-PCR, multiplex PCR etc are now being used routinely for detection of CTV, ICRSV, CiMV, CEVd and HLB in citrus plant samples as well as their potential insect vectors either single or as mixed infection. Similarly genomes of these pathogens have been cloned, sequenced and their phylogenetic and evolutionary relationships has been established. Standardized molecular diagnostic tools has been successfully utilized to implement citrus budwood certification program and to develop about half million certified virus-free planting material every year for the citrus growers of India.

**P16.030 Molecular characterization of Citrus yellow mosaic badna virus (CMBV) isolates prevalent in citrus orchards of central India**

*D.K. Ghosh*
*National Research Centre for Citrus (ICAR), Nagpur - 440010, Maharastra, India*
*Email: ghoshdk@hotmail.com*

Citrus yellow mosaic badnavirus (CMBV) is a non-enveloped, bacilliform DNA-containing virus and the etiologic agent of yellow mosaic disease of citrus, the third most important fruit crop after banana and mango in India. The disease was initially reported from Sathgudi sweet orange (*Citrus sinensis*) cultivars in the southern parts of India where it causes significant yield losses has now spread to other parts of the country. During a recent survey of citrus groves in the Nagpur region, central India, characteristic mosaic symptoms were observed in mandarin orange (*C. reticulata*) and sweet orange (*C. sinensis*). Virus transmission studies, electron microscopy, PCR amplification and sequencing of cloned PCR products from samples displaying mosaic symptoms confirmed the presence of a badnavirus. In contrast to the CMBV isolates reported earlier from India, the CMBV-Nagpur isolate could be transmitted to the Rangpur lime (*C. limonia*) and acid lime (*C. aurantifolia*) trees upon graft inoculation. Sequence analysis of a part of the ORF-III region and the intergenic region of the viral genome revealed that CMBV-Nagpur isolate is distinctly different from the previously reported isolates. Based on the transmission studies and phylogenetic analysis it was concluded that at least two strains of CMBV exist in India currently.

**P16.031 MYCORRAY: a microarray tool for simultaneous detection of grapevine trunk fungi. Evaluation of the best DNA extraction method**

*G. Marchi[1], T. Cinelli[1], B. Ginetti[1], M. Baleani[1], D. Bossio[1], D. Rizzo[2] and L. Mugnai[1]*
*[1]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Sezione di Patologia Vegetale ed Entomologia, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy; [2]Servizio Fitosanitario Regionale, Via Pietrapiana 30, 50121 Firenze, Italy*
*Email: guido.marchi@unifi.it*

Fungal pathogens causing grapevine decline and trunk diseases are widespread in different countries of the world causing significant reductions in vineyard longevity and productivity. Established vines can become infected through pruning wounds and during their lifetime can be re-infected multiple times with one or more causal agents of wood diseases. Therefore, it is common to isolate several pathogens from the discolored woody tissues of the trunk, including, among the most frequent, *Phaeomoniella chlamydospora*, several species of *Phaeoacremonium* and the Botryosphaeriaceae, *Fomitiporia mediterranea* and *Eutypa lata*. In order to set up a microarray tool (MYCORRAY) for simultaneous identification of the main fungal pathogens involved, a comparative evaluation of seven DNA extraction methods

from grapevine sawdust collected by drilling the tissues surrounding pruning wounds of naturally infected vines, was carried out Measurements of total DNA concentration ($A_{260}$) as well as real-time and end-point PCR amplifiability were used to determine quantity and quality of the extracted DNA. Meanwhile its suitability for indexing purposes was assessed by the mean of four independent nested-PCR assays specific for *P. chlamydospora*, *Neofusicoccum parvum*, *E. lata* and *F. mediterranea*, respectively. Probably due to the presence of PCR inhibitors that co-purified, total DNA concentrations were unsuitable for globally predicting PCR success. Meanwhile real-time PCR amplifiablity and relative Ct values were the best indicators of extract quality. However, when the presence of the targeted fungi was ascertained, not all methods were found to be equally suitable for detecting multiple infections of the wood.

**P16.032 Molecular, Serological and Biological Characterization of Two Flexuous Viruses Infecting Stone Fruit Trees in China**

*J.F. Zhou, G.P. Wang, L.N. Qu and N. Hong*
*The Key Laboratory of Plant Pathology of Hubei Province, College of Plant science and Technology, Huazhong Agricultural University, Wuhan 430070, P.R. China*
*Email: whni@mail.hzau.edu.cn; zhoujufang0918@yahoo.cn*

*Cherry green ring mottle virus* (CGRMV) and *Cherry necrotic rusty mottle virus* (CNRMV) are unassigned members in the family *Betaflexiviridae.* These two viruses are known to infect several *Prunus* species. Previously, we reported both viruses infecting stone fruit trees grown in China. Those two viruses showed high similarities in virion morphology with *Apple stem pitting virus* (ASPV, a type member of genus *Foveavirus* in the family *Flexiviridae*). However, their genomic organization is different from that of ASPV by having two more ORFs (ORF2a and ORF5a), of which functions are unknown. In this study, CGRMV and CNRMV from six different stone fruit species were molecularly characterized. Phylogenetic analyzes for both complete CP and TGB genes showed that these two viruses are highly divergent, sharing 80.2-100% of nt identities intra virus, and 66.9-71.9% of nt identities inter viruses. Although they are closely related to the members in genus *Foveavirus*, they had the highest divergence up to 39.7-41.2% for CP and 48.7-50.1% for TGBs with that of ASPV. In western blots, antisera against CGRMV and CNRMV reacted strongly with CPs of homologous virus and ASPV, and failed to recognize CPs of ACLSV in genus *Trichovirus* and ASGV in genus *Capilloviru*. For the first time, we confirmed that CGRMV and CNRMV can infect some herbaceous plants by mechanical inoculation, and found that they had similar woody and herbaceous host ranges to those of ASPV. The results obtained will help to get insight into their taxonomic assignment.

**P16.033 Identification and characterization of *Pestalotiopsis* spp. causing twig blight disease of bayberry (*Myrica rubra* Sieb. & Zucc) in China**

*H.Y. Ren[1], G. Li[2], X.J. Qi[1], J.G. Wei[3] and S.B. Zhong*
*[1]Institute of Horticulture Sciences, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China; [2]State Key Lab Breeding Base for Zhejiang Sustainable Plant Pest Control; MOA Key Lab for Pesticide Residue Detection; Institute of Quality and Standard for Agro-products, Zhejiang Academy of Agricultural Sciences; Hangzhou 310021, China; [3]College of Agriculture, Guangxi University, Nanning 530005, China; [4]Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA*
*Email: haiyingren@aliyun.com*

Bayberry (*Myrica rubra*) is a fruit tree native to the subtropical regions of China. It produces fruit with a unique taste and pharmacological characteristics, thus making it a widely cultivated tree commercially in many regions of China, including Zhejiang, Fujian, Guizhou, Sichuan and Yunnan Provinces. Recently, a twig blight disease occurred on the fruit tree and caused destructive damages of plantings in the Zhejiang Province. However, the etiology of the disease was unclear. This study was carried out to identify the causal agent(s) of the blight disease on bayberry. Fungal isolates were obtained from blighted twig samples collected from bayberry fields in Xianju, Ruian, and Huangyan of Zhejiang Province. The majority (87.9%) of the 257 fungal isolates were identified as *Pestalotiopsis* spp. based on their conidial morphology. DNA sequences of the internal transcribed spacer (ITS) region of the ribosomal RNA genes and the β-tubulin gene were obtained from six representative strains (XJ27, XJ42, RA2-1, YS26, YS44 and RA1-2) of the *Pestalotiopsis* spp. Phylogenetic analysis showed that three of the strains (XJ27, XJ42, and RA2-1) grouped with *P. versicolor* (Speg.) Steyaert while the other three strains (YS26, YS44 and RA1-2) grouped with *P. microspora* (Speg.) Batista & Peres. Pathogenicity tests in the greenhouse showed that all these six isolates of *Pestalotiopsis* spp. caused twig blight disease symptoms on bayberry plants, which were the same as observed in naturally infected plants in the field. Our results clearly indicated that *P. versicolor* and *P. microspora* were the major pathogens causing the twig blight disease on bayberry in southern China.

**N16.001 Identification of Phomopsis species Associated with Grapevine Trunk Disease in China**

*M. Liu, J.Y. Yan, W. Zhang and X.H. Li*
*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, P.R. China*

*Email: lxh1962@yahoo.com.cn*

Phomopsis causing grapevine disease is a serious problem in most countries where grapevines are grown. The fungus can infect all green parts of the grapevine and overwinters in the bark of older canes. Sixteen isolates were collected from 8 different grape growing provinces of China. The isolates were primarily identified based on mycelia growth rate in vitro, alpha conidium, conidiophore morphology and other cultural characteristics. In addition, translation elongation factor 1- α, calmodulin genes, ITS sequences and β-tublin genes were also used for phylogenetic analysising. The results showed that *Phomopsis caccinii* and *Phomopsis longicolla* were the main species of phomopsis caused grapevine disease in China. (The study was supported by CARS-30).

## Concurrent Session 17-Genomics and Proteomics

### O17.001 Accessory genomes and host-specific pathogenicity in *Fusarium oxysporum*

*M. Rep*
*University of Amsterdam, The Netherlands*
*Email: m.rep@uva.nl*

*Fusarium oxysporum* is a fungal species complex that harbours innummerable strains which appear to predominantly propagate asexually, often in close association with plant roots. It is likely that many strains can penetrate plant roots and live endophytically to various degrees. A small minority of strains colonizes a particular host plant to such an extent or in such a manner that disease ensues. Such strains commonly only cause disease in one or a few related plant species, and are hence considered together as '*forma specialis'* for that host. We have found that all strains that cause wilt disease in tomato (i.e. belonging to *forma specialis lycopersici*) harbour a common 'pathogenicity chromosome'. This chromosome harbours most 'effector genes', encoding small *in planta* secreted proteins that promote colonization and/or disease development. Horizontal transfer of this chromosome to a non-pathogenic strain occurs during co-cultivation and results in pathogenicity of the recipient strain towards tomato. We are currently investigating our hypothesis that similar chromosomes in other *formae speciales* confer pathogenicity towards other plants.

### O17.002 Septin-mediated plant tissue invasion by the rice blast fungus *Magnaporthe oryzae*

*Y.F. Dagdas, L.S. Ryder, M.J. Kershaw and N.J. Talbot*
*School of Biosciences, University of Exeter, Geoffrey Pope Building, Exeter EX4 4QD, United Kingdom*
*Email: N.J.Talbot@exeter.ac.uk*

*Magnaporthe oryzae* is the causal agent of rice blast, one of the most serious diseases affecting rice production. During plant infection, *M. oryzae* develops a differentiated infection structure called an appressorium. This unicellular, dome-shaped structure generates cellular turgor, which is translated into mechanical force to cause rupture of the rice cuticle and entry of the fungus into plant tissue. We have shown that a hetero-oligomeric septin GTPase complex is necessary for re-organisation of a toroidal F-actin network at the base of the appressorium which allows re-establishment of polarised fungal growth. Re-modeling of F-actin is necessary for cortical rigidification and localisation of proteins associated with membrane curvature to the appressorium pore. Septin-mediated cytoskeletal re-modeling is necessary formation of a penetration hypha that breaches the host cuticle and leads to plant tissue colonization by biotrophic invasive hyphae of *M. oryzae*. We will present evidence that septin-mediated plant infection is regulated by NADPH oxidase activity and a regulated burst of reactive oxygen species. A specialised Nox2 NADPH oxidase-tetraspanin complex is necessary for septin-mediated control of actin dynamics. We will also describe the potential operation of a pressure-mediated checkpoint, mediated by a cell wall mechanosensor protein that is necessary for initiation of septin activation and the re-orientation of the cortical F-actin cytoskeleton to facilitate plant tissue invasion.

### O17.003 Comparative genomics of nematode-trapping fungi

*K.Q. Zhang*
*Laboratory for Conservation and Utilization of Bio-Resources, and Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming, Yunnan, 650091, P. R. China*
*Email: kqzhang@ynu.edu.cn*

Nematode-trapping fungi are natural enemies of nematodes. They are capable of developing specific trapping devices such as adhesive networks, adhesive knobs, and constricting rings to capture nematodes and then extract nutrients from their nematode prey special natural enemies of nematodes. The morphological development of these traps is the key indicator of their switch from saprophytic to predacious lifestyles. Here, we report the genome of the nematode-trapping fungus *Arthrobotrys oligospora* Fres. (ATCC 24927). The genome contains 40.07 Mb assembled sequence with 11,479 predicted genes. Based on the combined genomic, proteomic and RT-PCR data, we propose a model for the formation of nematode trapping device in this fungus. In this model, multiple fungal signal transduction pathways are activated by its nematode prey to further regulate downstream genes associated with diverse cellular processes such as energy metabolism, biosynthesis of the cell wall and adhesive proteins, cell division, glycerol accumulation and peroxisome biogenesis. In addition, we also sequenced the genomes of the typical nematode-trapping fungi with other different types of traps for comparison. *Dactylellina entomopaga* (*Drechsler*, CBS624.8) forms adhesive knobs. *Dactylellina cionopagum* (*Drechsler*, YMF1.00569) develops adhesive columns and *Drechslerella brochopaga* (*Drechsler*, YMF1.01829) foemes constrict rings. Besides, the genome of *Dactylella tenuis* (*Drechsler*, CBS325.70), a close relative to the nematode-trapping fungi without the capability of trapping nematodes, has also been sequenced for comparison. RNA-seq data analyses suggested that there were 24 genes were common shared by the four species, considering to play a very important role in induced trap formation. Functional enrichment analysis showed that the 21 genes were enriched in stress response, transmembrane transport and proteolysis. Pathway enrichment analysis showed that the 21 genes

were enriched in catalysts, gene regulation and stimulus response. Our results provide the first glimpse into the genomes of carnivorous fungus. The data here should facilitate future investigations into the molecular mechanisms of nematode infection and the transition between saprophytic and predacious lifestyles in nematode-trapping fungi, ultimately leading to our enhanced ability to manipulate the biocontrol potential of these fungi.

### O17.004 Genomic tillage and the harvest of fungal phytopathogens

*R. Oliver*
*Department of Environment & Agriculture, Curtin University, Perth, Australia*
*Email:* richard.*oliver@curtin.edu.au*

Genome sequencing has been carried out on a small selection of major fungal ascomycete pathogens. These studies do not support the notion that pathogens evolved from phylogenetically-related saprobes by the acquisition or modification of a small number of specific genes. The genomes show that pathogens do not into three clearly delineated classes (biotrophs, hemibiotrophs and necrotrophs) but rather into a complex matrix of categories each with subtly different properties. It is clear that the evolution of pathogenicity is ancient, rapid and ongoing. Fungal pathogens have undergone substantial genomic rearrangements that can be appropriately described as "genomic tillage". Genomic tillage underpins the evolution and expression of large families of genes – known as effectors – that manipulate and exploit metabolic and defence processes of plants so as to allow the proliferation of pathogens.

### O17.005 Comparative transcriptome analysis between the necrotrophic pathogens *Sclerotinia sclerotiorum* and *S. trifoliorum*

*D. Qiu, G. Vandemark and W. Chen*
*USDA-ARS, Grain Legume Genetics and Physiology Research, Washington State University, Pullman, WA 99164, USA*
*Email: w-chen@wsu.edu*

*Sclerotinia sclerotiorum* and *S. trifoliorum* cause white mold on many economically important crops. *S. trifoliorum* mainly infects cool season legumes with a host range of about 40 plant species, whereas the host range of *S. sclerotiorum* encompasses more than 400 plant species including all the host species of *S. trifoliorum*. *S. sclerotiorum* has been extensively studied and its genome sequences are available. However, relatively little is known about *S. trifoliorum*. We compared the transcriptome of *S. trifoliorum* with that of *S. sclerotiorum* in order to gain a better understanding of the biology of both species. A total of 23133 unique transcripts with average length of 439 bases (10.1 Mb genome coverage) were obtained from *S. sclerotiorum*, whereas 21043 unique transcripts with average length of 418 bases (8.8 Mb genome coverage) were obtained from *S. trifoliorum*. Approximately 60% of the transcripts were common between the two species, and about 43% of the transcripts were genes with known functions for both species. Among 1411 orthologous contigs (transcripts with more than one read), 147 (10%) were highly (> 3 folds) expressed in *S. trifoliorum* than in *S. sclerotiorum*, and 173 (12%) were highly expressed in *S. sclerotiorum* than in *S. trifoliorum*. Approximately 140 transcripts from each species were found in the noncoding regions in the annotated genome of *Sclerotinia sclerotiorum*. Fifteen transcripts in the noncoding region were shared by both species. Additionally, differences in expressed genes involved in pathogenesis like oxalate biosynthesis and endopolygalacturonases were detected between the two species.

### O17.006 Identification of effector candidates through comparative genomics and proteomics of *Zymoseptoria tritici* (*Mycosphaerella graminicola*)

*M.C. McDonald, A.W. Milgate and P.S. Solomon*
*Australian National University, RSB-Plant Sciences 134 Linnaeus Way, Acton, ACT 2617, Australia*
*Email: megan.mcdonald@anu.edu.au*

*Zymoseptoria tritici* is a hemi-biotrophic pathogen of wheat, with an early biotrophic period, followed by necrosis and active pathogen growth. During latent infection, the fungus persists without causing host symptoms in the apoplastic space for an average of eight days. In order to persist in the apoplast, *Z. tritici* must produce proteins or metabolites, known as effectors, which protect it from plant defense responses. We used a combined proteomic and comparative genomic approach on 10 Australian isolates having different pathogenicity profiles (pathotypes) on common Australian wheat cultivars to identify these effectors. The genome of each isolate was sequenced using the Illumina HiSeq platform and assembled to the available reference genome. *Z. tritici* contains 13 essential chromosomes and eight dispensable chromosomes of unknown function. Each isolate was also assembled *de novo* to examine potential large insertions or deletions in both core and dispensable chromosomes. Additionally, a proteome dataset isolated from apoplastic fluids during *in planta* infection was generated. Proteomic analysis includes samples extracted from the apoplast at three, six and nine days post infection (dpi). Sequenced peptides were identified using both the available reference genome and the genome of each of the 10 pathotypes. Comparative results from these ten pathotypes will be presented.

### O17.007 Genomic approaches to research on *Pectobacterium* and *Dickeya* species

*L. Pritchard[1], S. Humphris[1], E. Douglas[1], P.R.J. Birch[1,2] and I.K. Toth[1]*
*[1]James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK; [2]Division of Plant Science, University of Dundee (at JHI), Invergowrie, Dundee DD2 5DA, UK*
*Email: ian.toth@hutton.ac.uk*

Sequencing the genome of the bacterial plant pathogen *Pectobacterium atrosepticum* (*Pba*); which is one of the most economically damaging bacterial plant pathogens in temperate regions, has uncovered many new pathogenicity and lifestyle determinants, and helped us to form new hypotheses about the way this group of pathogens attack plants. We are currently undertaking a related investigation with 29 strains of *Dickeya* species (formerly *Erwinia chrysanthemi*) and, following next generation sequencing, genome assembly, gene calling and annotation, we have undertaken comparative genomics to identify the pan-genome. With this information we have been able to identified strain, species and clade-specific genes that we are investigating in terms of their roles in pathogenicity and host range. We have also begun the process of identifying metabolic networks, through both genomic and phenotypic approaches, in an attempt to better understand the role of such networks in virulence, host range and adaptation to life on plants. As a practical spin-out of the work, a new genome-based method has been developed to identify primer pairs and probes for use in diagnostics. Particular effort has been on identifying primers for *D. dianthicola* and '*D. solani*', the latter of which is an increasing problem in Europe, although strains, species, clades and genus- specific primers have also been developed. These primers are currently being validated for molecular diagnostic testing by a number of European plant health laboratories, and the method has recently been applied to tracing pathogen movements in medical disease outbreaks.

### O17.008 Burn, baby burn: genomic view of how the fire blight pathogen *Erwinia amylovora* uses small RNAs and cyclic di-GMP to regulate distinct virulence pathways

*G.W. Sundin, Q. Zeng and L. Castiblanco*
*Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI USA*
*Email: sundin@msu.edu*

Pathogenesis of *Erwinia amylovora*, causal agent of fire blight, requires type III secretion, translocation of the type III effector DspE into plant cells, and production of the exopolysaccharide (EPS) amylovoran. Amylovoran is the major EPS component of biofilms, which are formed in planta in xylem vessels. Initial infection of apple flowers and leaves by *E. amylovora* involves induction of expression of the type III secretion system (T3SS) and effector translocation which function to kill host cells. The production of all of these major pathogenicity factors of *E. amylovora* is regulated by small RNAs and c-di-GMP. We used RNA-seq to identify regulatory sRNAs in *E. amylovora*; in that experiment, 34 sRNAs were successfully identified and the expression ratio of sRNAs in different conditions was confirmed by Northern blot. We have shown that two of these sRNAs, ArcZ and RprA, function with the sRNA chaperone Hfq to regulate the production and translocation of type III effectors, cellular motility, amylovoran production, and biofilm formation. The major impact of ArcZ on pathogenesis appears to occur through regulation of the master flagellar regulator FlhDC, and we have shown through mutagenesis and physical interaction studies that ArcZ interacts with the 5-untranslated region of *flhDC*. We identified five diguanylate cyclase enzymes in the *E. amylovora* genome and showed that three of them were active in synthesizing c-di-GMP. C-di-GMP positively regulated amylovoran production and biofilm formation, while negatively regulating motility and type three secretion. Similarities and differences among these two regulatory pathways will be discussed.

### O17.009 Omics approaches to understand *Erwinia amylovora* virulence

*Y.F. Zhao*
*Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, U. S. A.*
*Email: zhao888@illinois.edu*

The apple and pear industry in the U. S. and around the world has been devastated by fire blight disease, caused by a bacterial plant pathogen *Erwinia amylovora*. Extensive genetic studies in the past decade or so have demonstrated that a functional hypersensitive response and pathogenicity (*hrp*) - type III secretion system (T3SS) and its associated effectors as well as production of the exopolysaccharide amylovoran are primary determinants in *E. amylovora* to cause fire blight disease. The recent revealing of the genetic composition for more than a dozen strains of *E. amylovora* and related *Erwinia* species associated with pome fruit trees has greatly increased our understanding about the pathogen, its ability to cause disease, and interaction with host plants. Genome sequencing also provides new opportunities for the fire blight research community to adopt novel omics approaches to study *E. amylovora* virulence and survival during host-pathogen interactions. Comparative genomic approaches have been utilized to determine genetic diversity and evolution of *E. amylovora* and related *Erwinia* species associated with pome fruit trees, and to identify potential host specificity determinants; functional genomics approaches such as genome-wide oligo microarray have been employed to reveal many novel virulence factors and determine gene regulatory networks; and proteomics approach has been

applied to demonstrate the role of protein posttranslational modification in virulence and metabolism. In this presentation, recent findings utilizing omics approaches to understand *E. amylovora* virulence will be summarized and highlighted. Future perspectives and research directions for this important pathogen will also be discussed.

**O17.010 Insights into bacterial soft rot gained from *Pectobacterium* genomics**

*A.O. Charkowski*
*Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Dr., Madison WI USA 53706*
*Email: acharkowski@wisc.edu*

*Pectobacterium* species cause soft rot, blackleg, and stem rot in potato and a wide range of other vegetable crops and ornamental plants. Diseases caused by *Pectobacterium* are controlled mainly through use of healthy planting material, sanitation and copper sprays. The environment has a large effect on development of diseases caused by *Pectobacterium* and disease incidence can be unpredictable. The pathogen is spread by water, seed, equipment and insects. Little is understood about plant resistance to soft rot bacterial pathogens and, unlike some wild potato species, no commercial potato cultivars are completely resistant to *Pectobacterium*. Multiple *Pectobacterium* species may be found in the same field and even on the same plant. *Pectobacterium* strains vary in aggressiveness and virulence genes encoded, but there are many similarities across the genus. Over the past decade, genomic studies have provided new insights into *Pectobacterium* biology. For example, some strains of this pathogen may elicit plant cell death to promote disease in leaves. This pathogen also produces a volatile compound that may affect the plant ethylene signaling pathway and may act as an insect attractant. Recent work with a machine learning program has identified several novel target genes likely to contribute to plant-microbe interactions, suggesting that there is still much to learn about how soft rot bacteria cause disease.

**O17.011 Molecular signaling in rice immunity**

*K. Shimamoto*
*Laboratory of Plant Molecular Genetics, NAIST, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan*
*Email: simamoto@bs.naist.jp*

We have been studying molecular signaling in rice immunity by studying the small GTPase OsRac1 and its interacting proteins by using a variety of methods. We have identified a number of OsRac1-interacting proteins and studied their functions and interactions with other proteins. We found that OsRac1 interacts with two types of receptors; membrane-bound receptor-like kinases and NB-LRR type receptors. OsRac1 forms a protein network with several cheperones and co-chaperones, SGT1, RAR1, Hsp90, Hsp70, and Hop/Sti1. The OsRac1 network includes enzymes such as NADPH oxidase and CCR which are important for immune responses. We revealed a pathway for chitin-induced immunity in rice. Based on genetic, protein-protein interaction, and biochemical studies we propose that OsRac1 is a "hub" of rice innate immunity where PTI and ETI pathways merge. We also propose that these proteins form complex termed 'defensome'. Based on the recent biochemical analysis we found PTI and ETI receptors form separate defensomes but contain the same chaperones in each defensome. Our results suggest that the defensome complex is a key regulatory system for rice immunity.

**O17.012 Comparative genomic analyses of '*Candidatus* Liberibacter spp' and proteomic study of citrus in response to huanglongbing**

*H. Lin*
*United States Department of Agricultur, San Joaquin Valley Agricultural Sciences Center, Parlier, CA 93648, USA*
*Email: hong.lin@ars.usda.gov*

'*Candidatus* Liberibacter' species are gram negative, phloem- restricted, unculturable α-proteobacteria associated with citrus huanglongbing (HLB) and potato zebra chip (ZC) diseases. Successful completion of genome sequences of four pathogenic Liberibacter species; '*Candidatus* Liberibacter asiaticus', '*Candidatus* Liberibacter africanus', '*Candidatus* Liberibacter americanus' and '*Candidatus* Liberibacter solanacearum' provides insights into genomic, genetic and evolutionary adaptation of the complex pathosystems. Comparative genomic analysis of multiple Liberibacter genomes identified common and unique putative functions of virulence genes/factors in Liberibacter species. To better understanding molecular basis of host response to hunaglongbing, proteomic analyses via 2-DE and mass spectrometry were employed to elucidate protein expression profiles of citrus plants that were infected or uninfected with '*Candidatus* Liberibacter asiaticus'. Proteomic analysis identified an array of genes that were differentially expressed in response to the infection. These include some defense responsive genes such as Cu/Zn superoxide dismutase, chitinases, lectin-related proteins, miraculin-like proteins, and peroxiredoxins that were significantly up-regulated in early asymptomatic and later symptomatic disease stages. Information of Liberibacter genomes and pathogen-host interactions derived from this study advance our knowledge in understanding Liberibacter associated diseases.

**O17.013 The fungal effector AvrPiz-t targets the ubiquitination proteasome system for its virulence and avirulence activities in rice**

*C.H. Park[1,*], G. Shirsekar[1,*], S. Chen[1,*], B. Zhou[1*], C.H. Khang[3], P. Songkumarn[1], A.J. Afzal[1], Y. Ning[2], M. Bellizzi[1], B. Valent[3] and G.L. Wang[1,2]*

*[1]Department of Plant Pathology, Ohio State University, Columbus, Ohio 43210 USA; [2]Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; [3]Department of Plant Pathology, The Kansas State University, Manhattan, KS 66506 USA*

*Email: wang.620@osu.edu*

Although the function of effector proteins of plant bacteria and oomycete pathogens has been elucidated in the recent years, the information for plant fungal effectors is still lacking. We found that the avirulence effector AvrPiz-t from the rice blast fungus *Magnaporthe oryzae* preferentially accumulates in the biotrophic interfacial complex (BIC), and is translocated into rice cells. Ectopic expression of *AvrPiz-t* in transgenic rice causes suppression of the flg22- and chitin-induced reactive oxygen species (ROS) generation and enhances susceptibility to *M. oryzae*, indicating that AvrPiz-t has virulence function to suppress the PAMP triggered immunity (PTI) in rice. Interaction analyses show that AvrPiz-t suppresses the E3 ligase activity of two rice RING E3 ligases, APIP6 and APIP10, and in return, the two E3 ligases ubiquitinate AvrPiz-t *in vitro*. We also found that AvrPiz-t promotes the degradation of APIP6 and APIP10 *in vivo*. Silencing of *APIP6* and *APIP10* in the non-*Piz-t* background leads to a significant reduction of flg22-induced ROS generation, suppression of defense gene expression and enhanced susceptibility to *M. oryzae*. Interestingly, silencing of *APIP10* in the *Piz-t* plants causes strong cell death, accumulation of Piz-t and enhanced resistance to virulent isolates. Taken together, our results demonstrate that AvrPiz-t suppresses PTI for its virulence through manipulating the E3 ligase APIP6 in the non-*Piz-t* plants but activates ETI through targeting the E3 ligase APIP10 that leads to the accumulation of Piz-t and activation of downstream defense responses in the *Piz-t* plants.

**O17.014 Aquaporin AtPIP1;4 is a ligand protein-interacting $CO_2$ transport channel in the plasma membrane**

*L. Li, Z.Z. You, H.T. Ji, S.L. Sang, S.T. and H.S. Dong*

*Laboratory for Plant Growth and Defense Signaling Research, State Ministry of Education Key Laboratory of Integrated Management of Crop Pathogens and Insect Pests, Nanjing Agricultural University, Nanjing 210095, China*

*Email: hsdong@njau.edu.cn*

In mammals, a newly discovered function of aquaporins is to regulate physiological responses through interacting with other proteins. Here, we show that a plant aquaporin, plasma membrane (PM) intrinsic protein AtPIP1;4 of *Arabidopsis thaliana*, utilizes a similar mechanism to facilitate $CO_2$ transport across the PM in response to Hpa1, a harpin protein from a bacterial plant pathogen. Interaction occurs in a receptor-ligand binding manner through a glycine-triplet motif located in an extracellular region of the AtPIP1;4 sequence and nitroxyl terminus of Hpa1. A proline- threonine-proline motif present in the same extracellular region of AtPIP1;4 directs the specificity in Hpa1 binding to AtPIP1;4, but not to its isoforms. AtPIP1;4 binds with Hpa1 at the PM to facilitate $CO_2$ transport by leaf stomata and mesophyll cells, resulting in enhanced photosynthesis and growth of the plant. Our findings suggest that AtPIP1;4 is a ligand protein-binding, physiologically relevant $CO_2$ channel in the PM.

**O17.015 Characterization of the Clp regulon in *Xanthomonas oryzae* pv. *oryzae***

*F. Tian, B. Li, H.M. Chen, M.S. Wu and C.Y. He*

*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China*

*Email: ftian@ippcaas.cn*

Cyclic dimeric guanosine monophosphate (c-di-GMP) is a widely conserved second messenger in bacteria to regulate diverse biological activities by interacting with various receptors. It has been shown in several *Xanthomonas* species that the CRP-like-protein (Clp), a global transcriptional regulator, is a c-di-GMP receptor. In this work, we characterized the Clp homolog in *X. oryzae* pv. *oryzae* (Xoo), the causal agent of bacterial blight disease on rice. Using isothermal titration calorimetry (ITC), we demonstrated that Clp binds to c-di-GMP with high affinity as a 1:1 stoichiometry *in vitro*. To identify the target promoters of Clp in Xoo, we expressed Clp-HA protein in $PXO99^A$ Δ*clp* strain and carried out chromatin immunoprecipitation followed by sequencing (ChIP-seq). 65 putative Clp binding sites were detected, including the previously reported *engXCA* gene promoter region. 40 of them were selected for further confirmation by electrophoretic mobility shift assays (EMSAs), and only 5 showed negative results. It was previously demonstrated in *X. campestris*, c-di-GMP allosterically inhibited the binding of Clp to the promoter of *engXCA*. To investigate the influence of c-di-GMP on binding between Clp and the target promoters in Xoo, we added c-di-GMP in EMSAs. Surprisingly, most of the binding became stronger in the presence of c-di-GMP. We also performed qRT-PCR experiments to study the gene expression. In the 14 gene tested, six of them were positively regulated by Clp, while eight were negatively regulated. Taken together, these results suggested the regulatory role of Clp might be very complicated.

**O17.016 Combining genomic and proteomic approaches in the identification of bacterial symbionts in the banana aphid (*Pentalonia nigronervosa*)**

*C. De Clerck, T. Tsuchida, S. Massart, P. Lepoivre, F. Francis and M.H. Jijakli*
*Plant Pathology Department - Liege University - Gembloux Agro-Bio Tech, Passage des Déportés 2, 5030 Gembloux, Belgium*
*Email: sebastien.massart@ulg.ac.be*

The Banana Bunchy Top Virus (BBTV), transmitted by the aphid *Pentalonia nigronervosa*, is one of the most harmful viral diseases of banana and plantains. BBTV is difficult to detect and manage and leads to significant production decline. The existing control methods are not efficient enough, making this virus a serious threat for the banana production worldwide. Previous studies have yet considered the possibility of insects' symbiont implication in the transmission of some plant viruses. But studies have been mostly related to Luteoviridae and the case of the BBTV (Nanoviridae) was never investigated in spite of its economic importance. As a first step in the characterization of a potential interaction between the virus and the vector, this work aimed to determine the symbiotic population of several strains of *P. nigronervosa* using complementary approaches. Five aphid's strains were studied, coming from Burundi, Madagascar and Gabon. For the genomic approach, bacteria's 16S DNA regions were amplified using universal primers then sequenced. Results obtained were confirmed using specific primers targeting the identified bacteria. For the proteomic approach, 2D DIGGE was used in order to check for differences in protein content between a total *P. nigronervosa* protein extraction, a symbiont protein extraction from the same aphid and a total protein extraction from an *Acyrthosiphon pisum* aphid only containing *B. aphidicola*. The two approaches led to the same results and to the detection of two bacterial symbionts in *P. nigronervosa*. Phylogenetic analyses were made to replace those among other insects and aphids's symbionts.

**O17.017 Comparative genomics of phytopathogenic and beneficial *Herbaspirillum* bacteria**

*M.Y. Chen, S.T. Ye, B. Zhu and Q.L. An*
*State Key Laboratory of Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou 310058, P. R. China*
*Email: chenmy2007525@163.com*

*Herbaspirillum* bacteria have been widely isolated from plants, particularly important cereals in the family *Poaceae*, such as rice, maize, sugarcane and sorghum. *H. seropedicae*, the type species of the genus *Herbaspirillum*, can colonize in plants and promote plant growth by biological nitrogen fixation. Like the most studied beneficial *H. seropedicae* strain SmR1, five *Herbaspirillum* strains Os34, Os38, Os44, Os45 and Os49 isolated from rice roots contain genes for nitrogen fixation, indoleacetic acid synthesis, siderophore production and 1-aminocyclopropane-1-carboxylate deaminase. However, they inhibited the growth of rice seedlings and induced hypersensitive response in tobacco leaves. We thus made a deep genome comparison among the five pathogenic *Herbaspirillum* strains and strain SmR1 to see the genetic variation related to the pathogenesis. All the six genomes contain gene clusters for pathogenesis-related secretion systems including type I, type II, type III, type V and type VI secretion systems and the twin-arginine translocase secretion system. Their genomes also contain genes that encode type IV pili components, putative RTX toxins and hydrolytic enzymes involved in degradation of plant cell walls. A major genetic variation has been found in the type III secretion systems. The deduced protein components of the type III secretion systems of the five pathogenic *Herbaspirillum* strains show lower degrees of similarities to those of strain SmR1 compared with a pathogenic *H. rubrisubalbicans* strain M1. This suggests that the type III secretion systems in the five pathogenic *Herbaspirillum* strains may determine their pathogenesis.

**O17.018 Phenotype variation of a rhizobacterium *Paenibacillus polymyxa***

*Y. M. Lee, S.H. Kim[2], Y.S. Kim[2] and Y.H. Jeon[2]*
*[1]Department of Biology, Andong National University, Andong 760-749, Republic of Korea; [2]Department of Bioresource Sciences, Andong National University, Andong 760-749, Republic of Korea*
*Email: yongbac@andong.ac.kr*

*Paenibacillus polymyxa* E681 isolated from a winter barley root was reported as a promising plant growth-promoting rhizobacterium (PGPR). During comprehensive study we found that two different colony types appeared when the strain was grown on tryptic soy agar at 28℃, and it was also found that the strain E681 displayed phenotype variation in colony type, that is, "type I colony" having a milky white, round, convex, and shiny morphology underwent colony type variation into "type II colony" having a translucent, diffuse, scalloped edge, and not shiny morphology. Interestingly, the type II colonies looked very similar to type I colony when they cultured on TSA added high dextrose. Probably exopolysaccharide of the type II colonies was seemed to be induced by high dextrose. To study differentiation mechanism between the two types, two-dimensional electrophoresis (2-DE) was performed. 2DE revealed that minimum 200 proteins showed at least a two-fold increase or decrease in type II colonies. Some of the most prominent proteins were identified based on peptide analysis by MALDI-TOF. The type II colonies produced endospores less than the type I colonies and showed reduced capacities for root colonization,

root elongation, and antagonism against several fungal plant pathogens. Further study indicated that type II colonies lacked the capacity of root elongation in cucumber at 20℃ but not at 30℃. Our results showed that the phenotypic variation affected the biological activity of the strain E681, therefore phenotype variation of the strain E681 should be carefully taken into account before field application of the PGPR.

**O17.019 High-throughput metabolomics for the study of pathosystems: Case study the soybean - *Rhizoctonia solani* pathosystem**

*S. Jabaji and K.A. Aliferis*
*Department of Plant Science, McGill University, 21111 Lakeshore Rd., Sainte-Anne-de-Bellevue, Quebec, H9X 3V9, Canada*
*Email: suha.jabaji@mcgill.ca; konstantinos.aliferis@mcgill.ca*

The high-throughput dissection of interactions in plant-pathogen pathosystems applying metabolomics is challenging. Our main goal is the establishment of a comprehensive and standardized metabolomics pipeline for the study of the interaction between *Rhizoctonia solani* anastomosis group 4 (AG4) and soybean (*Glycine max* (L.) Merr). Analyses were performed by an original metabolomics approach integrating Orbitrap MS with GC/MS analyses. Key element in our approach is the construction of species-specific metabolite library, which accelerates the identification step, a restricting factor for large-scale metabolomics experiments and the use of bioinformatics software for the biological interpretation of results. The developed metabolomics/bioinformatics protocols enabled a global and comprehensive overview of the soybean metabolome and its perturbations in response to *R. solani* 24 and 48 h post-infection, and the detection of more than 4,000 features in Orbitrap chromatograms in positive (ESI+) and negative (ESI-) electrospray modes. The activation of the plant defense mechanism was expressed as a general disturbance of its metabolic status leading to increased biosynthesis of compounds that are bioactive, act as precursors for the biosynthesis of bioactive plant secondary metabolites or act as chemical signals. Metabolites belonging to the flavonoid, isoflavonoid, phenylpropanoid, and jasmonic acid biosynthetic pathways were up-regulated or *de novo* produced in response to pathogen attack, which is indicative of their importance as primary components of the soybean defense mechanism against *R. solani*. Understanding the global biochemical mechanisms occurring during soybean-*R. solani* interaction provides valuable information that could be exploited in applications in biotechnology, biomarker-assisted selection, and agrochemical industry.

**O17.020 Genes and Proteins differentially expressed in cassava during *Enterobacter cloacae* infection**

*J. Faks[1], N. Diez[1] and M. Santana[2]*
*[1]Laboratorio de Genómica y Proteómica, Fundación Instituto de Estudios Avanzados (IDEA), Valle de Sartenejas, Baruta, Edo. Miranda, 1015-A, Venezuela; [2]Laboratorio de Biotecnología agrícola, Departamento de Biología Celular, División de Ciencias Biológicas, Universidad Simón Bolívar, Valle de Sartenejas, Baruta, Edo. Miranda, 1080, Venezuela.*
*Email:josefaks@gmail.com*

Cassava (*Manihot esculenta* Crantz) is a major root crop widely grown in the tropics, and currently the sixth world food staple. In Venezuela, big losses in cassava production are caused by phytopathogenic bacteria. *Enterobacter cloacae* (*Ecl*), a recent reported bacterial specie infecting different plants like onions, coconut, ginger, maize, macadamia, mulberry and cassava. The aim of this work was to study the response of resistance and susceptible varieties of cassava to *Enterobacter cloacae* infection. In order to study genes differentially expressed during *Ecl* infection, a microarray hybridization was performed using cDNA from cassava resistant and susceptible varieties, 1 and 7 dai. Proteins differentially expressed were studied by bidimensional gels using total proteins isolated from cassava resistant and susceptible varieties at 0.5, 1, 2 and 7 dai. Non inoculated plants were used as control. A total of 109 genes were found to be differentially expressed (85 up-regulated and 24 down-regulated) mainly involved in metabolism, signaling and defense such as thaumatin, β-1,3 glucanase, NADP oxidoreductase, flavonoide 3´5´ hidrolase and AvrRpt2-induced AIG2. A total of 118 proteins were found to be differentially expressed (72 up-regulated and 46 down-regulated) mainly involved in metabolism, signaling and defense such as ribulose biphosphate carboxylase, RABD1 GTPase, auxin-responsive protein, defensins and anthocyanidin 3-O-glucosyltransferase, mostly expressed at 0.5 and 7 dai. Several genes and proteins with unknown function or showing no similarity to other proteins described previously were also expressed. In general, the results obtained showed an early defense response in resistance but no in susceptible varieties.

**O17.021 Profiling arrays of disease resistance-related genes in *Arabidopsis* and *Brassica* lineages**

*S.Y. Liu, J.Y. Yu, C.B. Tong and J.Y. Huang*
*Oil Crops Research Institute of CAAS, Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Wuhan 430062, P. R. China*
*Email: liusy@oilcrops.cn*

Diseases caused by fungi, bacteria and viruses are one of main constraints in plant growth and crop production. To counteract these constraints, plants have evolved a multiple-layer defense system. This system is largely

controlled by a set of genes. In this set of genes, however, some are conserved – inherited from ancestral species – and some are acquired through mutation/divergence of tandem duplicated genes or through other evolutionary mechanisms. The duplication-derived genes are consequently clustered on chromosomes. In this study, we identified all possible resistance-related genes in the sequenced genomes of the family Brassicaceae *Arabidopsis thaliana*, *A. lyrata*, and the relatives *Brassica rapa*, *B. oleracea* and their synthetic species *B. napus*. Further we compared differences of these gene arrays and traced these gene origins (for example, whole genome duplication/triplication, tandem duplication or homeologous recombination, paralogous gene conversion). Finally, we analyzed evolution of known function genes associated resistance to specific disease categories between species. These results will provide genome-wide fundamental information for the resistance-related gene evolution and thus promote more gene function identification.

**O17.022 Proteomic analysis of the compatible interaction between Non-heading Chinese cabbage and downy mildew**

*C.Z. Sun,T.K. Liu, X.L. Hou and Y. Li*
*College of Horticulture, Nanjing Agricultural University, 1# Weigang, Nanjing, Jiangsu, 210095, P.R. China*
*Email: yingli@njau.edu.cn*

Downy mildew (DM) is a serious fungal disease in non-heading Chinese cabbage (*Brassica campestris* L. ssp. *chinensis* Makino) caused by *Peronospora parasitica* Pers. Ex Fr., which infects members of the *Brassicaceae* family. It is important to understand the defense mechanism to downy mildew in non-heading Chinese cabbage. However, the proteins and their involved pathways in the interaction between non-heading Chinese cabbage and *P. parasitica* are still not largely understood. To determine the proteins, proteomes from non-heading Chinese cabbage seedling leaves that had or had not been infected with *P. parasitica* were characterized at different time points post infection by 2-DE and by MALDI-TOF/TOF MS/MS and database- searching protein identification. Approximately 600 protein spots and more than 400 highly reproducible protein spots ($P<0.05$) were detected on 2-DE gels. Mass spectrometry identified 95 differentially expressed proteins with significant intensity differences (>1.5-fold, $p<0.05$) in mock- and *P. parasitica*-infected leaves at least at one time point. These proteins were involved in 13 cellular responses and metabolic processes including carbohydrate metabolism, signal transduction, protein biosynthesis, photosynthesis, protein folding and assembly, energy pathway, cell rescue and defense, cell cycle, nitrogen metabolism, lipid metabolism amino acid metabolism, transcription regulation, and secondary metabolite biosynthesis. The identification of such differentially expressed proteins provides new targets for future studies. Our study indicated a pathogen-respon- sive protein network in *P. parasitica*-infected non-head- ing Chinese cabbage leaves. The study will enable future detailed investigation of gene expression and function linked with the interaction between non-heading Chinese cabbage and *P. parasitica*.

**P17.001 Phylogenomics of efflux – evolutionary diversification of membrane transporters in plant pathogenic fungi**

*G. Koczyk, D. Popiel, A. Dawidziuk and J. Kaczmarek*
*Institute of Plant Genetics Polish Academy of Sciences, Poznań, Poland*
*Email: gkoc@igr.poznan.pl*

Efflux of natural and artificial toxic compounds is one of the main tools in the molecular repairtoire of eukaryotic microorganisms competing for resources in a limiting environment. Increasing amount of data on both sequence and function of novel genes, permits large-scale inquiries into modes of evolution and diversification of function among previously difficult targets such as membrane transporters of ABC (ATP-Binding Casette) and MFS (Multidrug Facilitator Superfamily) transporter classes. We created MetaSites, a database combining sequential, structural and functional annotations for over 150 model fungal genomes as well as well as 'gold standard' structurally and functionally characterised proteins from PDB and UniProt/SwissProt. The developed tool enabled us to trace the evolution of function-determining residues (e.g. active sites, pores, exon-intron junctions and domain boundaries) across both protein and DNA levels. We demonstrate phylogenetic reconstruction and analysis of Maximally Parsimonious Reconciliations (between species phylogeny and histories of individual gene superfamilies) for duplication-loss-transfer scenarios likely in evolution of ABC and MFS transporters. As supporting evidence we considered exon-intron architecture and genomic context (synteny of conserved orthologs). Several scenarios leading to emergence and functional overlap of novel efflux specificities for both natural (mycotoxins, phytoalexins) and artificial (fungicides) substances are discussed in detail, with particular focus on plant pathogenic *Sordariomycetes* and *Dothideomycetes*.

**P17.002 Distribution and evolution of non-self recognition genes in Basidiomycota**

*M.A. van der Nest, Å. Olson, M. Lind, H. Velez, K. Dalman, M. Brandström-Durling, M. Karlsson and J. Stenlid*
*Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, SE-750 07, Sweden*
*Email: magriet.vandernest@fabi.up.ac.za*

In dikaryotic fungi, vegetative incompatibility is controlled by a regulatory signaling network involving proteins encoded at the *het* (heterokaryon incompatible) loci. Despite the wide occurrence of vegetative incompatibility, the molecular identity and structure of only a small number of *het* genes and their products have been characterized. The aim of this study was therefore to use the information available for the model fungi *Neurospora crassa* and *Podospora anserine* to identify homologs of *het* genes in the genomes of other fungi, especially the Basidiomycota. Putative *het-c*, *het-c2* and *un-24* homologs, as well as sequences containing the NACTH, HET or WD40 domains present in the *het-e*, *het-r*, *het-6* and *het-d* genes were identified. Putative genes that share significant similarity with *het-e*, *het-r* and *het-d* that encode HET, NACTH and WD40 domains, *i.e.*, HNWD family proteins, were only identified in some members of the Ascomycota. However, certain representatives of the Basidiomycota encoded these domains in the same genomic region. Although homologs of *het-S* are known only from the Sordariomycetes, we also identified a putative homolog of this gene in *Gymnopus luxurians* in the class Agaricomycetes. Furthermore, with the exception of *un-24*, all of the putative *het* genes identified occurred in a multi-copy fashion, some with species-specific expansions. Overall our results indicated that gene duplication followed by gene loss and expansion, as well as multiple events of domain fusion and shuffling played an important part in the evolution of *het* gene homologs of Basidiomycota and other filamentous fungi.

**P17.003 Characterisation of the mating type loci in the fungus, *Amylostereum areolatum***

*C.M. Botha, M.A. van der Nest, M.P.A. Coetzee and B. Slippers*
*Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*
*Email: magriet.vandernest@fabi.up.ac.za*

*Amylostereum areolatum* is a white-rot fungus involved in an obligate symbiosis with the woodwasp *Sirex noctilio*. *A. areolatum* has a tetrapolar mating system, thus mating is controlled by two mating type loci, mat-A and mat-B. Effective spread of asexual spores by the *Sirex* woodwasp has resulted in the presence of clonal populations in certain regions of the world. In this study we utilized the recently sequenced genome of *A. areolatum* to investigate the structure and organization of the mating type loci. We were able to identify the two mat loci by using sequences of the pheromone receptor (RAB1) located within mat-B and the mitochondrial intermediate peptidase (MIP) linked with mat-A in local BLAST searches. Further research using BLAST analyses as well as gene prediction and domain identification computer software allowed the genetic architecture of the mat-A and mat-B loci to be inferred. The structure of both the mat-A and the mat-B loci was highly similar to that of the model Basidiomycetes. Despite the close symbiosis with the woodwasp and a predominant asexual mode of reproduction, the mating type loci of *A. areolatum* thus appear to be similar in structure and function to those of other Agaricomycetes.

**P17.004 A genomic approach to study mating type genes of sugarcane smut disease fungus *Sporisorium scitamineum***

*M.C.P. Kmit[1], L.M. Taniguti[1], G.Carvalho[1], G. Gasparin[1], L.L. Coutinho[1], S.A.C. Souza[2], L.E.A. Camargo[1] and C.B. Monteiro-Vitorello[1]*
*[1]ESALQ, University of São Paulo, Av. Pádua Dias, 11, CEP 13418-900, Piracicaba, Brazil; [2]Centro de Cana, Rodovia Antonio Duarte Nogueira km 321, CEP 14001-970, Ribeirão Preto, Brazil*
*Email: cbmontei@usp.br*

*S. scitamineum* has a bipolar mating type system, where only compatible or incompatible reactions are found. Smut species need to produce dikaryotic hyphae before successfully infecting the host cell and completing its sexual cycle. Two loci are described as involved in mating type assignment: locus a and locus b. In bipolar species, the two loci are tightly linked in the same chromosome segregating after meioses only two mating types (+ or -). A BAC genomic library was constructed to characterize mating type genes of *S. scitamineum*. The library is composed of 2880 clones distributed in 23 plates of 96 wells. The insert average size is 92 kbp as determined by a DNA sample of 80 clones digested with *Hin*dIII. Clones of each one of the 30 plates were pooled in one tube and used in PCR screening for regions of interest. Primers amplifying a fragment of locus b were used to select BAC inserts that were completely sequenced. Gene content and organization of both loci were compared to ortholog genes in close related fungus *S. reilianum* and *S. hordei*. The library constructed will be also a useful resource for comparative genomic data by BAC-end sequencing and anchoring to other sequenced genomes, and for sequencing assembly of the *S. scitamineum* complete genome.

**P17.005 Comparison and proteome analysis of *Puccinia psidii* uredospores from eucalyptus and guava**

*M.C. Quecine, T.F. Leite, A.P. Bini, T.R. Capaldi, L.M. Franchischini, S. Guidotti, F.G. Marques and C.A. Labate*
*Laboratory Max Feffer of Plant Genetics, Department of Genetics, Escola Superior de Agricultura "Luiz de Queiroz" – University of São Paulo (ESALQ-USP). 11, Padua Dias Av., 13418-900, Piracicaba-SP, Brazil*
*Email: mquecine@usp.br*

*Puccinia psidii* is a very destructive eucalyptus rust, it attacks also a wide range of plant species, specially the myrtle family. It is unique based on the fact that the spermogonia stage was never found in other life stages, which conferee the status of hemicyclic and autoecious to the species. The uredospores are crucial to the dissemination of this pathogen. In this work we report the first proteome analysis of *P. psdii* uredospores structure from two contrasting sources: guava's fruits (PpGuava) and eucalyptus's leaves (PpEucalyptus). NanoUPLC-MSE was used to generate peptide spectra which were matched with a partial proteome, predicted from *P. graminis, P. striiformis* and *P. triticina* using the *Puccinia* Group Database (Broad Institute), partially sequenced *P. psidii* genome and UniProt *Puccinia* genera sequences. The best results were obtained from UniProt and *P. psidii* databases, out of which, 515 and 233 protein spectra, were detected, respectively. Concerning the expression analysis from ProteinLynx, around 300 proteins were differentially expressed, according to the sample source. A significant amount of exclusive proteins, from PpEucalyptus our PpGuava, were found, demonstrating the variability between the two fungal populations. This data was corroborated with a high number of variants and/or isoforms found for some protein groups. Many structural proteins such histones, beta-tubulins, among others, were identified from both databases. Moreover, some genes, as malate dehydrogenase, peptidases, correlated with fungal virulence, were identified; interestingly, almost all of them were over-expressed in PpGuava. These results lead to new proteomic approaches to study plant–pathogen interactions.

**P17.006 PsGcn5, a histone acetyltransferase of *Phytophthora sojae*, is involved to cyst germination, the stressful conditions and virulence**

*W. Zhao, T. Wang and R.D. Qi*
*Institute of Plant Protection and Agro-products Safety, Anhui Academy of Agricultural Sciences, No. 40 Nongkenan Road, Hefei, P. R. China*
*Email: rende7@126.com*

Microorganisms must rapidly induce and repress various transcriptional networks in order to adapt to the stressful conditions of the infected host. One aspect of this rapid transcriptional response is the ability to remodel chromatin, allowing transcription factors access to the promoters of important stress response genes. Acetylation of specific lysine residues of histone proteins is one mechanism for chromatin remodeling that leads to altered transcription. In eukaryotes, histone proteins are acetylated by the Gcn5 protein as part of larger, multi-subunit, chromatin remodeling complexes. In this study, using the homologue sequence blast, we identified the *Phytophthora sojae* PsGcn5 histone acetyltransferase, which has a conserved histone acetyltransferase domain and a conserved bromodomain. Real-time PCR analysis showed that *PsGcn5* was upregulated in cyst germination and during early infection. To elucidate the function, the expression of *PsGcn5* was silenced using stable transformation of *P. sojae*. The silencing of *PsGcn5* displayed a reduced cyst germination rate when compared to the recipient strain. *PsGcn5*-silenced mutants displayed defectes in osmotic response mediated by NaCl and reduced pathogenicity. Our results indicate the importance of chromatin remodeling by the conserved histone acetyltransferase PsGcn5 in regulating the expression of specific genes that is involved to cyst germination, the stressful conditions and virulence in *P. sojae*.

**P17.007 New insights in an old tale of structure for fungal NADPH oxidase gene families**

*N. Dátry, J. Choi, F.O. Asiegbu, J.P.T. Valkonen and Y.H. Lee*
*FiDiPro Group, Department of Forest Science, University of Helsinki, 00014 Helsinki, Finland*
*Email: nicolas.detry@helsinki.fi*

NAPDH oxidase (Nox) genes are known to play essential biological functions in cell signaling and transduction, production of reactive oxygen species (ROS) and pathogenicity in fungi. The general Nox structure has been deduced from the human Nox2 ($gp91^{phox}$) and bioinformatic identification of Nox has always been compromised due to the fact that ferric reductases (Fre) and ferric-chelate reductases (FRO) share high structural similarities. In order to better distinguish between the different three Nox isoforms (NoxA, NoxB and NoxC) and Fre/FRO in fungi, we used a combination of four different bioinformatic analyses to examine 310 putative fungal Nox. Our results showed that the fungal Nox structure is more diverse and differing than previously thought and we are providing the community with a new set of rules and requirements for the Nox identification by bioinformatic analyses. We further conclude that the average number of Nox genes within fungal genomes is 2-3 and that NoxC is restricted to species within the Ascomycota phyla.

**P17.008 Comparative proteomic analysis of differentially expressed proteins in mycelium, conidia and germinating conidia of *Fusarium oxysporum* f. sp. *conglutinans* between different races**

*E.F. Li, G.Wang,Y.H. Yang and B.Y. Xie*
*Institute of Vegetables and Flowers, CAAS, No.8 South Zhongguancun Street, Haidian District, Beijing, 10010, P. R. China*
*Email: xiebingyan@caas.cn*

*Fusarium oxysporum* is a soilborne fungus with a wide variety of hosts. It induces wilt and root rots on plants

and causes severe losses. Two physiological races (52557$^{-TM}$ and 58385$^{-TM}$) of the *Fusarium oxysporum* f. sp. *conglutinans* have been identified according to their differential reactions with the same cabbage genotypes. In this study, we provided the first high resolution of proteome reference maps related to mycelium, conidia and germinating conidia of *Fusarium oxysporum* f. sp. *conglutinans*, and revealed the differentially abundant proteins by analyzing the comparative proteomic responses of mycelium、conidia and germinating conidia between races. In total, 1162,1117 protein spots were detected in mycelium of two isolates, 461,626 protein spots in conidia, and 682,648 protein spots in germinating conidia. By comparing the DIGE gels, 288 protein spots were significant variation existed in two isolates. These proteins were identified by mass spectrometry (MS) and MASCOT database searches, of which, 74 protein spots identified from mycelium in the race1(38) and race2(36),82 from conidia in the race1(21) and race2(61) and 132 from germinating conidia in the race1(91) and race2(41). The majority of them were posttranslational modification, carbohydrate transport and metabolism related proteins, followed by energy production, conversion, amino acid transport and metabolism related proteins. These proteome-level differences suggest posttranslational modification, carbohydrate transport and metabolism associated proteins may play important roles in the differences about growth, development and pathogenicity of the two physiological races. Among the identified proteins, 31 were predicted to be secreted proteins, one of which belongs to Six effectors. Six effectors were well known in *Fusarium oxysporum* f. sp. *lycopersici* which were required for virulence on tomato. It was studied further on *Fusarium oxysporum* f. sp. *conglutinans* in our work.

**P17.009 Mitochondrial genomics of Oomycetes**

*F.N. Martin*
*USDA-ARS, 1636 East Alisal St., Salinas, CA 93905, USA*
*Email: frank.martin@ars.usda.gov*

The mitochondrial genomes of a range of *Phytophthora* and *Pythium* have been sequenced and while they encode a similar number of genes their sizes can differ by as much as 75% (size range from 37.5 to 73.5 kb). This is due to a large inverted repeat that is present in *Pythium* (representing as much as 85% of the genome) but absent in *Phytophthora* (several species have an inverted repeat but these are much smaller). While some intrageneric differences in gene order are reflective of phylogeny, many are not. Comparative genomics has proved useful for identifying gene order differences in *Phytophthora* compared to *Pythium* and plants for designing a highly specific real time PCR assay for *Phytophthora.* This marker system generates a single amplicon with annealing sites for a genus as well as species specific TaqMan probe. Based on polymorphisms observed in sequence alignments this marker system should provide a systematic approach for designing species specific probes for a wide range of species. Comparative analysis of mitochondrial genomes within the genus *Phytophthora* and among multiple isolates of the same species has also identified polymorphisms useful for mitochondrial haplotype analysis. Comparative analysis of the mitochondrial genomes of *Pythium* suggest a similar approach could be used for developing diagnostic markers and identifying mitochondrial haplotypes.

**P17.010 Comparative secretome analysis reveals perturbation of host secretion pathways by a hypovirus**

*J.Z. Wang, Q. Chen, L.M. Shi and B.S. Chen*
*State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources and Key Laboratory for Microbial and Plant Genetic Engineering, Ministry of Education, College of Life Science and Technology, Guangxi University, Nanning, 530004, P. R. China*
*Email: chenyaoj@gxu.edu.cn*

To understand the impact of a hypovirus infection on the secretome of the chestnut blight fungus (*Cryphonectria parasitica*), a phytopathogenic filamentous fungus, two-dimensional electrophoresis (2-DE). and isobaric tag for relative and absolute quantitation (iTRAQ) technology were employed to identify and quantify the secreted proteins. A total of 403 unique proteins were identification from the secretome of the wild type virus-free strain EP155. Of these proteins, 326 were predicted to be involved in known secretory pathways and they are primarily composed of metabolic enzymes, biological regulators, responders to stimulus and components involved in plant-pathogen interactions. When infected with the hypovirus CHV1-EP713, 99 proteins were found to be differentially expressed as compared to the wild type strain EP155. These proteins were mainly related to plant cell wall degradation, response to host defense, fungal virulence and intracellular structure. The changing tendency of these secreted proteins indicated potential relationship between special physiological pathways and hypovirulence.

**P17.011 Whole-genome sequencing and genomic variation between *Microdochium nivale* and *M. majus***

*L.E. Jewell and T. Hsiang*
*School of Environmental Sciences, University of Guelph, Guelph, Ontario, N1G 2W1, Canada*
*Email: ljewell@uoguelph.ca*

*Microdochium nivale* and *M. majus* are fungal plant pathogens causing pink snow mold on cereals and grasses. Both species are among the causal agents of Fusarium Head Blight, and *M. nivale* is also responsible for Fusarium patch on turfgrasses in the absence of

snow cover. Although these species are closely related, *M. majus* has not been observed growing on turfgrass in the field, whereas *M. nivale* is known to infect both cereals and grasses. To investigate the genetic variation between *M. nivale* and *M. majus*, and to better understand the variation between isolates of *M. nivale* originating from different host species, the genomes of two isolates of *M. nivale* (one from turfgrass and one from wheat) and one isolate of *M. majus* were sequenced using Illumina next-generation sequencing technology. The raw reads from each isolate were assembled using the freely-available programs SOAPdenovo, ABYSS, and velvet, and genes were predicted using AUGUSTUS. Whole-genome comparisons were performed using BLAST and BLAT searches to compare the genomes to each other and to a selection of other filamentous Ascomycete genomes. Putative genes that were unique to each group were identified, as well as genes that may be unique to wheat-derived isolates as opposed to turf-derived isolates, and unique to these species compared to other Ascomycetes. These experiments elucidate whole-genome differences between and among closely-related species and further demonstrate the usefulness and accessibility of next-generation sequencing technology and results to plant pathologists.

**P17.012 Genome sequencing of sordariomycetous plant pathogens to obtain primers for detecting mating type genes**

*T. Hsiang, K. Watanabe, L. Lah and Y. Deng*
*School of Environmental Sciences, University of Guelph, Guelph, Ontario, N1G 2W1, Canada*
*Email: thsiang@uoguelph.ca*

We sequenced the genomes of over 20 Sordariomycetes, including species from the orders Diaporthales, Glomerellales, Hypocreales, Magnaporthales, Ophiostomatales, and Xylariales, with important plant-pathogenic genera such as *Colletotrichum, Microdochium, Gaeumannomyces, Nectria* and *Pestalotiopsis*. For most of these fungi, we used the Illumina HiSeq2000 to produce paired-end reads of 100 bp and over 100 times coverage. The genomes were assembled with three different assembly programs, ABYSS, SOAPdenovo and VELVET. We searched among the contigs from each assembly using previously identified Sordariomycete mating type genes as query sequences in StandAlone BLAST analysis (TBLASTN). The regions 20 kb upstream and downstream of the mating type gene (MAT) loci were also examined for conserved or predicted genes. In addition to the various types of MAT gene idiomorphs encountered, many other genes were found including APN2 (DNA lyase) and SLA2 (cytoskeleton assembly control gene) which commonly flank mating type loci, as well as other genes such as APC5 (ubiquitin ligate) and COX13 (cytochrome oxidase). Rather than using conserved primers based on related species, complete genome sequencing is a more efficient approach to obtaining primers from flanking genes which can be used to detect the presence of mating type idiomorphs in other isolates of the same species. This allows for inferences about the sexual reproductive process of the pathogen and epidemiology of the disease.

**P17.013 An overview of gene family related to DNA methylation and histone modification in Oomycetes**

*X.W. Wang, M. Han and L.Y. Guo*
*College of Agriculture and Biotechnology, China Agricultural University , No.2 West Yuanmingyuan Road, Haidian District, Beijing, 100193, P. R. China*
*Email: xiaowenwang@cau.edu.cn; ppguo@cau.edu.cn*

In eukaryote, phenotype is regulated not only by genome sequence, but also regulated by the mechanism of Epigenetics, such as DNA methylation and histone modification. The histone modification includes histone acetylation, methylation, phosphorylation, and ubiquitination. To investigate if similar mechanism of Epigenetics existe in the organism of Oomycetes, the geneome of published species of Oomycetes were BLAST searched and predicted using the SMART, Pfam databases, Signal 3.0, and TMHMM. Results of the study showed that all three known classes of HDACs, HATs, HMTs, HDMTs, phosphorylases and ubiquitin family protein associated with histone modification were found in the genome of *Phytophthora infestans*, *P. sojae*, *P. capsici*, *P. ramorum*, and *Albugo laibachii*. However, no C-5 cytosine-specific DNA methyltransferase was found in these Oomycetes. All these sequences shared high similarity with those of close related organisms, Diatoms, Diplomonads and Ciliates, and those in yeast, human and Arabidopsis. These results indicated that gene regulation through histone modification, but not DNA methylation is involved in Oomycetes. In general, this study provides an overview of gene family related to DNA methylation and histone modification in sequenced Oomycetes and set up a foundation for future functional analysis of HDACs in gene regulation in Oomycetes.

**P17.014 Genome structure and sequence of the sugarcane smut fungus**

*Z.Q. Wang[1], Y.M. Liao[1,2] ,G.N. Zhu[3], C.W. Zou[4],Y. Ren[5], Z.M. Zhou[5],Y. Feng[5], Q. Chen[4], L.M. Shi[4], P. Li[4], L. Wang[5] and B.S. Chen[1,4]*
*[1]State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Guangxi Univieristy，Nanning, 530005, P. R. China; [2]College of Agriculture, Guangxi University，Nanning, 530005, P. R. China; [3]Guangxi Academy of Agricultural Sciences, Nanning, 530007, P. R. China; [4]College of Life Science and Technology，Guangxi University，Nanning, 530005, P. R. China; [5]TEDA School of Biological Sciences and*

*Biotechnology, Nankai University, Tianjin 300457, P. R. China*
*Email: chenyaoj@gxu.edu.cn*

Smut caused by *Sporisorium scitaminea* Sydow is a major sugarcane disease that can cause considerable yield loss. To better understand the pathogenesis mechanism of *Sporisorium scitaminea*, the haploid strain JG36 isolated from Guangxi province of China, was used to construct the physical map. The 19.60Mb assembled genomic sequence were determined by Roche 454, Solexa, and AB3730xl platforms. Twenty-two chromosomes with a total of 19.02Mb were identified by optical mapping. Transcriptomes from haploid strains JG36 and JG35 and a diploid strain were sequenced by Solexa technology and a total of 6,558 coding genes were annotated. Genes specifically expressed at haploid and diploid stages were identified. Our sequencing project lays a solid foundation for future study of the molecular biology of *Sporisorium scitaminea*.

**P17.015 *Shk1* gene of *Sclerotinia sclerotiorum* is required for the pathogen stress response and sclerotial formation**

*Y.B. Duan, C.Y. Ge and M.G. Zhou*
*College of Plant Protection, Nanjing Agricultural University; Key Laboratory of Monitoring and Management of Crop Diseases and Pest Insects, Ministry of Agriculture; Key Laboratory of Pesticide, Jiangsu Province, Nanjing, 210095, P. R. China*
*Email: mgzhou@njau.edu.cn*

Fungal histidine kinases (HKs) are involved in osmotic and oxidative stress responses, hyphal development, fungicide sensitivity, and virulence. Members of HK class III are known to signal through the HOG mitogen-activated protein kinase (MAPK). In this study, we characterized *Shk1* gene, which encodes a putative class III HK, from the plant pathogen *Sclerotinia sclerotiorum*. Disruption of *Shk1* resulted in resistance to phenylpyrrole and dicarboximide fungicides and in increased sensitivity to hyperosmotic stress and $H_2O_2$-induced oxidative stress. The *Shk1* mutant showed a significant reduction in vegetative hyphal growth and was unable to produce sclerotia. qRT-PCR and glycerol determination assays showed that the expression of *SsHOG1* (the last kinase of the Hog pathway) and glycerol accumulation were regulated by the *Shk1* gene. In addition, the *Shk1* mutant was unable to infect leaves of rapeseed, strawberry, tomato, and cucumber plants. All the defects were restored by genetic complementation of the *Shk1* deletion mutant with the wild-type *Shk1* gene. These findings indicate that *Shk1* is involved in vegetative differentiation, sclerotial formation, glycerol accumulation, adaption to hyperosmotic and oxidative stresses, and sensitivity to fungicides in *S. sclerotiorum*. These results, which are inconsistent with the results obtained with some filamentous fungi, suggest that the role of two-component histidine kinases in fungal development and virulence can differ among filamentous fungi.

**P17.016 The effect of JS399-19 to proteome of *Fusarium graminearum***

*Y.P. Hou* and *M.G. Zhou*
*College of Plant Protection, Nanjing Agricultural University, Nanjing, 210095, P. R. China*
*Email: mgzhou@njau.edu.cn*

JS399-19 (2-cyano-3-amino-3-phenylancryic acetate), a novel cyanoacrylate fungicide, had powerful inhibitory against Fusarium species, especially to *Fusarium graminearum*. In order to investigate the effect of JS399-19 to protein expression profiles of *F. graminearum*, total protein of *F. graminearum* treated by JS399-19 or nothing was extracted and proteomic analysis was performed using two-dimensional gel electrophoresis in conjunction with MALDI-TOF MS/MS. The expression levels of 38 proteins varied quantitatively with twofold and 33 proteins were identified successfully by MALDITOF MS/MS. Through classification of physiological functions from Conserved Domain Database analysis, among the identified proteins, 19, 2, 5, 3, 2 and 2 proteins were associated with metabolism, motility, regulatory, defense, signal transduction, and unkonwn function respectively. The expression levels of the genes were further confirmed by quantitative real-time PCR analyses. This study represents the first proteomic analysis of *F. graminearum* treated with JS399-19 and will contribute to a better understanding of the mode of action of JS399-19 against *F. graminearum*.

**P17.017 Genome sequence of the peach leaf curl pathogen *Taphrina deformans***

*H. Liu[1], C. Wang[1] and J.R. Xu[1,2]*
*[1]NWAFU-PU Joint Research Center, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P.R.China; [2]Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA*
*Email: jinrong@purdue.edu; liuhuiquan@nwsuaf.edu.cn*

Peach leaf curl, caused by the Archiascomycete fungus *Taphrina deformans*, is one of the most common diseases in peach growing areas around the world. *T. deformans* is a dimorphic plant parasite, with a biotrophic filamentous phase that colonizes and produces asci on infected plant tissues and a saprophytic yeast phase that grows by budding. To better understand this primitive dimorphic plant pathogenic fungus that is most closely related to the common ancestor of higher ascomycetes and basidiomycetes, we generated a high-quality draft

genome of *T. deformans* by paired-end Illumina sequencing in this study. The *T. deformans* genome has at least 17 chromosomes that vary from approximately 260- to 820-kb in length by CHEF gel electrophoresis. The genome assembly is 13.2-Mb and 96% of it is contained in the largest 83 scaffolds. Overall, 3.3% of the genome was identified as repetitive DNA. Gene prediction shows 6,380 gene models of 20 amino acids or more. Among these, 230 encode carbohydrate-active enzymes (CAZymes) and 108 encode candidate effectors. Transcriptome analysis revealed that most of the top up-regulated genes during plant infection encode putative effectors. Similar to what has been observed in the corn smut fungus *Ustilago maydis*, many of these up-regulated effector genes are in clusters in the *T. deformans* genome. Motif analyses revealed that 20 of these putative effectors harbor three novel motifs with similar arrangements, which may function as translocation signals similar to the RxLR motif characterized in Oomycetes. Because of the evolutionary status of *T. deformans* and its compact genome, it will be important and highly efficient to functional characterize these putative effectors and candidate motives likely involved in effector delivery.

### P17.018 Identification of a fungi-specific lineage of protein kinases closely related to metazoan tyrosine kinases

*Z.Z. Zhao*[1], *H.Q. Liu*[1], *C.F. Wang*[1] *and J.R. Xu*[1,2]
[1]*Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, P. R. China;* [2]*Department of Botany and Plant Pathology, Purdue University, USA*
*Email: jinrong@purdue.edu; zhzht621@126.com*

Tyrosine kinases (TKs) specially catalyze the phosphorylation of tyrosine residues in proteins. In animals, TKs play essential roles in cell proliferation, differentiation, immune response, organ development, and other cellular processes. Previous studies have demonstrated that TK genes underwent significant duplication and loss during the evolution of metazoans. More recently, tyrosine kinases were demonstrated to be established before the divergence of filastereans from the Metazoa and Choanoflagellata clades. Many organisms out of the Metazoa clade, including a few fungi, were also found to encode TK or TK-like genes. To better understand their evolution, it is important to identify and analyze TKs from as many organisms as possible, including fungi. In this study, we systematically searched for TKs across the fungal kingdom. A total of 71 candidate TKs from 32 fungi were identified. Furthermore, we constructed phylogenetic trees to determine the evolutionary relationships of these candidate TKs with the known TKs. The results showed there is a fungi-specific linage of protein kinases that located out of the metazoan and protozoan TK cluster but are most closely related to them. This result confirmed that ancestral TK genes emerged before the split of eukaryotes and expanded in animals especially in metazoans, but lost in fungi during evolution. In addition, analysis of TK-specific motifs showed that the fungi-specific linage of protein kinases probably have no TK activities.

### P17.019 Maize kernels resistance mechanisms against *Aspergillus flavus* identified by comparative proteomics

*L. Balzano-Nogueira*[1] *and N. Diez*[2]
[1]*Laboratorio de Biometría y Estadística. Fundación Instituto de Estudios Avanzados IDEA. Valle de Sartenejas, Caracas 1080, Venezuela;* [2]*Laboratorio de Genómica y Proteómica. Fundación Instituto de Estudios Avanzados IDEA.*
*Email: leobalzano@gmail.com*

*Aspergillus flavus* infection kernel and preharvest aflatoxin contamination of maize (*Zea mays*) kernels represent a major problem of crop production, not only in Venezuela but throughout the world, but little progress has been made in identifying proteins and metabolic pathways associated with pathogen resistance. In developing countries, major efforts to solve this problem have been made, mainly by deep molecular research. This work aims to identify proteins with differential expression pattern that could confer resistance to maize lines against *A. flavus*. Protein extracts from whole grains of four (4) lines of genetically infected previously identified as resistant lock (2) and susceptible (2) to infection by *A. flavus* was obtained by extraction with buffers of different pH values. In each case, samples of soluble proteins were quantified and characterized by their profiles obtained by silver stained two dimensional gel electrophoresis (2D) after isoelectric focusing (IEF) and then identified by MALDI-TOF MS. As a result, 40 proteins were identified with at least two-fold differential expression patterns between resistant and susceptible lines after infection with *A. flavus*, suggesting that they may play an important role in plant-pathogen resistance, role confirmed by statistical analysis and bioinformatics tools that eventually could be used as a predictive resistant model against a pathogen.

### P17.020 The genome organization of lucerne transient streak and turnip rosette sobemoviruses

*M. Sõmera and E. Truve*
*Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia*
*Email: merike.somera@ttu.ee*

A sobemovirus genome is a small (4 kb) polycistronic positive sense ssRNA molecule that differs from the cellular RNAs by having no cap structure or a polyA tail.

Instead, it has a genome-linked viral protein (VPg) covalently attached to the 5' end of the viral RNAs. A typical sobemovirus possesses diverse assortment of strategies (e.g. a ribosomal leaky scanning, a ribosomal frameshifting, a polyprotein processing and a usage of subgenomic RNAs) to regulate its' genome expression. In general, sobemoviral genome consists of 3 open reading frames (ORFs): ORF1, ORF2a/2a2b and ORF3. ORF1 encodes an RNA silencing suppressor. ORF2a encodes a polyprotein that is processed at least into 4-5 different proteins: N-terminal anchor, viral serine protease, VPg and C-terminal protein(s). A viral RNA-dependent RNA polymerase is expressed via a -1 frameshift from ORF2a frame into ORF2b frame after the VPg-encoding region. ORF3 encodes viral coat protein. However, unlike other sobemoviruses, lucerne transient streak virus (LTSV) and turnip rosette virus (TRoV) have been reported to contain two successive ORF1s (denoted as ORF1a and ORF1b) instead of the single ORF1. Also, their ORF2a/2a2b has been mapped to a region ca. 200 nucleotides downstream from that of other sobemoviruses, leading to the lack of transmembrane segments at the N-termini of P2a/2a2b. In the current study, we resequenced this region for TRoV and LTSV. The hypothetical beginning of ORF1b was mapped as the beginning of ORF2a/2a2b for both TRoV and LTSV. Computional analysis revealed transmembrane segments at the N-termini of the TRoV and LTSV polyproteins.

**P17.021 Genome-wide association analysis *in Oryza sativa* L. identifies resistance genes against *Magnaporthe oryzae* with high correlation to biotic stress tolerance QTLs**

*M.A. Franco[1], Y, Jayaro[2], A. Gonzalez[2] and I. Galindo-Castro[1]*
*[1]Laboratorio de Genómica y Proteómica. Fundación Instituto de Estudios Avanzados (IDEA). Caracas, Venezuela; [2]Fundación DANAC. Yaracuy, Venezuela*
*Email: igalindocastro@gmail.com; marialefranco@gmail.com*

Pathogen resistance traits screening has been especially important in breeding programs, since a large part of the cultivars worldwide are continually affected by different pests and diseases. In Venezuela, a devastating pest of rice crops is the fungus *M. oryzae*, the causal agent of rice blast disease. Recently many breeding programs have been directed toward the use of molecular markers to identify QTLs and their incorporation into elite lines despite of the lack of knowledge about the genes located within these regions. However, the genetic study of QTLs has benefited with the massive generation of complementary information through genomics and transcriptomics. In this work, after a genome-wide association study (GWAS) to identify candidate resistance genes against *M. orizae* in *O. sativa*, we then evaluated their potential quantitative significance by associating biotic stress tolerance QTLs reported by the *Gramene* database as located in the neighborhood of the gene loci identified by GWAS. Our results suggest a reliable method to evaluate the potential quantitative contribution of candidate genes, previously identified by their differential expression of mRNAs, to the resistance phenotype. Currently, experiments are being designed to validate the association of candidate genes in a larger group of both resistant and susceptible materials to provide more data to substantiate the methodology and to allow its incorporation into the rice breeding programs.

**P17.022 Generation of recombinant inbred lines (RILs) for genetic mapping and studies of the inheritance of metabolic synthesis in sesame (*Sesamum indicum* L.)**

*R.N. Syed[1], R. Splivallo[2] and P. Karlovsky[2]*
*[1]Department of Plant Pathology, Sindh Agriculture University Tandojam, 70060, Pakistan; [2]Insitute of Phytopathology and Plant Protection, Georg August University of Goettingen, Grisebachstasse 6, 37077, Goettingen, Germany*
*Email: syedarehanashah@yahoo.com*

The spectrum of secondary metabolites produced by plants constitute a central part of the chemical phenotype, which plays an important role in defense against pathogens and pests. The relationship between metabolic and genomic diversity has rarely been studies before. No linkage map of sesame is available, which hampers studies of the genetics of metabolic synthesis in this crop. The present study was conducted to advance a cross of two sesame accessions, generate preliminary AFLP data for the characterization of the RILs, and to generate metabolic profiles of a subset of the segregating population. A cross of two parent accessions that showed significant polymorphism revealed by AFLP was performed. Single seed descendents were selfed for five generations. AFLP markers were used to assess the relationship between the parents and RILs. Three primer combinations produced 123 polymorphic bands between the parents. AFLP data obtained will be used for the construction of a linkage map by collaborating sesame breeders in Antalya University, Turkey. Metabolic fingerprinting was done by using high performance liquid chromatography (HPLC) with full-scan mass spectrometric (MS) detection. 15 reproducible polymorphic signals were detected in parents. 6 RILs were used for the analysis of polymorphic metabolic signals.

**P17.023 Proteomics-based anlysis reveals *Verticillium dahliae* toxin induces cell death by modifying host proteins synthesis**

*C.J. Xie, C.Y. Wang, X.K. Wang and X.Y. Yang*
*The College of Life Science, Chongqing Normal University, Chongqing 401331, P. R. China*

*Email: yangxy94@swu.edu.cn*

*Verticillium dahliae* is one of the most destructive soil-borne fungal pathogens that cause vascular wilt diseases in a wide range of important crop plants, including cotton. However, the mechanisms employed by this pathogen to infect this crop have not been fully described. In the present study, we firstly investigated changes in protein abundance during the initial interaction between cotton roots and *V. dahliae*. Among the proteins that were upregulated upon infection, some were related to reactive oxygen species (ROS), whereas among those downregulated upon infection were proteins involved in normal metabolism or cell structure. Further experiments confirmed that a sudden release of ROS and cell death accompany *V. dahliae* infection in the cotton vasculature. The further analysis indicated that a *V. dahliae* supernatant induced lesion formation in tobacco leaves required de novo protein synthesis but not active gene expression. Lesion formation is dependent on the age of leaves, but the known ROS burst or the ubiquitin/26S proteasome system is not a prerequisite.

**P17.024 Proteomic analysis of banana roots of different resistance cultivars inoculating with *Furarium oxysporum* f. sp. *cubense* race 4**

*X.S. Li , X.L. Ruan, Y.F. Li, W.J. Shen and H.P. Li*
*College of Natural resources and Environment, South China Agricultural University, No.483 Wushan Road, Tianhe District, Guangzhou, 510642, P. R. China*
*Email: huaping@scau.edu.cn*

Banana fusarium wilt is one of the most destructive disease and results in critical losses in main banana production region distributed in the world. Until now, the effective methods to control the disease are still unknown. To investigate the interacted factors between different resistant banana cultivars with *Fusarium oxysporum* f. sp. c*ubense* race 4 (FOC4) at translational level and to understand the resistant mechanism against the infection of pathogen, we performed proteomic profiling analysis on resistant and susceptible banana cultivars against pathogen at 3 days after the treatment of inoculation with FOC4. Proteomic analysis of FOC4-innoculated roots showed that 45 of differentially expressed proteins were discovered after infection, and the function of 40 of proteins is annotated. The different proteins were associated with abiotic or biotic stress induced proteins, antifungi compound synthesis, signal translation, antioxidation and cell wall fortification. By compared the defense proteins between resistant and susceptible banana cultivars, we found that the abiotic or biotic stress proteins and antifungi proteins were more induced in susceptible cultivars, while antifungi proteins and lignin biosynthetic enzymes were more induced in resistant cultivars. Majority of these proteins except antioxidative enzymes, were positively regulated after infection. In addition, our results indicated that FOC4 coordinated other cellular activities such as photosynthesis and metabolism including the fixation of nitrogen. Taken together, the findings revealed the complexity of pathogen-induced resistance mechanisms and provided important clues for designing strategies to control fusarium wilt diseases on banana.

**P17.025 Characterization of quantitative trait loci (QTLs) in cultivated wheat contributing to resistance to sheath blight (*Rhizoctonia solani*)**

*M.Z. Che, Z.J. Zhang, H.Y. Zhang and J. Chen*
*Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing, 100193, P. R.China*
*Email: cauchemingzhe@163.com*

Sheath blight, caused by *Rhizoctonia solani*, is one of the most important diseases of wheat in the world. For many years it occurs in many areas and greatly affected the yield. Despite extensive searches of disease resistance, the major gene(s) which give complete resistance to the fungus have not been identified. However, many results have proved that there was much variation in quantitatively inherited resistance to *R. solani*, and this type of resistance can offer adequate protection against the pathogen under field conditions. Using 53 $F_8$ populations from a cross between Luke and AQ24755-83, including 35 resistant strains and 19 susceptible strains, one year of greenhouse disease evaluation and 155 well-distributed SSR markers, we identified one quantitative trait loci (QTLs) contributing to resistance to *R. solani* by composite interval mapping with threshold LOD $>2.5$. This QTL is located on the long arm of chromosome 4A and 4 SSR markers were significantly associated with it.

**P17.026 Reactions of the leading wheat cultivars, planted in six provinces of China, to stripe rust fungus race CYR33**

*Z.Y. Du, H.Y. Zhang, Y. Guo and Z.J. Zhang*
*College of Agriculture and Biotechnology, CAU, No 2 Yuanmingyuan West Road, Haidian District, Beijing, 100193, P. R. China.*
*Email: dp123705@163.com*

Stripe rust constitutes a severe damage to wheat in China. Use of host resistance is an important strategy to manage the disease. In recent years, the stripe rust fungus race CYR33, virulent to YrSu as well as other Yr genes, became extensively predominant over the fungus population. The objective of this study was to examine the reactions of the leading wheat cultivars planted in the six provinces to CYR33. In 2010, we collected 312 wheat cultivars which were widely grown in these provinces, comprising more than 90% of the wheat acreage. In 2011 and 2012, these cultivars, together with the susceptible

reference wheat Ming Xian 169 (MX), were tested in greenhouses on their infection types at both seedling and adult plant growth stages. The results showed that 20% of the cultivars performed high resistance at all growth stages, as represented by such cultivars as Chuanmai 53, Tian 9473, Yumai 58, Yumai 69, Zhoumai 17, and Zhongliang 26. Approximately 25% of the cultivars were as susceptible as MX, including such cultivars as Chuanyu 20, Jimai 22, Luomai 23, Shannong 15, Taikong 6, Wenmai 18, Sinong 389, Xinong 2611, Xinxiang 9178, Yannong 21, and Yumai 49-198. The remaining 55% of the cultivars exhibited moderate resistance to moderate susceptibility. More than three-fourths of the moderate cultivars had susceptible infection types at seedling stage, while they became more resistant with aging. These results indicate that a considerable number of cultivars possess adult plant resistance. Analysis of quantitative resistance is being under way on these cultivars.

**P17.027 The construction of a BAC-based physical map of wheat of durable resistance to stripe rust**

*Y. Guo, M.X. Che, Z. Wang and Z.J. Zhang*
*Department of plant pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing, 100193, P.R. China*
*Email: guoyan001@cau.edu.cn*

Wheat is one of most principle grain crop in the world, BAC-based physical maps of which are of crucial importance for the research of wheat genomics. A whole-genome BAC library-physical map for *Triticum aestivum*, one hexaploid wheat with durable resistance genes of stripe rust, leaf rust, sharp eyespot and powdery mildew diseases from USA, is being constructed with the basis of high density genetic map. In contrast with the construction of λ DNA BAC library, the construction of wheat BAC library is more difficult with different and complicated genetic composition. We have developed the PFGE to purify the quality of HWM DNA to ligation and transformation, and the higher the purity of DNA selected, the higher the efficiency of electrotransformation (3-5 times of PFGE depuration ). The weight of DNA has a remarkable effect on the ligation. The efficiency of BAC library construction was improved by the edulcoration of consistent 150kb DNA. The BAC library-physical map is shown here to be an efficient resource and platform for the development of wheat genome, the clone of resistant genes of diseases, and molecular assisted selection.

**P17.028 Diversity arrays technology (DArT) for constructing genetic linkage map of wheat**

*Z. Wang, F. Lin M.Z. Che, J.D. Ren and Z.J. Zhang*
*Department of plant pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing, 100193, P.R. China*
*Email: zhangzj@cau.edu.cn*

Diversity arrays technology(DART)is a microarray hybridization-based molecular marker technique, and discover genetic polymorphic markers by different site distribution of particular restriction enzyme in different genotype materials. Furthermore DArT can detect and type DNA variation at several hundred genomic loci without relying on sequence information. Here we show that it can be effectively applied to genetic mapping and diversity analyses of wheat. In previous work of our laboratory, a linkage genetic map of chromosomes anchored with SSR DNA markers and covering all 21 chromosomes was constructed by using the intervarietal mapping population derived from two wheat lines Luke (LU) and AQ24755-83 (AQ) of quantitative resistance. At present, there are more then 500 anchored markers in this linkage genetic map. However，there is still lack of anchored markers in some chromosome section up to affect the accuracy of QTL analysis. To increase the number of anchored markers, a mapping population of 90 recombinant inbred lines (RILs) developed using single-seed descent from a cross between LU and AQ was characterised by DArT analysis. The total number of DArT marker loci was combined with the previous genetic linkage map to increase genetic length and a marker density of DNA marker loci. The wheat stripe rust resistance QTL will be identified effectly using the composite interval mapping facility of Window QTL Cartographer 2.5.

## Concurrent Session 18-Global Seed Health Concerns and Solutions

### O18.001 Improved detection and monitoring of seed-borne plant pathogens in China

*J.Q. Li[1, 2], L.X. Luo[1, 2], N. Jiang[1, 2], X.L. Liu[1, 2], P.F. Liu[1, 2] and Y.S. Cao[1, 2]*
*[1]Department of Plant Pathology, China Agricultural University; [2]Beijing Engineering Research Center of Seed and Plant Health (BERC-SPH) / Beijing Key Laboratory of Seed Disease Testing and Control (BKL-SDTC), Beijing, P. R. China*
*Email: lijq231@cau.edu.cn*

China is one of the most important seed production countries in the world. Seed production industry makes significant contribution to local economic development. However, seed-borne diseases have been proved to be a serious bottleneck constraining seed quality and the seed industry, and caused substantial economic losses. The most economical and effective way to control seed-borne diseases is to ensure that seeds are pathogen-free via seed health testing. During the past ten years, with the support from government, many seed health and disease testing centers have been established in China. Seed health testing has been widely noted by seed companies and institutes. More detection methods for seed-borne pathogens have been increasingly used in China. For seed-borne fungi, washing test, blotter test, agar plate methods, and PCR techniques were used for the detection of *Tilletia* spp., *Ustilago maydis*, *Peronospora* spp., *Fusarium* spp., and *Phoma* spp. etc, from cereal and vegetable seeds. For seed-borne bacteria, such as *Clavibacter michganensis* subsp. *michiganensis* (Cmm) on tomato and *Acidovorax citrulli* on cucurbits, the Bio-PCR methods were used commonly and improved by semi-selective media modification. Moreover, the Bio-ELISA was developed for Cmm detection from seeds by combining mSCM liquid medium with ELISA. For seed-borne viruses (e.g., *Tobacco mosaic virus*, *Pepper mild mottle virus*, *Cucumber green mottle mosaic virus*, *Squash mosaic virus*), Bio-assay combined with DAS-ELISA and RT-PCR methods were established and used for seed assay. Detection and monitoring of seed-borne plant pathogens have been made great strides in China.

### O18.002 Development of chemical seed treatments, a future outlook

*S.C. Knight, S.E. Roberts and R.E. Zeun*
*Syngenta Asia Pacific Pte. Ltd., No.1 Harbour Front Avenue #03-03 Keppel Bay Tower, Singapore 098632*
*Email: susan.knight@syngenta.com*

Farmers throughout the world are increasingly sowing improved, high-yielding seed varieties. As a consequence, they are seeking more advanced seed treatment products that will protect their costly investment, ensuring that each seed produces a healthy seedling that is free from pest and disease damage. Several other factors are driving the adoption of new seed treatments. The increasing cost and decreasing availability of farm labour is favouring the use of products that give long-lasting disease and pest protection, reducing the need for intervention in the early stages of the crop. Trends in pathogen populations, particularly the evolution of strains with fungicide resistance, will also shape future strategies for developing chemical seed treatments. In some markets, older fungicide seed treatments are being superseded by newer products that offer broader disease spectrum and higher potency at lower use rates. A recent innovation is the introduction of seed treatments based on active ingredients from the succinate dehydrogenase inhibitor (SDHI) class of fungicides. Seed treatments that contain SDHI fungicides, such as sedaxane, offer extended disease spectrum and longer duration of protection against seedling, soil borne and seed borne pathogens. They also represent another tool for the management of fungicide resistance. Trends in the development of chemical seed treatments are not confined to the introduction of new active ingredients. Innovation in the development of seed treatment formulations will be equally important in ensuring that seeds are protected with products that deliver the correct combinations of active ingredient to the seed, with optimal coverage and low dust-off.

### O18.003 Non-chemical seed treatment in the control of seed-borne pathogens

*E. Koch*
*JKI, Institute for Biological Control, Darmstadt, Germany*
*Email: eckhard.koch@jki.bund.de*

In certain situations the use of chemical seed treatments may not be possible or even unwanted, like in organic farming or if effective chemicals are difficult to get or too expensive. Among the physical methods thermal treatment in water is probably the one that is mostly used. Apart from broad spectrum activity against various seed-borne fungal pathogens, hot water treatment also provides a comparatively high level of control of seed-borne bacterial pathogens. For this purpose it is commercially used in vegetable production. In order to overcome the need for re-drying the seeds after treatment a method based on hot, humidified air (Thermoseed®) has been developed. It is in practical use for the treatment of cereals in Scandinavia. Another fairly new technology (e-ventus®) uses electrons to kill pathogens located on the seed surface or in the outer layers of the seed coat. A number of other physical seed treatment methods have been tried but generally not exceeded the stage of experimentation. Different organic materials like plant extracts or powders (*e.g* Tillecur®)

provide protection against seed-borne pathogens. However, often their use is limited by low efficacy, phytotoxicity or technological properties of the formulations that are not compatible with modern farming equipment. A number of micro-organism preparations are recommended as seed treatments, but few have been developed specifically for the control of seed-borne pathogens. Among the latter are products (*e.g*. Cedomon®) based on *Pseudomonas chlororaphis* that are used in Scandinavia and other parts of Europe for control of seed-borne fungal pathogens of cereals.

**O18.004 Advancements of Seed Pathology in Latin America and the Caribbean: Development and international impact**

*I.N. Salas-Torres*
*Institute of Colombian Agriculture (ICA), Carrera 41 No. 17-81 Zona Industrial de Puerto Aranda, Bogotá D.C. Colombia*
*Email: isabel.salas@ica.gov.co; ciat-ica@cgiar.org; isabelnatalia@hotmail.com*

Training or capacity building in tropical Latin America and the Caribbean (LAC) in agriculture and related sciences received an important boost in 1967 with the establishment of two important international organizations: the former Danish Government Institute of Seed Pathology for Developing Countries (DGISP), later the Danish Seed Health Centre for Developing Countries (DSHC), and the International Center for Tropical Agriculture (CIAT) headquartered in Palmira, Colombia. During the last 25 years, Danida's support has been key to several major achievements in the Colombian and LAC seed sectors. Five Colombian professionals have received training in seed pathology at the former DGISP, and the CIAT Seed Health Laboratory and the Seed Pathology Laboratory of the Institute of Colombian Agriculture (ICA) were established. Routine seed health tests were also implemented within Colombia's and CIAT's seed certification and plant quarantine schemes. Four national training courses in seed pathology were held directed towards professionals of ICA and the private and academic sectors. The ICA-Danish Seed Health Center (DSHC)-CIAT initiative to establish a Seed Health Center for Latin America, to be headquartered in Colombia, was officially recognized, and the Regional Capacity-Strengthening Platform for Seed Systems was established and three workshops on seed systems held in Colombia and Bolivia.

**O18.005 Technical challenges for specific, sensitive detection of seed-borne bacterial pathogens**

*N.W. Schaad[1*], C.N. Mortensen[2], J.Q. Li[3], L.X. Luo[3], J.J. Feng[4], A. Mazzaglia[5] and G. Balestra[5]*
*[1]USDA, ARS, Foreign Disease Weed Science-RU, Ft. Detrick, MD USA; [2]Danish Seed Health Centre, Dept. of Plant and Environmental Sciences, University of Copenhagen, Denmark; [3]Dept. Plant Pathology, China Agric. University, Beijing, P.R. China; [4]Animal & Plant Quarantine Technology Center, Shenzhen, P.R. China; [5]Dept. Agric. Forestry, Nature and Energy, University of Tuscia, Italy.*
**Retired, 2414 Rikkard, Thousand Oaks, CA 91362*
*Email: lionschaad@gmail.com*

Seed-borne pathogens are a major threat to agriculture production and security in a fast-moving global economy. As global trade increases so does the threat of accidental and deliberate introduction of seed-borne pathogens. Seed transmitted diseases can result in severe economic losses. The challenge is for government and industry to cooperate in providing pathogen-free seeds. Seeds can be assayed and infested lots destroyed or treated by chemical and/or physical means and re-assayed. Considerable progress has been made in developing reliable sensitive and specific assays. However, technical challenges remain. Seeds are challenging because they are often heavily contaminated with saprophytic bacteria. This makes agar plating difficult and often inhibits molecular-based protocols such as PCR. Use of DNA sequence information for designing highly specific and sensitive PCR primers is especially challenging. To avoid false negative results in classical PCR, internal controls of primers targeting bacterial 16S rDNA or plant 26S mitochondrial rDNA can be applied. Perhaps the most reliable and sensitive PCR protocol is real-time PCR using probe-based protocols such as TaqMan. The biggest challenge to PCR has been problems with PCR inhibitors present in seeds. These inhibitors can be partially removed using such treatments as heat, DNA extraction, immunocapture, and BIO-PCR. BIO-PCR not only reduces inhibitors but greatly increases sensitivity by allowing the target bacterium to multiply. This expands our ability to detect low numbers of pathogens even in seeds contaminated with large numbers of saprophytes. A major challenge for plant quarantine is dealing with PCR positive results without cultures for confirmation.

**O18.006 Global standards in seed health testing**

*T.A.S. Aveling and G.P. Munkvold*
*ISTA SHC chair, Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa*
*Email: terry.aveling@fabi.up.ac.za*

Routine seed health testing is carried out in most countries for seed certification and plant quarantine. However, the majority of seed health tests used throughout the world have never been subject to rigorous validation. Discrepancies between testing methods can occur, leading to costly phytosanitary disputes or liability claims.

These issues can be avoided by working toward a system of universally accepted, standardized testing methods on a global level. To ensure that seed health tests are standardized and give reliable and reproducible results in accordance with the given specifications of the test methods, methods should go through a peer review system and/or collaborative study among laboratories. Three primary organizations publish standardized seed health tests: the International Seed Testing Association (ISTA), the International Seed Health Initiative (ISHI), and the U.S. National Seed Health System (NSHS). In 1957, the ISTA Plant Disease Committee (PDC) established a comparative seed health testing program to standardize techniques for detection of seed-borne pathogens. In 1993, the Seed Health Committee (formerly the PDC) began development of published guidelines for comparative testing. All ISTA validated methods are published in the Annexe to Chapter 7 of the International Rules for Seed Testing. TESTA, an EU FP7 project, focuses on the development of innovative new seed health tests which will also be validated by ISTA. Additionally, some ISHI-Veg methods have been accepted as ISTA Rules and as Standards by the NSHS. The procedures followed by ISTA, ISHI and the NSHS to achieve global standards in seed health testing will be discussed.

### O18.007 Seed-borne pests and phytosanitary issues: the role of EPPO

*F. Petter, M. Suffert, A.S. Roy, D. Griessinger and M. McMullen*
*European and Mediterranean Plant Protection Organization, 21 Boulevard Richard Lenoir 75016, Paris, France*
*Email: petter@eppo.int*

One of the main aims of EPPO is to help its member countries to prevent entry or spread of dangerous pests (plant quarantine). The Organization has therefore been given the task of identifying pests which may present a risk (early warning), evaluating their risk for the region and making proposals on the phytosanitary measures which can be taken against them (Pest Risk Analysis). Once a pest has been identified as presenting a risk for the EPPO region, recommendations on how to detect and identify the pest may be developed (diagnostic protocols and phytosanitary procedures for inspection) as well as recommendation on how to eradicate and control this pest. To perform these activities, much information on pests presenting a risk to the EPPO region is required and is collected by the Organization and made available to its member countries. Different databases have been developed including PQR (Plant Quarantine data Retrieval system) and the EPPO database on Diagnostic expertise. In addition to pest specific activities, EPPO has also developed recommendation for quality assurance in laboratories, in order to promote harmonization of procedures in the EPPO region. The different activities conducted in this framework are presented with a special focus on activities conducted for seed-borne pests.

### O18.008 Detection and quantification of *Verticillium dahliae* in spinach seed

*D. Duressa, G. Rauscher, S.T. Koike, B. Mou, R.J. Hayes, K. Maruthachalam, K.V. Subbarao and S.J. Klosterman*
*United States Department of Agriculture, Agricultural Research Service, 1636 E. Alisal St. Salinas, CA 93905*
*Email: Steve.Klosterman@ars.usda.gov*

*Verticillium dahliae* is a soilborne fungus that causes Verticillium wilt on multiple crops in central coastal California. Although spinach crops grown in this region for fresh and processing commercial production do not display Verticillium wilt symptoms, spinach seeds produced in the United States and Europe are commonly infected with *V. dahliae*, and may contribute to Verticillium wilt epidemics on crops grown in rotation with spinach. A sensitive, rapid, and accurate method for quantification of *V. dahliae* in spinach seed may help identify highly infected lots and curtail their planting, and thereby minimize the spread of exotic strains via spinach seed. A quantitative real-time polymerase chain reaction (qPCR) assay was employed for detection and quantification of *V. dahliae* in spinach germplasm and commercial spinach seed lots. The assay used *V. dahliae*-specific primer pair (VertBt-F and VertBt-R) and an analytical mill for grinding the tough spinach seed for DNA extraction. The assay enabled quantification of *V. dahliae* in spinach seed, with a sensitivity limit of ≈1 infected seed per 100 (1.3% infection in a seed lot). Quantification was reproducible between replicate samples of a seed lot and among different real-time PCR thermocyclers. A pathogen DNA content corresponding to a quantification cycle value of ≥31 corresponded with a percent seed infection of ≤1.3% when tested on commercial seed lots. The assay is useful in qualitatively assessing seed lots for *V. dahliae* infection levels, and the results of the assay can be helpful to guide decisions on whether to apply seed treatments.

### O18.009 Electron treatment of seed – an effective, environmental friendly, physical plant protection measure

*M. Mehlstäubl and F.H. Rögner*
*Fraunhofer-Institute for Electron Beam and Plasma Technology FEP, Winterbergstrasse 28, 01277 Dresden, Germany*
*Email: Frank-Holm.Roegner@fep.fraunhofer.de*

The chemical effects of ionizing radiation on polymers have been known for a long time. The effects essentially

result from the breaking open of multiple bonds in the polymer due to the energy input and the subsequent generation of highly reactive chain ends and radicals which in turn undergo secondary reactions. These effects are the key for the sterilizing effect of accelerated electrons, because amongst other things harmful germs can be killed. By using accelerated electrons the penetration of electrons into materials can be accurately adjusted via the kinetic energy of the impinging electrons. Thus, the sterilizing effect can be restricted to the surface and a defined outer layer of the seed grains, without affecting the seed embryo or the endosperm inside the seed grain. The technological breakthrough of the accelerated electron treatment came with the development of band emitters with high power density at Fraunhofer FEP. Thus, from the year 2000 onwards it became possible to treat seed in air via a continuous process. In Germany more than 300,000 hectares have been sown with electron treated cereal seed so far. The Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA) - now Julius-Kühn-Institut (JKI) - and the European and Mediterranean Plant Protection Organization (EPPO) recommend this treatment method for both conventional and organic farming. Advantages of the treatment with electrons are: chemical free, environmentally-friendly, over-produced seed can be used as animal feed, no chemical dust at sowing, no resistance, cost-efficient, high throughput up to 30 t/h, seed can be long term stored.

**O18.010 Integrated control of potato pathogens through seed potato certification and provision of clean seed potatoes**

*K.E. Frost[1], R.L. Groves[2] and A.O. Charkowski[1]*
*[1]Department of Plant Pathology and [2]Department of Entomology, University of Wisconsin-Madison, 1630 Linden Dr., Madison WI 53706, USA*
*Email: acharkowski@wisc.edu*

Long term data sets are rare in agriculture and the impact of plant diseases on food production is challenging to measure, which makes it difficult to assess the impact of policy changes or research-based disease control efforts. Despite this, it is clear that one of the largest impacts on food security of biological research over the past century is in production of vegetatively propagated crops. Of these systems, seed potato production and certification is among the most developed. We analyzed a dataset from a century-old seed potato certification program in Wisconsin to assess the efficacy and cost of potato disease control through certification. Over the past century, certification has gradually reduced the incidence of mechanically transmitted vascular potato pathogens that lack insect vectors to undetectable levels and much of this reduction occurred prior to the use of tissue culture and the development of immunoassays. Rejection of seed lots from certification is now rare, with *Potato virus Y* (PVY), a virus spread non-persistently by numerous, non-colonizing aphid species being the main causes of rejection. PVY level increases occurred in 2000, coincident with the first detection of a new invasive vector, soybean aphid, in the Midwest. The increased PVY incidence was more pronounced in varieties that exhibit mild foliar symptoms. Starting in 2004, a decrease in PVY incidence occurred following comprehensive science-based changes to early generation seed potato production. The cost of the certification program has not increased two decades and the fees charged are comparable to those in 1913.

**O18.011 Does the informal seed system threaten maize seed health?**

*P.C. Biemond[1,2], O. Oguntade[3], T.J. Stomph[2], P. Lava Kumar[3], A.J. Termorshuizen[4] and P.C. Struik[2]*
*[1]International Institute of Tropical Agriculture (IITA), Sabo Bakin Zuwo Road, PMB 3112, Kano, Nigeria; [2]Centre for Crop Systems Analysis, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; [3]International Institute of Tropical Agriculture (IITA), PMB 5320, Ibadan, Oyo state, Nigeria; [4]BLGG Research, Postbus 170, 6700 AD Wageningen, The Netherlands*
*Email: christiaan.biemond@wur.nl*

The majority of smallholder maize farmers in sub-Saharan Africa depend for their seed on seed-producing farmers, the informal Seed System, despite the presence of seed companies selling hybrid maize seed. Overhead costs force seed companies to charge a relatively high seed price, while farmers have limited budget for seed, request small quantities, and live in remote areas far from seed company outlets. The informal SS can meet this seed demand, but seed quality remains unknown. The objective of this research was to test seed quality (germination, off-types and seed health) of 87 farmer-produced seed samples from Northern Nigeria, and to compare it with six seed company and six foundation seed samples. Seed health was quantified by plating disinfected seeds onto agar, and identifying all bacteria and fungi present after three days. The most prevalent seed-borne pathogen was *Fusarium verticillioides*, identified in all samples and infecting over 50% of the 49.500 seeds tested. Twelve seed-borne pathogens were identified. *Bipolaris maydis* (found in 45% of the farmer-produced samples), *Botryodiplodia theobromae* (97%), *Curvularia lunata* (38%), and *Macrophomina phaseolina* (74%) were the four most devastating pathogens detected. Seed company samples had lower infection incidences than farmer produced seed for three out of four of these pathogens, and had significantly less off-types ($P<0.01$) and higher germination ($P<0.01$). However, none of the 99 samples tested met the requirements for certified seed of the National Agriculture Seed Council (NASC) in Nigeria. Seed companies and

seed-producing farmers are recommended to improve disease control and seed cleaning.

**O18.012 Quality enhancement in sunflower seed by seed coating with polymer and fungicides**

*V. Bharathi, M. Sudha Rani, R. Sudhakar, A. Vishnuvardhan Reddy and K.V.S. Meena Kumari*
*Seed Research and Technology Centre, Acharya N G Ranga Agricultural University Rajendranagar, Hyderabad, A P, India*
*Email: bharathiv@yahoo.com*

Studies for seed quality enhancement through various seed coating techniques were carried out in sunflower up to six months storage period at SRTC, Rajendranagar for two years 2011-13 to minimize the seed borne pathogens which causes enzyme degradation and nutritive loss in seed germination during storage period. An experiment was conducted with six treatments in four replications with different seed coating techniques. Seed coating with polymer (polykote @ 4 ml/kg seed + thiram 75% WP @ 2.5 g/kg seed) induced maximum suppression of the fungal mycroflora followed by polymer coating across the storage period upto six months in both gunny bags and HDPE bags. Irrespective of bag, maximum germination percent (92.37) and seedling vigour index (2068) (Germination X seedling length) was recorded with polykote + thiram 75 WP when compared to control and other treatments. Polycoat seed treatment was the next best in maintain seed quality which induced maximum suppression of fungi but the response was relatively lower compared to polykote + thiram treatment. Coated seed with polykote and thiram 75 WP was proved to be the best with gunny bag up to 4 months storage period.

**O18.013 *Fusarium fujikuroi* is strictly associated to rice seeds: detection and pathogenicity**

*D. Spadaro, S. Matic, M.L. Gullino and A. Garibaldi*
*AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*
*Email: davide.spadaro@unito.it*

*Fusarium fujikuroi* is the causal agent of bakanae, an important rice disease worldwide distributed. The pathogen is primarily seed transmitted. Economic losses generally never exceed 20%, but the disease is spreading rapidly, becoming a serious problem especially for the seed companies. During 2006-2010, 421 isolates of *Fusarium* spp. were obtained from Italian diseased rice plants and seeds. These isolates were identified, based on the translation elongation factor sequence and pathogenicity tests to assess their aggressiveness. *F. fujikuroi* was the most abundant *Fusarium* spp. isolated and the only species able to cause bakanae disease. Other species of the *Gibberella fujikuroi* species complex (GFSC) can also be isolated from rice. Multiple alignment of translation elongation factor (*TEF*) gene sequences of different *Fusarium* spp., showed a deletion of six nucleotides in *F. fujikuroi* sequence and a two nucleotide polymorphism in the same region of *F. proliferatum* sequence. These elements of variability were used to develop a conventional and Real-Time PCR assay for diagnosis. Two species specific primer pairs gave a product of 179 and 188 bp for *F. fujikuroi* and *F. proliferatum* respectively. Primer specificity was confirmed by analyzing the DNA of the most representative species of the GFSC and 298 strains of *Fusarium* spp. isolated from rice plants and seeds in Italy. The specific primers were also successfully used to detect fungal presence directly from infected rice tissues and seeds, providing a rapid tool for the early detection of pathogen contamination.

**O18.014 Blackleg (*Leptosphaeria maculans*) pathogen in canola seed and dockage**

*W.G. Dilantha Fernando and B.T. Demoz*
*Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada*
*Email: D_Fernando@umanitoba.ca*

Blackleg, caused by *Leptosphaeria maculans*, is a major threat to canola production in Canada. Although the pathogen can infect all parts of the plant, yield losses occur when the pathogen migrates to the stem base causing girdling. With the exception of China, *L. maculans* is present in areas around the world where cruciferous crops are grown. The pathogen can cause trade barriers due to the risk of spread into unaffected areas via seeds, as it was with the temporary ban of Canadian canola export into China. Hence, it is important to assess the level of blackleg infection in Canadian canola seeds and dockage. Canola seed and dockage samples collected from farmers' fields in the Prairie Provinces were tested for the presence of the aggressive *L. maculans* and the less aggressive *L. biglobosa* using species specific primers. Results showed that both *L. maculans* and *L. biglobosa* are present in seeds in very low proportions (0-0.4% and 0-0.2%, respectively). In contrast, dockage had higher levels of *L. maculans* (0-37%) and *L. biglobosa* (0-6%). Although both pathogens can be found in seeds, they are present in very low levels. Dockage appears to harbour more *L. maculans* compared to seeds and is also more likely to be a source of infection than infected seeds.

**O18.015 A flow cytometric method for counting viable *Clavibacter michiganensis* subsp. *Michiganensis***

*N. Jiang[1, 2], J.Q. Li[1, 2] and L.X. Luo[1, 2]*
*[1]Department of Plant Pathology, China Agricultural University; [2]Beijing Engineering Research Center of Seed and Plant Health (BERC-SPH) / Beijing Key Laboratory of Seed Disease Testing and Control*

*(BKL-SDTC), Beijing, P. R. China, 100193*
*Email: jn2009@139.com; luolaixin@cau.edu.cn*

Bacterial canker of tomato, caused by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), is a quarantine seed-borne disease worldwide. Copper-based chemicals and hydrochloric acid are responsible for bacterial diseases control for decades. Meanwhile, copper and acid were reported to be inducers for viable but non-culturable (VBNC) state of some microbes. To study the VBNC state of Cmm, a rapid and reliable flow cytometric method based on the permeability of the cell membrane was established for counting the viable Cmm cells. BD TruCount tube and the BD FACSCalibur flow cytometer (FCM) were used for counting. The total and dead bacterial cells were enumerated by means of the SYTO and PI dyes staining, respectively. The VBNC cell numbers were calculated by subtracting the number of culturable cells (by plating) from viable cells (subtracting the number of PI-stained cells from that of SYTO-stained cells). The results showed that the optimal population range of Cmm for FCM detecting was $10^5$-$10^7$ CFU/mL. The SYTO 9 was the better dye and with the final concentration of 50 μmol/L. The final concentration of PI was 150 μmol/L. The incubation times were 40 min for SYTO 9 and 50-60 min for PI. The settings of FCM were as follows: amplifier mode, Log; voltage of FSC, E00; voltage of SSC, 440; voltage of FL1 for SYTO 9, 640; voltage of FL2 for PI, 625; speed, medium. This method can be used to monitor the VBNC state of Cmm and TruCount tube was first used for counting plant pathogenic bacterial cells.

### O18.016 High-throughput and multiplex detection of plant pathogenic bacteria in *Leguminosae* seeds using padlock probes and Bio-Plex system

*J.J. Feng[1,2], G.M. Zhang[1,2], S.J. Wu[1], F.R. Li[1,2], N.W. Schaad[3*], X.Y. Ling[1,2] and Z.N.Chen[1]*
*[1]Shenzhen Academy of Inspection and Quarantine, Shenzhen, 518010, P. R. China; [2]Animal & Plant Inspection and Quarantine Technology Center, Shenzhen Entry-exit Inspection and Quarantine Bureau, Shenzhen, 518045, P. R. China; [3*]USDA, ARS, Foreign Disease Weed Science-RU, Ft. Detrick, MD USA; [*]Retired, 2414 Rikkard, Thousand Oaks, CA 91362, USA*
*Email: sccfjj@yahoo.com.cn*

Several bacterial pathogens affect *Leguminosae* spp. worldwide, including *Curtobacterium flaccumfaciens* pv. *flaccumfaciens, Pseudomonas syringae* pv. *phaseolicola*, *P. syringae* pv. *pisi*, *P. syringae* pv. *glycinea*, and *Xanthomonas campestris* pv. *phaseoli*. The first three pathogens also have been listed by China as an official import plant quarantine pest. These bacteria often contaminate or infect *Leguminosae* seeds, and a few infected seeds are sufficient to initiate a general epidemic under favourable conditions. Economic losses due to these pathogenic bacteria result from crop yield reductions and loss of seed marketability. In our work, a padlock probe (PLP)-based multiplex assay was developed for detection and identification of these bacteria simultaneously in a single sample using Bio-Plex system. PLPs are long oligonucleotides containing target complementary sequence regions at both ends which can be ligated into a circular molecule upon hybridization to the target. Ligation of species-specific PLPs is conducted following PCR amplification of the five circularized PLPs using a pair of universal primers. A 96-well-based Bio-Plex platform with magnetic beads is then used to detect and identify the five different pathogens. Preliminary tests on genomic DNA of the five pathogens and DNA from inoculated *Leguminosae* seed samples showed the specificity and reliability of PLPs for multiplex detection in a single assay.

### P18.001 New pathogens transmitted through basil and rocket seeds in Italy

*G. Giovanna[1], G. Ortu[1], A. Garibaldi[1] and M.L. Gullino[1,2]*
*[1]Centre of Competence for the Innovation in the Agro-environmental Sector (AGROINNOVA), University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy; [2]DISAFA, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy*
*Email: marialodovica.gullino@unito.it*

Two new leaf spot caused by *Alternaria alternata* on basil and *Plectosphaerella cucumerina* on rocket were recently observed for the first time in Italy. Circumstantial evidence from surveys in the area interested by these diseases suggested that then sudden appearance was possibly due to the transmission of the pathogens by seeds. Eight seed samples of rocket and eleven seed samples of basil, obtained from farms affected by these diseases were assayed on a medium containing Potato dextrose agar (PDA, Difco, Detroit, Michigan, USA) amended with 25 $mgl^{-1}$ of streptomycin sulphate. Subsamples represented by 400 seeds (disinfected in 1% of sodium hypochloride for 1 min or not) were tested on Petri plates (10 seeds/plate) in four trials. The obtained *Alternaria* spp. and *P. cucumerina* isolates were tested for their pathogenicity under artificial inoculation of healthy basil and wild rocket plants. Four out of eight samples of wild rocket seeds, used for sowing in farms severely affected by *P. cucumerina*, were contaminated by the pathogen and eleven isolates of this pathogen were obtained out of 7,200 not disinfected seeds. From disinfected seeds it was not possible to isolate any strain of *P. cucumerina*. *Alternaria* sp. was isolated respectively from 7.3% and 2.6% of not disinfected and disinfected seeds belonging to commercial varieties of basil. The possibility of isolating the two pathogens from seeds, although from a low percent of them, supports the hypothesis that the rapid spread of these new diseases of rocket and basil recently observed in Italy is due to the

use of infected propagative material.

**P18.002 Efficacy of bioagents on seed quality enhancement and yield in chickpea**

*V. Bharathi, K.K. Durga, R. Sudhakar and A.V.V. Reddy*
*Seed Research and Technology Centre, Acharya N G Ranga Agricultural University Rajendranagar, Hyderabad, A P, India*
*Email: bharathivatyahoo.com*

Studies revealed that seed treatment with *Pseudomonas fluorescens* at 10 g / kg seed along with soil application of *P. fluorescens* at 3 kg/acre was found to be the best and effective as it recorded least incidence of wilt disease (11.26 per cent) and root rot (1.43 per cent) followed by seed treatment with Tebuconazole at 1 ml / kg seed with less incidence of wilt (13.62 per cent) and root rot (0.44 per cent) under field conditions. Among all the treatments studied, seed treatment with Benomyl at 2 ml/kg seed recorded maximum yield (7.17 q/ha) and is on par with treatment *P. fluorescens* at 10 g/kg seed along with soil application of *P. fluorescens* at 3 kg/acre (7.02 q/ha) and Tebuconazole at 1 ml/kg seed (6.62 q/ha). Maximum germination percentage was recorded with Benomyl at 2 ml/kg seed (treated check) and Tebuconazole at 1 ml/kg seed (95.0 per cent) followed by treatment with *P. fluorescens* at 10 g/kg seed along with soil application of *P. fluorescens* at 3 kg/acre (94.0 per cent). Maximization of seedling parameters like root length, shoot length and total seedling length were observed with Benomyl at 2 ml/kg seed as 17.0 cm, 10.3 cm and 27.3 cm, respectively. Considering seedling vigour index as an important seed quality character, *P. fluorescens* and Benomyl at 2ml/kg seed recorded high seedling vigour index. The per cent recovery of infested seeds from the harvested produce was found to be low with treated seeds compared to the control.

**P18.003 Initial seedlot infection by seedborne bacterial pathogens affects germination and yield of rice**

*D.L. Adorada[1], B.J. Stodart[1], C. Vera Cruz[2] and G.J. Ash[1]*
*[1]EH Graham Centre for Agricultural Innovation (an alliance between CSU and DPI NSW), Charles Sturt University, Locked Bag 588 Wagga Wagga NSW 2678 Australia; [2]Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines*
*Email: dadorada@csu.edu.au*

Rice is the most important staple food for half of the world's population. The effect of seedborne bacterial pathogens on rice production has received scant attention. Generally, it is accepted that the effect of seedborne bacterial pathogens is on seed quality rather than on yield reduction. The reported 1% to 5% yield loss seems negligible to farmers. But, considering the large area of land planted to rice worldwide, a this yield loss translates to millions of dollars lossed production. It is imperative then to understand the effects of these seedborne bacterial pathogens. One aspect of seedborne bacterial infection that has not been carefully investigated is their effect on rice germination and yield components. In this study, the effects of different seedborne bacteria on root and shoot growth of rice is reported, as well as the effect of initial seedlot infection by seedborne pathogens on germination and yield components of rice. Using the seed-soaking inoculation technique, seedborne bacteria were found to have significant effects on percent germination, root number, root length and shoot height of rice. Subsequently, the initial seedlot infection by seedborne pathogens was found to have significant effects on percent germination, tiller number and grain weight. In conclusion, the yield loss caused by seedborne bacterial pathogens can be attributed not only to the reduction in grain quality but also to their effects in reducing germination and yield components of rice.

**P18.004 Detection of seed-born fungi of rice commercial varieties in Hunan**

*D. Xue and B.D. Gao*
*College of Bio-Safety Science & Technology, Hunan Agricultural University; Hunan University Key Laboratory for Control and Utilization of Plant Diseases and Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Changsha 410128, P. R. China*
*Email: bdgao@aliyun.com*

Several significant diseases of rice can be spread by seeds. On the other hand, seed-born fungi often reduce seed germination rate of rice. In order to clarify the current status of fungus-carrying in rice seed the method of moisturizing culture combined with microscopic examination was used to investigate 150 commercial cultivars (cvs.) in Hunan Province. As a result, we found that the fungus-carrying rate of Luoyou 8 was the highest (11.65%) among the Three-line hybrid rice cvs., while Jinyou 463, Zhongyou 177 and Yiyou 701 were the lowest (0%). The fungus-carrying rate of Zhuliangyou 819 was the highest (7.13%) among the two-line hybrid rice cvs., while Aoliangyou 69 and Baliangyou 18 were the lowest (0.27%). Among the conventional rice cvs., the fungus-carrying rate of Xiangzaoxian 17 was the highest (6.35%) while Huanghuazhan were the lowest (0.18%). The rate of fungus-carrying is not related to the parental species. Contamination of Seed husks is much higher than kernels, Pathogenic fungi, such as *Cochliobolus miyabeanus*, *Sarocladium* spp., *Tilletia horrid*, *Curvularia* spp., *Fusarium* spp. *and Nigrospora* spp., detected but rice blast fungus did not. Storage stage fungi such as *Aspergillus* spp., *Mucor* spp., *Alternaria* spp., *Penicillium* spp. were also detected, most of which cause seedling rot during early season rice seedling,

especially under the condition of greenhouse seedlings.

**P18.005 Evaluation of methods of seed disinfection for quarantine treatment of *Acidovorax citrulli***

*Y.M. Ma[1], P. Wu[2] and B.D. Gao[1]*
*[1]Hunan University Key Laboratory for Control and Utilization of Plant Diseases, Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Hunan Agricultural University, Furong District, Changsha 410128; [2]Vegetable Research Center of Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, P. R. China*
*Email: bdgao@yahoo.com.cn*

*Acidovorax citrullI* (AC), the causal agent of watermelon fruit blotch, is a quarantine pest in China, which requires zero tolerance of viable bacterium in the seed disinfection treatments. This paper reports a reliable evaluation system of disinfection methods, i.e. mixing streptomycin-resistant AC with watermelon seed or inoculating watermelon fruits with AC, then evaluating of effects of individual treatments by counting colonies on LBS plate after isolation. The identity of those colonies was confirmed by specific primer colony-PCR technique. The evaluation system was applied to screen the reported seed disinfection methods. The results showed that 4% hydrochloric acid for 20min, 2% formaldehyde for 30min, and high temperature at 75℃ for 48h, gave desirable result that disinfection effect reached 100%, without any adverse effect on seed germination and seedlings viability of watermelon. Those 3 methods are recommendable in the watermelon production. On the other hand, irradiation at the intensity of 8 K and 10 K can completely kill the bacteria, but the seed germination was significantly inhibited. Irradiation at the intensity 6 K and below, and chemical such as sodium hypochlorite, calcium hypochlorite, and hydrogen peroxide at recommended concentration and time, can not completely kill bacteria so that they are not suitable for application in practice.

## Concurrent Session 19-Induced Resistance

### O19.001 Induced systemic resistance triggered by beneficial microbes

*C. Zamioudis, G. Stringlis, R.L. Berendsen, S.C.M. Van Wees, P.A.H.M. Bakker and C.M.J. Pieterse*
*Plant-Microbe Interactions, Utrecht University, P.O. Box 80056, 3508 TB Utrecht, the Netherlands*
*Email: C.M.J.Pieterse@uu.nl*

In the rhizosphere, plant roots interact with complex communities of commensal and mutualistic microbes, which structure a functional microbiome that improves plant fitness and health. In the past years, we intensively investigated the complexity of the plant immune system and discovered that plant defense signaling networks finely balance plant responses to beneficial microbes, pathogens, and insects to maximize both profitable and protective functions. Cross-communicating plant hormones play pivotal roles in the regulation of the defense signaling network. However, a major gap in our knowledge is how recognition of beneficial rhizosphere microbes drives the whole-plant body towards enhanced growth and elevated diseases resistance. Therefore, we study the mutualistic interaction between Arabidopsis roots and the plant growth-promoting rhizobacterium (PGPR) *Pseudomonas fluorescens* WCS417. WCS417 triggers an induced systemic resistance (ISR) that is effective against a broad spectrum of pathogens. In addition, WCS417 promotes plant growth and drives developmental plasticity in the roots of Arabidopsis by stimulating lateral root and root hair formation. The root-specific transcription factor MYB72 was identified as an essential component of the ISR signaling pathway. Confocal laser scanning microscopy revealed that MYB72 is strongly activated in root epidermal and cortical cells upon colonization of the roots by WCS417. Whole-genome analysis of the MYB72-dependent root transcriptome revealed that WCS417 hijacks iron-limitation induced signaling in the roots to trigger systemic resistance in above-ground plant parts. Moreover, a novel MYB72-dependent β-glucosidase was identified as a key signaling component for the establishment of rhizobacteria-mediated ISR in the Arabidopsis root.

### O19.002 Prospect and application of plant immunity inducer

*D. Qiu*
*Institute of Plant Protection, Chinese Academy of Agricultural Sciences,Beijing, 100081, P. R. China*
*Email: qiudewen@caas.cn*

We report the purification, characterization, and gene cloning of a novel hypersensitive response-inducing protein elicitor (MoHrip1) secreted by *M. oryzae*. The protein fraction was purified and identified by de novo sequencing, and the sequence matched the genomic sequence of a putative protein from *M. oryzae* strain 70-15 (GenBank accession No. XP_366602.1). The elicitor-encoding gene mohrip1 was isolated; it consisted of a 429 bp cDNA, which encodes a polypeptide of 142 amino acids with a molecular weight of 14.322 kDa and a pI of 4.53. MoHrip1-treated rice seedlings possessed significantly enhanced systemic resistance to *M. oryzae* compared to the control seedlings. a novel protein elicitor from the pathogenic cotton verticillium wilt fungus, *Verticillium dahliae*, induced a hypersensitive response in tobacco plants. The protein-encoding pevD1 gene consists of a 468-bp open reading frame that produces a polypeptide of 155 amino acids, with a theoretical molecular weight of 16.23 kDa. The protein elicitors significantly induce plant immunity to obviously suppresses TMV on tobacco leaf, grey mould on tomato and greatly increases plant growth in rice, tomato, tobacco and cucumber. The 3% Pu Lv-tong WP can be dissolved in water and diluted into 1000-fold solutions, which could be applied in seed soaking, roots perfusion and foliar application. The protein elicitor is a kind of novel biological pesticide with highly activity, broad spectrum and multi functions and an environmental friendly product in agricultural production system. It was safe to human and animals. This formulation is stable for more than two years by packing.

### O19.003 Systemic signaling in plant defense

*K. Yu, J.M. Soares, C.Wang, M.El-Shetehy, M.K. Mandal, A. Kachroo and P. Kachroo*
*Department of Plant Pathology, University of Kentucky, Lexington, KY 40546, USA*
*Email: pk62@uky.edu*

Identified as a form of plant immunity nearly 100 years ago, systemic acquired resistance (SAR) is a highly desirable form of resistance that protects against a broad-spectrum of pathogens. SAR involves the generation of a mobile signal at the site ofprimary infection, which moves to, and arms distal portions of a plant against subsequent secondary infections. The last decade has witnessed considerable progress and a number of signals contributing to SAR have been isolated and characterized. Among the signals contributing to SAR are salicylic acid (SA) and several components that feed into the SA pathway, the nine carbon dicarboxylic acid azelaic acid (AA), the phosphorylated sugar glycerol-3-phosphate (G3P), and two lipid transfer proteins DIR1 (Defective in Induced Resistance) and AZI1 (AA insensitive). The diverse chemical natures of the SAR inducing molecules have led to the growing belief that SAR might involve the interplay of multiple diverse and independent signals. More recent evidence suggests that coordinated signaling from diverse signaling components facilitates systemic immunity in plants. We further demonstrate that an intricate feed-back regulatory

loop between G3P, DIR1, and AZI1 regulates SAR and that AA functions upstream of G3P in this pathway. Relationship among recently identified mobile inducers of SAR will be discussed.

### O19.004 Genetic modification of rice for enhancement of induced resistance

*K. Xie, E.E. Helliwell, W. Liu, T.A. Bailey, Q. Wang and Y. Yang*
*Department of Plant Pathology and Environmental Microbiology, and Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA 16802, USA*
*Email: yuy3@psu.edu*

Rice is a model for monocots and one of the most important food crops for over half of the world's population. Rice diseases such as blast (*Magnaporthe oryzae*), sheath blight (*Rhizoctonia solani*) and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) are a major constraint for achieving optimal crop yield and grain quality. During the past decade, increasing efforts have been made to elucidate the complex network of signaling pathways and molecular mechanisms that lead to the activation of rice innate immunity against microbial infections. To enhance host resistance, various molecular and transgenic strategies have been devised to genetically modify rice genes and components critical for pathogen recognition (e.g., pattern recognition receptors and resistance proteins), disease susceptibility (e.g., host proteins targeted by microbial effectors), signal transduction (e.g., reactive oxygen species, hormones, protein kinases and transcription factors), or defense responses (e.g., pathogenesis-related proteins and phytoalexins). Since plant defense response often antagonizes growth and development due to crosstalks among various signaling pathways, increasing attention is being paid to fine tune molecular strategies for improving rice disease resistance without negatively impacting crop yield and abiotic stress tolerance. In addition, novel genome editing tools such as the TALEN and CRISPR-Cas systems will significantly enhance our ability to precisely edit rice genome for genetic improvement of induced resistance.

### O19.005 Reduction of disease incidence by *Pseudomonas syringae* pv. *actinidiae* on kiwifruit using elicitors

*J.L. Vanneste[1], F. Spinelli[2], L. Fiorentini[2], J. Yu[1], A. Cellini[2], D.A. Cornish[1], I. Donati[2], G. Costa[2]and C. Felman[1]*
*[1]The New Zealand Institute for Plant & Food Research Ltd. Ruakura Research Centre, Private Bag 3123, Hamilton 3240 New Zealand; [2]PO Box 84, Alma Mater Studiorum, Università di Bologna, Dipartimento di Colture Arboree, viale Fanin 46, 40127, Bologna, Italy*
*Email: Joel.Vanneste@plantandfood.co.nz*

*Pseudomonas syringae* pv. *actinidiae* (Psa) causes bacterial canker on kiwifruit plants (*Actinidia deliciosa* and *A. chinensis*). Strains of Psa biovar 2 (so far mostly strains isolated from Korea) produce coronatine, a non-host-specific toxin which mimics jasmonic acid (JA). The production of a JA analogue by Psa suggests that this bacterium is not affected by JA-mediated defence reactions. Furthermore, the JA and the salicylic acid (SA) pathways are mutually antagonistic; therefore, by eliciting the JA pathway, Psa may be suppressing the SA pathway. To determine how Psa is affected by the SA or the JA-mediated defence reactions, *A. deliciosa* 'Bruno' seedlings were sprayed with methyl jasmonate (MeJA) or SA seven days before inoculation. The plants treated with MeJA were as susceptible to Psa as water-treated control plants, while the plants treated with SA were more resistant to this pathogen. When the experiments were repeated using acibenzolar-S-methyl (ASM), a derivative of SA commercialised as Bion™ or Actigard™, the incidence of Psa was significantly lower on kiwifruit seedlings that had been treated as a spray or a root drench. Although only Psa biovar 2 produces coronatine, a reduction of disease incidence after applying SA or ASM was observed for strains all biovars of Psa and in particular biovar 3, which has recently colonised Europe and New Zealand. Over 18 other elicitors have been tested on kiwifruit seedlings in the glasshouse. Some elicitors that significantly reduced the incidence of Psa on one cultivar of *A. deliciosa* significantly increased the incidence of Psa on *A. chinensis*.

### O19.006 Use of arabinoxylan polymers for plant defence

*J. Rätsep[1], N.D. Havis[1], G. Loake[2] and D. Walters[1]*
*[1]Crop & Soil Systems, Scotlands Rural College (SRUC), Edinburgh EH9 3JG, UK; [2]Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh EH9 3JH*
*Email: Jaan.Ratsep@sruc.ac.uk*

Successful control of plant diseases is a key priority in agricultural production. Effective control might require multiple fungicide applications. These often become ineffective due to the development of resistance by pathogens. However, every plant possesses an innate ability to defend itself. A range of biotic and abiotic factors, including naturally-derived and synthetic compounds, are known to elicit plant resistance to pathogens. A project was establsihed to study the ability of a plant cell-wall derived arabinoxylan polymer to induce local or systemic resistance or "prime" plant defence mechanisms for rapid and effective response to pathogen challenge. This ability was examined in a number of field and controlled environment experiments. The results obtained from the field trials in three consecutive seasons showed a significant effect on both disease expres-

sion and severity and also green leaf area and yield of barley (*Hordeum vulgare*). Further experimental data suggests a priming effect of the polymer on plant resistance to pathogens, which could be beneficial for overall plant fitness under conditions of pathogen challenge. Confirmation and further characterisation of the resistance-inducing properties of the polymer would be of great benefit to agriculture. The use of non toxic substances to induce plant defences could minimise environmental impact and provide a novel, cost-effective and environmentally benign measure for crop disease control. Furthermore, this approach could also contribute to current anti-resistance strategies and help to prolong the life of existing agrochemicals.

**O19.007 A soybean nuclear localized type III DnaJ domain-containing HSP40 is a positive regulator of cell death and disease resistance**

*J.Z. Liu[1] and S.A. Whitham[2]*
*[1]College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua, 321004, P. R. China; [2]Department of Plant Pathology and Microbiology, Iowa State University, Ames, IA 50011, USA*
*Email: jzliu@zjnu.cn*

Heat shock proteins such as HSP70 and HSP90 are important molecular chaperones that play critical roles in biotic and abiotic stress responses. However, the involvement of their co-chaperones in stress biology remains largely uninvestigated. In a screen to identify potential positive regulators of cell death in soybean (*Glycine max*), we used agroinfiltration to transiently over-express full length cDNAs of soybean genes that are highly induced during soybean rust infection in *N. benthamiana* leaves. A type III DnaJ domain-containing HSP40 (*GmHSP40*.1) caused hypersensitive response (HR)-like cell death when over-expressed in *N. benthamiana* leaves. The HR-like cell death was dependent on MAPKKKα and WIPK, because silencing each of these genes suppressed the HR. Consistent with the presence of a nuclear localization signal (NLS) motif within the *GmHSP40.1* coding sequence, GFP-GmHSP 40.1 was exclusively present in nuclear speckles. Nuclear localization of GmHSP40.1 was necessary for its function because deletion of the NLS or addition of a nuclear export signal (NES) abolished its HR-inducing ability. GmHSP40.1 co-localized with HcRed-SE (SERRATE), a protein involved in pri-miRNA processing, which has been shown to be co-localized with SR33-YFP, a protein involved in pre-mRNA splicing, implying a role for GmHSP40.1 in mRNA splicing or miRNA processing and a link between these processes and cell death. Silencing *GmHSP40.1* enhanced the susceptibility of soybean plants to *Soybean mosaic virus* confirming its role as a positive regulator of pathogen defense. Together, the results reveal a critical role of a nuclear-localized DnaJ domain-containing GmHSP40.1 in cell death and disease resistance in soybean.

**O19.008 The *Arabidopsis* OXI1 kinase regulates salicylic acid-dependent immunity and cell death programs**

*A.V. Garcia, C. Forzani, K. Yoshimoto, C. Balagué, E. Bueso, J. Colcombet and H. Hirt*
*URGV Plant Genomics Research (INRA/UEVE/CNRS), 2 rue Gaston Crémieux, 91057 Evry cedex, France*
*Email: garcia@evry.inra.fr*

Whereas animal AGC kinases are key regulators of growth and cell death programs in response to environmental stresses and growth factors, little is known about the function of AGC kinases in these processes in plants. Arabidopsis OXI1 is an AGC protein kinase that is rapidly activated by oxidative stress and various signals mimicking pathogen attack. OXI1 is required for complete resistance against biotrophic pathogens and for the growth promotion conferred by the basidiomycete *Piriformospora indica*. Furthermore, *oxi1* mutant plants display reduced activation of the stress-induced MAPKs MPK3 and MPK6. To further assess the function of Arabidopsis OXI1, we generated transgenic lines expressing tagged *OXI1* under its own promoter and selected lines with different levels of protein accumulation. Interestingly, several independent transgenic lines displaying high OXI1 protein accumulation had reduced growth and severe leaf necrosis. The appearance of necrotic lesions correlates with the misregulation of MAPK activities, over accumulation of the defense hormone salicylic acid, transcriptional reprogramming of defense genes and increased disease resistance to *Pseudomonas syringae* bacteria. Our results suggest that, similarly to the function of AGC kinases in animals, the OXI1 kinase plays an important role in controlling plant growth, disease resistance and cell death programs in Arabidopsis.

**P19.001 *Cypereus rotundus* rhizomes induce sexual reproduction in some fungi**

*A. Singh and U.P. Singh*
*22, Ganesh Dham, Newada, Sunderpur, Varanasi-221005, India*
*Email: amitabhs777@gmail.com*

*Cyperus rotundus* is a perennial obnoxious weed of tropics. Its root bears rhizomes in the soil which can survive for a long time. They germinate under suitable environmental conditions and affect the yield of the crop. During the course of a field visit sclerotia of *Sclerotium rolfsii* were found growing on rhizomes of *C. rotundus*. Such rhizomes were brought to the laboratory and the fungus (*S. rolfsii*) was isolated on potato dextrose agar (PDA) medium. *S. rolfsii* was again grown on PDA supplemented with rhizome meal on which sexual stage

was formed. Similarly, two other fungi, viz., *Ustilago cynodontis* and *Cintractia limitata* were also grown on this medium on which they also formed sexual stage. Methanolic extract of *C. rotundus* rhizomes also induced sexual stage in *S. rolfsii* while aqueous extract induced sexual stage in *U. cynodontis* and *C. limitata*.

**P19.002 Function of class II TGA transcription factors in oxylipin signalling**

*H.U. Stotz[1], S. Berger[2] and M.J. Mueller[2]*
*[1]School of Life and Medical Sciences, University of Hertfordshire, Hatfield, AL10 9AB, UK; [2]Julius-von-Sachs-Institute, Pharmazeutische Biologie, Julius-Maximilians Universität, 97082 Würzburg, Germany*
*Email: hustotz@gmail.com*

Oxylipins constitute a class of signalling molecules that are generated via oxygenation of polyunsaturated fatty acids. Plant oxylipins include the hormonal precursor jasmonic acid (JA), its physiologically active amino acid conjugates, the chemically reactive JA-precursor 12-oxo phytodienoic acid (OPDA) and a group of phytoprostanes that are structurally and functionally related to prostaglandins from animals. These molecules control developmental processes as well as resistance to microbial pathogens and herbivorous insects. We previously showed that responses to OPDA and phytoprostanes are mediated via basic region/leucine zipper (bZIP) transcription factors that belong to the class II of TGA factors, namely TGA2, TGA5 and TGA6. To further characterize the regulation of plant responses to oxylipins, root growth and target gene expression were analysed in *tga* mutant and *TGA*-overexpressing lines. Class II TGA factors interfered with oxylipin-responsive root growth inhibition, but expression of the target genes *CYP81D11* vs. *GST25* and *OPR1* was activated by TGA2, TGA5 and/or TGA6. As the molecular mechanism of oxylipin-dependent TGA2 action is not known, two biochemical approaches were pursued: expression of a functionally active TAP-tagged TGA2 in the background of the *tga2 tga5 tga6* triple mutant to permit the isolation of proteins that interact with this transcription factor *in vivo*, modification of TGA2 with radioactively labelled OPDA or biotinylated oxylipins to determine functional and physiological consequences of this modification. Based on these two approaches, we hope to obtain a more detailed understanding of the oxylipin signalling pathway.

**P19.003 Induction of resistance in tea against root pathogens by plant growth promoters and arbuscular mycorrhizal fungi**

*U. Chakraborty, B. Chakraborty, A. Chakraborty and U.K. De*
*Immunophytopathology Laboratory, Department of Botany, University of North Bengal, Siliguri-734 013, West Bengal, India*
*Email: chakrabortyusha@hotmail.com*

The present study was undertaken to explore the potential of microorganisms from the rhizosphere of tea (*Camellia sinensis*) for growth improvement and biological control of diseases. The selected microorganisms which showed positive PGPR traits *in vitro* such as phosphate solubilization, siderophore production, antagonism to pathogens and IAA production were - *Bacillus amyloliquefaciens, B. pumilus, B. megaterium* and *Ochrobactrum anthropi*.16S rDNA sequencing of the bacteria was done and their phylogenetic relationships determined. *Glomus mosseae* and *G. fasciculatum*, which were the dominant arbuscular mycorrhizal fungi (AMF) which colonized tea roots were selected for mass multiplication and application in nursery grown plants. Under *in vivo* conditions, the PGPR and AMF, applied either singly, or jointly, enhanced seedling growth of tea varieties in the nursery as well as in the field. Biocontrol of root diseases of tea caused by *Sclerotium rolfsii, Phellinus noxius, Poria hypobrunnea* and *Sphaerostilbe repens* was achieved by application of PGPR and AMF. Sustainability of the applied bacteria in soil was tested by PTA-ELISA and Dot immunobinding. Localization of AMF hyphae in root cells was determined by indirect immunofluorescence. Application of the PGPR and AMF led to enhancement in activities of defense related enzymes-phenyl alanine ammonia lyase, peroxidase, chitinase and β-1,3 glucanase, in tea leaves. Total phenols also increased quantitatively along with increase in isoforms of catechins. It is evident from the results of the present study that application of PGPR and AMF in the soil lead to biopriming of the plants through growth promotion, induced systemic resistance and other mechanisms.

**P19.004 Potential modes of action of *Paenibacillus* sp. for biocontrol of black rot in cabbage**

*H. Ghazali-Biglar, J. Hampton, E. van Zijll de Jong, A. Holyoake and A. Stewart*
*Bio-Protection Research Centre, Lincoln University, Lincoln 7647, New Zealand*
*Email: hoda.ghazalibiglar@lincolnuni.ac.nz*

Black rot caused by the seed-borne pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*) is a widespread disease of brassicas. *Paenibacillus* sp. strain P16 is a potential biocontrol agent (BCA) as it can reduce the incidence of this disease in cabbage. The aim of this study was to investigate whether the control was provided by plant growth promotion and/or induced systemic resistance. In the plant growth promotion study, the BCA was co-applied with *Xcc* as a seed treatment. In the presence of *Xcc*, BCA-treated seedlings had significantly ($P<0.05$) greater growth than the control. However, there was no significant difference in plant growth parameters between these treatments in the absence of

the pathogen. Hence it appears that the BCA, by reducing *Xcc* infection, enables the plants to survive and grow normally. In the induced systemic resistance study, the BCA was applied as a seed treatment and plants were challenged with *Xcc* at 2 and 4 weeks after sowing. There was a significant ($P<0.05$) reduction in disease severity in BCA-treated plants 4 weeks after sowing compared to the non-treated control. Further research is being conducted using molecular markers to confirm that induced systemic resistance is a biocontrol mechanism of P16 to control black rot.

**P19.005 Systemic control of rice blast by redox compounds**

*A.A. Aver'yanov[1], T.D. Pasechnik[1], V.P. Lapikova[1], T.S. Zakharenkova[1] and C.J. Baker[2]*
*[1]Research Institute of Phytopathology, B. Vyazemy, Moscow region, 143050 Russia; [2]Agricultural Research Service USDA, Beltsville, Maryland 20705, USA*
*Email: aaveryanov@post.ru*

Systemic acquired resistance is induced by many agents through oxidative burst in treated plant tissues. The resistance may be realized through new bursts occurring in distant parts in response to infection. In this regard, we studied substances favoring reactive oxygen production in the organism but unknown as disease controllers. Those were inhibitors of catalase (aminotriazole) and superoxide dismutase (diethyldithiocarbamate) leading to accumulation of hydrogen peroxide and superoxide respectively. Light-activated compounds bengal rose and methylene blue yielding singlet oxygen as well as mercaptopyridine oxide yielding hydroxyl radical were also tested. It was found (probably, for the first time) that the compounds applied to rice lower leaf of the susceptible cultivar reduced blast symptoms (caused by fungus *Magnaporthe grisea*) on the inoculated upper leaf. Exogenous antioxidants combined with these treatments reduced the protection effectiveness. The latter was also reduced if plants had been darkened for a day just after application of the light-activated compounds. All treatments increased systemically superoxide production (assayed with epinephrine) and fungitoxicity in diffusates of inoculated leaves. The toxicity was sensitive to antioxidants. In addition, mercaptopyridine oxide suppressed the ability of the diffusates to decompose hydrogen peroxide. Therefore, several novel compounds to control rice blast are offered. Apparently, they cause systemic resistance; its induction and realization might involve reactive oxygen species. The work was supported by the grant 4071p of ARS USDA mediated by International Science and Technology Center.

**P19.006 Systemic control of cucurbit scab by redox compounds**

*T.D. Pasechnik[1], A.A. Aver'yanov[1], V.P. Lapikova[1] and C.J. Baker[2]*
*[1]Research Institute of Phytopathology, B. Vyazemy, Moscow region, 143050 Russia; [2]Agricultural Research Service USDA, Beltsville, Maryland 20705, USA*
*Email: beefarmer@yandex.ru*

The work deals with scab caused by fungus *Cladosporium cucumerinum* on cucumber leaves. We attempted to systemically protect susceptible plants by compounds shifting pro-/antioxidant balance to overproduction of reactive oxygen species (ROS). Superoxide radical formation in drop diffusates of treated or infected leaves was assayed with epinephrine. Potassium secondary phosphate, β-aminobutyric acid (BABA), photodynamic dyes bengal rose and methylene blue were taken as known anti-infectional agents. As unknown in this regard, hydroxyl radical source mercaptopyridine oxide (MPNO), inhibitor of superoxide dismutase (diethyldithiocarbamate), and inhibitor of catalase (aminotriazole) were examined. Droplets of the solutions were applied to the first true leaf, yet alone. When the second leaf unfurled, it was inoculated with droplets of spore suspension. All tested substances diminished disease severity in comparison with a water-treated control. They stimulated superoxide generation in the 1st leaf diffusates collected a day after its treatment. One-day darkening plants treated with the dyes reduced the disease control as against normal illumination. All (but aminotriazole) treatments enhanced superoxide formation in the 2nd leaf diffusates collected a day after the inoculation. After treatments with BABA, dyes, or MPNO, these diffusates acquired fungitoxicity, which increased by a diffusate illumination. Therefore, induction and realization of systemic acquired resistance caused by some known compounds were associated with extracellular ROS. Several compounds novel as to systemic disease control, possibly with the same mode of action, were offered. The work was supported by the grant 4071p of ARS USDA mediated by International Science and Technology Center.

**P19.007 Control of *Peronospora belbahrii* of basil by using resistance inducers, fungicides and natural products**

*G. Gilardi[1], S. Demarchi[1], M.L. Gullino[1,2] and A. Garibaldi[1]*
*[1]Centre of Competence for the Innovation in the Agro-environmental Sector (AGROINNOVA), University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy; [2]DISAFA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*
*Email: giovanna.gilardi@unito.it*

To control downy mildew of sweet basil (*Ocimum basilicum* L.), incited by *Peronospora belbahrii*, products(acibenzolar-S-methyl, mineral and organic fertilizers, plant extracts), known for their capability of inducing resistance in plants to several pathogens, were tested

in 2011 and 2012, in comparison with registered fungicides, copper-based fungicides and biocontrol agents. Four experimental trials were carried out under glasshouse conditions in the presence of a high disease incidence. The tested compounds were applied alone and in rotation in three treatments at 6 days interval except of systemic chemical fungicides that were applied once. One artificial inoculation with the pathogen was carried out 24 hours after the last treatment applications. In all trials the best results, in terms of reduction of disease incidence and disease severity were offered by metalaxyl-M + copper hydroxide, by the phosphite-based products, by mandipropanid, by azoxostrybin, and by acibenzolar-S-methyl. Such products did significantly reduce disease incidence and severity also 20 days after the last treatment. Among the copper-based products, the best results were provided by copper hydroxide with terpenic alcohols and copper oxychloride + copper hydroxide. *B. subtilis* QST 713 and thyme oil extract gave results significantly different from the untreated control, but were only partially effective. When different combinations of various products used in rotation were tested, it was possible to reduce disease incidence and severity with different strategies, based on the rotation of fungicides and resistance inducers as well as with the rotation of resistance inducers.

**P19.008 Alterations of gene expressions in lily as affected by *Bacillus cereus*-treatment and subsequent inoculation with *Botrytis elliptica***

*Y.H. Liu, C.J.Huang and C.Y. Chen*
*Department of Plant Pathology and Microbiology, National Taiwan University, Taiwan, China*
*Email: cychen@ntu.edu.tw*

There are evidences showing that *Bacillus cereus* effectively induces systemic resistance in *Liliumformosanum* and lily cultivar Star Gazer against *Botrytis elliptica*. A cDNA-AFLP (amplified fragment length polymorphism) analysis of *B. elliptica*-infected leaves and the leaves of *B. cereus*-treated lily plants with or without subsequent *Botrytis* infection revealed alterations of gene expressions related to metabolism, signal transduction, cell defense, transport, energy, protein synthesis, and transcription occurred in the ISR response of *Lilum* 'Star Gazer'. By semi-quantitative reverse transcriptase-polymerase chain reaction, the genes encoding GTPase-binding protein, calmodulin (CAD), and glutamine synthetase (GS) were shown under positive regulation in lily leaves after application of *B. cereus* to the rhizosphere of lily plants. The expression of these genes also increased following *B. elliptica* infection, but under negative regulation in *B. cereus*-treated lily plants after subsequent inoculation with *B. elliptica*. Since abscisic acid (ABA) could increase disease resistance to *B. elliptica* and caused opposite gene expressions of *GS* and *PR-1*, we presumed that *B. cereus*-induced systemic resistance is positively correlated with an ABA-driven defense signalling pathway. In addiiton, differentially expressed sequences with low expression, such as *GS* and *CAM*, were identified, and gene expression increased post-treatment with beneficial rhizobacterium but decreased following subsequent pathogen attack were addressed in a non-model plant system.

**P19.009 Methyl jasmonate induces systemic resistance against Botrytis bunch rot in wine grapes**

*D. Errampalli, P.H. Goodwin and A. Sharon*
*Agriculture and Agri-Food Canada, 4902 Victoria Ave N., Vineland Station, Ontario L0R 2E0, Canada*
*Email: Deena.Errampalli@agr.gc.ca*

Botrytis bunch rot in grapevine (*Vitis vinifera*) is caused by *Botrytis cinerea*, a necrotrophic pathogen. Methyl jasmonate (MeJA) occurs naturally in host plant tissues and has signalling role in eliciting induced systemic resistance (ISR) against diseases. The effect of exogenous MeJA, on the suppression of postharvest botrytis in green grape cultivars 'Chardonnay' and 'Vidal.' and in red grape cultivars 'Merlot' and 'Cabernet Sauvignon' was investigated. The grape bunches (15 grapes/bunch and three replicate treatments) were spray-treated with 1mM of MeJA, air dried for 3 hours. Three days after the MeJA treatment, each of the grape berry in the bunch was wounded with a needle and inoculated with $1\times10^4$ spores of *B. cinerea* B05.10 and incubated in the dark at 20 ℃ and 85% RH. Control treatment did not receive MeJA. The lesion diameter was recorded at 7 and 14 days after inoculation. The elicitor, MeJA induced defense response by significantly suppressing the *Botrytis* bunch rot in all the grape cultivars tested. Defense response, expressed as *PAL* gene, in grapevine berries towards *B. cinerea*, was studied. Maximum levels of induction of *PAL* gene was observed at 48 hours post inoculation in *B. cinerea* infected, MeJA treated, or MeJA treated and *B. cinerea* infected grapevine berries. A significantly lower level of *PAL* gene expressed in MeJA treated and *B. cinerea* infected grapevine berries, as compared to *B. cinerea* only infected berries. Postharvest treatment with MeJA may be incorporated as a potential tool in the grape postharvest disease management strategies.

**P19.010 Elicitor induction of defence genes and reduction of bacterial canker in kiwifruit**

*K. Wurms, E. Gould A. Ah Chee J. Taylor and T. Reglinski*
*The New Zealand Institute for Plant & Food Research Limited, Ruakura, Private Bag 3123, Waikato Mail Centre, Hamilton 3240, New Zealand*
*Email: kirstin.wurms@plantandfood.co.nz*

*Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of bacterial canker of kiwifruit, is the most serious

global pathogen of this crop. Like most bacterial pathogens, control options are limited, but elicitors have been shown to reduce disease significantly, particularly those that induce the salicylic acid (SA) pathway. Acibenolar-S-methyl (ASM), an analogue of SA, is one of the most effective elicitors for Psa control. In this study, real-time PCR (qPCR) was used to measure the expression of putative defence genes in *Actinidia chinensis* 'Hort16A' in response to Psa and ASM. Application of acibenolar-S-methyl (ASM) or inoculation by Psa led to up-regulation of RIN4, RPM1, phenylalanine ammonia lyase (PAL), a hypersensitivity-induced response protein, and chalcone-flavanone isomerise (CHI), and expression was further enhanced when elicitor application and Psa-inoculation were combined. Elevated expression was correlated with decreased disease expression and supports the hypothesis that elicitor-treated plants react more rapidly and/or strongly to pathogen attack. Further qPCR studies are underway to look at the role of defence genes in five different kiwifruit cultivars and to examine cultivar-specific responses to ASM and methyl jasmonate. In addition to the candidate gene approach, we are also adopting unbiased (hypothesis free) next generation sequencing approaches to identify additional key genes that respond to elicitors. The information will be used to tailor elicitor application strategies to different cultivars to maximise control of Psa.

### P19.011 Plant growth promotion and induced systemic resistance in tobacco by volatiles from *Pseudomonas fluorscens* SS101

*K. Park[1], S.H. Lee[1], M.N. An[1], K. Balaraju[1], S.W. Lee[1], J.E. van de Mortel[2] and J.M. Raaijmakers[2]*
*[1]Microbial Plant Activation Lab, Division of Agricultural Microbiology, National Academy of Agricultural Science, RDA, Suwon 441-707 Republic of Korea; [2]Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands.*
*Email: kspark3383@korea.kr*

Plant growth-promoting rhizobacterium (PGPR) can induce various metabolic changes in plants that lead to changes in growth, development and resistance to plant pathogens. Here we show that *Pseudomonas fluorscens* strain SS101 (Pf.SS101) protects tobacco plants (*Nicotiana tobacum* L.) from infection by soft rot pathogen *Pectobacterim carotovorum* SCC1 through induction of systemic resistance (ISR). Strain Pf.SS101 also enhanced biomass of tobacco plants when grown under in vitro conditions using I-plate assay. To determine whether the volatiles from Pf.SS101 are involved in ISR and/or plant growth promotion of tobacco, we performed solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) analysis and bioassays. SPME-GC-MS revealed that Pf.SS101 released at least 12 different volatiles with 13-tetradecadien-1-ol being highly represented. When compared to water-treated control, Pf.SS101 grown on different media showed greater appearance of plant growth promotion and ISR against soft rot in tobacco. However, among various media tested, for the growth of Pf.SS101 to release volatiles for growth promotion and ISR activity, potato dextrose agar (PDA) and King's B agar (KBA) media were found to have greater effect on plant growth promotion, while nutrient agar (NA) and KBA were found to have greater effect on ISR activity when compared to tryptic soy agar (TSA), PDA, and luria bertani agar (LBA) media.

### P19.012 Decyl alcohol, a bacterial volatile from *Bacillus subtilis* BS15 can induce plant growth and systemic resistance in tomato through Bio-priming

*K. Park[1], S.H. Lee[1], E.Y. Cho[1], K. Balaraju[1] and K.W. Nam[2]*
*[1]Microbial Plant Activation Lab, Division of Agricultural Microbiology, National Academy of Agricultural Science, RDA, Suwon 441-707 Republic of Korea; [2]Department of Horticulture, Hankyung National University, Ansung, Republic of Korea*
*Email: kspark3383@korea.kr*

Bio-priming is one of the new techniques for enhancing the plant growth and disease suppression through induced systemic resistance (ISR). In the previous research, seed priming been described for the control of various diseases as alternative chemical fungicides. Of many priming agents, bacterial volatiles play an important role in disease control through ISR and growth promotion as well. Various metabolites from plant growth-promoting rhizobacterium (PGPR) can act as barriers against biotic and abiotic stresses. In our study, the volatile decyl alcohol was identified from *Bacillus subtilis* BS15 by solid-phase micro extraction (SPME) and gas chromatography-mass spectrometry (GC-MS). Decyl alcohol showed greater effective for plant growth promotion and protection of the tomato plants from *Pectobacterium carotovorum* SCC1 (SCC1) and *Phytophthora capsici* through ISR. Commercially available decyl alcohol (Sigma) was tested for plant growth promotion and ISR activity against SCC1 and *P. capsici*. *In vitro* growth promotion by I-plate assay showed greater plant growth promotion in terms of fresh weight at 1.0 ppm of decyl alcohol, and there was an increased plant height under greenhouse conditions when compared to water-treated control. There was greater reduction (57.5%) of SCC1 in the leaves of tomato plants by treatment with decyl alcohol at 1.0 ppm, while in water-treated control the disease incidence was 100% under greenhouse and field conditions. Therefore, our study suggests that low dosages of decyl alcohol might be the volatile Bio-priming mediated systemic resistance and growth promotion.

**P19.013 Biocontrol agent *Pythium oligandrum* induces resistance against a bacterial pathogen in rice seedlings**

*S. Hase, S. Takenaka, M. Maeda, T. Hori, S. Tsushima and T. Namai*
*Faculty of Agriculture, Yamagata University, 1-23 Wakaba-machi, Tsuruoka,Yamagata 997-8555, Japan*
*Email: s.hase@tds1.tr.yamagata-u.ac.jp*

*Pythium oligandrum* (PO), a non-pathogenic soil-inhabiting oomycete, has an ability to activate jasmonic acid (JA)-mediated induced resistance against pathogens in dicot plants. The cell wall protein (CWP) fraction of PO acts as a potent elicitor of the induced resistance. To elucidate the ability of PO to activate the defense reaction in monocot plants, we studied the induction of defense gene expression in rice treated with oospore suspension or CWP of PO, and the induced resistance to a bacterial pathogen, *Burkholderia glumae*. The global gene expression analysis of the roots treated with CWP indicated that CWP enhanced expression of genes encoding lipoxygenase and allene oxide synthase, which are key enzymes of JA synthesis, and JA-responsive *PR* genes, e.g. *OsPR6*, *OsPR10a/PBZ1* and *OsPR10b*. However, the expression of salicylic acid-responsive genes was not included in the up-regulated genes. The expression of the JA-responsive *PR genes* was also enhanced in hastened seeds treated with oospore suspension or CWP. Treatment of seeds with oospore suspension following inoculation with *B. glumae* significantly reduced disease severity of the seedling rot as compared with an untreated control. These findings suggest that PO seems to activate JA signaling-mediated defense reaction and induce resistance against the infection of bacterial pathogens in rice.

**P19.014 Influence of salicylic acid on physiological responses of 'Red Sun' kiwifruit to the soft rot disease**

*Y. Zhou, S.T. He, Y.L. Cui, G.S. Gong, N. Liu and J.J. Wang*
*Department of Plant Pathology, Agricultural College, Sichuan Agricultural University, Chengdu 611130, P. R. China*
*Email: guoshugong@126.com*

Kiwifruit soft rot caused by *Botryosphaeria dothidea* is one of the most serious diseases in kiwifruit cv. 'Red Sun' producing regions and can cause rapidly fruit rot during storage period. Most evidences have showed that salicylic acid (SA) can induce plant resistance to pathogen. The protective enzymes, proline (Pro) and malondialdehyde (MDA) were reported to play important roles in plant defense against to disease. For kiwifruit, little is known about physiological resistance induced by SA to kiwifruit soft rot. In our study, 'Red Sun' kiwifruit was pre-treated with SA, and water as the control. After inoculated with *B. dothidea*, Pro, MDA, catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) were measured per 24h. Compared to control, the activities of CAT and SOD significantly decreased, and the maximum value of CAT and SOD reduced about 31.29% and 27.73%, respectively. However, the POD activity was always higher than that in control during the process of disease development. The activities of CAT and SOD reached the maximum value at 4d (15.087 $U.min^{-1}.g^{-1}$) and 3d (18.940 $U.g^{-1}$), respectively. The content of Pro and MDA also significantly decreased, which maximum value reduced about 34.88% and 23.18%, respectively, as compared to control. The content of Pro and MDA reached the maximum value at 4d (36.790 $mg.g^{-1}FW$) and 5d (8.057 $mmol.g^{-1}FW$) with SA treated, respectively. Furthermore, SA was found to alleviate significantly symptom of soft rot. The results indicated that SA can improve kiwifruit's physiological properties against the pathogen.

**P19.015 The *Trichoderma harzianum* LTR-2 confers *Mentha piperita* L. enhanced tolerance to salt stress by promoting endogenous NO production**

*K. Guo, Y. Qu, Z. Li, H.Z. Zhou, Y.L. Wei, J.S. Li, X.Q. Wu and H.T. Yang*
*Biotechnology Center, Shandong Academy of Sciences, Shandong Provincial Key Laboratory for Applied Microorganism , No. 19 Keyuan Road, Lixia District, Jinan, 250014, P. R. China*
*Email: yanght@sdas.org*

The *Trichoderma harzianum* LTR-2 is a registered biocontrol fungi, which can colonize of diverse plant roots, attack pathogens and improve plant performance by inducing of systemic resistance. Laboratory and field results show that *Mentha piperita* has strong tolerance to salt stress. Depending on our preliminary research, LTR-2 displayed strong salt resistance and increase *Mentha piperita* slat tolerance after colonization of roots. Our results showed that elongation of adventitious root and root hair number of *Mentha piperita* colonizated by LTR-2 were increased 20 % and 50% compared to control. Reactive oxygen species generation was reduced and antioxidant enzyme activity was increased after LTR-2 colonization under salt stress. Remarkably, the trypan blue staining showed that the undergoing programmed cell death of *Mentha piperita* was alleviated following LTR-2 colonization under salt stress. Nitric oxide (NO), an important signaling molecule, plays vital role in plant response to salt stress. We first detected the changes of endogenous NO production and enzyme activities of NO production pathway in LTR-2. It was found that under salt stress salt tolerance and endogenous NO of *Mentha piperita* were enhanced after LTR-2 colonization. The similar observation was noted in *Mentha piperita* pre-treated with NO donor SNP under salt stress. Furthermore, addition of NO scavenger

cPTIO effectively inhibited the enhanced salt tolerance of *Mentha piperita* conferred by LTR-2. In all, these results indicated that the LTR-2 colonization increased the salt tolerance of *Mentha piperita*, and NO played vital roles in the process.

**P19.016 Real-time imaging of the sub-cellular redox potential in living plant cells infected by plant virus**

*R. Lei, F. Hu, Y.X. Ma, J.J. Li, X.Wang and S.F. Zhu*
*Institute of Plant Quarantine of China, Chinese Academy of Inspection and Quarantine , Beijing, 100029, China*
*Email: Leirong2009@gmail.com*

Dynamic analysis of glutathione (GSH) and reactive oxygen species (ROS) in living plant cells is pivotal to the pathogen infection and defense mechanism, since both GSH and ROS are indispensable for plant survival. Conventional measurements of redox pools are destructive, non-specific and complicated, tending to underestimate the redox state. In this study, redox-sensitive GFPs(roGFP) are targeted to mitochondria, chloroplast and cytosol to *in vivo* measure the redox potential in tobacco and Arabidopsis infected by *cucumber mosaic virus (CMV*). The symptom development after virus infection is associated with redox changes that are obviously subcellular compartment specific. The real-time imaging of the sub-cellular redox potential demonstrated that roGFP is an ideal candidate for developing a redox reporter in different subcellular compartments.

**P19.017 Proteomic analysis of soybean leaf induced by bacteria against atrazine**

*L. Chen, X.F. Zhu, Y.Y. Wang, L.J. Chen, F. Tian and Y.X. Duan*
*Nematology Institute of Northern China, Shenyang Agricultural University, Shenyang, 110866, P. R. China*
*Email: Duanyx6407@163.com*

Soybean (*Glycine max*) is an atrazinet-sensitive crop, and its production is severely affected by atrazine residual soils. Our previous research showed that SnebYK (*Klebsiella pneumoniae*) can induce soybean resistant to atrazine even though the inactivated one(121℃, 30min Sterilization). In order to clear the molecular mechanisms of this induced resistance, we carried out proteomic analysis of soybean leaves. Soybean seeds were treated with bacteria fermentation broth ($10^9$ cfu • $mL^{-1}$) and inactivated bacteria fermentation broth ($10^9$ cfu • $mL^{-1}$), the proteins extracted from 20d soybean leaves were separated using two-dimensional gel electrophoresis (2-DE). The 2-DE gel analysis revealed that 3 and 21 protein spots from the treated samples were differentially expressed respectively compared with controls, and then were selected for peptide mass fingerprintied. Of these differentially expressed proteins, 3 and 12 protein spots were identified by MALDI-TOF-MS separately. Further analysis showed that the majority of these were involved in defense, energy and metabolism, suggesting that these proteins might have important roles in defense mechanisms against atrazine stress during soybean seed germination.

**P19.018 Activation of tomato plant defence responses against bacterial spot caused by *Xanthomonas axonopodis* pv. *vesicatoria* using thymol and acibenzolar-S-methyl (ASM)**

*K.A.M. Abo-Elyousr*
*Plant Pathology Department, Faculty of Agriculture, Assiut University, 71526, Assiut, Egypt*
*Email: kaaboelyousr@yahoo.com*

In this study, we investigated the ability of thymol and acibenzolar-S-methyl (ASM) to protect tomato against bacterial spot caused by *Xanthomonas axonopodis* pv. *vesicatoria*. This was combined with studies of accumulation of total phenolic compounds, and activity of enzymes related to plant defence, i.e., polyphenol oxidase (PPO). Under greenhouse conditions, tomato plants pre-treated by thymol and acibenzolar-S-methyl (ASM) profoundly reduced disease severity of bacterial spot compared to plants treated with water. A reduction of bacterial spot were observed in plants treated by thymol and acibenzolar-S-methyl (ASM) (62.1 %) and (69.2%) respectively. Not only thymol and acibenzolar-S-methyl (ASM) reduced bacterial spot but also significantly reduced the population of *Xanthomonas axonopodis* pv. *vesicatoria* in leaves of tomato plants compared with the inoculated control. Application of thymol and acibenzolar-S-methyl (ASM) resulted in a high increase in PPO activity both in plants with and without inoculation. Compared to water-treated plants, treatment with thymol and acibenzolar-S-methyl (ASM) also induced a significant increase (66.1 and 72.2%, respectively) of total phenolic compounds in leaves inoculated tomato plants. These findings suggest that thymol and acibenzolar-S-methyl (ASM) treatment resulted in induction of resistance to bacterial spot in tomato.

**P19.019 AtROP1 negatively regulated potato resistance to *Phytophthora infestans* via NADPH oxidase mediated the production of $H_2O_2$**

*Z.W. Zhang, F. Yang, X.L. Zhang, R. Na, M.S. Fan and J. Zhao*
*Agronomy Department of Inner Mongolia Agricultural University, Hohhot 010019, P. R. China*
*Email: zzw_217@163.com*

Small GTPases are monomeric guanine nucleotide binding proteins. In plant, *ROPs* have multiple roles on regulating the plant cell polarity, plant cell differentiation and development and biotic and abiotic stress signaling pathway. To investigate whether *ROPs* also regulate

defense response of potato to *P. infestans* infection, a dominant negative form of the *Arabidopsis* AtRop1 was overexpressed in potato cultivar Shepody. Both transient expression and DN-Rop1transgenic stable potato lines showed the smaller lesion size, high level of $H_2O_2$ production, and less development of mycelium and less number of zoospores on potato leave surface after infection with *P. infestans*. RT-PCR was also performed by using specific primer derived from rboh-D gene which encodes NADPH oxidase homologus, compared with control plant, the transcript profile of rboh-D gene was induced and lasted for rather long period in DN-Rop1 transgenic plants after infection with *P. infestans*. Based on above results our conclusion is AtROP1 negatively regulated potato resistance to *P. infestans* via increasing the production of $H_2O_2$ and this is achieved via targeting on rboh-D gene, a homologue of NADPH oxidase gene in potato.

**P19.020 Genotype-based variation in induced systemic resistance in *Agrostis* species**

*J. Tung, P.H. Goodwin and T. Hsiang*
*School of Environmental Sciences, University of Guelph, Guelph, Ontario, N1G 2W1, Canada*
*Email: thsiang@uoguelph.ca*

Cultivars of *Agrostis stolonifera* L. and *A. capillaris* L. were screened for their level of responsiveness to the induced systemic resistance (ISR) activator, 2R, 3R-butanediol (BD), by measuring the reduction in disease symptoms caused by the fungal pathogen *Waitea circinata* Warcup & Talbot var. *circinata* compared to a water control. Induced resistance in cultivars 'SR7100' and 'SRP1GMC', as measured by reduced yellowing and mycelial coverage, was strongly responsive to BD. In contrast induced resistance of cultivars 'Penn A4' and 'Providence' was weakly responsive, and BD treatment even led to increased yellowing and mycelial coverage. Following BD treatment, expression of the ISR marker genes, *AsGNS-5, AsOPR-4* and *AsAOS-1*, was determined by RT-PCR before inoculation to measure gene induction, and after inoculation to measure gene priming. Expression of both *AsOPR-4* and *AsAOS-1* was induced by BD only in 'SR7100', and expression of *AsGNS-5* was induced only in 'SRP1GMC'. The two weakly responsive cultivars did not show significant induction of the marker genes. None of the genes showed significant priming of expression in the tested cultivars, and the two weakly responsive cultivars showed significant suppression of marker gene expression after inoculation. Variation among cultivars in their responsiveness to ISR activation by BD clearly indicates that this is a trait that could be used in plant breeding programs, and future work is needed to determine if the induced expression segregates with strong BD responsiveness.

**P19.021 Hydrogen peroxide as inductor of acquired resistance of tomato plants to Fusarium oxysporum**

*N.L. Pshybytko, N.B. Zhavoronkova, L.A. Zenevich, L.F. Kabashnikova and E.A. Lysenko*
*Institute of Biophysics and Cell Engineering, National Academy of Sciences of Belarus, Akademicheskaya str. 27, 220072 Minsk, Belarus*
*Email: pshybytko@rambler.ru*

The main physiological processes in tomato plants under pathogenesis of Fusarium oxysporum under normal and high temperature conditions were studied. Pathogens were inoculated in 4-mounts-old tomato plants trough root system. The pathogenesis carried out about 30-40 days and drying up of tomato plants was watched after them yellowing. The pathogenesis was caused by toxins of Fusarium oxysporum, which suppressed the physiological processes in tomato plants and inhibited biosynthesis of main cell components. Moreover the fungi proliferated actively in root tissues and mycelium grow, enlarged and corked the phloem. The decrease of xylem flow and dehydration of plant tissue were displayed. The activation of destructive processes (lipid peroxidation and generation of reactive oxygen) was obtained. Some peaks of ROS generation were shown after Fusarium oxysporum inoculation. First peak was identificated in half an hour after infection, and physiological processes in tomato plant were not changed. Repeated increase in ROS generation was after 5-7 days. In this case destructive processes was accelerated and some physiological functions were limitated. The PR proteins accumulation was indicated after 24 h. Thus, we surmise that first ROS burst is signal for activation protective mechanisms while second ROS accumulation is destructive process. For the purpose of induction of acquired resistance of plants to pathogens the pretreatment of plants growing in hydroponics by exogenous hydrogen peroxide was tested. Exogenous hydrogen peroxide induced 4 times increase in endogenous ROS content after 3 h after treatment. Then ROS level decreased but it was at high level over a long period of time. In this time the PR proteins synthesis accelerated and pretreatment plants had high level of chitinase. Under following infection of plants pathogen could not penetrate through defence barriers or it development was suppressed. The mechanisms of plant – fungi interaction, signal transduction and hydrogen peroxide role in plant protection are discussed.

**P19.022 The Wound-induced resistance activated with proteins CED-3 via pathogen triggered hypersensitive response in rice**

*W. Hu, W.B. Xie and Y.P. Zou*
*The Key Lab of Natural Medicinal Research of JiangXi Province, YiChun University, No. 576 XueFu Road, YiChun JiangXi, 336000, P.R.China*
*Email: zyping66@scau.edu.cn*

Hypersensitive reaction (HR), as the result of self-protection caused by programmed cell death (PCD) is often found at the pathogen infection site in resistant rice. *Caenorhabditis elegans* is one model organism for cell development research. The mechanism of regulation of *C. elegans* cell programmed cell death (PCD) is mainly regulated and actuated by the genes coding two proteins CED-3 and the CED-4. There is astonishing similarity between the protein domain of CED-3/CED-4 and the R protein domain of plant resistance. Synthetic inducible plant promoters W-box and Tub-1 containing defined regulatory elements provide novel insights into pathogen- and wound-induced signaling. We had construct expression vectors by fusing sequence of inducible promoters and the genes of protein CED-3/CED-4, and transform it into rice. After the transgenic rice is inoculated with plant nematodes, a pathogen triggered response activated coordinately with multiple defense functions. Differential expression patterns of the wound-inducible transgenic *ced-3* in rice following infection with either *Magnaporthe grisea* or roots nematodes, it have been show induced resistance to its disease and HR caused by PCD of epidermis cell in rice. This further show the relationship among the apoptosis programmed cell death and the hypersensitive response, the degree of PCD of epidermis cell and the degree activated by HR in transgenic rice. We found that wound-response regulation of the rice cell PCD gene inducible promoter region coordinately expression in the rice hypersensitive response. It tested that enhanced expression level of interesting protein and HR in transgenic rice, wound-induced disease resistance in rice.

**P19.023 Characteristics of expression of high temperature resistance to stripe rust in Luke**

*H.Y. Zhang, Z.J. Zhang, X.H. Bi and Z. Wang*
*College of Agriculture and Biotechnology, CAU, No 2 Yuanmingyuan West Road, Haidian District, Beijing, 100193, P. R. China*
*Email: huyan7768@yahoo.com.cn*

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most destructive diseases in many regions of the world. High-temperature adult-plant (HTAP) stripe rust resistance induced by the higher environmental temperature has proven to be race non-specific and durable. This type of resistance has remained effective against all races of *P. striiformis* in the northwestern United States for several decades with no loss of resistance. Luke is one of the characteristic representative cultivars. It was susceptible in low temperatures (e.g. 14℃) at all stages of growth, and showed varying degrees of resistance in high temperatures at different growth stages. Luke in the seedling stage can be induced obvious resistance in need of sustained higher temperatures (e.g. 27℃， last for 36h), and there was no significant differences among different leaf ages of seedling stage. Expression of the resistance enhanced at jointing and later growth stages. The under lowest temperature of resistance induction was 16-18℃, and the time requiring of resistance expression could be shorten with the rise of treatment temperature (e.g. 18℃, last for 36h; 29℃, last for 8h). The differences of resistance in seedling and adult stage had no relation with whether plants were vernalized or not. The optimal period for resistance expression of Luke was symptom appearance phase. Express unobvious too early or too late.

## Concurrent Session 20-Invasive and Emerging Diseases

### O20.001 Emergence of the sudden oak death pathogen *Phytophthora ramorum*

N.J. Grünwald
*Horticultural Crops Research Laboratory, USDA Agricultural Research Service, Corvallis, OR 97330, USA*
*Email: grunwaln@science.oregonstate.edu*

*P. ramorum* has emerged repeatedly in North America and Europe despite concerted efforts to eradicate the pathogen. Here, I explore the repeated emergence of *P. ramorum* in the US reconstructing the pattern and process of emergence since discovery of the pathogen in North America based on research published to date in several research groups. The NA1 clonal lineage was first introduced into California, most likely on nursery crops. The pathogen subsequently migrated to Oregon, Washington and British Columbia via nursery shipments. Furthermore, several shipments from the West coast also moved the pathogen East to a range of States. The EU1 clonal lineage was moved from Europe into the Pacific Northwest. From its initial introduction to either British Columbia or Washington, this lineage was further distributed to Oregon and California. The NA2 clone was first introduced into British Columbia or Washington and has to date only spread to California. Thus, the EU1 and NA1 clonal lineages remain restricted to the West coast states and provinces, while the NA1 lineage is now found in many states in the East and West. While both mating types have been found in nurseries, to date sexual reproduction has not been detected. It is apparent that the nursery shipments and imports are the cause for repeated introductions and movement of the pathogen across continental North America.

### O20.002 Bacterial fruit blotch— a big threat on cucurbit crops in China

*Y.L. Tian and B.S. Hu*
*[1]College of Plant Protection and Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Nanjing Agricultural University, Nanjing, 210095, P. R. China; [2]National Engineering Research Center For Cucurbits, Changji, 831100, P. R. China*
*Email: hbs@njau.edu.cn*

Bacterial fruit blotch (BFB), caused by *Acidovorax citrulii*, was introduced into china in the 1980s, and spread rapidly on watermelon, melon and other cucurbits crops and caused unacceptable losses on fruit and seed production of cucurbits. BFB has been the biggest threat on cucurbits production in china. In this report, the disease spreading and distribution, epidemiology, economic losses, disease control and research progress of BFB in mainland China were discussed.

### O20.003 *Phytophthora sojae Avr1d* genes encode an RxLR-dEER effector with presence and absence polymorphisms

*W.X. Yin, S.M. Dong, L.C. Zhai, Y.C. Lin, X.B. Zheng and Y.C. Wang*
*Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095, P.R.China*
*Email: 2011202022@njau.edu.cn*

Root and stem rot of soybean is caused by the oomycete pathogen *Phytophthora sojae*. The interaction between *P. sojae* and soybean fits "gene-for-gene" hypothesis. Our previous research showed that we identified a *P. sojae* RxLR effector *Avr1d* encoding 125 amino acids. In this study, we found Avr1d was absence in the genome of virulence isolates. Sequencing of *Avr1d* genes in different *P. sojae* strains revealed two alleles. Although polymorphic to each other, both two *Avr1d* alleles could trigger *Rps1d* mediated HR. *P. sojae* strains carrying either of the alleles were avirulent on *Rps1d* soybean lines. *Avr1d* was up-regulated during germinating cyst and early infection stages. Furthermore, transient expression of Avr1d in *N. benthamiana* could suppress BAX induced cell death, and enhanced *Phytophthora capsici* infection, suggesting a potential role in suppressing plant immunity. Transient expression of *GFP*::*Avr1d* construct in *N. benthamiana* showed that Avr1d might be localized in both cytoplasm and nucleus, however, expression of both *Avr1d*::NLS and *Avr1d*::NES does not affect Avr1d recognition and virulence phenotype.

### O20.004 Early detection of emerging diseases on urban trees using Next Generation DNA sequencing

*J.A. Berube[1], G. Nicolas[1] and J. Ponchart [2]*
*[1]Laurentian Forestry Centre, Canadian Forest Service, 1055 du PEPS, Quebec, QC, Canada, G1V 4C7; [2]Faculty of Forestry, Université Laval, Quebec city, QC, Canada*
*Email: jberube@cfl.forestry.ca*

Urban trees are the frontline against alien pests. Increasing international trade leads to increasing number of introductions and alien invasive fungi with potential to become emerging diseases usually establish themselves first on urban trees located near ports and transport nexus. An early warning system based on Next Generation DNA Sequencing (454 Roche pyrosequencing) is used to identify newly introduced or already established alien invasive fungi on urban trees. More than 800 urban trees from Vancouver, Victoria, Montreal and Quebec City were sampled in 2011. More than 500,000 DNA sequences were generated and permitted to identify

more than 5000 fungal species. Fungi with some degree of potential impact among these fungal species are presented, along with the 10 most common fungal species found. Analysis of data shows the first ten most common fungi are typically common cosmopolitan pathogens and none of them represent an added risk to Canadian forests. Less than 20 of the species presented could have some potential impact (usually low) to Canadian forests and were found at very low frequency. Alien pests can be molecularly monitored using Next Generation DNA Sequencing, leading to early warning and development of new management options.

**O20.005 Downy mildew of basil in US: what have we learned so far?**

*S. Zhang[1], Z. Mersha[1, 2], R. N. Raid[3], X. Liao[1, 4], Y. Fu[1] and J. S. Patel[1]*
*[1]Tropical Research and Education Center, University of Florida, IFAS, 18905 SW 280th Street, Homestead, FL 33031-3314, USA; [2]Lincoln University Cooperative Extension, Jefferson City, MO, USA; [3]Evereglades Research and Education Center, University of Florida, IFAS, Belle Glade, FL, USA; [4]College of Bio-Safety Science and Technology, Hunan Agricultural University, Changsha 410128, P. R. China*
*Email: szhang0007@ufl.edu*

Basil downy mildew caused by *Peronospora belbahrii* is a devastating foliar disease of basil worldwide. Since its first discovery at Homestead of Florida in October 2007, *P. belbahrii* has been detected in over 20 US states and this disease became a major threat to the US basil production. Strategies for management of basil downy mildew are very limited because of the very recent discovery of the disease, lack of resistance in commercial sweet basil, limited choice of fungicides currently labeled on basil, and leaf symptoms often confused with nutritional deficiencies. Results from our studies indicated that disease severity was greater at 20 or 25℃ than 15 or 30℃, and the most severe disease occurred when wetness duration exceeded 24 hours. Less disease was observed in 5-, 6- and 7- week-old plants at inoculation compared to 2-, 3- and 4- week seedlings. High levels of resistance were detected in Lemon and Indian basil, but not in sweet basil or Genovese, the most popular fresh-cut types of basil worldwide. Biological products alone were generally insufficient in controlling downy mildew of basil. Many fungicides were evaluated to be effective in suppressing this disease, but most are not yet registered on basil. The SAR inducer acibenzolar-S-methyl (Actigard®) significantly reduced this disease under greenhouse and field conditions. In addition, a simplified protocol was developed for rapid detection of *P. belbahrii* from seed and leaves of basil. No genetic variation was detected in *P. belbahrii* isolates collected from US states.

**O20-006 Unwanted dead or alive – weeds revealed as hosts for pathogenic and cryptic *Diaporthe* species in broad-acre cropping systems of eastern Australia**

*S.M. Thompson[1,2], S.M. Neate[1], E.A.B. Aitken[2], Y.P. Tan[3] and R.G. Shivas[3]*
*[1]Department of Agriculture, Fisheries and Forestry, Toowoomba, Queensland, 4350, Australia; [2]School of Agriculture and Food Science, University of Queensland, St. Lucia, Qld. 4067, Australia; [3]Department of Agriculture, Fisheries and Forestry, Ecosciences Precinct, Dutton Park, Qld. 4102, Australia*
*Email: sue.thompson@daff.qld.gov.au*

Several novel *Diaporthe* species have been isolated from weed hosts in the broad-acre cropping regions of eastern Australia. Three recently described species pathogenic on sunflower, *D. gulyae, D. kongii and D. novem* have been isolated from weeds as well as from sunflower. This is the first record of *D. novem* on sunflower in Australia. Additionally, five of a further nine undescribed species collected from weeds or weed stubble have also been indentified from crops including soybean, mungbean and lupin. Six of these cryptic species are associated with sunflower, two with soybean, two with mungbean and one with lupins. Up to five species have been identified from the stubble of one weed species at a single site indicating a high level of taxonomic diversity of this genus in the cropping environment. These findings are highly significant, as low and zero till cropping systems which retain crop and weed stubble and their colonising fungi on the soil surface have been favoured in many regions in Australia for more than twenty years. Soil moisture retention, decreased compaction and cost advantages are benefits of low tillage practices but in recent years stubble borne pathogens have become more prevalent. It is clear that weeds and weed stubble play a pivotal role as alternative hosts assisting the survival of a number of *Diaporthe* species and that many of these species have multiple hosts amongst the crops and associated weed populations. Strategic burial of stubble will need to be part of many tillage operations in the future.

**O20.007 Invasive and emerging threats of *Phytophthora* diseases to vegetable production in India**

*P. Chowdappa*
*Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore-560 089, India*
*Email: pallem22@gmail.com*

India is the second largest producer of vegetables in world with an annual production of 87.53 million tonnes from 5.86 million hectares, accounting 14.4% to the world production. Diseases caused by *Phytophthora* spp. are emerging as a major production constraint for sustainable vegetable production in India. Since 2008, severe outbreaks of Phytophthora diseases such as late

blight on tomato (*P. infestans*), fruit rot on brinjal (*P. parasitica*), leaf blights and wilts in chili (*P. capsici*) were noticed. *P.infestans* isolates causing late blight of tomato, collected from south-west India, were assessed for metalaxyl sensitivity, mating type, mitochondrial DNA (mtDNA) haplotype, DNA fingerprinting patterns based on simple sequence repeats (SSR) and RG 57 probe and aggressiveness on tomato. All isolates were metalaxyl resistant, A2 mating type, mtDNA haplotype Ia and had identical SSR and RG57 fingerprints and highly aggressive on tomato. The phenotypic and genotypic characters of isolates examined in this study were found to be similar to that of 13_A2 genotype of *P. infestans* population reported in Europe. The 13_A2 MLG is unrelated to *P. infestans* populations prevalent in other Asian countries suggesting migration as a more likely cause of the emergence of this pathogen in India. India imported potatoes from U.K and from Netherlands during 2006 and 2007 where 13_A2 genotype was found since 2005 and this genotype might have migrated to India along with potatoes. This study clearly indicates that migration of 13_A2 genotype is responsible for outbreaks of destructive late blight epidemics and stresses the importance of bio-security in agricultural trade.

**O20.008 Implication of glutamate biogenesis in regulation of plant fungal virulence**

*Z.T. Yao, C.W. Zou, H. Zhou, J.Z. Wang, Y. Li, L.D. Lu* and *B.S. Chen*
*Sate Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, College of Life Sciences and Technology, Guangxi University, Nanning 530004, P.R.China*
*Email: chenyaoj@gxu.edu.cn*

Proline dehydrogenase and P5C dehydrogenase are two key enzymes in the biogenesis of glutamate in the cells. The *Prodh* and *P5Cdh* that encode proline dehydrogenase and P5C dehydrogenase in the chestnut blight fungus *Cryphonectria paracitica*, were able to complement the *put1*- and the *put2*- null mutants of the yeast, respectively, to allow the proline utilization-defect yeast mutants to grow in medium with proline as the sole source of nitrogen. Deletion of the *Prodh* gene in *C. parasitica* resulted in hypovirulence and significantly lower level of conidiation. But deletion of *P5Cdh* resulted only in hypovirulence but not lower level of conidiation, suggesting a discrepancy in function of these two genes. In the wild-type strain, the intracellular level of proline and the activity of Prodh and P5Cdh increased after supplementation of exogenous proline to the medium, but the intracellular P5C content kept unchanged, suggesting that P5C was tightly controlled in the cell. The transcript levels of *Prodh* and *P5Cdh* in *C. parasitica* were both down-regulated upon infection by the hypovirus CHV1-EP713. Combined together, we demonstrate that glutamate biogenesis is required for fungal virulence and this pathway is targeted by the hypovirus in *C. parasitica*.

**P20.001 Invasive alien species between Europe and Asia: results from a EU Asia-Link Project**

*M. Pugliese[1,2], H.W. Dehne[3], R. Albajes[4], F.H. Wan[5], S.S. Liu[6], M. Thungrabeab[7], M.L. Gullino[1,2] and A. Garibaldi[1]*
*[1]Agroinnova – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy; [2]Disafa – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy; [3]University of Bonn, Germany; [4]University of Lleida, Spain; [5]China Academy of Agricultural Science, China; [6]Zhejiang University, China; [7]Rajamangala University of Technology Lanna, Thailand*
*Email: massimo.pugliese@unito.it*

The expanding of international travel and trade in the 21st century made national borders more porous and dramatically increased the risk of introduction of invasive plant pests and diseases – the so-called Invasive Alien Species (IAS), which could cause economically disastrous disease outbreaks, resulting in reduction of yields and food quality, higher cost of pest and disease control, thus hindering the international markets and trade. This is particularly true when the increasing commercial relationships between Europe and Asia are considered. Either in Europe or in Asia, there is the emerging need to re-orient scientific and technical capabilities towards the market and social requirements, in order to meet the new challenges of sustainable development. For these reasons, a partnership between China, Thailand, Italy, Spain and Germany has been established, with a total of eight institutions involved as main partners and associates, funded by the European Union under the Asia-Link Programme (CN/Asia-Link/028 108-962). Given the trans-boundary characteristic of IAS management, this project aimed to develop human resources by upgrading the relevant skills of University faculty staff, with particular emphasis on young faculty and future teachers, within a global perspective. Throughout the development of human resources and educational materials the integration of the academic, economic and policy aspects of IAS management have been also addressed. Criteria for the evaluation of the level of risk of accidental introduction of IAS have been individuated, together with a scoring model for risk assessment, and used to develop a list of IAS capable to negatively influence food production in and trade between Europe and Asia.

**P20.002 Investigating the effects of invasive fungal plant pathogens and phytophagous insects on native plants, pathogens, phytophagous insects and symbionts: toward a holistic understanding of biological invasions**

*P. Gonthier, N. Luchi, E. Petrucco Toffolo, R. Balestrini, S. Colazza, M. Faccoli, M. Garbelotto, S. Guarino, L. Giordano, F. Guglielmo, G. Lione, F. Loreto, A. Mello, M. Michelozzi, A.L. Pepori, A. Santini, F. Sillo, A. Vizzini and E. Zampieri*
*University of Torino, Department of Agricultural, Forest and Food Sciences, I-10095 Grugliasco, Italy*
*Email: paolo.gonthier@unito.it*

The effects of biological invasions have been mostly studied in terms of financial losses and ecological impact on native species. There is little information on whether and in which extent invasive organisms may determine physiological responses and genetic changes in native components of ecosystems. A research project named DEFINE (Deciphering the Effects of invasive Fungi and Insects on Native Ecosystems) was recently granted by the Italian Ministry of Education, University and Research, within the FIRB program. The project aims at investigating the potential impacts of invasions by fungal plant pathogens and phytophagous insects on the main components of the native ecosystems: plants, their pathogens, pests and symbionts. Three model systems will be investigated, each including either an exotic pathogen or exotic insect in Europe (the tree pathogen *Heterobasidion irregulare* and the phytophagous insects *Hyphantria cunea* and *Bagrada hilaris*) and its main host in the invasion area (*Pinus pinea*, *Populus nigra*, *Brassica oleracea*). Effects of invasive organisms on a range of native components will be determined by performing comparative inoculation/infestation experiments with other native host-associated pathogens and insects. These effects will be also evaluated on neighbouring healthy plants (inter-plant signalling). Performance of pathogens and insects will be tested, while host responses will be assessed analysing the Volatile Organic Compounds (VOCs), including terpenes stored in conifer tissue. Effects will be also determined on the occurrence and gene expression of native ecto- and endomycorrhizal fungi, and on the genomics of native species that may have experienced introgression of alleles from the invasive organism.

**P20.003 Element analysis of grapevine leaves infected by *Plasmopara viticola* using energy dispersive X-ray fluorescence**

*T. Phiwthong[1], J. Nikaji[1], W. Kaewchanuan[1], M. Sompong[1], K. Thumanu[2], W. Tantanuch[2], A. Auvuchanon [3], D. Athinuwat[4] and N. Buensanteai [1]*
*[1]School of Crop Production Technology, Institute of Agriculture Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand; [2]Synchrotron Light Research Institute (Public organization), Ministry of* Science a*nd Technology, Nakhon Ratchasima, 30000 Thailand; [3]Faculty of Agriculture, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom, 73140 Thailand; [4]Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, Pathumthani, 12121 Thailand*
*Email: nid.nueng@hotmail.com*

Grapevine cv. marroo seedless leave infected by *Plasmopara viticola*, the causal agents of downy mildew disease, were investigated the chemical element using Micro-beam Synchrotron X-ray Fluorescence (μ-SXRF). The result indicated that significant alteration lower content of P, K, Ca, and Mg elements while Fe was increased in infected grapevine leaves when compared with healthy leaves, suggesting that the fungal infection process might disturb these elements accumulation by element consumption of pathogenic fungi. This finding might lead to knowledge adaptation to the management of disease control in the near future.

**P20.004 Evolutionary relationship and adaptation to *Solanum betaceum* of newly emerging *Phytophthora infestans sensu lato* strains reported in Colombia**

*M.F. Mideros[1], L.M. Jaime[1], J.F. Tabima[3], L.S. Lagos[2], N.J. Grunwald[3] and S. Restrepo[1]*
*[1]Laboratorio de Micología y fitopatología LAMFU. Universidad de los Andes. Bogota, Colombia; [2]Grupo Genetica de patosistemas. Universidad de Nariño. Pasto, Colombia; [3]Horticultural Crops Research Laboratory, USDA ARS, Corvallis, OR, USA*
*Email: mf.mideros35@uniandes.edu.co*

Recent research has provided new insights into the global impact of emerging plant diseases caused by oomycete species, but in most cases the phylogenetic relationships of emerging pathogens, their taxonomic status and their adaptation to new hosts are still unclear. In South America emerging *Phytophthora* populations are causing high economic impacts in wild and recently domesticated hosts. Here, we studied new populations of *Phytophthora infestans* attacking *Solanum betaceum* in Colombia, identifying their taxonomic status through phylogenetic and population analyses to determine the evolutionary history. Additionally, we used cross-host pathogenicity tests and microscopic approaches to study host specificity and adaptation during the infection process on several *Solanum* hosts. Nuclear and mitochondrial phylogenetic reconstructions did not show a clear separation between these populations and *Phytophthora infestans* isolates. From a phylogenetic species concept, the hypothesis of a new species attacking tree tomato cannot be supported as previously shown in Ecuador. Furthermore, high differentiation of of these populations was observed respect to *Phytophthora infestans* populations from potato. Cross-pathogenicity and microscopic approaches assays showed a high degree of host specificity. We believe that new hosts, such as tree tomatoes may offer alternative niches, which allow the adaptive emergence of new populations of *Phytophthora infestans* on this host. Both gain and loss of virulence determinants

are probably the main molecular mechanism involved in adaptation processes to new hosts. Results of this research could be used to understand the processes that lead to the emergence of new pathogens, and this can help manage emerging epidemics in South America.

**P20.005 Recent outbreak of *Sclerotinia sclerotiorum* in Bangladesh and its management through eco-friendly phytopathological intervention**

*M.M.E. Rahman[1,2,3], T.K. Dey[1], M.D. Hossain[2] and N. Harada[3]*
*[1]Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh; [2]Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; [3]Graduate School of Science & Technology, Niigata University, Niigata 950-2181, Japan*
*Email: mmer_bari@yahoo.com*

*Sclerotinia sclerotiorum* is a plant pathogenic fungus and can cause a disease called white mold of different crops. In Bangladesh, *S. sclerotiorum* was found to cause cottony rot, stem rot, leaf drop, crown rot and blossom blight of many plants including different fields, and horticultural crops and even weeds. Its diverse host range with increasing trend made a very serious disease. It has found to affect young seedlings, mature plants, and fruits in the field or in storage at different colder parts of the country. It is being considered as an immerging problem for crop production in those regions in recent years. Before 2007, only 5 crops was noted to be infected by *S. sclerotiorum*, but at present around 40 plant species have been identified as hosts of the fungus. In this study, twelve fungal isolates were collected from infected plant parts of different hosts and their morpho-physiological and molecular characteristics were examined. Growth of mycelia was the maximum when it was incubated at 15 to $20^0$C and pH 6 regimes. PCR amplifications of internal transcribed spacer (ITS) region of rDNA of selected isolates were conducted and the sequences were determined. NCBI BLAST searches revealed the highest homologies (>99%) with the sequence of *S. sclerotiorum*. Different management options including eco-friendly cultural means were practiced to control the disease. A *Trichoderma*-based biofungicide was found effective against the disease in *in-vitro* and pot house assay. Intercropping with short duration crops was found to be best in field condition.

**P20.006 Distribution of blackleg disease on oilseed rape in China and its pathogen identification**

*Q.S. Li, B.C. Hu, S.B. Rong, Y.F. Jiang, S.M. Hou, W.X. Fei, F.X. Chen, X.J. Wu, Z.X. Fan and W.X. Lei*
*Crop Research Institute, Anhui Academy of Agricultural Sciences, Anhui provincial key lab of crop quality improvement, Hefei, 230031, Anhui, P.R.China*
*Email: baochenghu2006@126.com*

Surveys for stem canker / blackleg on oilseed rape (*Brassica napus*) were conducted from 60 locations throughout provinces in China covering both winter oilseed rape and spring oilseed rape during 20008-2012. Infected plants were found in 42 locations from 14 provinces out of 16 provinces investigated. One tenth of the surveyed fields were found to be blackleg-infested with incidence up to 92% and 5% of plant death in the most severely affected field. The diseased plants or plant debris with blackleg symptoms were collected and the causal fungi were isolated from the lesions. In total, 348 purified isolates were collected. Both cultural identification and multiple PCR detection methods were used to identify the pathogen species. Results showed that only the less aggressive *Leptosphaeria biglobosa*, but no aggressive *Leptosphaeria maculans*, was identified from the samples. The pathogenicity of 22 *L. biglobosa* isolates (collected from 11 provinces) was evaluated in vivo cotyledon using inoculation method with pycnidiospore suspension. Results showed all 22 isolates were pathogenic to *Brassica napus*. All the tested isolates showed higher pathogenicity than the control Polish *L. biglobosa* isolate（PL-Lb）, with only one exception of isolate CN-21, collected from Hailer, Inner Mongolia. 14 isolates were considered high pathogenic and 8 isolates were moderately pathogenic.

**P20.007 Studies on infection conditions of *Leptosphaeria biglobosa***

*P.L. Song[1,2], X.Z. Li[1,2], L.F. Hao[1,2], Y.Y. Bao[1*] and Z.Q. Li[2*]*
*[1]School of Life Science, Inner Mongolia University, Hohhot 010020,P.R.China; [2]Plant Protection Institute, Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot 010031 P.R.China*
*Email: peiling8721@126.com*

Phoma stem canker (blackleg) is an internationally important disease of oilseed rape and vegetable brassicas. The pathogen populations comprise two main species: *Leptosphaeria maculans* and *Leptosphaeria biglobosa*. The species identity of the pathogen of Phoma stem canker was determined through a combined study of rDNA-ITS sequences. Phylogenetic analysis showed that the isolate clustered together with the isolates of *L. biglobosa* and was distinct from those of *L. maculans*. ITS sequence analysis indicated the isolate was *L. biglobosa*. Infection conditions and pathogenicity of *L. biglobosa* were conducted in controlled environment. The studies indicated that blackleg of oilseed rape frequently occurred under high temperature and high humidity conditions, and the pathogen couldn't infect the host once the temperature and humidity were unsuitable; Temperature and wetness period could greatly affect the infection of *L. biglobosa*, the favorite combination of temperature and wetness period for its infection was

21-24℃ with over 48 h wetness, below 48 h, disease index decreased with wetness decreasing and temperature increasing or decreasing. As wetness period from12-72h, disease index increased with temperature increasing from15-21℃, but it reduced with temperature increasing from21-27℃.

**P20.008 Citrus canker: status in Bangladesh with the threat on export and overcome through taking pathological strategies**

*T.K. Dey[1], M.M.E. Rahman[1], R. Islam[1] and M.A. Razzaque[2]*
*[1]Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh; [2]Krishi Gobesona Foundation, Bangladesh Agricultural Research Council, Dhaka, Bangladesh*
*Email: mmer_bari@yahoo.com*

Commercially growers and exporters of citrus were facing impending production and exporting debacle due to citrus canker disease in Bangladesh. Considering the situation, a phytopathological effort was initiated to solve the problem. An intensive survey and monitoring of citrus canker disease were performed. Disease incidence was varied depending upon season, varieties of lemon and location of garden. After field experimentation, it is concluded that only fungicides can not control the disease successfully. Integrated approach was found to be effective for controlling the canker disease. Some important steps were taken for integration to manage the citrus canker such as a) proper sanitation, b) application of cow dung and balanced chemical fertilizers, c) pruning of older branches followed by application of Bordeaux paste, d) application of copper fungicide (Sunvit or Cupravit 50 WP at the rate of 7 gm/l water) with the initiation of disease at 15 days interval, and e) monthly application of Imidacloropid (Admire @ 0.5 ml/l water) for controlling leaf miner to reduce wounds for infection. For post-harvest treatment, healthy lemon soaked in the solution of Sodium Ortho Phenyl Phenate (SOPP) (@ 2.3%) for 1 minute reduced the bacteria from the skin surface of lemon. With judicious execution of such pre and post technology our large sized (Jara) lemon exports from Bangladesh to Europe started again. A phytopathological strategy helped us to overcome an export crisis successfully.

## Concurrent Session 21-Management of Forest Diseases

### O21.001 Plantation health management in the tropics and southern hemisphere: Challenges and opportunities

*M.J. Wingfield, J. Roux, B.D. Wingfield and B. Slippers*
*Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0028, South Africa*
*Email: Mike.Wingfield@fabi.up.ac.za*

During the course of the last 100 years, plantation forestry has grown rapidly in the tropics and southern hemisphere, chiefly to sustain large solid timber and pulpwood industries. These plantations are almost exclusively based on non-native species of *Pinus*, *Eucalyptus* and *Acacia*. Consistent with the fact that the trees were separated from their natural enemies, plantations were initially relatively free of health challenges. This is a situation that has changed with time, due to the accidental introduction of pests and pathogens, but also through challenges arising from native disease agents and insets adapting to infect/infest non-native tree species. Conceptually unexpected tree health problems continue to arise and these are presenting growing challenges to plantation owners. In some cases, entire industries failed and forestry companies are increasingly aware that pests and pathogens arguably present the single greatest threat to the sustainability of plantation forestry. While the long-term challenges are substantial, there are also many opportunities to reduce the impact of pests and diseases in plantations. These rest on new technologies, long-term investments and generally require intensive research and development. In contrast to what is required, many business leaders in forestry tend to rely on promises of short-term options and so-called "silver bullet" solutions. Yet those prepared to adopt a longer-term view have already and will continue to reap the benefits of their investments.

### O21.002 Managing invasive pathogens: *Puccinia psidii* in Australia

*A.J. Carnegie[1] and G.S. Pegg[2]*
*[1]Biosecurity NSW, NSW Department of Primary Industries, PO Pox 100, Beecroft NSW 2119 Australia; [2]Horticulture & Forestry Science, Department of Agriculture, Fisheries & Forestry, GPO Box 267, Brisbane 4001, Australia*
*Email: angus.carnegie@dpi.nsw.gov.au*

*Puccinia psidii* was described in Brazil over a century ago and began to gain prominence following its invasion of new countries, as well as exotic hosts in its endemic range, where it caused devastating disease. The common theme was the susceptibility of naive hosts, either as *P. psidii* spread outside its native range (e.g. to Jamaica, Florida, Hawaii) or as exotic species (e.g. *Eucalyptus*) were planted on a large scale in Brazil. *P. psidii* is a rust of Myrtaceae, and as such was recognised as a biosecurity threat to Australia's Myrtaceae-rich ecosystems and industries reliant on Myrtaceae. In 2010 it was detected in Australia, and has since spread along the eastern seaboard from temperate to tropical environments. Prior to reaching Australia there were approximately 125 known hosts of *P. psidii*; this figure has more than doubled in the short time the rust has been present in Australia. Although the host range is wide, and expanding, significant impact has currently only been observed on a handful of individual species. However, for some of these species the impact is devastating, with gradual decline over an entire species range (e.g. *Rhodamnia rubescens*). Some rare and threatened species are on a "watch list" as they are highly susceptible. Impact on key species includes reduced foliage production and growth, seedling death and flower and fruit collapse, thus affecting species fecundity. We briefly discuss the taxonomy of this rust, the emergency response in Australia, and focus on the impact in the native environment and commercial industries and potential management strategies.

### O21.003 Current research on the biology and management of laurel wilt in the United States

*J.A. Smith, R.C. Ploetz, J. Hulcr, J. McCutcheon, T.J. Dreaden, M.A. Hughes, D. Spence, K. Shin, S.Inch, J. Ploetz and A. Campbell*
*University of Florida, School of Forest Resources and Conservation, Gainesville, FL 32611-0410, USA*
*Email: jasons@ufl.edu*

Laurel wilt (LW), caused by the fungal pathogen, *Raffaelea lauricola*, is a highly destructive exotic disease that threatens trees in the Lauraceae, including avocado, redbay and sassafras. Since 2002, the disease has nearly wiped out redbay in much of the southeastern USA and threatens the avocado industries in Florida (valued at > $60 million/year) and California ($400 million). The pathogen is transmitted to trees by an insect vector, the Asian redbay ambrosia beetle (*Xyleborus glabratus*). Typically, ambrosia beetles carry nonpathogenic fungal symbionts that the beetles consume, rather than wood. LW is unique in that the symbiont of *X. glabratus*, *R. lauricola*, is highly virulent to host trees. Very few spores of *R. lauricola* are needed to incite LW. Microscopic observations and studies with secondary metabolites indicate that hosts over-react to the presence of the pathogen by excessive tylose formation and, ultimately, vascular dysfunction, but toxins are probably not involved in pathogenesis. Genetic analyses of *R. lauricola* indicate that a single clone occurs in the USA. Comparisons of microsattelite loci of the pathogen in the USA indicate a close match with isolates in Taiwan, but differences with Japan; however, there were no differences

in virulence detected. Lateral transfer of the pathogen to other ambrosia beetle species has been documented. Current efforts to manage LW focus on systemic fungicides, judicious sanitation in avocado orchards and host resistance. Genomic analyses of the pathogen and closely related, nonpathogenic symbionts are underway to gain insight into what makes *R. lauricola* a plant pathogen.

**O21.004 Population genetics and GIS based analyses as essential tools to model biological invasions: the case of *Heterobasidion irregulare* in Italy**

*P. Gonthier and M. Garbelotto*
*University of Torino, Department of Agriculture, Forest and Food Sciences, I-10095 Grugliasco, Italy*
*Email: paolo.gonthier@unito.it*

The North American forest pathogen *Heterobasidion irregulare* is currently present in pine stands along over 100 km of coastline west of Rome. We used population genetics and GIS based analyses to infer introduction pathways, spread patterns and habitat preference of this destructive root rot fungus in Italy. Until now the notion that *H. irregulare* may have been introduced through infected wood by the US Army in the area of Castelporziano/Castelfusano was based on circumstantial evidence. In a comparative analysis of *H. irregulare* isolates from six sites, sequence-based diversity indices indicate the highest diversity is found right in Castelfusano/Castelporziano, and that diversity progressively decreases with increasing distance from that site; this finding is congruent with the above suggested introduction pathway. Bayesian clustering analysis based on 12 SSR loci indicate the same three genetic clusters are present in all sites, suggesting the current infestation is the result of a single rather than multiple introductions. Spatial autocorrelation analyses using SSR data indicate a significant under-dispersion of alleles up to 0.5-10 km and a significant over-dispersion of alleles over 80 km. A GIS based analysis of infectious airspora of *H. irregulare* and the native *H. annosum* in relation to different vegetation types indicates that while the native species is positively associated with pines and negatively associated with deciduous oaks, the invasive one is present irrespective of vegetation type, and may be found in pure oak forests. This information is essential to both predict and hinder the progress of the exotic pathogen in Europe.

**O21.005 Can genome sequencing transform our understanding of fungal forest pathogens?**

*J. Stenlid, M. Cleary, K. Dalman, M. Brandström Durling, M. Elfstrand, M. Lind, M. van der Nest and Å. Olson*
*Department of Forest Mycology and Plant Pathology, Box 7026, 75646 Uppsala, Swedish University of Agricultural Sciences (SLU), Sweden*
*Email: jan.stenlid@slu.se*

The recent technical developments with dramatically lowered costs for full genome sequencing have opened up an exciting field of discovery of the genetic basis for fungal pathogenicity to trees. One important feature is that non-model organisms can be analyzed in an amazing depth. When sequencing the root rot pathogen *Heterobasidion annosum sensu lato* found quantitative trait loci critical for pathogenicity, and rich in transposable elements, orphan and secreted genes. A wide range of cellulose-degrading enzymes are expressed during wood decay. By contrast, pathogenic interaction between *H. irregulare* and pine engages fewer carbohydrate-active enzymes, but involves an increase in pectinolytic enzymes, transcription modules for oxidative stress and secondary metabolite production. Our results show a trade-off in terms of constrained carbohydrate decomposition and membrane transport capacity during interaction with living hosts. Our findings establish that saprotrophic wood decay and necrotrophic parasitism involve two distinct, yet overlapping, processes. Furthermore, by resequencing the genomes of a population of *H. annosum*, we were able to identify a number of genes associated with pathogenicity. The sequencing of genomes of a number of other forest pathogens, including *Hymenoscyphus pseudoalbidus*, the cause of recent ash dieback in Europe, indicates key factors important for pathogenicity.

**O21.006 *Bursaphelenchus xylophilus*: opportunities in comparative genomics and molecular host–parasite interactions**

*C. Vicente, M. Espada, F. Nascimento, P. Vieira, J. Jones and M. Mota*
*Depat. Biologia, Universidade de Évora, Portugal*
*Email: mmota@uevora.pt*

*Bursaphelenchus xylophilus*, the pinewood nematode (PWN), is the causal agent of the pine wilt disease (PWD), which is a major threat to European and Far East forestlands. Knowledge concerning various aspects of the PWN ecology and interaction with different species of the insect-vector, a broad range of susceptibility/resistance of the tree hosts, coupled with associated bacteria, play a decisive role in the development of the disease. However, these mechanisms are not totally understood. The first insights into PWN genomics were based upon expressed sequence tags (EST) providing the identification of putative parasitism genes, or effectors. Moreover, the recent release of the whole-genome sequence of *B. xylophilus* will open new opportunities to further studies on nematode interactions, in particular with ecto-mutualistic bacteria that may contribute synergetically for PWN pathogenicity. A review of the current know-how on *B. xylophilus* is presented with recent developments in molecular biology of PWN which may

bring new insights into its pathogenicity and interactions with the other factors governing PWD.

**O21.007 Emerging plant diseases: combining genotypic and phenotypic data to improve our predictions of invasive pathogens**

*M. Garbelotto, P. Gonthier, G.D. Rocca and T. Kasuga*
*Department of ESPM, University of California, Berkeley, USA*
*Email: matteog@berkeley.edu*

While technological advances currently allow to genotype pathogen strains relatively easily, the connection between genotype (the unit of genetic inheritance) and phenotype (the unit upon which selection operates) is complicated by the fact that genetic, environmental, and epigenetic factors are all important contributing factors in phenotype determination. However, the genetic structure of a potentially invasive species can help in at least assessing the complete range of risks it may pose. Three cases are well known from the literature: i) An invasive species comprises morphologically undistinguishable but evolutionarily distinct lineages, each posing a distinct threat; ii) Introduction was limited to a single population source, thus leading to a strong founder effect and to a narrower range of phenotypes than in the area where the invasive species is native, and, iii) An individual genotypes may be particularly destructive in the new naïve ecosystem, hence not all genotypes should be regarded equal. In spite of common beliefs, we show that phenotypic plasticity is not always correlated to breadth of genetic variability of an invasive species, but that often genetically narrow invasive populations will display broader phenotypic variability than source populations. Using *Phytophthora, Seiridium* and *Heterobasidion spp*. as case studies, we will discuss the points above. Finally, we present data showing that invasions may lead to (i) interspecific hybridization and gene introgression with unpredictable outcomes, and to, (ii) different epigenetic effects on an identical genotype of a pathogen. While epigenetic control is well known for plant and animal hosts, it has only been indirectly postulated for pathogens: our direct evidence shows that phenotype can permanently be affected by the *history* of a genotype, further complicating predictions.

**O21.008 Management of forest pathogens in a drying climate – a case study in Western Australia**

*G.E. Hardy, G. Matusick, K. Ruthrof, W. Dunstan, N. Brouwers and T.I. Burgess*
*State Centre of Excellence for Climate Change, Woodland and Forest Heath, School of Veterinary and Life Sciences, Murdoch University, Murdoch, 6150, Western Australia*
*Email: g.hardy@murdoch.edu.au*

The south-west of Western Australia (SWWA) is considered a global 'guinea-pig' with regards to impacts of climate change on forest health and function. The SWWA has experienced a pronounced shift in rainfall since the 1970s along with temperature rises of 0.15 ℃ per decade over this period. As a result, a number of endemic *Eucalyptus* forest species have been undergoing significant declines in health and mortality since the early 1990s. Most recently, a record dry and hot year in 2010 resulted in significant canopy collapse of several endemic eucalypt species. In response, the incidence and severity of some forest pathogens have increased, whilst others such a *Phytophthora cinnamomi* have declined. The incidence and severity of opportunistic pathogens have also increased, together with outbreaks of pests like wood borers (e.g. *Phorocantha semipuncta).* We will discuss our approaches to monitoring the impact and severity of these declines, including the use of Digital Multi-spectral Imagery, eco-physiological studies, changes in mycorrhizal symbioses, the importance of correct diagnostics, and the need to develop models to predict likely impacts on the forest ecosystems into the future. Without this detailed background knowledge, managers and policy makers cannot make informed decisions on how to manage these forest ecosystems into the future.

**O21.009 Latest developments in pine decline disease development and management**

*L.G. Eckhardt, G. Matusick, J. Zanzot, Y. Zeng and R.D. Menard*
*Forest Health Dynamics Laboratory, School of Forestry and Wildlife Sciences, AuburnUniversity, Auburn, AL 36849, USA*
*Email: eckhalg@auburn.edu*

Pine decline and mortality syndromes have been increasingly reported in the past twenty years in many areas in the southeastern US. Pine tree decline, like forest declines is described as resulting from complex interactions of biotic and abiotic stressors. Current studies of pine stands in the southern U.S. with trees expressing symptoms of decline show strong associations with the presence of root-feeding bark beetles and the presence ofophiostomatoid fungi. Ophiostomatoid species are commonly associated with various species of root-feeding bark beetles, which attack healthy and stressed trees. These insects serve as vectors introducing these fungi into tree roots or as wounding agents creating infection courts which permit infection by these fungi. In addition, some ophiostomatoid fungi are known to be pathogens under appropriate field conditions. *Leptographiumalacris, L. procerum, L. terebrantis, Grosmanniahuntii* and several undescribed*Ophiostoma* spp. have been reported from the roots of declining southern yellow pines in the southeastern U.S. The level of pathogenicity and the specific role of these fungi are uncertain.

New studies are focused on not only fungal pathogenicity and fungal-insect relationships, but also the affect that management strategies have on these relationships. Other studies include (1) how thinning, biomass removal, burning, fertilization, and the management of cogongrass affect the vector and vector-predator populations in these areas, (2) ophiostomatoid fungi isolated from the snouts of wild pigs that root and feed in pine stands and (3) two new *Ophiostoma* spp. have been associated with the *Hylastes* populations in Alabama and Georgia.

### O21.010 *Hymenoscyphus pseudoalbidus* and *Hymenoscyphus albidus*: viridiol concentration and virulence do not correlate

*C. Junker[1], F. Mandey[2], A. Pais[2], R. Ebel[2] and B. Schulz[1]*
*[1]Institute of Microbiology, Technical University of Braunschweig, Spielmannstr. 7, 38106 Braunschweig, Germany; [2]Marine Biodiscovery Centre, Department of Chemistry, University of Aberdeen, AB24 3UE Aberdeen, UK*
*Email: b.schulz@tu-bs.de*

*Hymenoscyphus pseudoalbidus* is the causal agent of ash dieback, a disease which is presently endangering *Fraxinus* spp. throughout most of Europe. The phytotoxin, viridiol, was previously isolated from culture extracts of *H. pseudoalbidus* and found to be toxic to leaves of *F. excelsior*. Thus, we were interested in learning to what extent viridiol is responsible for pathogenicity of *H. pseudoalbidus* and investigated this using twelve isolates *H. pseudoalbidus*. We also included five isolates of the closely related avirulent species, *Hymenoscyphus albidus,* in our studies. Some, but not all, isolates of *H. pseudoalbidus* and *H. albidus* produced measurable quantities of viridiol in culture. Three tests were used to determine to what extent viridiol concentration correlates with virulence: culture extracts were tested for activity in leaf segment tests and for inhibition of germination of seedlings of *Fraxinus excelsior*; virulence of the isolates was tested following infection of axenically cultured ash seedlings. Activity of the culture extracts varied, as did virulence of the isolates following inoculation into seedlings. No correlations were found between viridiol concentration and activities of culture extracts in leaf segment tests or in the germination test, nor between viridiol concentration and disease symptoms when inoculated into seedlings. However, activities of culture extracts in leaf segment and in the germination test correlated, as did the results of each of these tests with virulence in the infection experiment. Apparently, as yet unidentified factors other than viridiol content play important roles in the virulence of *H. pseudoalbidus.*

### O21.011 Infecting structure of *Melampsora pruinosae* in *Populus euphratica* and *P. pruinosa*

*S.C. Gu[1, 2], Q. Li[1, 2], Y.L. Wang[3], Z.J. Li[3] and J.Q. Li[1, 2]*
*[1]Department of Plant Pathology, China Agricultural University / Key laboratory of plant pathology, Ministry of Agriculture P. R. China, Beijing 100193, P. R. China; [2]Beijing Engineering Research Center of Seed and Plant Health (BERC-SPH) / Beijing Key Laboratory of Seed Disease Testing and Control (BKL-SDTC), Beijing 100193 P. R. China; [3]College of Plant Science, Tarim University / Xinjiang Production & Construction Corps Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin, Alar, Xinjiang 843300, P. R. China*
*Email: gsc2007118698@126.com;lijq231@cau.edu.cn*

*Populus euphratica* and *P. pruinosa* are the most ancient and primitive species of genus *Populus*. Residing in arid and semi-arid areas, they have functions of climatic regulation and water conservation, but endangered due to climate change and land desertification. Rust disease caused by *Melampsora pruinosae* is serious in *populus* afforestation. Studies on morphology and germination of uredospores, as well as infecting structure of *M. pruinosae* in *P. euphratica* and *P. pruinosa* were conducted by means of dry examination, microscopy, and ultrastructure observation. The results showed that the uredospores and uredia of *M. pruinosae* in both *P. euphratica* and *P. pruinosa* appeared to be similar in morphology and structure. The uredia revealed hemispherical or half ellipsoidal masses with the visible color of orange or golden yellow on both sides of diseased leaves. Uredospores were unicellular and thick-walled spherical or ovoid shape, transparent and orange yellow. The testing of the uredospores germination of two isolates of *M. pruinosae* respectively from *P. euphratica* and *P. pruinosa* indicated that there were no significant differences in four parameters of uredospores, including length, width, height of projection, and intervals of projections. The hyphae, formed by germ tubes from germinated uredospores, infected the leaves through stoma directly, and expanded in epidermal and mesophyll cells of the diseased leaves in both *P. euphratica* and *P. pruinosa*.

### O21.012 Response of living tissues of *Pinus sylvestris* to the saprotrophic biocontrol fungus *Phlebiopsis gigantea*

*H. Sun[1,2], L. Paulin[3], E. Alatalo[3] and F.O. Asiegbu[1]*
*[1]Department of Forest Sciences, University of Helsinki, PO Box 27, Helsinki, Finland; [2]Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 66, Helsinki, Finland; [3]DNA Sequencing and Genomics Lab, Institute of Biotechnology, University of Helsinki, PO Box 56, Helsinki, Finland*
*Email: hui.sun@helsinki.fi*

The saprotrophic fungus *Phlebiopsis gigantea* has been used for several years as a biocontrol agent against the

conifer pathogen *Heterobasidion annosum*. Although the effectiveness of *P. gigantea* in biocontrol has been shown empirically, the long-term effect on living conifer trees as well as the mechanism underlying its antagonistic activity is still unknown. An additional concern is the potential of *P. gigantea* to acquire a necrotrophic habit through adaptation to living wood tissues. By using a combination of histochemical, molecular and transcript profiling (454 sequencing), we investigated under *in vitro* conditions the necrotrophic capability of *P. gigantea* and induced localized resistance as a mechanism for its biocontrol action. *Pinus sylvestris* seedlings (10 years old) were challenged on the xylem surface with *P. gigantea* or *H. annosum*. Both fungi provoked strong necrotic lesions, but after prolonged incubation, *P. gigantea* lesions shrank and ceased to expand further. Tree seedlings pre-treated with *P. gigantea* further restricted *H. annosum*-induced necrosis and had more lignified cells. The 454 sequencing revealed elevated transcript levels of genes important for lignification, cell death regulation and jasmonic acid signalling. The results suggest that induced localized resistance is a contributory factor for the biocontrol efficacy of *P. gigantea*, and it has a limited necrotrophic capability compared with *H. annosum*.

### O21.013 Plantation forestry diseases in Ghana: Causes and management strategies

M.M. Apetorgbor[1], P.P. Bosu[2], E. Nkrumah[1] and J. Roux[2]
[1]*Forestry Research Institute of Ghana, P. O. Box 63, KNUST, Kumasi, Ghana;* [2]*Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa*
*Email: mapetorgbor@yahoo.com; mape@csir-forig.org.gh*

Ghana is on the verge of exhausting its timber resources and has thus embarked on reforestation with indigenous and exotic tree species. However, with the establishment of pure stands of exotic and indigenous tree species the threat of disease problems has increased. Large scale planting of high value indigenous tree species such as Odum (*Milicia excelsa* and *Milicia regia*), Mahogany (*Khaya* and *Entandrophragma* species), Kokrodua (*Pericopsis elata*) in Ghana were unsuccessful as a result of insect pest and disease problems. These failures, together with the urgent need to establish plantations to meet decreasing timber from naturally managed forests, partly account for the widespread planting of Teak (*Tectona grandis*) and other exotic species in Ghana today. Disease outbreaks in exotic tree plantations such as *Tectona grandis*, *Cedrela odorata* and *Ceiba pentandra* which were generally low have been reported to be increasing with the expansion of the plantation estate. A study was initiated to identify key diseases occurring in tree plantations, determine their distribution, and evaluate management strategies to contain them. This paper presents a preliminary review of diseases affecting plantation forestry and highlight factors favouring their development in Ghana. Diseases encountered include root rot caused by *Armillaria* sp. on *Tectona grandis* and *Cedrela odorata*, stem cankers initiated by stem borers which predispose *Cedrela odorata* to *Lasiodiplodia theobromae* infection, leaf spot and dieback on *Ceiba pentandra* seedlings and saplings caused by *Colletotrichum capsici*, *Fusarium solani* and *Lasiodiplodia theobromae* and dieback and stem canker on *Eucalyptus* spp. caused by *Botryosphaeria* sp. These diseases are imparting serious economic losses on plantation development and successful plantation management will require training of foresters regarding tree health issues, more effective quarantine and silvicultural practices, establishment of sound breeding and selection programmes with a considerable commitment from Government, commercial companies and research organizations in the country.

### O21.014 Testing and protecting butternut (*Juglans cinerea*) trees putatively resistant to *Ophiognomonia clavigignenti-juglandacearum*

D. Rioux[1], P. DesRochers[1], N. Nadeau-Thibodeau[2], M. Lagacé[1], C. Overton[1], K. Klimaszewska[1] and L. Bernier[2]
[1]*Natural Resources Canada, Laurentian Forestry Centre, Québec, QC, Canada, G1V 4C7;* [2]*Centre d'étude de la forêt, Université Laval, Québec, QC, Canada, G1V 0A6*
*Email: danny.rioux@rncan-nrcan.gc.ca*

*Ophiognomonia clavigignenti-juglandacearum*, the causal agent of a canker, is considered the major pest of butternut and is threatening its survival all over its native range in North America. In Canada, butternut was listed as endangered under the *Species at Risk Act* in 2005. A project aimed at identifying, propagating and testing trees putatively resistant to butternut canker in the province of Quebec was initiated in 2010. As butternut is shade intolerant, another goal was to release trees from competition within natural stands in order to improve their vigour and, hopefully, their resistance to butternut canker. Some 30 trees that were released in 2011 still appear healthy and seedlings were seen under these trees. In all, 202 putatively resistant trees were located in Quebec where the disease is well established. Only two of these trees appear to be hybrids between butternut and Japanese walnut (*J. ailanthifolia*). Putatively resistant trees were propagated using nuts, cuttings and cultures of axillary buds. Material from susceptible trees served as control. The propagated material was stem-inoculated in 2012 and is currently being assessed for resistance. However, it is already possible to observe that some trees have reacted strongly to the inoculation by compartmentalizing their invaded tissues in which suberized barriers and phenol accumulation are particularly noticeable in microscopy. Should resistant trees be

found, they will be propagated and used to restore suitable forest sites.

### O21.015 Western gall rust pathology and Monterey pine conservation

*M.M. Chen*
*University & Jepson Herbaria, Valley Life Science Building, Berkeley, CA 94720, USA*
*Email: momeichen@berkeley.edu*

Along the North California coast especially at Point Reyes Park areas a large number of deaths of Monterey pine (*Pinus radiata*) occurred in the 1980's caused by western gall rust. The Monterey pine supplies the world's best and perfect lumber, but under the deadly attack of the rust disease, the genetic pool of Monterey pine has been facing risky declines. Therefore, forest geneticists and forest pathologists proposed a cooperative internationally research project. The results show a positive correlation between the pathogen rust virulence and the tree susceptibility. Obvious display of experimental results show, the most virulent strains of rust were collected from the most susceptible original Monterey pine clones. All rust are obligate parasites. In particular, I discovered and researched the Telia sexual reproductive stage on the evergreen Live Oak (*Quercus agrifolia*) and the Pycnia sexual stage on the Monterey pine, advancing our understanding of the rust life cycle basis of heteroecism. We are now well aware that the western gall rust not only has an aeciospores stage. The life cycle of rust must have coevolved genetically on fast-growing Monterey pine and evergreen Live Oak in the Californian coast ecosystem. Therefore, we should consider transfer the Monterey pine to the southern hemisphere where there are no oak needed for the heteroecious Monterey Pine rust and allow the pines to escape the disease without the expense of preservation and control treatments.

### O21.016 Diseases of eucalypts in China

*S.F. Chen[1,2], J. Roux[2], Y.J. Xie[1] and M.J. Wingfield[2]*
*[1]China Eucalypt Research Centre (CERC), Chinese Academy of Forestry (CAF), ZhanJiang 524022, GuangDong Province, China; [2]Forestry and Agricultural Biotechnology Institute (FABI), Department of Microbiology and Plant Pathology, Private Bag X20, University of Pretoria, Pretoria 0028, South Africa*
*Email: shuaifei.chen@gmail.com; shuaifei.chen@fabi.up.ac.za*

In order to meet a rapidly growing need for pulp and paper, more than 3.5 million hectares of eucalypt plantations have been established in South China. The sustainable development of the eucalypt plantations is under increasing threat due to pathogens, while information on the species identification, origin and impact of fungal pathogens in China is limited. During the past several years, a number of eucalypt disease surveys have been conducted in South China. The causal agents of these diseases have been identified by using morphology and DNA sequence data. The diseases encountered in China include stem canker/wilt caused by species of *Celoporthe*, *Ceratocystis*, *Chrysoporthe*, *Fusicoccum*, *Lasiodiplodia*, *Neofusicoccum* and *Teratosphaeria*. Leaf blight caused by species of *Calonectria*, *Mycosphaerella* and *Quambalaria*, and bacterial wilt caused by *Ralstonia solanacearum* are also common. Field trials have been conducted to test the pathogenicity of these fungi on commercially grown eucalypt clones. Results showed that there are significant differences in the susceptibility of these clones to fungal species, indicating that selection of resistant material for commercial planting in the future can be achieved. Population studies of *Teratosphaeria zuluensis* indicated that it has a very high genetic diversity in China, suggesting the presence of sexual recombination in the region. The origin of many of the pathogens are still unknown, but they most likely represent a combination of native and introduced fungal species. The number of disease problems on eucalypt plantations in China will continue to grow. A combination of management strategies and close interaction between foresters, tree breeders and pathologists will be needed to ensure a sustainable eucalypt industry in China.

### P21.001 First report of oak anthracnose caused by *Apiognomonia errabunda* on oriental white oak in Korea

*S.H. Lee[1] , J.H. Park[1] and H.D. Shin[2]*
*[1]Division of Forest Diseases and Insect Pests, Korea Forest Research Institute, Seoul 130-712, Republic of Korea; [2]Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-701, Repulic of Korea*
*Email: shlee4@forest.go.kr*

Oriental white oak (*Quercus aliena*) is native to East Asia including Korea. It is one of the major deciduous tree species in natural forests in Korea. In May 2012, several hundred trees were found to be heavily damaged by a previously unknown leaf disease in a forest near Songjiho Lake in Goseong County of central Korea. Leaf symptoms began as small, water-soaked, pale greenish to grayish lesions, which enlarged to follow the veins or midribs and to be bounded by them, often killing part of the leaf. Leaf distortion and blight might be resulted in the later stage of disease development. A number of grayish brown to nearly black acervuli formed on the lesions, especially on the midribs and veins. Morphological characteristics of fungal structures (e.g. conidia, acervuli) were consistent with the description of conidial state of *Apiognomonia errabunda*. Fungal DNA was extracted for sequencing of the complete internal transcribed spacer (ITS) region with the primers

ITS1/ITS4. BLAST search against GenBank database found >99% similarity with sequences of *A. errabunda* (AJ888475–888477). Pathogenicity test was conducted with six seedlings; three for inoculating with a conidial suspension ($10^6$ conidia/ml) and three for serving as controls. Plants were covered with plastic bags to maintain 100% relative humidity for 24 h and then kept in a greenhouse (20 to 26℃ and 60 to 80% RH). After 26 days, typical leaf spot symptoms, identical to the ones observed in the field, developed on the inoculated plants. No symptom was observed on controls.

**P21.002 First Report of Frosty Mildew on *Salix koreensis* Caused by *Mycopappus alni* in Korea**

*S.H. Lee[1] , J.H. Park[2] and H.D. Shin[2]*
*[1]Division of Forest Diseases and Insect Pests, Korea Forest Research Institute, Seoul 130-712, Republic of Korea; [2]Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-701, Republic of Korea*
*Email: shlee4@forest.go.kr*

A frosty mildew was observed on leaves of *Salix koreensis* in two localities of Korea during 2011 and 2012. Themain signs and symptoms were expressed as conical white to cream colored tufts of the causal fungus on the brown lesions, followed by premature defoliation. Based on morphological observations, cultural characteristics and gene analyses of rDNA-ITS, the fungus was identified as *Mycopappus alni*, which has been known to be associated with frosty mildews on *Alnus* spp., *Betula* spp., *Crataegus chlorosarca*, and *Pyrus pyrifolia*. Pathogenicity test was conducted twice with the same results, fulfilling Koch's postulates. This is the first case of *Salix-Mycopappus* association as well as the first report of frosty mildew on *S. koreensis*.

**P21.003 Community structure of dsRNA viruses infecting the conifer pathogenic fungus *Heterobasidion parviporum***

*E.J. Vainio, M. Müller, K. Korhonen, T. Piri and J. Hantula*
*Finnish Forest Research Institute, Jokiniemenkuja 1, Vantaa, Finland*
*Email: eeva.vainio@metla.fi*

Fungal viruses (mycoviruses) with RNA genomes are considered to lack extracellular infective particles. Instead, their entire life cycle takes place within the cytoplasm or mitochondria of their host fungi. Lateral virus transmission may occur during hyphal fusion between somatically compatible host strains, or in some cases during transient anastomosis contacts between somatically incompatible fungal strains. Vertical virus transmission occurs *via* sexual or asexual spores. We assessed mycovirus community dynamics at a Norway spruce dominated forest plot heavily infected by the conifer root rot pathogen *Heterobasidion parviporum* by investigating the presence and identity of dsRNA viruses at two time points: years 2005 and 2012. The *H. parviporum* population hosted three distinct partitivirus strains: HetRV1-pa2, HetRV2-pa1, and HetRV7-pa1, and several strains of the yet unassigned virus species HetRV6. Distribution of the viruses was associated both to the *Heterobasidion* genet and location at the study site, and identical viruses were found from neighboring, somatically incompatible host genets. Based on sequence analysis, the partitivirus infections seemed highly stable, while HetRV6 showed numerous minor polymorphisms. Overall, the virus infection frequency of this *H. parviporum* population was high, and double or triple virus co-infections were commonly found from single fungal isolates.

**P21.004 Diversity of partitiviruses infecting the fungal genus *Heterobasidion***

*E.J. Vainio, M. Kashif, R. Hyder, K. Korhonen and J. Hantula*
*Finnish Forest Research Institute, Jokiniemenkuja 1, Vantaa, Finland*
*Email: eeva.vainio@metla.fi*

The *Heterobasidion annosum* complex includes some of the most destructive conifer pathogenic fungi in the Boreal forest region. These fungi are commonly infected by partitiviruses that are composed of two genomic segments encoding an RNA-dependent RNA polymerase (RdRP) of 585-722 aa and a capsid protein of 510-659 aa. *Heterobasidion* partitiviruses represent taxonomically distant groups of *Partitiviridae*, many of them sharing less than 40% RdRP sequence similarity. In contrast, many *Heterobasidion* partitiviruses share high sequence similarities with viral species hosted by unrelated fungal hosts. Thus, the partitivirus species *Heterobasidion* RNA virus 1 (HetRV1), HetRV4 and HetRV5 are closely related to alphacryptoviruses of plants (subgroup 4 of *Partitiviridae*), while HetRV3 resembles the *Helicobasidion mompa* dsRNA mycovirus and HetRV8 is similar to *Fusarium poae* virus 1and *Pleurotus ostreatus* virus 1. The partitivirus species HetRV1and HetRV3 occur in several *Heterobasidion* species, including species of the *H. insulare* complex. Overall, the partitivirus community infecting *Heterobasidion* species seems to be polyphyletic and characterized by horizontal transmission between species.

**P21.005 Understanding the role of and determining practical management solutions for *Quambalaria coyrecup* canker disease in *Corymbia calophylla* (marri) decline in the South West of Western Australia**

*T. Paap, J. McComb, T.I. Burgess and G.E. Hardy*

*Centre of Excellence for Climate Change Woodland and Forest Health, School of Veterinary and Life Sciences, Murdoch University, Western Australia 6150, Australia*
*Email: G.Hardy@murdoch.edu.au*

*Corymbia calophylla* (marri) a keystone tree species in Western Australian woodlands and forests is suffering a major decline syndrome associated with the canker fungal pathogen *Quambalaria coyrecup*. The disease was first recorded on marri in 1939, and by the 1960s cankers were found to occur throughout the south west of WA. Since the 1990s, mortality attributed to the pathogen has increased significantly, with recommendations that immediate attention be given to determine predisposing factors and develop options for disease control. While the cause of the disease has been determined, the incidence and severity has continued to increase, with the resulting decline and loss of marri having major economic, social and ecological implications, due to the costs associated with lost honey and pollen production, tree removal, wildlife habitats, conservation of roadside verges, amenity values such as shade, and the control of salinity and erosion by reforestation. We will discuss the use of on-ground assessments to quantify the extent, incidence and severity of the decline; examine the diversity and pathogenicity of *Q. coyrecup*; the role of additional biotic and abiotic factors in the decline syndrome, and methods being trialed for control.

### P21.006 Leaf, shoot and fruit blight of marri (*Corymbia calophylla*) caused by *Quambalaria piterika*

*C. Marbus, T. Paap, B. Dell and G. Hardy*
*State Centre of Excellence on Climate Change, Woodland and Forest Health, School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, 6150, Australia*
*Email: g.hardy@murdoch.edu.au*

Decline of *Corymbia calophylla* (marri) in the south-west of Western Australia (WA) has been an increasing source of concern in recent years, due to a canker disease caused by *Quambalaria coyrecup*, and more recently *Q. pitereka* which causes a leaf, shoot and flower blight. The blight affects flowering parts at all stages of development. This symptomology is not typical of Quambalaria shoot blight (QSB) in its origin of eastern Australia, and this study is the first investigation of the disease on flowering structures. Marri flowers are invaluable to the WA beekeeping industry and to native fauna, for which reason this study was conducted to ascertain the extent, causes and possible impacts of the disease. Six surveys conducted over a 13 month period determined that QSB was widespread over the *C. calophylla* distribution range, causing deformities and early termination of flowering structures. DNA sequences of 23 isolates obtained in this study confirmed the presence of *Q. cyanescens* and *Q. pitereka* with the latter identified as the primary blight pathogen of both vegetative and reproductive tissues.

### P21.007 Resistance of Korean chestnut cultivars to chestnut ink disease and fungicide efficacy for disease control

*S.H. Lee , S.K. Lee, S.T. Seo , J.H. Park and C.Y. Park*
*Division of Forest Diseases and Insect Pests, Korea Forest Research Institute, Seoul 130-712, Republic of Korea*
*Email: shlee4@forest.go.kr*

Chestnut ink disease is one of the most damaging diseases of chestnut worldwide. In 2006, the occurrence of chestnut ink disease was first observed in Hadong County, Gyeongnam Province and Yeonggwang County, Jeonnam Province, in Korea. Several *Phytophthora* species are known to be a common cause of the disease worldwide (e.g. *P. cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. katsurae*) but a single species, *P. katsurae*, was isolated from affected chestnut trees in Korea. The same fungus was also reported on chestnut trees in Japan in 1969. Resistance of Korean chestnut cultivars to chestnut ink disease was investigated by inoculating the fungus on both cut branches (30 cm × 5 cm) and 3-year-old branches of asymptomatic trees. Chestnut cultivars tested include Pakmi2, Daebo, Eunsan, Manseki, Ibuki, Idea, Riheiguri, and Arima. All the cultivars developed typical symptoms of chestnut ink disease when inoculated, indicating that none of them are immune to the disease. Riheiguri appeared most resistant, showing the most delayed disease onset (5 weeks after inoculation) and the lowest mortality rate (30%). Efficacy of two fungicides (dimethomorph and metalaxyl) was determined by injecting 1000 ml of each chemical at different dilutions into 15-year-old trees naturally infected. Metalaxyl appeared highly effective at a 1:500 dilution with the lowest mortality rate (25%) compared to either dimethomorph treatments (1:500 and 1:1000 dilutions) or the other metalaxyl treatment at a 1:1000 dilution.

### P21.008 Effect of different pre-planting soil preparation and post-harvest wood debris utilization on the soil microbiota and its effect on *Armillaria* and *Heterobasidion*

*J.B. Borowczyk and H. Kwaśna*
*Department of Forest Pathology, Poznan University of Life Sciences, Wojska Polskiego 71c, 60 – 625 Poznań, Poland*
*Email: jbehnke@up.poznan.pl*

Structures of fungal communities and their effect on two dangerous butt and root rot pathogens, i.e. *Armillaria* and *Heterobasidion* were studied in 12 soils in 10-year old Pinus sylvestris in Poland. Pre-planting soil preparation and post-harvest wood debris utilization were different in each soil. Two methods were used: one based

on the pure-culture isolation and morphotyping, and second based on extraction of environmental DNA, cloning and sequencing of rDNA. The first method favoured the fast-growing, spore-producing and low-substrate-specific fungi. The second method favoured non-culturable, slow-growing and poor competitive fungi. Two different fungal communities were identified using the two methods. Knowledge of the activity of the individual components of a fungal community helps in selecting the most favourable (from a phytopathological point of view) procedures for pre-planting preparation of forest soil and post-harvest wood debris utilization. Ploughing the soil and shredding post-harvest wood debris and leaving all organic matter on the surface or mixing it with the soil stimulated the growth of fungi that are antagonistic to *Armillaria* and *Heterobasidion*, in the first 10 years after clear cut. Increased density of *Penicillium, Tolypocladium geodes, Trichoderma,* Mortierellales and Mucorales suggests that natural and spontaneous protection against conifer butt and root rot pathogens can be expected.

**P21.009 Effects of different pre-planting soil preparation and post-harvest wood-debris utilization treatments on occurrence of *Penicillium adametzii*, antagonist of *Armillaria* and *Heterobasidion***

*H. Kwaśna*
*Department of Forest Pathology, Poznan University of Life Sciences, Wojska Polskiego 71c, 60 – 625 Poznań, Poland*
*Email: kwasna@up.poznan.pl*

*Armillaria* and *Heterobasidion* are dangerous butt and root rot fungi. *Penicillium adametzii* is a natural antagonist of *Armillaria* and *Heterobasidion* in forest soils, although its effect in tests depended on pathogen species and isolate of the antagonist. Effects of different pre-planting soil preparation and post-harvest wood-debris utilization treatments on the occurrence of *P. adametzii* in forests soil were studied in 10-40-year- old *Pinus sylvestris* plantations in Poland. Two methods were used for detection of fungi: one based on pure-culture isolation and morphotyping, and one based on extraction of environmental DNA, cloning, and sequencing of rDNA. Only the first method allowed detection of *P. adametzii* in soils. Its detection helps in selecting those pre-planting and post-harvest procedures likely to be most beneficial for disease suppression (= biocontrol]). Ploughing the soil stimulated growth of *P. adametzii*. The presence of coniferous-tree wood eliminated the fungus from soil. Removing or leaving all organic matter on the surface of the soil, instead of mixing it with the soil, was associated with an increased *P. adametzii* population in soil. The results suggest that natural and spontaneous protection against conifer butt and root rot pathogens in forests can be expected most after pre-planting deep ploughing and practices that eliminate coniferous wood from the soil.

**P21.010 Screening of G3 clone of *Populus deltoides* against toxin of *Bipolaris* spp., the leaf blight pathogen**

*S. Mishra, K. Uniyal, Y.P. Singh, U.S. Singh[1] and R.C. Dhiman[2]*
*Forest Research Institute, Dehradun 248 006, Uttarakhand, India; South Asia Regional Project Coordinator, IRRI, New Delhi Office, NASC Complex, Delhi 110 012, India; WIMCO Seedlings Limited, Kashipur Road, Baghwala, Rudrapur 263 153, Uttarakhand, India*
*Email: singh_yp@rediffmail.com*

Poplar is amongst world's fastest growing multi-purpose tree species and considered as model tree species for bioenergy, feed stock, phyto-remediation and carbon sequestration. Poplar suffers from number of abiotic and biotic agents. The blight pathogen i.e. *Bipolaris* spp. has been identified as one of the major pathogens of poplars especially G-3clone of *P. deltoides*. Shoot juveniles were collected from the nursery and seedlings of G3 clone were raised in root trainers of 250ml capacity. Toxin of sixty fungal isolates of *Bipolaris* spp. was prepared. Shoot juveniles and stem cuttings were then dipped in bottle containing 30ml culture filtrate + 20ml sterilized water. Check was raised in medium only. Three replicates were maintained. Symptoms were expressed in differential time period, for example, juvenile showed them in 3hr while stem cutting in 24hr. Initially, flecking (black minute, irregulars spots) appeared that spread to varying extent on the petiole and later, intervenial brightening covered the entire petiole leaving mid rib and main side ribs unaffected. In certain isolates, blightening was uniform over the entire leaf blade starting from margin inwards. Further, the entire shoot juvenile was blighted giving a burning appearance. In case of stem cutting, the flecking was more defined and close to field symptoms. Most of the aggressive isolates (B2, B19, B31 and B32) exhibited cent percent foliage blight. This is a quick and robust field screening method for resistance of *P. deltoides* genotypes against blight disease.

**P21.011 Resistance of Norway spruce to *Heterobasidion parviporum* based on lesion size and monoterpene levels**

*S. Keriö, S. Niemi, M. Haapanen, J. Holopainen and F.O. Asiegbu*
*Department of Forest Sciences, University of Helsinki, Latokartanonkaari 7 (P.O. Box 27), FI-00014 University of Helsinki, Finland*
*Email: susanna.kerio@helsinki.fi*

In Europe, *Heterobasidion parviporum* causes the majority of root and heart rot on Norway spruce. Trees with infection are prone to windfall, grow slower and produce less timber, with significant economic losses. Records of natural infection frequencies in infected Norway spruce stands and clonal trials suggest that host characteristics

have importance in limiting the disease incidence. However, results from the inoculations are affected by climatic conditions, and the relative resistance is not necessarily correlated with natural infection frequencies. This makes it important to study the responses of Norway spruce to *H. parviporum* under diverse conditions and on different genotypes. We inoculated Norway spruce clones representing different genotypes with variable geographic origins with a homokaryotic strain of *H. parviporum*. Based on lesion size, we characterized a Novgorodian spruce clone to be most resistant and a clone from Central Finland as most susceptible. This difference between the two clones was reflected by the mycelial growth as determined by re-isolations and PCR diagnostics. We also confirmed by compatibility tests that the homokaryotic strain had remained a homokaryon in most of the inoculations, suggesting potential for homokaryons to be source of infection in nature. The concentrations of monoterpene and sesquiterpene compounds in phloem and xylem samples of infected and wounded trees was determined. We observed induction of terpene levels in infected trees compared to wounded trees and higher accumulation of b-pinene and a-pinene in resistant and susceptible clones respectively. The importance of these findings in the hunt for potential biomarkers is being explored.

## Concurrent Session 22-Molecular Diagnostics of Plant Pathogens

### O22.001 The results of the EU project QBOL deposited in the Q-bank database to support plant health diagnostics

*P.J.M. Bonants*[1] *and M.J. Edema*[2]
[1]*Plant Research International, PO BOX 69, 6700 AB Wageningen and* [2]*NVWA, National Plant Protection Organization, Wageningen, The Netherlands*
*Email: peter.bonants@wur.nl*

The rate of introduction and establishment of damaging plant pests and diseases has increased steadily over the last century as a result of expanding globalisation of trade in plant material, climate change, EU expansion, and by a recognised decline in the resources supporting plant health activities. Furthermore there is a constant decline in the number of taxonomic specialists in the different disciplines (mycology, bacteriology, etc.), capable of identifying plant pathogens, and funds to support this kind of work are very hard to obtain. Also the number of other specialists in phytopathology and other fields, which are vital for sustaining sound public policy on phytosanitary issues, are diminishing. These problems affect all countries. In this context QBOL (www.qbol.org), an EU project on DNA barcoding, started in 2009 to generate DNA barcoding data of quarantine organisms and their taxonomically relatives to support plant health diagnostics. The data are included in a database, called Q-bank (www.Q-bank.eu), which now consists of a dynamic open-access database of quarantine plant pests and look-alikes, linked to curated and publicly accessible reference collections. It contains sequence and morphological data including photographs, nomenclatural and diagnostic data of specimens available in reference collections. Within Q-bank curators from many countries with expertise on taxonomy, phytosanitary and collection issues for the different groups have been appointed and links with other databases have been made; this in order to provide Q-bank an international role in supporting plant health agencies. The results of the QBOL project will be presented as well as the Q-bank database.

### O22.002 Bringing nucleic acid based detection of pathogens to the masses: the use of isothermic amplification approaches by practitioners

*W. Mahaffee, J. Keune, L. Thiessen and G. Grove*
*Horticulture Crops Research Lab, USDA-ARS Corvallis, OR 97330, USA*
*Email: walt.mahaffee@ars.usda.gov*

The nucleic acid based detection assays have utilized for decades by researchers and diagnostic laboratories to detect presence of plant pathogens. However, the wide spread application of these technologies has been hindered by the need for specialized and expensive equipment in conjunction with highly skilled labor PCR or quantitative PCR assays. The advent of isothermic nucleic acid amplification strategies is helping to remove barriers to practitioners using molecular diagnostic tools to detect and monitor plant pathogens. Over the past seven years, we have shown that viticulturist can utilize knowledge of the presence of air borne *Erysiphe necator* inoculum to initiate fungicide application and reduce the number of pesticide applications required to manage grape powdery mildew. However, the commercial implementation of inoculum detection for management decision was too expensive or cumbersome for most viticulturists to utilize. To remove this barrier, we developed and demonstrated that viticulturist can utilize Loop mediated isothermal AMPlication (LAMP) assays as a robust method for the detection of *E. necator* DNA with less than $2000 in equipment. Others are beginning to implement such approaches to monitor and detect a wide range of pathogens. There are also hand held devices being develop that will increase our ability to bring these technologies to field.

### O22.003 Q-DETECT - Developing pest and pathogen detection methods for national plant protection organizations

*N. Boonham*
*Fera, Sand Hutton, York, YO41 1LZ, UK*
*Email: neil.boonham@fera.gsi.gov.uk*

Detection methods are the first tools used by national plant protection organizations (NPPO) and inspection services in order to find incursions of quarantine pathogens or pests across borders. This is done visually in the first instance, with support from a laboratory for confirmatory testing and monitoring. Reliance on laboratory testing can cause significant delays when action is only taken on the results from the laboratory. Thus, there is a real need for rapid, simple and robust detection methods that can be deployed by NPPOs in the field with inspection services, in the first instance to guide inspectors to high risk consignments as well as enabling confirmation and identification of pests to facilitate rapid decision making. The Q-detect consortium developed detection methods based on biochemical (detecting volatile organic compounds [VOC]), acoustic (including resonance), remote imaging (incorporating spectral and automated data analysis) and pest trapping (insect pests and pathogen vectors) techniques. In addition methods were developed for rapid on-site confirmation and identification based on LAMP amplification techniques and the Genie II platform. How best to deploy detection methods however provides a potential conundrum for policy makers and other stakeholders. Deploying simplified detection and identification methods remotely helps to speed up inspection and facilitates trade. However,

without care this approach may risk a blinkered inspection approach and a 'winding down' of laboratory expertise which is needed in the case of outbreaks of new pests. This paper will discuss the new techniques developed and the context in which they may be deployed.

**O22.004 Detection of plant quarantine pathogens using surface plasmon resonance technology**

*R. Di, L.M. Zhao and L. Levy*
*Department of Plant Biology and Pathology, Rutgers, the State University of New Jersey, 59 Dudley Road, New Brunswick, NJ, USA 08901*
*Email: di@aesop.rutgers.edu*

Surface plasmon resonance (SPR)-based technology provides rapid, sensitive and specific detection methods for plant quarantine pathogens. SPR technology uses biosensors with different capturing molecules to bind analytes such as pathogenic microorganisms and converts the binding to a measurable signal in real-time. We have used a handheld SPR device to detect one of the quarantine plant pathogens, *Ralstonia solanacearum* (Race 3 biovar 2), which is of high consequence and importance in the U.S. We have shown biosensors coated with *R. solanacearum*-specific antibody can be used to detect this pathogen and these biosensors can be re-used. Biosensors coupled with oligo DNA molecular probes targeting to different races and biovars of *R. solanacearum* will be discussed for the detection and differentiation of this important plant quarantine pathogen.

**O22.005 Molecular diagnostics and genomic studies on some filamentous plant viruses in China**

*J. Chen and J.P. Chen*
*Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, P. R. China*
*Email: jpchen@zaas.ac.cn; jpchen2001@yahoo.com.cn*

In the past, plant viruses in China were mainly identified by biological characteristics, which were sometime not reliable. There is a need for more powerful and accurate approaches for plant virus identification and diagnostics. In this study, a molecular strategy of virus detection, identification and genomic cloning for the complete sequencing of 6 genera with filamentous virions was established, and 49 species of these 6 genera were investigated, including 5 *Allexiviurses*, 3 *Bymoviruses*, 6 *Carlaviruses*, 2 *Potexviruses*, 34 *Potyviruses* and 1 *Tritimovirus*. Of those, 11 were new species [*Garlic allexivirus E* (GarVE), *Narcissus common latent carlavirus* (NCLV), *Narcissus symptomless carlavirus* (NSV), *Scallion potexvirus X* (ScaVX), *Butterfly flower mosaic potyvirus* (BFMV), *Fritillary potyvirus Y* (FVY), *Isis potyvirus Y* (IVY), *Lily potyvirus Y* (LiVY), *Scallion mosaic potyvirus* (ScaMV), *Thberose mild mottle potyvirus* (TuMMoV), *Thunberg fritillary mosaic potyvirus* (TFMV)], 11 were first records in China, 6 identified before were proved to be wrongly named and 20 were fully sequenced for the first time. The filamentous plant viruses occurring in China were found to be widely distributed, greatly variable and including various species. Detailed analysis of sequence variations, genome organization and species classification of these 49 viruses were carried out. It was noticed that complex infections of different viruses commonly occurred on vegetable propagated crops such as sugarcane, garlic, narcissus and scallion in China.

**O22.006 siRNA deep sequencing and assembly: piecing together viral infections**

*J.F. Kreuze, Z. Fei, G. Shan, D.L. Gutierez, W. Cuellar and S. Fuentes*
*International Potato Center (CIP), Avenida la Molina 1895, La Molina, Lima, Peru*
*Email: j.kreuze@cgiar.org*

RNA silencing constitutes a fundamental antiviral defense mechanism in plants in which host enzymes cut viral RNA into pieces of 20-24 nt. When isolated, sequenced en-mass and properly assembled or aligned these virus-derived small RNA (sRNA) sequences can reconstitute genomic sequence information of the viruses being targeted in the plant. This approach is independent of the ability to culture or purify the virus and does not require any specific amplification or enrichment of viral nucleic acids as it automatically enriches for small RNAs of viral origin by tapping into a natural antiviral defense mechanism. Using this technique known and novel DNA and RNA viruses as well as viroids have been identified at sensitivity levels comparable to PCR. Results from the application of this technique and the development of a bioinformatics pipeline to identify novel plant viruses as well as map variability and distribution of viruses will be presented.

**O22.007 Comparison of rolling-circle amplification and direct-PCR based methods for diagnosis of banana streak virus infection in East Africa**

*A.P. James, L. Karanja, J. Kubiriba, C.M. Changa, J.A. Mugini, W.K. Tushemereirwe, R.M. Harding and J.L. Dale*
*Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, Queensland, 4001, Australia*
*Email: ap.james@qut.edu.au*

BSV diagnosis is complicated by significant serological and genomic variability between isolates and the presence of endogenous virus sequences in the *Musa* genome. We tested samples of banana from Uganda, Kenya and Tanzania for BSV and compared the results obtained using

PCR with species-specific primers, and rolling-circle amplification (RCA). For BSV with no known endogenous counterpart, both PCR and RCA were suitable methods for BSV detection, irrespective of the host cultivar genotype. For four BSV with endogenous counterparts, namely *Banana streak* OL *virus* (BSOLV), *Banana streak* MY *virus* (BSMYV), *Banana streak* GF *virus* (BSGFV) and *Banana streak* IM *virus* (BSIMV), PCR and RCA were suitable in cultivars with a pure *Musa acuminata* genetic background. However, in bananas with a *M. balbisiana* (B-genome) genetic background, many more samples were positive using PCR compared to RCA, suggesting that the positive results were due to integrated BSV sequences and not episomal virus infection. In these cases, RCA was needed to diagnose BSV infection. For generic BSV indexing, RCA is the most suitable method as it is able to detect a broad range of BSV species while avoiding detection of integrated sequences. However, the faster and cheaper PCR serves as a suitable alternative for BSV diagnostics in specific combinations of host plant cultivar and BSV species.

**O22.008 Multiple gene target multiplex PCR diagnostic approach can effectively detect xanthomonads in culture and plant material**

*J. Adriko*
*National Agricultural Research laboratories, National Agricultural Biotechnology Centre, Uganda*
*Email: adrikoj@yahoo.com*

Molecular diagnostic tools detecting xanthomonads in culture and plant material were developed. Three gene targets were evaluated for detection; Internal transcribed spacer region (ITS) of the ribosomal DNA, TonB dependent receptor gene (*fyuA*) and a xanthan biosynthetic gene (*gumD*). To determine effectiveness of *Xanthomonas* diagnosis in culture, primer sets were evaluated using DNA extracted from 45 *Xanthomonas* strains from 25 different species broadly covering the genus. Fifteen non-*Xanthomonas* strains of plant-associated bacteria including the phylogenetically closely related species, *Stenotrophomonas maltophilia* and *Xylella fastidiosa* were also tested. X-ITS primers consistently amplified an expected 254 bp DNA fragment of all 45 *Xanthomonas* strains whereas no amplification was observed for non-xanthomonads. Primers targeting *fyuA* amplified DNA from all xanthomonads except *X. theicola*. The X-GumD primers allowed efficient amplification of DNA in 38 out of 39 isolates from Group II (represented by *X. campestris*) whereas no or very weak amplification was observed for Group I members. To validate use of the tools in diagnosing xanthomonads in plants, the tools were tested in banana, rice, cabbage and tomato hosts; primers X-GumD and X-ITS effectively amplified *Xanthomonas* DNA in artificially inoculated samples of these plants without giving amplification in the non-diseased plants. To avoid false negative results, internal controls of primers targeting bacterial 16S rDNA or plant 26S mitochondrial rDNA were successfully applied in multiplex PCRs for testing pure bacterial cultures or plant tissue, respectively. Our results offer the possibility for the use of these improved diagnostic tools in a wide range of xanthomonas-plant interactions.

**O22.009 Development of rapid in-field loop mediated isothermal amplification (LAMP) assays for phytoplasmas and other plant pathogens**

*M. Dickinson[1], S. Millington[2], N. Yankey[3], J. Tomlinson[4], J. Hodgetts[4] and N. Boonham[4]*
*[1]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK; [2]OptiGene Limited, Unit 5 Blatchford Road, Horsham, West Sussex, RH13 5QR, UK; [3]Council for Scientific and Industrial Research (CSIR-OPRI), PO Box 245 Sekondi, Ghana; [4]The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK*
*Email: matthew.dickinson@nottingham.ac.uk*

Loop-mediated isothermal amplification (LAMP) is an isothermal amplification technique that can be undertaken with minimal equipment to obtain amplification of target DNA within 30 minutes. We have developed primers for a range of assays for specific 16Sr phytoplasma groups and for a range of other plant pathogens including fungi and viruses. These assays have been combined with a real-time isothermal amplification system and the OptiGene GenieII portable lightweight detection machine to develop a rapid in-field diagnostic test for plant diseases. When combined with a 2-minute DNA extraction method from plant material, including leaves and coconut trunk borings, the method can be used to detect pathogens in the field within 30 minutes of sampling. Use of the system in remote locations has been piloted for detection of the Cape St Paul wilt phytoplasma disease of coconuts in Ghana, and results of these trails will be presented.

**O22.010 Deep sequencing: a powerful tool for detection and characterization of known and new plant viruses and viroids**

*A. Olmos[1], E. Bertolini[1], M.C. Martínez[1], T. Candresse[2], M. Glasa[3], J.A. Pina[1] and M. Cambra[1]*
*[1]Instituto Valenciano de Investigaciones Agrarias (IVIA). Plant Protection and Biotechnology Center, Moncada-Naquera km 5, 46113 Moncada, Valencia, Spain; [2]UMR1332 BFP, INRA & University of Bordeaux, CS 20032, 33882 Villenave d'Ornon cedex, France; [3]Institute of Virology, Slovak Academy of Sciences, Dubravska cesta 9, 84505 Bratislava, Slovakia*
*Email: aolmos@ivia.es*

The accumulation of virus-derived small interfering (si)

RNAs (21-24 nucleotides) generated by Dicer upon recognition of dsRNA formed during viral replication was analyzed by next generation sequencing technologies. Different platforms (Solexa, 454 Roche, Illumina and Ion Torrent) are available and some were used with sweet cherry, citrus and grapevine field samples, with the aim of screening the virus and virus-like nucleotide sequences present in the analyzed plant material. Large contigs with homology to viral sequences, allowed the detection and/or full genome reconstruction of various virus species by mapping or even using *de novo* analyses. *Cherry virus A* and *Little cherry virus 1*, were identified in cherry samples. Complete sequences, including intra isolate variability, of *Hop stunt viroid*, *Citrus exocortis viroid*, *Citrus viroid III* (CVdIII), *Citrus viroid IV* (CVdIV) were obtained, as well as the complete sequence of a new virus causing vein enation in citrus (proposed name Citrus vein enation virus). The complete genomes of *Arabis mosaic virus*, *Grapevine fleck virus* and *Grapevine leafroll associated virus 1* isolates were recovered from grapevine samples. Next generation sequencing is a very powerful technology that could greatly simplify the screening, routine diagnosis and characterization of plant pathogens. Different approaches to purify targets as well as the suitability of different platforms for detection purposes are discussed and compared with conventional technologies. Deep sequencing has the potential to replace conventional screening by biological indexing of graft-transmissible agents.

**O22.011 Molecular plant pathogen detection and high-throughput sequencing: toward a paradigm shift**

*S. Massart and M.H. Jijakli*
*Laboratory of Phytopathology (Gembloux Agro-BioTech - University of Liège), Passage des déportés, 2, 5030 Gembloux, Belgium*
*Email: sebastien.massart@ulg.ac.be*

This review focus on the recent technological, scientific and bioinformatic developments of high-throughput sequencing and how they will impact the diagnostic and characterization of plant pathogens. The ongoing sequencing revolution has paved the way for an exponential growth of the sequenced genomes available; quickly building the microbe genes and genomes catalogues. This accumulated genomic knowledge is leading to paradigm switches which can be synthetised in the advent of the "Genome-based" approach in research, epidemiology and diagnostic. This approach will deeply impact the way of diagnosing and characterizing plant pathogens taking into account the specificities of virus, bacteria or fungal diagnostic. Through didactic examples based on the recent applications of these technologies for human, animal or plant pathogens, we analyze their potential, their current impact and their future use in plant pathogen molecular diagnostic. We also underline their limitations and the key factors to promote their use for developing new molecular diagnostic tools. Eventually, we envision that high-throughput sequencing will become a mandatory tool to develop and apply molecular diagnostic tests and will lead to significant improvements in their utility and use.

**O22.012 Rapid detection of *Acidovorax avenae* sub sp. Citrulli based on antibody microarray**

*J. Wang and Y. Bi*
*College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, P.R. China*
*Email: wangjing@gsau.edu.cn*

*Acidovorax avenae* sub sp. *Citrulli* (*Aac*) is a seed borne pathogen harmed Cucurbitaceae plants seriously. Based on chemi-luminescence method; an antibody microarray was developed for rapid, sensitive, and specific detection of *Aac*. The major parameters were optimized such as sample concentration, incubation condition, hybridization reaction conditions and chromogenic time. The lowest detection capability could reach$2.4\times10^4$ cfu /mL -$2.4\times10^5$c fu /mL. In the protein chip system, only 1/25 dosage of the capture antibody and 1 /8 of the detection time could reach the same detection capability that achieved by ELISA. In addition, the cost of detection was low and the procedure was simple and convenient.

**O22.013 Global virus survey in tomato using small RNA deep sequencing technology**

*K.S. Ling[1], R. Li[1] and Z. Fei[2]*
*[1]U.S. Department of Agriculture-Agricultural Research Service, U.S. Vegetable Laboratory, Charleston, SC, USA; [2]Boyce Thompson Institute for Plant Research, USDA-ARS, Robert W. Holley Center for Agriculture and Health, Cornell University, Ithaca, NY, USA*
*Email: Kai.ling@ars.usda.gov*

Tomato is one of the most important and widely grown vegetable crops in the world, growing in more than 173 countries under various eco-systems. Viral diseases are one of the major limiting factors in tomato production, with up to 136 described viral species. Emerging viral diseases are especially difficult to control as *no priori* knowledge on the causal virus or no proper detection method available. Effective disease management is dependent on an accurate identification of the causal agent(s) for a disease. In recent years, next generation sequencing technologies, including deep sequencing of small RNA (sRNA) and assembly, have been developed for plant virus identification. Small RNAs (including miRNA and siRNA) are produced abundantly in plants and animals in regulating gene expression or in defense against virus or viroid infection. Analysis of a sRNA profile upon virus infection in plant may allow for a *de novo* assembly of known or unknown virus genomes. In

collaboration with several vegetable seed companies, we are conducting a global survey for viruses and viroids across major tomato producing regions in the world and using deep sequencing of sRNA to generate a comprehensive tomato virus inventory and a global virus distribution map. The ultimate goal for this global survey is to develop molecular detection methods for the identified tomato viruses and viroids. Public availability of such tools and information will help to understand global virus distribution, to guide phytosanitory requirements, to predict risk of future epidemics, and to devise regional disease management strategies.

**O22.014 Detection and quantification of the root-lesion nematode *Pratylenchus penetrans* using real-time PCR**

F. Mokrini[1,2], L. Waeyenberge[2], N. Viaene[2,4], F. Abbad Andaloussi[5] and M. Moens[2,3]
[1]National Institute of Agricultural Research, (INRA), Km 9, 14000 Kenitra, Morocco; [2]Institute for Agricultural and Fisheries Research, Plant, Crop Protection, Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium; [3]Laboratory for Agrozoology, Ghent University, Coupure links 653, B-9000 Ghent, Belgium; [4]Department of Biology, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium; [5]National Institute of Agricultural Research, Scientific Division, BP415RP, Rabat, Morocco
Email: fouad_iav@yahoo.fr

*Pratylenchus penetrans* is one of the most economically damaging plant-parasitic nematodes and is found on a wide variety of crops. Correct identification and quantification of this nematode are necessary for providing advice to farmers, and can be used for studying the plant-nematode relationships. We developed a real-time PCR assay to detect and quantify *P. penetrans*. A real-time PCR primer set, including two primers and a probe, was designed based on the sequence of a parasitism gene. The assay was optimized by using the primers in a real-time PCR assay with SYBR green I dye and setting the real-time PCR program to different annealing temperatures ranging from 60 ℃ to 65 ℃. Based on the Ct-values, we retained the program with an annealing temperature of 63 ℃. The assay was able to detect a single individual of *P. penetrans*. The specificity of the reaction was confirmed by the lack of amplification of DNA from 52 populations of 20 other *Pratylenchus* species. DNA extraction from 80 individuals was repeated 4 times; Ct-values showed consistent results (Ct = 24.4 ±0.4). A dilution series from DNA of *P. penetrans* resulted in a standard curve showing a highly significant linearity between the Ct-values and the dilution rates ($R^2$=0.98; slope = -3.2). The tests showed a high correlation between real numbers of second stage juveniles in a DNA sample and expected numbers detected by real- time PCR. The developed real-time PCR assay provides a sensitive means for the rapid detection and reliable quantification of juveniles of this pest. This method does not require expertise in nematode taxonomy and morphology, and can be used as a rapid diagnostic tool in research, as well as in diagnostic labs and extension services advising farmers for pest management.

**O22.015 Practical genomics: geographic mapping of the race structure in the Canadian *Leptosphaeria maculans* pathogen population**

S.H. Liban, D.J. Cross, W.G. Dilantha Fernando[1], G. Peng and H.R. Kutcher
Department of Plant Science, University of Manitoba, 66 Dafoe Road, Winnipeg, Manitoba, R3T 2N2, Canada
Email: s.h.liban@gmail.com

Phoma stem canker (aka blackleg) is the most destructive disease affecting oilseed rape and Canola (*Brassica napus* L.) and is caused by the fungal pathogen *Leptosphaeria maculans.* This dothideomycete fungus has led to major limitations in Canola production and is responsible for epidemics in Australia and France. Genetic resistance has proven to be an effective means of disease control in western Canada and a cultivar carrying a specific resistance gene will be resistant to the pathogen carrying the corresponding avirulence gene. However, host genetic resistance can be overcome with population shifts and the emergence of new races of the pathogen. Canola is the most economically important crop in Canada and in an effort to maintain genetic resistance the Canola industry has funded research to geographically map the avirulence genes in the pathogen population across Western Canada. The information collected on the genetic variability in the pathogen population will be used to avoid susceptible reactions in farmer's fields. This study sampled isolates of *L. maculans* in 2010 and 2011 across Alberta, Saskatchewan, and Manitoba. The race structure was assessed by differentials and/or PCR on avirulence alleles *AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4*, *AvrLm6*, *AvrLm7*, *AvrLm9*, *LepR1*, *LepR2*, and *AvrlmS*. Overall, certain alleles were more prevalent in the pathogen population with *AvrLm6* and *Avrlm7* present in >90% of isolates and *AvrLm3*, *AvrLm9*, and *AvrLepR2* present in <10% of isolates. However, some loci differed greatly across geographic locations. Selection pressure from different race-specific resistance genes is a significant contributor to the variation in virulence observed.

**O22.016 *Alternaria* leafspot pathogens: genetics, evolutionary history and diagnostics**

A.D. Armitage, J. Woodhall, C. Lane and J. Clarkson
Warwick Crop Centre, University of Warwick, Wellesbourne Campus, Wellesbourne, Warwickshire, United Kingdom, CV35 9EF
Email: A.D.Armitage@warwick.ac.uk

*Alternaria* black spot diseases on pome fruits are economically important in Asia and the Americas. Movement of infected apple and pear material risks establishment of these and other host specific *Alternaria* diseases in Europe. EU countries are obliged to take action on intercepted non-native pathogenic *Alternaria*. However, identification based on morphology is unreliable and conventional genetic loci show little resolution between species. Fast and reliable diagnostics need to be developed to identify *Alternaria* pathogens. Phylogenetic and morphological studies were performed on over 100 small-spored *Alternaria* isolates. Highly variable loci (EndoPG, Alta1, L152 and three novel loci) showed resolution within the small spored *Alternaria*. Host-specific toxin genes on conditionally dispensable pathogenicity chromosomes were detected using PCR. Isolates carrying the pear specific pathogenicity chromosome were phylogenetically distinct. Isolates carrying the apple specific pathogenicity chromosome were not distinct and were polyphyletic. This may indicate that presence of toxin genes is a better molecular marker for pathogenicity than morphology (primarily sporophore shape) or phylogeny. Twelve isolates of *Alternaria* have been genome sequenced. This data is being used to characterize the pathogenicity chromosome and its evolutionary history. In addition, it allows the development of diagnostics based on presence of pathogenicity chromosomes, which are more direct markers for pathogenicity than morphology or gene sequence on essential chromosomes.

### P22.001 Identification of superelongation disease in *Manihot esculenta (Crantz*.) by spm molecular markers

*A.T. Alleyne and J.M. Gilkes*
*Department of Biological and Chemical Sciences, Faculty of Science and Technology, University of the West Indies, Cave Hill campus, St. Michael, Bridgetown, Barbados*
*Email: angela.alleyne@cavehill.uwi.edu*

Cassava (*Manihot esculenta* Crantz), is a tropical tuber root-crop grown in West Africa, Latin America and the Caribbean, and an important dietary starch alternative to cereal grains. One of the more important endemic cassava diseases in the Caribbean is Super-elongation caused by the fungus *Sphaceloma manihoticola.* Superelongation is characterized by necrotic leaf spots, hypertrophy, stem and leaf cankers and eventually exaggerated stem elongation caused by overproduction of the hormone, gibberellic acid A4 (GA4). The Giberellic acid biosynthesis gene cluster in *S. manihoticola* consists of five genes encoding P450 monooxygenase. One of these genes, SmP450-2 converts GA14 to GA4 without accumulation of intermediates. Disease severity in cassava plants was measured by pathogenicity tests and DNA amplification of the molecular target in the SmP-2 gene cluster in infected plants. These markers identified the disease in cassava varieties grown in Barbados but did not do so in uninfected plants. Early detection based on molecular targets is important for disease diagnosis in Barbados before hyperelongation is seen. This study is the first to apply the *Spm 2* marker as diagnostic tool for early detection of super-elongation disease and an early warning signal of the disease and can aid in pre-emptive disease management as well as a method for characterization of disease free planting material.

### P22.002 Development of probes and real time PCR assay for detection of *Rhizoctonia solani* infecting pulse crops

*S.C. Dubey, A. Tripathi and B.K. Upadhyay*
*Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012, India*
*Email: scdube2002@yahoo.co.in*

*Rhizoctonia solani* Kühn (teleomorph - *Thanatephorus cucumeris* (Fr.) Donk) is a destructive soil-borne plant pathogen causing web blight/wet root rot in a wide range of pulse crops. The present study aimed to determine the variability in ITS region and to develop a real time PCR based assay for the detection of *R. solani* isolated from different pulse crops. Eighty-nine representative isolates of *R. solani* infecting various pulse crops belonging to 7 anastomosis groups (AGs) originating from 16 agro-ecological regions of India covering 21 states were analyzed for genetic diversity using ITS-5.8S rDNA sequences. The isolates were variable in their nucleotide sequences of the ITS region. Phylogenetic tree constructed from bootstrap neighbour-joining analysis indicated high level of genetic similarity among the isolates representing different AGs, crops and areas. Five sets of molecular markers (ARSF1&R1, ARSF2& R2, ARSF3&R3, ARSF4&R4 and ARSF5&R5) were designed from ITS sequences of the pathogen. The markers produced variable sizes of amplicons (174-563 bp) specific only to *R. solani* isolates. The markers were highly sensitive in detecting both genomic DNA of the pathogen and infected plants up to the level of 1.24 pg. The marker ARSF4&R4 proved the most sensitive in detecting the pathogen at 6 h and 24 h post inoculation under *in vitro* and field conditions, respectively. The probes developed from the markers were validated using real time PCR analyses and found to be highly sensitive and specific to the pathogen.

### P22.003 Fungal pathogens occurring in eucalypt plantations and woodlots in Zimbabwe

*L. Jimu[13], M.J. Wingfield[2], E. Mwenje[3] and J. Roux[3]*
*[1]Forestry Department, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa; [2]Department of Microbiology and Plant Pathology,*

*Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa; [3]Bindura University of Science Education (BUSE), P. Bag 1020, Bindura, Zimbabwe*
*Email: Luke.Jimu@fabi.up.ac.za*

Eucalypt plantations were introduced into Zimbabwe in the early and late 20th century respectively to meet the increasing demand for wood and the related products. In-spite of the high utilitarian value of eucalypts in the country, very little attention has been paid to the threat posed by diseases which reduce timber quantity and quality. In this study, surveys were conducted across the country's 5 agro-ecological regions to identify pathogens negatively impacting on the productivity of eucalypt plantations and woodlots. The pathogens were identified basing on morphology, DNA sequence comparisons as well as symptomatic responses on the eucalypt host. The diseases identified were Teratosphaeria stem canker, Botryosphaeria stem canker, Armillaria root and stem rot, Mycosphaerella and Teratosphaeria leaf spot diseases. Other important pathogens identified were Microascales and Ophiostomatales which were commonly found on freshly cut stumps and logs. This study is the first to identify most of the eucalypt pathogens to species level and will act as a foundation for disease and pest management and future research in Zimbabwe.

**P22.004 Exploratory studies of *Ganoderma* species in the Garden Route of South Africa**

*J. Roux[1], M.J. Wingfield[1] and M.P.A. Coetzee[2]*
*[1]DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB), Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; [2]DST/NRF Centre of Excellence in Tree Health Biotechnology (CTHB), Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa*
*Email: Jolanda.roux@fabi.up.ac.za*

Basidiomycete fungi in the polypore family Ganodermataceae include agents of wood rot and important pathogens of woody plants globally. Of the eight genera that are recognised in the Family, *Ganoderma* is especially well-known as a tree pathogen and a source of traditional medicine. The taxonomy of genera and species in the Ganodermataceae has been problematic for many years, largely due to the historic use of morphological features to classify species. However, contemporary taxonomic studies using DNA sequence data have revealed a number of species complexes resulting in significant changes in the classification of these fungi. Knowledge regarding the *Ganoderma* species from South Africa is based almost exclusively on identifications applying morphological characteristics. The aim of this study was to identify fruiting bodies resembling species of *Ganoderma* on trees in the Garden Route area of South Africa. Isolates were obtained from both living and dead native trees in forests in that region, as well as from dying non-native *Acacia cyclops* trees growing along the coast of this area. All isolates were characterized based on DNA sequence data of the ITS regions and studies are underway to add additional gene regions to support the description of probable new species. Based on these preliminary studies, four species of *Ganoderma* have been identified from the area. A single species, different from those occurring on the native trees, was collected from large areas of dying *A. cyclops* trees. Future studies will promote an enhanced understanding regarding the ecology of these fungi in the region and they will determine whether any of the recovered species, especially those causing tree death, represent introduced species.

**P22.005 A specific and sensitive method for the detection of *Colletotrichum musae* in banana fruit**

*M.F. Peeran, P. Kuppusami, P.R. Thangamani, N. Krishnan, K. Gandhi and R. Thiruvengadam*
*Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore – 641003. Tamil Nadu, India*
*Email: faisal.tnau@gmail.com*

Banana is one of the most popular fruits in India, for national and international trading banana fruits are usually harvested before ripening and stored at relatively low temperature during transportation and market process. Long distance transport and extended storage period in the market may make them sensitive to diseases. Anthracnose disease caused by *Colletotrichum musae* (Berk. and Curt) Arx. occurs in almost all the banana growing countries. Detection of specific fungus from the fruit surface of banana is difficult because of latency. Therefore, rapid, reliable and accurate diagnostic tools are required to detect and identify causal organism. In this study the molecular variability generated from fourteen isolates of *C. musae* by RAPD-PCR technique was utilized to determine the percent similarity between the isolates. The genetic similarity coefficient within each group and variation between the groups was observed. Phylogenetic analyses, conducted on RAPD banding patterns, indicated *C. musae* isolates, however shares a 71 per cent genetic similarity among themselves. Decamer OPA-01 generated a RAPD polymorphic profile that distinguished *C. musae* from the other organism. Cloning and sequencing of the specific band yielded 588 bp sequences, to which forward CM-SCAR-FP and reverse CM-SCAR-RP were designed. The SCAR primer pair amplified a single SCAR of 490 bp from each of the 14 isolates of *C. musae*. Different dilutions of the purified DNA extracted from infected and healthy banana fruits (ranging from 3 ng to 250 ng/µl)

were prepared and amplified with SCAR primers. The results revealed that the amplification of infected fruit has stopped at 30 ng/µl with an amplicon size of 490 bp, below this the amplification did not occur, whereas the healthy fruit remain without any amplification.

**P22.006 Production of cocktail of polyclonal antibodies using bacterial expressed recombinant protein for multiple virus detection**

*R. Kapoor[1], B. Mandal[1], P.K. Paul[2] and R.K. Jain[1]*
*[1]Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012, India; [2]Amity Institute of Biotechnology, Amity University, Noida- 201303, India*
*Email: reetikakapoor@yahoo.co.in; rakeshjain56@yahoo.co.in*

Field and horticultural crops suffer from multiple virus infection and immuno-based diagnostic reagents for simultaneous detection of multiple viruses are not available. An attempt has been made to develop cocktail of antibodies that will help in multiple virus detection and overcome the limitation of individual virus purification, protein expression and purification as well as immunization in multiple rabbits. Conserved coat protein (CP) sequences from *Cucumber mosaic virus* (CMV) and *Papaya ringspot virus* (PRSV) and conserved nucleocapsid protein (N) sequences from *Groundnut bud necrosis virus* (GBNV) were identified, amplified by PCR and cloned into pGEM-T Easy vector. A triple construct was designed by fusing conserved coat protein (CP) sequences of CMV (~444 bp) and PRSV(~528 bp) and nucleocapsid protein (N) of GBNV (~252 bp) in frame in an expression vector, pET-28a(+).The recombinant protein with His-tag (~50 kDa) was expressed and purified from *E. coli* BL21(DE3) using His-Bind resin. Polyclonal antibodies to individual virus CMV, PRSV and GBNV recognized the fusion protein in Western blot indicating retention of desired epitopes for binding with target antibodies.The recombinant protein was used to produce cocktail of polyclonal antibodies by immunizing rabbit, which simultaneously detected natural infection of CMV, PRSV and GBNV in several Cucurbitaceous, Solanaceous and other hosts in DAC-ELISA at 1: 500 dilution. This forms the first report on production of cocktail of polyclonal antibodies for simultaneous detection of three distinct viruses.

**P22.007 Phenotypic, molecular, and pathological characterization of *Colletotrichum acutatum* associated with Andean lupine and tamarillo in the Ecuadorian Andes**

*C.E. Falconi[1], R.G.F. Visser[2] and A.W. van Heusden[2]*
*[1]Agropecuarian Science Faculty, Life Science Department, Army Polytechnic School, P.O. Box. 171-5-231-B, Sangolqui, Ecuador; [2]Wageningen UR Plant Breeding, P.O. Box. 386. Droevendaalsesteeg 1. 6708PB Wageningen, The Netherlands*
*Email: cefalconi@espe.edu.ec*

Anthracnose is a serious problem of both Andean lupine and tamarillo in Ecuador. Morphological features, internal transcribed spacer (ITS) sequences, and host specificity tests were used to characterize *Colletotrichum* isolates from lupine and tamarillo. Based on phenotypic and molecular characterization, the causal agent of anthracnose on both hosts was *Colletotrichum acutatum.* All the isolates were identified in a *C. acutatum*-specific polymerase chain reaction (PCR) assay. Colony diameter, spore shape, and insensitivity to benomyl also placed isolates from both hosts in the *C. acutatum* group. However, a detailed analysis of the ITS sequences placed the lupine and tamarillo isolates from the Ecuadorian Andean zone in two clades with high bootstrap support. In each clade both lupine and tamarillo isolates were represented. *C. acutatum* isolates from Andean lupine were distinct from other *C. acutatum* isolates collected on lupine around the world. In cross-infection studies, the diameter of lesions produced by isolates from each host was compared on the main stem of two tamarillo and three lupine cultivars. Some isolates produced larger lesions on the host from which they were isolated, but others showed similar aggressiveness on their alternate host. Isolates from both hosts were biotrophic on lupine stems, producing little necrosis and abundant sporulation while on tamarillo stems isolates from both hosts produced dark lesions with few spores. The population of *C. acutatum* from lupine and tamarillo provides an interesting model for the study of quantitative host adaptation.

**P22.008 Genetic diversity of Western Australian isolates of *Sclerotinia sclerotiorum* in Canola**

*B.N. Hidayah[14], B. Dell[1], R. Khangura[2], T.I. Burgess[1] and L. Brau[3]*
*[1]School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch WA 6150 Australia; [2]Department of Agriculture and Food, Western Australia; [3]School of Life and Environmental Sciences, Deakin University, Victoria 3125 Australia; [4]Balai Pengkajian Teknologi Pertanian (BPTP) Nusa Tenggara Barat, Narmada, Lombok Barat, NTB, Indonesia*
*Email: B.Hidayah@murdoch.edu.au*

Stem rot disease caused by *Sclerotinia sclerotiorum* has emerged as a serious problem on canola (*Brassica napus* L.) production in Western Australia (WA) over the past few years where crop losses can be up to 40% in the worst affected crops. Hundreds of isolates of *S. sclerotiorum* have been collected from different canola growing regions of WA. As the majority of WA isolates of *S. sclerotiorum* have not been analysed for their genetic characterization, analysis of genetic variation of WA

isolates will be undertaken using classical and molecular techniques such as mycelial compatibility groups (MCGs), next generation sequencing, and cluster analysis. The experiments which started in February 2013, aim to use classical and molecular tools to identify groups of WA isolates of *S. sclerotiorum* from which isolates will be selected for the main studies on the management of *S. sclerotiorum* in canola. Accurate information of genetic diversity through research on characterization of the pathogen will lead to better understanding of the pathogen and will also benefit the breeding programs particularly aiming at breeding for disease resistance and moreover, could lead to developing better techniques for managing the disease. The poster provides an outline of the experiments and preliminary results.

**P22.009 Detection of *Pythium myriotylum* by real-time PCR using SYBR green assay**

*B.R. Kim[1], S.S. Hahm[1], K.S. Han[1], M.K. Kwon[1] Y.G. Nam[1] and S.H. Yu[2]*
*[1]Chungcheongnam-do Agricultural Research and Extension Services, Yesan 340-861, Republic of Korea; [2]College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305-764, Republic of Korea*
*Email: brkim@korea.kr*

Rhizome rot of ginger caused by *Pythium myriotylum* is the most serious diseases affecting ginger plant in Korea. This disease easily spread under high soil water. During 2001-2011, the ginger yield was between 18,000 and 30,000 tons per year in Korea. *P. myriotylum* is a major pathogen of ginger in summer, which has caused the annual yield of ginger in Korea to decrease by 30%. For this reason, fast detection of pathogen is important to establish control strategy for rhizome rot disease in ginger fields. We developed a rapid real-time PCR assay using SYBR green for *P. myriotylum* that can simultaneously detect and quantify this pathogen in the soil. The specific PCR primers were designed based on the rDNA ITS sequence of several representative isolates from Korea. Genomic DNA of *P. myriotylum* isolate was serially diluted from 100 pg/ul to 0.1 fg/ul with a genomic DNA solution to test the sensitivity of the real- time PCR assay. There was a strong linear ($R^2$ = 0.970) inverse relationship between Ct and DNA concentration over seven orders of magnitude from 100pg to 0.1fg, with little variation in dilution series. The ability to quantify pathogen populations using real-time PCR will lead to a reduction in pesticide application.

**P22.010 Molecular characterization of RNAs 1 and 2 of the first Arabis mosaic virus Spanish isolate**

*A. Olmos[1], E. Bertolini[1], L.B. Torres[1,2], L. Dupuis-Maguiraga[2], A. Bassler[2], I. López-Fabuel[1] and T. Wetzel[2]*
*[1]Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), Carretera Moncada a Náquera km 5, 46113 Moncada, Valencia, Spain; [2]RLP Agroscience, AlPlanta – Institute for Plant Research, Breitenweg 71, 67435 Neustadt an der Weinstrasse, Germany*
*Email: aolmos@ivia.es*

The *Arabis mosaic virus* (ArMV) is one of the causative agent of the grapevine fanleaf disease, one of the most widespread and damaging viral diseases of grapevine. Recently, the ArMV has been detected in Spanish vineyards, and its determination and molecular characterization was undertaken. To this aim, the nucleotide sequence of the genomic RNAs 1 and 2 of the first isolate of ArMV infecting grapevine detected in Spain (ArMV-DU13) has been determined. The ArMV-DU13 genomic sequences were compared to the corresponding sequences of other isolates of ArMV, or nepoviruses. The most divergent genes among ArMV isolates were the X1 and VPg genes on the RNA 1, and the 2A gene on the RNA 2, with identity levels at the amino acid level of 78% (X1 and VPg) or 69% (2A) between the most distant isolates. Interestingly, the VPg genes were identical between the two grapevine isolates ArMV-DU13 and –NW, suggesting a possible implication of the host. The phylogenetic analysis of the RNA 2 showed that the Spanish isolate was close to *Grapevine fanleaf virus* isolates. The analysis of the full length RNA 2 suggests a recombination event between ArMV-DU13 and GFLV-GHu isolates between nucleotides 54 and 586 in the ArMV-DU13 isolate. Altogether, these results confirm the high variability between isolates of ArMV, and will be helpful to design more appropriate and reliable molecular diagnostic techniques for the control of this emerging virus in Spain.

**P22.011 Monitoring of *Pythium aphanidermatum* and *P. helicoides* causing root rot of poinsettia in ebb-and flow irrigation system using real-time PCR**

*Y. Ishiguro, K. Otsubo, N. Miyake, H. Nagai, H. Suga and K. Kageyama*
*River Basin Research Center, Gifu University, Gifu 501-1193, Japan*
*Email: ishiguro@green.gifu-u.ac.jp*

Recently, root rot of poinsettia caused by *Pythium aphanidermatum* and *P. helicoides* is serious in ebb-and-flow irrigation system. Poinsettia is cultivated from early summer to early winter and the disease occurs in summer. Although different potted ornamentals are cultivated in other season, the disease affect poinsettia in next cropping period again. While both of the pathogens are high-temperature tolerant species, zoospore formation is enhanced at lower temperature, 20 to 25℃, suggesting that the pathogens will be also in activity even if the disease does not appear. In this study, a population dynamics of the pathogens in nutrient solution

was determined by real-time PCR. Solution samples were periodically collected in three farmer's greenhouses (A, B, C) from August 2011 to September 2012. DNA was extracted from a filtrate through 1L of the solution on a membrane with 0.45 m pore size, and inoculum densities were evaluated by species-specific real-time PCR. In the greenhouse A, although the disease did not occur or different ornamentals were cultivated, *P. helicoides* was continuously detected, and *P. aphanidermatum* was rarely detected. In the greenhouse B, *P. aphanidermatum* was detected in summer 2011 when the disease occurred. In 2012, *P. myriotylum* and *P. aphanidermatum* were detected in June and July, respectively, and then the disease occurred in August. In the greenhouse C, *P. aphanidermatum* was detected in July 2011 before the disease occurrence. The events indicate that the monitoring of the pathogen will be essential to assess a disease risk.

**P22.012 Monitoring of *Pythium aphanidermatum* and *P. myriotylum* causing root rot of tomato in Hydroponic culture using real-time PCR**

*K. Kageyama, Y. Ishiguro, K. Otsubo, H. Suzuki, T. Tsuji, F. Hashizume, A. Fujita and H. Suga*
*River Basin Research Center, Gifu University, Gifu 501-1193, Japan*
*Email: kageyama@green.gifu-u.ac.jp*

Recently, root rot of tomato plant caused by *Pythium aphanidermatum* and *P. myriotylum* is serious in hydroponic culture. The disease mainly occurs in summer even in the same greenhouse. Although both of the pathogens are high-temperature tolerant species with more than 35℃ of the optimum temperature, zoospores are released at lower temperature, 20 to 25℃, suggesting that the pathogens will also be in activity even if the disease does not appear. Therefore, a monitoring of the pathogens in nutrient solution can provide valuable information to develop an efficient disease control strategy. In this study, a population dynamics of the pathogens in nutrient solution was determined by real-time PCR. Solution samples were periodically collected in three farmer's greenhouses (A, B, C) from August 2011 to September 2012. DNA was extracted from a filtrate through 1L of the solution on a membrane with 0.45 m pore size, and inoculum densities were evaluated by species-specific real-time PCR. *P. aphanidermatum* and *P. myriotylum* were detected in one (A) and two (B and C) greenhouses, respectively, regardless of the disease occurrence, although the densities were not always high. Furthermore, the pathogens were detected after the nutrient solution was exchanged and the culture panels for growing tomato plants were disinfested by sodium hypochlorite. The events indicate that the monitoring of the pathogen will be essential to assess a disease risk.

**P22.013 Development of a novel multiplex PCR for simultaneous detection of *Prunus necrotic ringspot virus* and *Prune dwarf virus***

*X.J. Zong, H.R. Wei, J.W. Wang, W.W. Wang and Q.Z. Liu*
*Shandong Province Key Laboratory of Fruit Tree Biotechnology Breeding, Shandong Institute of Pomology, Shandong Academy of Agricultural Sciences, Tai'an Shandong 271000, P. R. China*
*Email: qzliu001@126.com*

A novel multiplex PCR assay was developed and subsequently evaluated for its effectiveness in simultaneously detecting multiple viral infections in sweet cherry trees. The specific primers were designed according to the genome sequences of *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) and used for amplifying the fragments of 538bp and 265bp, respectively. The result showed that the two viruses can be detected from the dilutions of $10^4$ by multiplex PCR. Sequence analysis showed 98.6% and 99.4% identities to the PNRSV and PDV isolates, respectively. No fragment was amplified from the cDNA templates which containing the other viruses nucleotides, such as ApMV, CGRMV, CVA and LChV2. This novel multiplex PCR method is more efficient in the routine molecular detection of sweet cherry virus disease.

**P22.014 Avirulence genes of *Leptosphaeria maculans* populations in Europe in autumn 2010 and 2011**

*J. Kaczmarek[1], W. Irzykowski[1], A. Brachaczek[2] and M. Jedryczka[1]*
*[1]Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland; [2]DuPont Poland Ltd., Postepu 17b, 02-676 Warsaw, Poland*
*Email: jkac@igr.poznan.pl*

The fungus *Leptosphaeria maculans* causes stem canker (blackleg) – one of the most damaging diseases of oilseed rape in Poland and worldwide. Numerous observations support high variability of isolates belonging to this species. The aim of this study was to characterize the composition of avirulence genes present in the current population of *L. maculans* in Europe. The study was done in autumns of 2010 and 2011. Over 400 isolates of *L. maculans* were obtained from infected winter rapeseed leaves collected at experimental fields in Poland, Germany, France and the United Kingdom. The DNA of each isolate was extracted using a CTAB method. The taxonomic identity of isolates was checked by RAPD using OPJ-10 primer and compared with specific banding patterns characteristic for the representatives of *L. maculans* and *L. biglobosa*, which belong to *Leptosphaeria* species complex and coexist on leaves of oilseed rape. Isolates of *L. maculans* were studied to identify a mating type and avirulence alleles *AvrLm1/avrLm1* and *AvrLm6/avrLm6*. The isolates were tested using a differential set of plants with known resistance

genes elaborated at INRA, Versailles (France). Mating types *MAT1.1* and *MAT1.2* were found in similar frequencies in all populations, suggesting that both groups are well adapted to the environment. Similarly to the previous studies done in 2002 and 2003 years, out of eight identified races of the pathogen the most prevailing was Av5-6-7.

**P22.015 Molecular diagnostics of fungicide resistance in phytopathogenic fungi**

*D. Popiel[1], A. Dawidziuk[1], G. Koczyk[1], J. Kaczmarekand[1] and A. Brachaczek[2]*
*[1]Institute of Plant Genetics Polish Academy of Sciences, Poznań, Poland; [2]DuPont Poland Ltd., Postepu 17b, 02-676 Warsaw, Poland*
*Email: dpop@igr.poznan.pl*

Fungicides are a key component in the integrated management of plant diseases. Fungicide resistances form stable and inheritable traits impacing fungal survivability and pathogenicity. There are several resistance mechanisms: an altered target site of reduced affinity to fungicide molecules, complementation by an alternative enzyme taking over target function, overexpression of the fungicide target, active efflux or reduced uptake of the fungicide. With the available information on the mechanisms of fungal resistance to fungicide substances, it is now possible to design molecular diagnostics procedures allowing rapid analysis of multiple, environmental samples. As part of the batch *in vitro* testing, we conducted bioassays, PCR and qRT-PCR experiments on fungicide sensitive (e.g. *Fusarium verticilioides*) and resistant (*Alternaria alternata*) fungal strains. Our experiments aim to correlate morphological changes in mycelia with polymorphism of candidate resistance genes and their expression in stress conditions (fungicide treatment). We demonstrate example polymorphisms in CYP51 sterol demethylase evolution and in two ABC transporter genes from *F. graminearum* (FGSG_02865 and FGSG_05318) which appear to provide one of the best candidate genes for wide specificity MDR pumps capable of exporting fungicides.

**P22.016 A combination of baits and molecular genetics methods for detection and identification of *Phytophthora* spp.**

*T. Surina, M. Kopina and E.S. Mazurin*
*All-Russian Plant Quarantine Center, Bykovo, Moscow oblast, 140150, Russia*
*Email: t.a.surina@yandex.ru*

There are various methods for diagnosing *Phytophthora* diseases. However, every method has a certain specificity and sensitivity. At present, combinations of different methods for identification of causative agents of the *Phytophthora* genus are applied, which increases identification efficiency of these pathogens. The purpose of the conducted research was to optimize the combination of baits and molecular and genetic identification methods for *Phytophthora* spp. Detection of causative agents of wood and shrubby plant diseases caused by *Phytophthora* spp. was carried out locating baits in natural conditions. For identifying most suitable plants as baits, rhododendron, guelder-rose and lilac leaves were used. These leaves were placed in a bag with holes and immersed in standing or running water for 7-10 days. Then pieces of necrotized baits were placed on PARPH selective medium. DNA was extracted from infected areas on baits and mycelium formed in the medium. Sequencing of ITS and Y pt1 gene regions was conducted for identification of *Phytophthora* pathogens. DNA cloning was performed on samples with two or more fungi species followed by sequencing. The studies have shown that for detection of diseases of wood and shrubby plants caused by *Phytophthora* spp. under natural conditions, rhododendron leaves are most appropriate. Using leaves of this plant as baits enabled to detect *Ph. megasperma* and *Phytopythium litorale* in water basins located in areas of natural plantations. Necroses were absent on lilac and guelder-rose leaves. Y pt1 and ITS sequencing allowed determining the species of the plant pathogens.

**P22.017 Polymorphic microsatellites in worldwide populations of *Sclerotinia sclerotiorum***

*W. Irzykowski[1], X.L. Duan[2], X. Zhang[1], Q.S. Li[2], B.C. Hu[2], Z.Q. Li[3]and M. Jedryczka[1]*
*[1]Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland; [2]Crops Research Institute, Anhui Academy of Agricultural Sciences, 40 South Nongke,230031 Hefei, P. R. China; [3]Institute of Crop Protection IMAAAHS, 22 Zhaojun, Yuquan, Hohhot, 010031 Inner Mongolia, P. R. China*
*Email: mjed@igr.poznan.pl*

*Sclerotinia sclerotiorum* (Lib.) de Bary is an omnivorous species, infecting numerous plants. The fungus is regarded as one of the most damaging pathogens due to its presence in many geographical regions and climatic zones and its ability to cause disease symptoms on more than 400 plant species. The pathogen belongs to the phylum *Ascomycota*, what implies formation of a generative stage. The sexual reproduction takes place in apothecia – fruiting bodies in which ascospores and produced and transmitted to high distances by air. The sexual reproduction generates new forms of the pathogen. The aim of this study was to evaluate the polymorphism of microsatellite fragments among isolates of *S. sclerotiorum* originating from different geographic zones and host plants. Examined collection consisted of 329 isolates of *S. sclerotiorum* collected from seven host plants in nine countries, including 144 isolates from China, originating from ten provinces and three autonomic regions. Investigation included DNA sequencing

of different product sizes. Polymorphism of studied microsatellite sequences was very high. Agarose or polyacrylamide gel separation did not allow to use the variation of microsatellite size fragments as the marker of a particular trait, due to the additional polymorphism present in or close to the studied region. In case of polymorphism present outside the microsatellite region the possible solution is to design starters located in close distance from the microsatellite region. Great number of product sizes and the additional polymorphism found in amplified PCR products allow to use these regions for fingerprinting.

**P22.018 Detection of azole insensitive *CYP51* overexpressing strains of *Mycosphaerella graminicola* using loop-mediated isothermal amplification (LAMP) assays**

*B.A. Fraaije and H.J. Cools*
*Department of Biological Chemistry and Crop Protection, Rothamsted Research, Harpenden, AL5 2JQ, UK*
*Email: bart.fraaije@rothamsted.ac.uk*

Septoria leaf blotch is currently controlled by programmed applications of multisite inhibitors (e.g. chlorothalonil), azoles (e.g. epoxiconazole and prothioconazole) and a new generation of Succinate Dehydrogenase Inhibitors (e.g. bixafen, isopyrazam and fluxapyroxad). Azole fungicides have been used for three decades but their efficacy has eroded over time due to the evolution of azole insensitive strains carrying alterations in the sterol 14α-demethylase (CYP51) target protein. Because of the importance of azoles as a mixing partner of SDHIs continued monitoring of azole sensitivity shifts is paramount. Recently, we have reported a new mechanism, a 120 bp insertion in the *CYP51* promoter which is linked with 10- to 40-fold *CYP51* overexpression. Other isolates carry promoter variants based on a larger insert of 868 bp but the impact of this insert on the regulation of *CYP51* expression remains unclear. Here we present the development and application of loop-mediated isothermal amplification (LAMP) assays for rapid, on the spot detection of *CYP51* promoter inserts in *M. graminicola* isolates and in infected leaves.

**P22.019 A quick and reliable diagnostic tool for the identification of *Colletotrichum kahawae***

*D.N. Silva, P. Talhinhas, V. Várzea and D. Batista*
*CIFC/IICT - Centro de Investigação das Ferrugens do Cafeeiro/BioTrop/Instituto de Investigação Científica Tropical, Quinta do Marquês, 2784-505 Oeiras, Portugal*
*Email: ptalhinhas@gmail.com; ptalhinhas@iict.pt*

*Colletotrichum kahawae* is an emergent fungal pathogen in the African continent and the causal agent of Coffee Berry Disease (CBD) on Arabica coffee (*Coffea arabica*). This disease represents the biggest threat to coffee production in African highlands, where most of the Arabica coffee is cultivated in this continent. Attending to the high economic and ecological impact of this disease, it is important to develop an easy, reliable and quick diagnostic tool. However, the development of such tool has been hindered by the fact that *C. kahawae* belongs to a complex of cryptic species collectively named *C. gloeosporioides sensu lato*, which can be nearly indistinguishable at the morphological, biochemical and even molecular level, rendering previous diagnostic tools ineffective. Pathogenicity tests on green Arabica berries remain the best method to diagnose the pathogen, but are extremely time-consuming. To address the current limitations in the diagnosis of *C. kahawae*, we have developed an efficient and easy-to-use molecular tool that successfully exploits a molecular polymorphism in the intronic region of the *gluthamine syntethase* (GS) gene, unique to *C. kahawae*. The robustness and reliability of this method was assessed in an extensive sampling of *Colletotrichum* isolates from 8 species from the *C. gloeosporioides s.l.* complex found on coffee, along with *C. acutatum*, revealing that *C. kahawae* could be unambiguously diagnosed in all cases with maximum specificity and sensitivity. This procedure will create unprecedented opportunities for monitoring campaigns of the CBD pathogen in the field that could ultimately provide valuable insights to understand of its epidemiology and population dynamics.

**P22.020 Nested PCR assays for the detection of *Venturia nashicola*, a causal agent of scab on Asian pears**

*H.S. Koh, Y.S. Lee, H.J. Kim, Y.J. Koh[1] and J.S. Jung*
*Department of Biology and [1]Department of Plant Medicine, Sunchon National University, Suncheon 540-950, Republic of Korea*
*Email: jjung@sunchon.ac.kr*

The fungus *Venturia nashicola* is the causal agent of scab on Asian pears. For the rapid and reliable identification and the sensitive detection of *V. nashicola*, a technique based on the PCR was developed. DNA fingerprints of three closely related species, *V. nashicola*, *V. pirina* and *V. inaequalis*, were obtained with random amplified polymorphic DNA (RAPD) analysis. Two pairs of sequence-characterized-amplified-regions (SCAR) primer were then designed from nucleotide sequences of *V. nashicola* specific RAPD markers. The SCAR primer pairs designated as D12F/R and E11F/R amplified a 535 bp and a 525 bp DNA fragments, respectively, only from genomic DNA of *V. nashicola*. The specificity of primer sets were tested with 9 strains of representing three species of *Venturia* and 20 fungal species of plant pathogens. The nested PCR primer pair specific to *V. nashicola* was developed based upon the sequence of species-specific 525 bp DNA fragment amplified by primer set E11F/R. The internal primer pair

Na11F/R amplified a 235-bp fragment from *V. nashicola*, but not from any other fungal species tested. The nested PCR assays were sensitive enough to detect the specific fragment in 50 fg of *V. nashicola* DNA. These primer pairs are highly specific and reproducible, and provide a rapid and sensitive tool for detection of scab pathogen by PCR.

**P22.021 Simultaneous detection of multiple lily-infecting viruses via reverse transcription polymerase chain reaction**

*J.Y. Kwon and S.H. Choi*
*Department of Horticulture, Biotechnology and Landscape Architecture. Seoul Women's University, Seoul 139-774, Republic of Korea*
*Email: paxs@swu.ac.kr*

The lily is one of the most popular flower crops cultivated throughout the world, including Korea. Virus detection is important for lily cultivation as the rapid of spread of viruses affects the susceptibility of cultivars or growing conditions. A detection system based on a multiplex reverse transcription (RT) polymerase chain reaction (PCR) was developed to simultaneously identify multiple viruses in the lily plant. The most common viruses infecting lily plants are the *cucumber mosaic virus* (CMV), *lily mottle virus* (LMoV), *lily symptomless virus* (LSV). Leaf samples were obtained from imported cultivars that were cultivating in the experimental fields in Korea and used to evaluate the detection system. Simplex and multiplex RT-PCR were performed using virus-specific primers to detect single- or mixed viral infections in lily plants. Target bands of CMV, MoV and LSV could be amplified by multiplex RT-PCR, and yielding three distinctive bands of three viruses with the band from internal control. Sequence identities of three amplified virus fragments were checked by comparison with corresponding viral sequences in database. Our results demonstrate the selective detection of 3 different viruses by using specific primers as well as the potential of simultaneously detecting 2 or 3 different viruses in lily plants with mixed infections. For the simultaneous detection and differentiation of all 3 viruses in the lily, 3 sets of primers for each target virus, and 1 set of internal control primers were used to evaluate the detection system for efficiency and reproducibility.

**P22.022 PCR Assays to detect *Pseudomonas syringae* pv. *atrofaciens*, causal agent of basal glume rot on wheat**

*J.H. An, Y.E. Kim and J.S. Cha*
*Department of Plant Medicine, Chungbuk National University, Cheongju, 361-763, Republic of Korea*
*Email: wise6111@gmail.com*

*Pseudomonas syringae* pv. *atrofaciens* that causes Basal glume rot on wheat is a plant quarantine bacterium in many countries including Korea. Not enough studies for detection of *P. syringae pv. atrofaciens* are currently available. In order to detect the target plant pathogen we designed and developed PCR assays. Since not many nucleotide sequences of *P. syringae* pv. *atrofaciens* strains useful for primer design has been known, next generation sequencing (NGS) was performed to obtain draft-genome sequence of *P. syringae* pv. *atrofaciens* LMG 5095. We obtained 1037 contigs and the similarity for each ORFs was compared to other known genes by BLATN. Total 52 primer sets were designed from the ORFs which showing low homology to genes in the GenBank. The primer set, Psa-42-F/Psa-42-R, was designed from ORF in contig specifically amplified a 477 bp fragment from all *P. syringae* pv. *atrofaciens* strains by the PCR. While the DNA was not amplified in non-target bacteria including *P. syringae* pathovars and *P. savastanoi* pathovars. TaqMan PCR with Psa-42-ne-F/Psa-42-ne-R and Psa-PBL-TaqMan probe generated CT values for positive detection in all *P. syringae* pv. *atrofaciens* strains. PCR and TaqMan PCR assays developed in this study could reliably applied to detect and used for identification of *P. syringae* pv. *atrofaciens*.

**P22.023 PCR assays for the detection of *Pseudomonas syringae* pv. *persicae*, causal agent of the bacterial dieback of peach**

*Y.H. Noh, M.J. Song and J.S. Cha*
*Department of Plant Medicine, Chungbuk National University, Cheongju, 361-763, Republic of Korea*
*Email: syungsou@gmail.com*

Bacterial dieback, caused by *Pseudomonas syringae* pv. *persicae*, is a serious disease on peach, nectarine and Japanese plum. No satisfactory detection method for plant quarantine service had been reported for *P. syringae* pv. *persicae*. That is a plant quarantine pathogen in Korea. PCR assays for the detection of *P.syringae* pv. *persicae* has been developed in this study. Since not enough nucleotide sequences of *P. syringae* pv. *persicae* strains have been reported to design the PCR primer, whole genome sequencing of *P. syringae* pv. *persicae* CFBP1569 was carried out with the Next Generation Sequencing (NGS). A total of 1799 contigs were obtained from the sequencing. The similarity of each ORF in the contigs was compared to the known genes in the NCBI GenBank by nucleotide BLAST (BLASTN) to select candidate genes to design primers for PCR assays. PCR primer set, Per17-F/Per17-R, and nested primer set, Per17-ne-F/Per17-ne-R were selected based on specificity to the target bacterium, *P. syringae* pv. *persicae*. PCR with Per17-F/Per17-R and the bacterial DNA amplified the target 485 bp DNA from the 15 strains of *P. syringae* pv. *persicae*. The target size DNA was not amplified from the non-target bacterium including 30 pathovars of *P. syringae*, 4 strains of *Pseudomonas* spp. and 16

strains of other plant pathogenic bacteria by the PCR with primers, Per17-F/Per17-R or the nested primers, Per17-ne-F/Per17-ne-R. The results obtained in this study indicate that *P. syringae* pv. *persicae* can be readily detected and identified by PCR assays with primer sets.

**P22.024 Characterization of the epitope recognized by a broad-spectrum monoclonal antibody against capsid proteins of plant potyviruses**

*H.L. Liu and Y.C. Chang*
*Department of Plant Pathology and Microbiology, National Taiwan University, No.1, Sec.4, Roosevelt Road., Taipei, 10617 Taiwan, China*
*Email: d98633001@ntu.edu.tw*

*Potyvirus* is the largest genus in plant viruses containing more than 146 species and numerous members cause significant damage to various crops. A high-specificity monoclonal antibody, named C4 (C4 MAb), which could detect at least 14 potyviruses was screened in our previous research. To clarify the binding of C4 MAb to which part of the capsid proteins (CP) of different potyviruses, epitope mapping was performed. After phage display library screening and alanine substitution analyses, a 12-residue epitope in the conserved domain of CP was identified. Two amino acids (W and Y) in this epitope region were most required for C4 MAb binding, and a D to F mutation reduced the binding affinity. The epitopes derived from *Dasheen mosaic potyvirus* (DsMV), *Konjak mosaic potyvirus* (KoMV) and *Zantedeschia mild mosaic potyvirus* (ZaMMV) contain similar sequences and were separately fused to the C-terminus of *Odontoglossum ringspot tobamovirus* (ORSV) CP, and then expressed in bacterial pET system. The epitope of KoMV fusing to ORSV CP showed better binding affinity than those of DsMV and ZaMMV by ELISA and western analyses. The results showed the 12-residue epitope recognized by C4 MAb which was proved to be broad-spectrum and genus-specific has great potential to develop as an epitope tag.

**P22.025 First report of witches broom phytoplasmas associated with coconut wilt disease**

*A. E. Prasetyo[1] and K. H. Mutaqin[2]*
*[1]Plant Protection Division, Indonesian Oil Palm Research Institute, PO BOX 37 Pematang Siantar 21100, Indonesia; [2]Plant Protection Departments, Bogor Agricultural University, St. Kamper, Dramaga, Bogor 16680, Indonesia*
*Email: prasetyo_marihat@yahoo.com*

Phytoplasmas are insect-transmitted, phloem-limited bacterial pathogens that are not cultivable in cell-free media at present. Phytoplasmas are small bacteria (±500nm in diameter) surrounded by a single cell membrane, which appear to have suffered extreme genome reductions compared with their Gram-positive relatives. In coconut plantation, phytoplasmas can cause devastating losses and natural ecosystems worldwide. Some phytoplasma diseases names are used, i.e. Lethal yellowing disease, Lethal wilt disease, Cape St Paul wilt disease, Tanzanian lethal disease, Nigerian Awka disease, Kaincope disease Togo, Kribi disease, Kalimantan wilt, Kerala wilt, Cocounut lethal decline, etc. Different strain of phytoplasmas had detected by nested polymerase chain reaction technique in each of these diseases, that are belong to Candidatus Phytoplasma palmae (16SrIV), Group 16SrXXII-A, Ca. Phytoplasma oryzae (16SrXI), Mexican periwinkle virescence (16SrXIII), and Ca. Phytoplasma cynodontis (16SrXIV). The last research in Indonesia, phytoplasmas has been detected in coconut trees namely Derawan wilt disease that were closely related to witches broom phytoplasma (16SrII). This phytoplasma was the first founded in the world.

**P22.026 Direct sample preparation methods for easy, simple and accurate detection of plant pathogens by complete kits based on real-time PCR**

*E. Bertolini[1], M.C. Martínez[1], G. Teresani[1], A.V. Sauer[2], W. Vicente[3], M. Colomer[4], A. Olmos[1], M. López[1] and M. Cambra[1]*
*[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Plant Protection and Biotechnology Centre, Carretera Moncada-Náquera Km 5. 46113 Moncada, Valencia, Spain; [2]Universidade Estadual de Maringá, Centro de Ciências Agrarias, Maringá, P.R. Brazil; [3]Phytopathology and Nematology Department ESALQ/USP. Piracicaba, S.P. Brazil; [4]PLANT PRINT Diagnostics S.L. www.plantprint.net, Guadassuar, Valencia, Spain*
*Email: ebertoli@ivia.es*

Real-time PCR has demonstrated to be the most sensitive and reliable molecular method for plant patho- gen detection. However, standard protocols of real-time PCR require a previous nucleic acids purification step that makes this procedure tedious, costly, time-consuming and not suitable for large-scale analyses. Fast and simple sample direct preparation procedures have been successfully used to detect plant pathogens such as virus, viroids, phytoplasmas, bacteria and fungi and some vectors. Direct methods for sample preparation prior to molecular analyses were validated and incorporated as the first step in several commercially available complete kits for detection of *Plum pox virus*, *Citrus tristeza virus*, *Peach latent mosaic virus*, *Potato spindle tuber viroid*, *Hop stunt viroid*, *Citrus exocortis viroid*, '*Candidatus* Liberibacter solanacearum', '*Ca.* Liberibacter' spp. causing huanglongbing (HLB) disease,vectors of these '*Ca.* Liberibacter' species, *Colletotrichum gloeosporioides and C. accutatum.* The direct methods, without RNA/DNA, purification are: i) spotting of crude extracts on nylon positively charged or Whatman paper membranes, ii) dilution of crude extracts in extraction buffer,

and iii) tissue-print or squash of plant or arthropod tissues on membranes. Detection by complete kits was compared with conventional procedures for each pathogen. The estimation of diagnostic parameters such as sensitivity, specificity, accuracy, Cohen kappa index, likelihood ratios and predictive positive and negative values, demonstrates the usefulness and robustness of these approaches and complete kits for the detection of a wide range of plant pathogens in different host species.

**P22.027 Phylogenetics and diagnostics of *Colletotrichum falcatum* causing sugarcane red rot**

*P. Malathi, R. Viswanathan and A. Ramesh Sundar*
*Division of Crop Protection, Sugarcane Breeding Institute, Coimbatore – 641007, Tamil Nadu, India*
*Email:emalathi @yahoo.com*

Red rot caused by *Colletotrichum falcatum* Went (Teleomorph: *Glomerella tucumanensis* (Speg.) Arx and Muller) is one of the oldest known diseases of sugarcane which challenges the sugarcane breeders for the continuous release of sugarcane cultivars to tackle newly emerging pathotypes under field conditions. To understand existing pathogen variation, representative isolates of *C. falcatum* collected from major red rot endemic regions in the country were characterized by cultural, pathological and molecular methods using conserved gene sequences. Phylogenetic analysis of 5.8s-ITS, actin, β-tubulin, calmodulin and glyceraldehydes-3-phosphate dehydrogenase sequences confirmed the divergent and unique nature of *C. falcatum* from other *Colletotrichum* spp. and separated the pathogenic Indian isolates from non-pathogenic resembling other country isolates. Since development of specific primers based on conserved gene sequences could be valuable for accurate, easy and rapid PCR based detection, the variable regions have been used in developing specific primers for diagnostics at species level. The specific primers were evaluated with other *Colletotrichum* species and confirmed the *C. falcatum* specificity particularly for actin and calmodulin primers. Among the primers, actin primers were found to be highly useful as they were able to recognize the existing variability among isolates. In continuation, the primers were validated for PCR-based diagnostics in plant tissue and soil under various situations Results of this study found to be highly helpful for quarantine, screening and field level diagnosis and also to develop molecular based high throughput system of screening.

**P22.028 Real time PCR and conventional PCR detection of *Colletotrichum coccodes***

*J.Y. Kim[1], H W. Kwon[1], M.A. Choi[1], H.J. Kim[2] and S.H. Kim[2]*
*[1]Department of Microbiology and [2]Institute of Basic Sciences, Dankook University, Cheonan 330-714, Republic of Korea; [2]Experiment & Analysis Division, Animal, Plant and Fisheries Quarantine & Inspection Agency, Incheon International Airport Regional Office 400-718, Republic of Korea*
*Email: piceae@dankook.ac.kr*

Anthracnose fungus *Colletotrichum coccodes* is a serious plant pathogen of tomato, pepper and potato not only in Korea but also other foreign countries. In the present study we tried to design a specific molecular marker for sensitive and easy detection of *C. coccodes*. The translation elongation factor 1 α(*tef*-1α) gene was amplified from genomic DNA of *C. coccodes* and several *Colletotrichum* species by PCR and sequenced. A *C. coccodes*-specific primer set, Coccodes-F and Coccodes-R, was designed based on the *tef*-1α gene sequences. To test the specificity of the primers, genomic DNAs of *C. coccodes, C. orbiculare, C. acutatum, C. gloeosporioides, C. lindemuthianum, C. higginsianum, C. liliacearum, C. caudatum, C. musae, C. dematium, C. boninense* were tested by PCR. The primer set amplified the target band only from the genomic DNA of *C. coccodes*, indicating it is *C. coccodes* specific. The primer set could specifically amply the target band with 100pg of genomic DNA by real time PCR method. When we used conventional PCR method, the amplification of target band was successful with 10ng of genomic DNA.

**P22.029 Monitoring of *Leptosphaeria* species causing phoma stem cancer on winter oilseed rape in the Czech Republic**

*J. Mazakova, J. Urban, M. Zouhar and P. Rysanek*
*Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague, Czech Republic*
*Email: mazakova@af.czu.cz*

*Leptosphaeria maculans* (Desmaz.) Ces. & De Not. and *L. biglobosa* Shoemaker & H. Brun are two closely related ascomycete fungi and important pathogens of cruciferous plants. These fungi that were originally considered as one species coexist in many countries, but differ in their biology and epidemiology. Both species may be differentiated by various methods, including plant assays, assessments of growth characteristics *in vitro*, isozyme analyses, secondary metabolite profiling, serology and molecular techniques. On the basis of molecular phylogeny studies *L. maculans* has been also divided into two subclades, and *L. biglobosa* into six subclades. *L. maculans* brassicae and *L. biglobosa* brassicae, canadensis, australensis, occiaustralensis originating from different region of the world are associated with phoma stem cancer (blackleg) of oilseed rape. During the years 2007-2012 *Leptospaeria* spp. infected tissue of oilseed rape were analyzed for species differentiation with a view to surveying the country-wide distribution of both species. The samples of winter oilseed rape leaves,

stems and root collars with disease symptoms were collected in different regions of the Czech Republic. Isolated DNA was tested by conventional PCR analysis using species-specific primers for the species detection and differentiation. Both *L. maculans* and *L. biglobosa* pathogens were detected in leaf spots, stem spots, stem and root collar cancer. Also coexistence of both species was confirmed in one plant and also in one spot.

### P22.030 Biological soil mapping (*BioSoM)* of soilborn pathogens in soils

*A. Jonsson, A.C. Wallenhammar and C. Dixelius*
*Department of Soil and Environment, Swedish University of Agricultural Sciences, PO Box 234, SE-53223 Skara, Sweden*
*Email: anders.jonsson@slu.se*

In future agriculture with high expectations of increasing yields of high quality (www.fao.org) in a changing climate, the level of infestation of pathogens must be effectively monitored to employ adequate crop management routines. New technologies used in Precision Agriculture such as Global Positions System (GPS) and Geographic Information Systems (GIS) makes it possible to keep track of and present infestation levels. New detections methods based on DNA–technologies makes it possible to determine infestation levels of many organisms in soil. The aim of this research program is to establish the scientific foundation for an in practice useful biological soil mapping of soil borne pathogens (BioSoM) to be used by farmers to optimize crop production. This is done by: 1) Develop and validate DNA-base PCR-methods for specific and quantitative detection of soil borne pathogens; 2) The development and validation of standard operation procedures (SOP) for field sampling and handling as well as for presentation and implementation of results; 3) Enhancing the understanding of correlation between level of infection and soil characteristics such as macro- and micro nutrients.

### P22.031 Molecular characterization of the 16SrII group phytoplasmas associated with faba bean (*Vicia faba* L.) in Saudi Arabia

*M. A. AL-Saleh[1] and M. A. Amer[1, 2]*
*[1]Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, P. O. Box 2460, Riyadh 11451, Kingdom of Saudi Arabia; [2]Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt*
*Email: malsaleh@ksu.edu.sa*

Faba bean is one of the most widely grown protein-producing food legumes. Plant samples showing symptoms of phyllody, yellowing and stunting were observed in fields of Agricultural Research Station, Riyadh region, Saudi Arabia during December, 2011. These samples were collected and tested for plant pathogenic phytoplasmas using the P1/P7 universal primer to amplify the phytoplasma 16S rRNA gene. Nested PCR screen was conducted using the R16F2n/R16R2 primer pair, which yielded fragment of approximately 1.2 kb. The tested samples were positive to the PCR, while no PCR products were obtained from the DNA extraction from asymptomatic plants. Phylogenetic analysis of the 16S rRNA gene of the obtained nucleotide sequence indicated that the two faba bean phytoplasmas isolates [faba bean phyllody (FBP) and faba bean stunting (FBS)] from Saudi Arabia were more closely related to the peanut witches'-broom phytoplasma group since its 16Sr RNA sequence showed a 97.2% to 99.3% identity with most members of this group. The nucleotide sequence for these isolates were deposited in the GenBank with accession no. JQ861532 and JQ861533 respectively. The cDNA probe was prepared and hybridized with DNA extraction from different part of symptomatic plants, but no hybridization with DNA extracts from asymptomatic plants. This is the first report of phytoplasma infecting this crop in Saudi Arabia.

### P22.032 Multiplex detection of *Phytophthora* using SNPs coupled to *Luminex* xMAP technology

*K. Kostov, E. Verstappen, J. Bergervoet, M. de Weerdt, C. Schoen and P. Bonants*
*Plant Research International, Wageningen UR, Wageningen, The Netherlands*
*Email: peter.bonants@wur.nl*

*Phytophthora* spp. is pathogenic on a wide range of different crops and the number of species is increasing. To detect and identify those species several molecular methods have been developed for single species only. A uniform method for detection of all *Phytophthora* species would be very useful for research and regulatory communities. Therefore we developed a diagnostic method to detect a range of *Phytophthora* species. The method includes a generic PCR amplification of different loci for all *Phytophthora* species combined with specific primer extension and detection on the Luminex platform. Several loci were analyzed for the presence of SNPs for specific species, clades or sub-clades: internal transcribed spacer 1 and 2, cyclooxygenase gene 1, β-Tubulin, translation-elongation factor 1α and nicotinamide adenine dinucleotide. We designed and tested several TSPE primers (5'- labelled with specific Tag sequences) for 25 species, 2 clades and 13 sub-clades using the discovered SNPs. The TSPE products can then be detected on the Luminex platform, since anti-Tags are coupled to specific Luminex beads. In this paper the specificity and sensitivity of the multiplex detection system was tested on a range of DNAs from reference *Phytophthora* cultures as well as mixed infected material collected from environmental studies.

**P22.033 Molecular identification of *Verticillium* sp. and *Fusarium* sp. isolated from cotton, pepper and tomato in Xinjiang, China**

*X.D. Lu[1], W. Dmytriw[2], L. Guo[1], P. Kharbanda[2], J. Yang[2] and R.M. Lange[2]*
*[1]Shihezi Vegetable Research Institute in Xinjiang, Shihezi, P. R. China; [2]Alberta Innovates - Technology Futures, Technology Futures, Alberta, T9C1T4, Canada*
*Email: lxdcom@yahoo.com.cn*

Soil-borne diseases caused by *Fusarium* spp. and *Verticillium* spp. on cotton, processing tomato, and peppers are widely prevalent in Xinjiang area in China. Fungal isolates were collected from these crops that showed wilt symptoms. The isolates were purified by the single spore technique and identified as *Verticillium* spp. and *Fusarium* spp. based on their cultural and morphological characteristics. To confirm identification, molecular work was done at the Alberta Innovates - Technology Futures, Canada. The genomic DNA of fungal isolates were extracted; the ribosomal DNA (rDNA) region consisting of part of the 18S rDNA, 5.8S rDNA, internal transcribed spacers (ITS) 1, 2 and part of the 28S rDNA of three isolates were amplified in a polymerase chain reaction (PCR) with the universal primer set ITS5/ITS4. The translation elongation factor 1-α gene region of one isolate from tomato was amplified using primer set EF1/EF2. PCR products were sequenced. The aligned DNA sequences were searched in the nucleotide database (BLASTN), National Centre for Biotechnology Information (NCBI). Three fungal isolates were identified as *V. dahliae* and one isolate from tomato was identified as *F. oxysporum* f. sp. *lycopersici*.

**P22.034 Identification and molecular characterization of *Milk vetch dwarf virus* from legumes crops in Bangladesh**

*M.K. Uddin, M.B. Meah, K. Okazaki and Y. Sano*
*Niigata University, Ikarashi, Niigata 950-2181, Japan; Bangladesh Agriculutural University, Mymensingh-2202, Bangladesh*
*Email: kamalbauctg@yahoo.com*

Viral diseases with yellowing, dwarfing, leaf curling and stunting symptoms diminish yield and quality of leguminous crop production in Bangladesh. Based on the generation of PCR amplicons utilizing nanovirus-specific primers it was shown to be evolved by *Milk vetch dwarf virus* (MDV). For characterizing MDV, all genomic components encoding DNA-R, DNA-S, DNA-M, DNA-N, DNA-C, DNA-U1, DNA-U2 and DNA-U4 of three MDV isolates from common bean (*Dolichos lablab* L.), yard long bean (*Vigna unguiculata* subsp. *sesquipedalis*) and cowpea (*Vigna unguiculata* L.) were cloned and sequenced. Only DNA-R and DNA-S were also cloned and sequenced for another two isolates from common bean (*Dolichos lablab* L.). In addition, MDV associated alpha satellites have also been characterized. Genomic components of five isolates from Bangladesh shared overall nucleotide and amino acid sequence identities of above 94-97% with the type isolate (MDV-N) from Japan. Identified alpha satellites shared overall nucleotide and amino acid sequence identities ranging from 97-99% with alpha satellites associated with MDV-N. These isolates were thus identified as MDV, so far known to cause significant disease of legumes in Japan. This is the first record of MDV in Bangladesh and only record of complete genome sequencing outside of Japan.

**P22.035 The RT-PCR detection of Cucumber green mottle mosaic virus in watermelon rootstock seeds**

*H.R. Zhao and B.D. Gao*
*Hunan University Key Laboratory for Control and Utilization of Plant Diseases, Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Hunan Agricultural University, Changsha 410128*
*Email: bdgao@yahoo.com.cn*

We detected 139 sets of rockstock seeds for watermelon graft from 4 seed produce cities of different provinces (Zhangye of Gansu, Changde of Hunan, Yulin of Shanxi and Turpan of Xinjiang) for presence of Cucumber green mottle mosaic virus (CGMMV) in January-April of year 2012. The results of RT-PCR with specific primers showed that seeds from all the 4 provinces proved to be CGMMV –contaminated and there was no report of CGMMV before in Shanxi. CGMMV was detected in 80.6% on average of seed sets from different provinces. RT-PCR products of Changde isolates share 100% nucleotide identities with those of Shaoyang isolates that previously reported. However, RT-PCR products of isolates from the other three provinces share 100% nucleotide identities with sequences of Yunnan isolates (accession number: HM363013) and Shandong isolates (accession number: HM008919 , GQ500898) after sequencing and BLAST of RT-PCR products. There should be at least 2 invasion sources of CGMMV in China.

**P22.036 Real-time Fluorescence PCR Identification of *Alternaria alternata* caused occurrence of fragrant pear black spot**

*F.J. Wang, X.L. Zang and X.Z. Feng*
*Korla Entry-Exit Inspection and Quarantine Bureau, 39# Petrochemical Boulevard, Korla, Xinjiang, 841000, P. R. China*
*Email: wfj0808@sohu.com*

The pathogen causing fragrant Pear black spot identified as *Alternaria alternata*（Fries）Keissler is one of the major diseases in plant, which damages the qualities and

quantities of fruits and flowers and spreads worldwide. Identification of emerging fungal pathogens by conventional methods is difficult and time-consuming. Fast identification and detection of the pathogen is the key way to diagnose and control its further spreading. In this study, a real-time fluorescence PCR method was established by using a pair of specific primers and double-fluorescence labeling probe according to the conserved sequence of the pathogen causing fragrant pear black spot. 12 blossoms, 30 fruits and 94 slides samples from fragrant pear were detected in this study. The real-time fluorescence PCR was used to detect other four kinds of plant disease pathogens on pear. Results show that only in the pathogen causing black spot, fluorescence signal can be collected with the specific primers and probe. The assay for specific detection was more sensitive than conventional PCR, which could detect the template concentration of DNA samples as low as 1.9 pg/μL and don't have to through the pathogen separation and purification culture. This reliable, sensitive, quick and easy-handling method is suitable for screening, dynamic monitoring, identification and prevention of plant disease.

**P22.037 Physiological races identification and detection study on cruciferous vegetables clubroot in Yunnan and Tibet**

*L.H. Zhou, L.F. Wei, L. Feng, L.H. Zhang, Y.Q. He and G.H. Ji*
*College of Plant Protection, Yunnan Agricultural University, Kunming, Yunnan, 650201, P. R. China*
*Email: jghai001@ yahoo.com.cn*

The study identified main physiological races of Cruciferous Vegetables clubroot by Williams identify system. We also adopted the rapid detection method of PCR technique of *Plasmodiophora brassicae*. The main results were shown below. The results of physiological race identified indicated that the pathogens which collected from 32 clubroot disease regions of Yunnan were divided into 9 physiological races. The predominant race was No. 4, and the proportion was 53.1%. The pathogens which collected from 9 clubroot disease regions of Tibet were divided into 4 physiological races. The predominant race was also the No. 4. Clear and definite the race distribution of populations of *P. brassicae* may be helpful in screening resistant varieties in different regions of Cruciferous Vegetables clubroot. The simple one-step polymerase chain reaction (PCR) and QPCR protocol was developed to specific detect the pathogen in *P. brassicae*-infested plant and soil samples. 3 pair of specific primers was designed for routine PCR which based on the *P. brassicae* partial 18S ribosomal RNA and internal transcribed space1 gene sequences of GenBank database. The primer PB10 was selected for fluorescence quantitative PCR, and the length of PCR amplification product was 109bp. The detection system of routine PCR and SYBR Green I fluorescence quantitative PCR in this study could provide a reliable, specific and rapid diagnosis of *P. brassicae* detection in plant and soil materials.

**P22.038 Detection of *Citrus psorosis virus* by ELISA and double-stranded RNA extraction in Mazandaran, Iran**

*F.P. Bandpey, S. V. Alavi and A. Massah*
*Department of Plant Protection, Isfahan University of Technology, Iran*
*Email: f_pourvali@yahoo.com*

Psorosis is one of the most important viral disease of citrus associated with *citrus psorosis virus* ($CP_SV$), type species of the genus *ophiovirus*, causes serious losses worldwide. Triple antibody sandwich (TAS) ELISA, employing the IgG monoclonal antibody (mab) 13c5 and dsRNA extraction were used to detect $CP_SV$ in orchards of different citrus varieties in Mazandaran, northern Iran. Sixteen and six out of 30 samples were determined as positive and suspected samples by ELISA, respectively. Electrophoretical patterns of extracted double-stranded RNA (dsRNA) showed two bands (larger than 10000 bp and 1700 bp) on agarose gel in all ELISA positive samples. The 1700 bp band seems to be dsRNA of CPsV RNA2 and the band larger than 10000 bp appears to be the dimer form of double stranded form of virus RNA1. observed symptoms in orchards and low rate of tree mortality suggest that psorosis A is the common type in Mazandaran province. This is the first report of CPsV detection in citrus varieties by ELISA and optimization of a method for CPsV dsRNA extraction in Iran.

**P22.039 Development of a real-time fluorescence LAMP method for rapid detection of *Fusarium oxysporum* f. sp. *cubense***

*L.L. Yang[1,2], X.L. Ruan[1] and H.P. Li[1]*
*[1]College of Natural Resources and Environment, South China Agricultural University, Guangzhou, 510640, P. R. China; [2]Inspection Technical Center of Zhongshan Entry-Exit Inspection & Quarantine Bureau, Zhongshan, Guangdong ,528400, P. R. China*
*Email: huaping@scau.edu.cn*

Banana fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* race 1 (FOC1) and race 4 (FOC4) respectively, is a destructive disease on banana in the world. Establishment and application of detection method is an important means to prevent the disease from spreading and occurring. A real-time fluorescent LAMP (Loop-mediated isothermal amplification) assay was developed based on the unique conserved primers for detecting FOC1 and FOC4 respectively. The results showed that the method can be used to detect FOC1 and FOC4 accurately and effectively in 1 hour. With high specificity,

the method could distinguish *F. oxysporum* f. sp. *cubense* from other allied species and forma, such as *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *momodicae*, *F. oxysporum* f. sp. *luffae* and other plant pathogens, *Penicillium*, *Phytophthora*, *Aspergillus*, *etc*. The detection limit of the total DNA template was approximately $6.2 \times 10^{-4}$ μg/μL, which was $6.2\times10^{-3}$ μg/μL in the ordinary LAMP detection, and it was approximately 3 times higher than that of the normal PCR detection. At the same time, the method did not need to open the reaction tubes during the detection process, in case to avoid aerosol problem and solve the major drawback of being easily contaminated by aerosol in ordinary LAMP. In conclusion, this real-time LAMP method could be utilized to assist in the implementation of quarantine measures for prevention and control of the banana fusarium wilt caused by *F. oxysporum* f. sp. *cubense*.

**P22.040 Management and automation of qPCR diagnostic and research workflows**

*M. Hren[2], K. Gruden[1], K. Zupančič[2], N. Mehle[1], U. Čepin[2] and M. Ravnikar[1]*
*[1]National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia; [2]BioSistemika Ltd., Tehnološki park 24, 1000 Ljubljana, Slovenia*
*Email: maja.ravnikar@nib.si*

Growing challenge in laboratories dealing with high throughput real-time PCR (qPCR) analyses for diagnostic or research purposes is how to make the complete process from sample preparation to data analysis and interpretation faster. We are presenting a case study on detection of plant pathogens with qPCR in difficult plant samples, focusing on automation, unification and simplification of the complete process. The new procedure combines automated simple and quick homogenization step and automated DNA extraction method based upon the binding of DNA to magnetic beads. Further sequence of dry-lab steps (experiment design, creation of templates for lab work, data analysis, results interpretation and reporting) that are complex, repetitive, time consuming and typically involve use of different softwares were connected into innovative software solution GENEIO®. This is easy-to-operate workflow environment installed in centralised server and accessible to all employees via internet browser from everywhere inside local network. It communicates with LIMS and different qPCR thermal cyclers and stores all the necessary data in one place (quality assurance compliant). This way an overview and control over all qPCR work inside the organizational unit (laboratory, department, organization etc.) is allowed for the lab managers. Manual wet-lab step–laborious loading of mastermixes and samples onto qPCR plates have been fastened using a smart pipeting assistant. Combining automation approaches can save huge amounts of time and relieve personnel which are of extreme importance in case of having high throughput qPCR analyses.

**P22.041 Detection of plant quarantine *Wheat streak mosaic virus* using RT-PCR and nested PCR**

*N.Y. Heo[1], S. Lee[1,2], E.H. Kang[1,3], S. Lee[3], T.Y. Ahn[2] and Y.G. Shin[1]*
*[1]Plant Quarantine Technology Center, Animal, Plant and Fisheries Quarantine and Inspection Agency, Suwon 443-440, Republic of Korea; [2]Department of Microbiology, Dankook University, Cheonan 330-714, Republic of Korea; [3]Department of Genetic Engineering, Sungkyunkwan University, Suwon 440-746, Republic of Korea*
*Email: syg1286@korea.kr*

*Wheat streak mosaic virus* (WSMV), a member of the family *Potyviridae*, shows severe impact on rice, wheat and corn. It is never existed plant virus in Korea, but influx through importation from the other place. To prevent WSMV flowing into the country, it is necessary to prepare a specific, sensitive and fast detection method for plant quarantine. For this reason, a two-step diagnosis system consists of RT-PCR and nested PCR is being used for the WSMV detection. In addition, also mutation positive control was made to be used as the positive control. WSMV has detected in seed sweet corn from Japan and seed wheat from U.S by a two-step diagnosis system, which is designed in this study. According the sequence analysis, the similarities were found between the 80.6 and 100.0% with other strains by BLAST. They showed the same topology, which was classified as 4 genotypes by various phylogenetic trees using poly protein encode sequence amplification. In this analysis, WSMV-JSweet-corn2868 (KC754961) is classified as clade B, while WSMV-Uwheat1944-1 (KC754959) and WSMV-Uwheat1944-2 (KC754960) belong to clade D.

**P22.042 Optimization for ISSR-PCR reaction system on *Leptosphaeria biglobosa***

*P.L. Song[1, 2], L.F. Hao[1, 2], X.Z. Li[1, 2], J. Zhang[3], Y.Y. Bao[1] and Z.Q. Li[2]*
*[1]School of Life Science, Inner Mongolia University, Hohhot 010020, P. R. China; [2]Plant Protection Institute, Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot 010031 P. R. China; [3]Agricultural College, Inner Mongolia Agricultural University, Hohhot 010018, P. R. China*
*Email: peiling8721@126.com*

ISSR (inter simple sequence repeat), a kind of DNA molecular markers, with the superiorities of simple, quick, reliable, and generating higher levels of DNA polymorphism. ISSR has a wide range of research applications, including the characterization of genetic relatedness among populations, genetic fingerprinting, gene tagging, detection of colonial variation, cultivar

identification, phylogenetic analysis, detection of genomic instability, and assessment of hybridization. Before using ISSR markers for genetic diversity of *Leptosphaeria biglobosa*, an orthogonal design was used to optimize ISSR reaction system for *L. biglobosa* on oilseed rape in five factors (Taq polymerase, $Mg^{2+}$, dNTPs and the concentration of primer or template) at four levels. A suitable ISSR-PCR reaction system for *L. biglobosa* was established. The optimized reaction system consists of 1 U Taq DNA polymerase, 2.0 mmol/L $Mg^{2+}$, 0.2 mmol/L dNTPs, 0.6 μmol/L primer, 40ng template DNA, and 2.5 μL 10×buffer. ISSR markers could be used as a useful tool for study the genetic diversity of the pathogen which was confirmed by testing on the stability of 24 strains of *L. biglobosa*.

**P22.043 Molecular and morphological diversity in *Rhizoctonia bataticola* isolates causing dry root rot of chickpea (*Cicer arietinum* L.) in India**

*R. Ghosh, M. Sharma and S. Pande*
*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, Andhra Pradesh, India*
*Email: r.ghosh@cgiar.org*

Dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butler. [Pycnidial stage: *Macrophomina phaseolina* (Tassi) Goid] is emerging as a serious biotic constraint for chickpea production. To find out the diversity in *R. bataticola* populations in India, a total of 94 isolates collected from *R. bataticola* infected chickpea plants from different agro climatic regions of India were analyzed with amplified fragment length polymorphism (AFLP), rDNA-internal transcribed spacer (ITS) region sequencing, ITS-RFLP and different morphological properties. *R. bataticola* populations collected from different agro-ecological zones were very diverse with respect to their different cultural and morphological parameters like colony color, growth pattern, growth rate, mycelial characters, sclerotial initiation time, intensity and morphology. Five AFLP primer combinations provided a total 121 fragments. All fragments were found polymorphic with an average polymorphic information content value of 0.213. The dendrogram based on AFLP and ITS sequencing analysis showed that the maximum number of *R. bataticola* isolates were diverse and did not depend on geographical origin. The AFLP and rDNA-ITS have the unique potential for providing information across an entire genome, while ITS-RFLP analysis can reveal variation only within small region of the genome. Both morphological and molecular data correlated each other and supported that the *R. bataticola* present in India were diverse and independent to their origin.

**P22.044 Development and evaluation of specific PCR and LAMP assays for detection of *Phytophthora melonis***

*B.J, Li, P.Q, Liu, C.Z, Lan, Q.Y, Weng and Q.H Chen*
*Institute of Plant Protection, Fujian Academy of Agricultural Science, No. 247 Wusi Road, Gulou District, Fuzhou, 350003, P. R. China*
*Email: chenqh@faas.cn*

*Phytophthora melonis* is a widespread and devastating pathogen for the Cucurbitaceae family. Early and accurate detection of *P. melonis* is essential to control the disease in the field. To establish a simple, visual, and rapid detection system for *P. melonis*, we developed nested polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) systems based on the Ras-related protein (*Ypt1*) gene. All 36 isolates of *P. melonis*, from geographically distinct counties in China, yielded positive detection results on LAMP or nested PCR assays. No cross reaction was observed with other oomycetes or fungal pathogens. A sensitivity assay showed that both methods had a detection limit of 10 fg genomic DNA per 25 μl reaction. We also detected *P. melonis* in diseased cucumber tissues and soils, and evaluated positive detection rates using LAMP, nested PCR, and conventional isolation methods. Our results suggest that the LAMP assay has the greatest potential for active detection of *P. melonis* in regions that are at risk of contracting the disease, and for use in resource-poor settings.

**P22.045 Real-time PCR and spore trap-based detection of the downy mildew pathogen, *Peronospora effusa***

*S.J. Klosterman, N. McRoberts, S.T. Koike, K.V. Subbarao, H. Voglmayr, M. Thines and F.N. Martin*
*United States Department of Agriculture, Agricultural Research Service, 1636 E. Alisal St. Salinas, CA 93905*
*Email: Steve.Klosterman@ars.usda.gov*

*Peronospora effusa* is an obligate pathogen and the causal agent of downy mildew on spinach. The pathogen can be dispersed by splashing rain and wind, and may overwinter as oospores. Outbreaks of downy mildew on spinach are common in the cool climate of central coastal California, including the Salinas Valley. The objective of this research was to develop a DNA-based assay for detection and quantification of *P. effusa* primarily for use in spore trap-based detection of airborne inoculum. A real-time PCR Taqman assay was developed using a DNA template of the intergenic transcribed spacer (ITS) region of the ribosomal DNA. Probe and primer sets were tested for specific detection of *P. effusa* with DNA derived from *P. effusa* and related oomycete species. Samples from spore traps in the Salinas Valley tested positive for detection of *P. effusa* using the ITS primers. Specific detection and quantification of *P. effusa* in airborne samples may be helpful to develop an early

warning system for downy mildew outbreaks on spinach in the Salinas Valley, California.

**P22.046 Rice new diseases internodes rot of the preliminary study**

*S.W. Wang and B.D. Gao*
*College of Bio-Safety Science & Technology, Hunan Agricultural University, Hunan University Key Laboratory for Control and Utilization of Plant Diseases and 3Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Changsha 410128, P. R. China*
*Email: bdgao@yahoo.com.cn*

A new rice disease was found in Fenghuang county, Hunan province, in August 2011. In order to find out the reasons, we have the plant pathogenic microscopy, separation, pathogenicity test and molecular identification. Examination found colorless, single cell and oval spore, suspected rice sheath rot bacteria, but it is different from the leaf sheath intact scabbard rot. At the beginning of the colony is white, plush form, from the petri dishes bottom observation colony pale orange; Then colony become pale orange , color from the center to the periphery become weak gradually. Use spore liquid vaccinate in booting stage rice produced white ear and black ear symptoms. The sequence of PCR product of the fungus rDNA-ITS was found to be the most homologous with those of *Sarocladium attenuatum* in GenBank after BLAST. Therefore, we identified the pathogen for *S. attenuatum.* This is the first report of rice internodes rot disease in the world.

**P22.047 A new rice disease symptom, stem-node rot, caused by *Sarocladium attenuatum***

*S.W. Wang and B.D. Gao*
*Hunan University Key Laboratory for Control and Utilization of Plant Diseases, Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, College of Bio-Safety Science & Technology, Hunan Agricultural University, Changsha 410128, P. R. China*
*Email: bdgao@yahoo.com.cn*

A new rice disease was found in Fenghuang county, Hunan province, in August 2011. Diseased rice plants showed stem-node rot from the top node to lower node, resulting dead head symptom but leaf sheaths intact, which is unique on rice. In order to find out the causal agent, we used microscope check, microbe isolation, pathogenicity test and molecular identification. Colorless, single-celled and oval conidia similar to rice sheath rot fungus (*Sarocladium oryzae*) were found under microscope. At the beginning, the colony on PDA plate is white plush form but pale orange from the Petri-dish bottom observation; By inoculation in rice booting stage with spore suspension, white panicle and black glume symptoms produced. The sequence of PCR product of its rDNA-ITS was found to be the most homologous to those of *S. attenuatum* in GenBank after BLAST. Therefore, we identified the pathogen for *S. attenuatum*. This is the first report of rice stem-node rot symptom.

**P22.048 RAPD reaction system optimization for molecular research of *Puccinia graminis* f. sp. *tritici***

*Y.Y. Cao and S. Chen*
*Institute of Plant Immunol ogy, Shenyang Agricultural University, Shenyang, Liaoning, 110866, P. R. China*
*Email: chensi19860324@yahoo.cn*

RAPD amplification technology is one of the most common analysis methods which were used in variety identification and polymorphism analysis of *Puccinia graminis* f. sp. *tritici*. This technique is rapid, inexpensive and does not require abundant quantities of high-quality DNA, but one disadvantage is poor reproducibility. In this paper, RAPD reaction system of *P. graminis* f. sp. *tritici* was optimized in order to acquire optimal PCR amplification system for researching of *P. graminis* f. sp. *tritici.* In this study，using races of *P. graminis* f. sp. *tritici* as material,$L_{16}(4^5)$ orthogonal experiment design was performed with 5 factors of the concentration of DNA, $Mg^{2+}$, TaqDNA polymerase, dNTPs and random primer for the RAPD reaction system in *P. graminis* f. sp. *tritici*. On the basis of this, annealing temperature and circulation times were optimized. The results indicated that the RAPD reaction system of *P. graminis* f. sp. *tritici* was 1×Buffer , 60 ng template DNA, 1.5 mmol/ L $Mg^{2+}$, 1.5 U TaqDNA polymerase, 0.25 mmol/L dNTPs and 0.30 μmol/L random primer in the 25 μL reaction solution; 36℃ annealing temperature and 43 cycles. This reaction system has great ability in detecting polymorphism，stable response system and good repeatability. It can be well applied to analysis genetic diversity of *P. graminis* f. sp. *tritici*.

**P22.049 Establishment of a triplex PCR diagnostic technology for three pathogenic fungi of wheat**

*Y.Y. Cao and S. Chen*
*Institute of Plant Immunol ogy, Shenyang Agricultural University, Shenyang, Liaoning, 110866, P. R. China*
*Email: chensi19860324@yahoo.cn*

*Puccinia graminis* f. sp. *tritici, P. recondita* f. sp. *tritici* and *Blumeria graminis* f. sp. *tritici* are three important plant pathogenic fungi of wheat, they often occur simultaneously, so, a timely identification method is required. In this study, three sets of specific primers were selected, a multiplex polymerase chain reaction was optimized from annealing temperature and primers, TaqDNA polymerase and dNTPs concentration to establish the method for the detection of *P. graminis* f. sp. *tritici, P.*

*recondita* f. sp. *tritici* and *B. graminis* f. sp. *tritici* simultaneously at early stage. The specificity and sensitivity of the multiplex PCR were tested. In result, the reaction system could amplify three specific bands of 395 bp, 151 bp and 464bp. The detection sensitivity of this multiplex PCR was 10 fg DNA of *P. graminis* f. sp. *tritici*,100 fg DNA of *P. recondita* f. sp. *tritici* and 10 fg DNA of *B. graminis* f. sp. *tritici*. It was the first time to establish the triplex PCR system for detecting *P. graminis* f. sp. *tritici*, *P. recondita* f. sp. *tritici* and *B. graminis* f. sp. *tritici* in infected plant tissues simultaneously. The PCR-based method developed here could direct the diagnosis of plant disease and pathogen monitoring.

**P22.050 A primer pair specific to a pill gene discriminates between groups I and II of *Acidovorax citrulli***

*Z.Y. Li[1], Y.M. Min[1], B.D. Gao[1], H.Q. Liu[2], T.C. Zhao[2] and N.W. Schaad[3]*

*[1]Hunan University Key Laboratory for Control and Utilization of Plant Diseases, Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Changsha 410128, P. R. China; [2]The Institute of Plant Protection (IPP), Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; [3]USDA ARS, Foreign Disease-Weed Science Reserch Unit, 1301 Ditto Avenue, Ft. Detrick, MD 21702-5023, USA*

*Email: bdgao@yahoo.com.cn; tczhao@ippcaas.cn*

*Acidovorax citrulli* can be divided into two genetic groups: group I and group II based primarily on PFGE and MLST. In order to distinguish more rapidly between strains of the two groups, a pair of specific primers were designed using bioinformatics and a *pil*L gene of a group II strain, AAC00-1 as a template. The results of classical PCR showed a 332 bp band was generated for 51 of 52 group II strains whereas only 3 of 93 group I strains were positive. Classification of these strains largely agreed with previous studies. Results of PCR showed the primers were able to detect group II strains of *A. citrulli* and distinguish between strains of groups I and II rapidly and accurately.

**P22.051 Identification of causal agent of a new rice disease, sheath black spot**

*W. Huang[1] , and B.D. Gao[1,2,3]*

*[1]College of Bio-Safety Science & Technology, Hunan Agricultural University, [2]Hunan University Key Laboratory for Control and Utilization of Plant Diseases and [3]Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Changsha 410128, P. R. China*

*Email: bdgao@yahoo.com.cn*

A new rice disease of rice sheaths was found in Huayuan county, Hunan province, in July 2011. An elliptical black spot, with obscure border, about 10×5mm in size, was observed on rice sheath. The leaf blades of infected sheaths became yellow and withered. Because the disease did not infect rice stems as did sclerotial diseases, it should be a new rice disease. Two types of colonies formed after tissue isolation. One, named as HNHY001, appeared as dark green velvet with branchy black stroma scattered on PDA plate. HNHY001 proved to be *Curvularia* sp. which showed 4-septate conidia with 3 dark central cells and subhyaline terminals, the measured average size of conidia of HNHY001 was 30.4×l2.5 μm. HNHY002 proved to be N*igrospora* spp. After artificial inoculation both in vitro and in vivo, HNHY001 caused black spots on rice sheaths while HNHY002 did not induce any lesions. In vitro mixture inoculation of those 2 isolates did not induce disease degree higher than that of HNHY001 alone. The sequence (JQ360963) of PCR product of HNHY001 rDNA-ITS was found to be the most homologous with those of *Cochliobolus geniculatus* in GenBank after BLAST. As sexual stage of HNHY001 have not been found after cultured in vitro for nine months, in view of the asexual stage characteristics i.e., conidia slightly straight, 4-septate and stroma branchy, we identified HNHY001 as *C. fallax*. This is the first report of rice sheath black spot disease in the world.

**P22.052 Molecular identification and rapid detection of three isolates of *Cucumber green mottle mosaic virus* in Guangdong**

*W.D. Zhang[1], L. Liao[1], Y.B. Quan[1], M.F. Xu[1], Y.L. Chi[1]and N.Z. Chen[2]*

*[1]Zhuhai Entry-Exit Inspection and Quarantine Bureau, Zhuhai, 519015, P. R. China; [2]Chinese Academy of Inspection and Quarantine, Beijing 100029, P. R. China*

*Email: zwdjlq@126.com*

The coat protein and movement protein gene of Cucumber green mottle mosaic virus (CGMMV-GZ, CGMMV-LZ, CGMMV-GF) in Guangdong province was amplified by RT-PCR. The results revealed that the three isolates contains complete coat protein gene and movement protein gene, which are 486 and 795 nucleotides respectively, and encodes the coat protein of 161 amino acids and the movement protein of 264 amino acids. Specific probe were designed based on movement protein gene, a rapid and effective method of cucumber green mottle mosaic virus detection were established by real-time RT-PCR, and it was used to detect 3 strains of CGMMV and 9 species of *Tobamovirus* in infected leaves. The results show that no signal was detected for the others except CGMMV. The method，which can also detect CGMMV in seeds，is not only rapid, accurate and sensitive, but only play as a well technical support for Entry-Exit Inspection and Quarantine and agricultural safety.

**P22.053 Development of species-specific PCR primers sets for detection of three *Botrytis* species infecting broad bean**

*X. Fan, J. Zhang, L. Yang and G.Q. Li*
*State Key Laboratory of Agricultural Microbiology and Key Laboratory of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan 430070, P. R. China*
*Email: 725fanxuan@webmail.hzau.edu.cn*

This study was conducted to establish a PCR-based method to detect three *Botrytis* species (*B. cinerea*, *B. fabae* and *B. fabiopsis*) infecting broad bean (*Vicia faba*) by designing species-specific primers and by determining the specificity, sensitivity and practicality of these primers in detection of these *Botrytis* species. The primer pairs for *B. cinerea* and *B. fabiopsis* were developed on the basis of SCAR markers, whereas the primer pair for *B. fabae* was designed on the basis of *NEP1* sequence. Using the three primers, three DNA fragments of 327, 613 and 762 bp in size were PCR-amplified for *B. cinerea*, *B. fabiopsis* and *B. fabae*, respectively. Even in the PCR reaction system containing the mixed DNA template of the three *Botrytis* species and the three sets of primers, the species-specific DNA products (327, 613, 762 bp) were consistently detected. On the other hand, these primer pairs could not generate any products when they were used to amplify the DNA template of *B. aclada*, *B. byssoidea*, *B. elliptica*, *B. porri*, *B. sinoallii*, *B. sinoviticola*, *B. squamosa*, *Amphobotrys ricini*, *Streptobotrys* spp. or *Verrucobotrys* sp. The minimum amount of the DNA template in each 25 μL-reaction system was 40 pg. They could be used to discriminate the three *Botrytis* species inoculated on leaves of broad bean. This study suggests that the three species-specific primer pairs can be used to detect three *Botrytis* species infecting broad bean and use of primers in investigating epidemics of chocolate spot of broad bean is underway.

**P22.054 A multi-locus phylogenetic evaluation of *Phomopsis* (*Diaporthe*) spp. isolates from blueberry in the northern China**

*L. Liu, X.R. Yan and J.F. Fu*
*College of Plant Protection, Shenyang Agricultural University, No.120 Dongling Road, Shen He District, Shenyang, Liaoning, 110866, P. R. China*
*Email: zbyxr@126.com*

Twenty isolates of *Phomopsis* were obtained from twigs and leaves with canker or dieback symptoms of highbush blueberry, *Vaccinium corymbosum*, isolated primarily from plants grown in the northern China. Molecular analysis was performed to identify the isolates by multi-locus sequence analysis. Amplification of rDNA ITS gene, and part of the translation elongation factor 1-α (EF 1-α) gene, β tubulin (TUB) gene and calmodulin (CAL) gene were performed using the ITS1/ITS4, EF1-728F/EF1- 986R, Bt2a/Bt2b and CAL228F/ CAL737R primers, respectively. Four utilizable loci were analyzed individually and in combination, and ITS, EF 1-α and multi-locus phylogenetic trees are presented. The phylogenetic tree inferred by combined analysis of four loci provided the best resolution for species as compared to single gene analysis based on the fact that most of the ex-type derived taxa are placed in terminal clades and higher supported, without conflict between well-recognized. The multi-locus phylogenetic trees definitely divided the twenty fresh isolates to at least four groups. Group 1 and group 4 stayed in the separate clade respectively, group 2 gathered with *Diaporthe cotoneastri* and group 3 with *D. castaneaemollissimae*. *Phomopsis* canker investigation on blueberry, indicated that there were high diversity among these pathogens.

**P22.055 Evaluation of PCR assay for detection of *Sclerotinia sclerotiorum* on oilseed rape petals and prediction of sclerotinia stem rot**

*Y.M. Liu, J. Lu, Q. Yuan, L. Zheng and J.B. Huang*
*The Key Lab of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan, Hubei, 430070, P. R. China*
*Email: lymliuyoumei@163.com*

Sclerotinia stem rot caused by *Sclerotinia sclerotiorum*, is a highly destructive disease in oilseed rape (*Brassica napus)*. From 2011 to 2012, specific primers XJJ21/XJJ222 were used to detect S. sclerotiorum on oileed rape petals in two types of fields (Cotton – oilseed rape fields and Rice – oilseed rape fields) of Huanggang and Wuxue, Hubei province, China. The relationships between percentage of petals infested (PPI) during different blossoming stage (early blossom stage, full-blossom stage and late blossom stage) and incidence of leaf disease or stem disease were analyzed further to evaluate this molecular detection technical system in the field by linear regression method. Regression analysis showed that the incidence of leaf disease and stem disease both have significant correlation with PPI during different blossoming stage ($P < 0.05$). It proves that detection of PPI is benefit for forecasting sclerotinia stem rot. The monitoring models for the two types of fields in the early-blossom stage are $Y = 0.8688\ x1 + 2.87123\ x2 + 1.19886\ x3 - 1.61886\ x4 + 6.8080$ and $Y = 0.99552\ x1 + 0.87286\ x2 + 0.05175\ x3 - 0.19556\ x4 + 5.474$, respectively (Y, stem incidence; x1, PPI; x2, number of apothecia; x3, rainfall capacity, x4, temperature). The models could be used for prediction of Sclerotinia stem rot of oilseed rape in blossom stage and useful for disease control in the field.

**P22.056 Molecular variability of coat protein gene of *Sweet potato chlorotic fleck virus* and preparation of antiserum**

*Q. Qiao[1,2,3], Y.H. Qin[1,2,3], D.S. Zhang[1,2,3], Y.T. Tian[1,2,3], S. Wang[1,2,3], Y.J. Wang[1,2,3] and Z.C. Zhang[1,2,3]*
*[1]Institute of Plant Protection, Henan Academy of Agricultural Sciences, No.116 Huayuan Road, Zhengzhou, 450002, P. R. China; [2]Henan Key Laboratory of Crop Pest Control, No.116 Huayuan Road, Zhengzhou, 450002, P. R. China; [3]IPM Key Laboratory in Southern Part of North China for Ministry of Agriculture, No.116 Huayuan Road, Zhengzhou, 450002, P. R. China*
*Email: zhangzhenchen@126.com*

The coat protein (CP) gene of *Sweet potato chlorotic fleck virus* (SPCFV) from four Chinese isolates was cloned by RT-PCR. Sequence analysis showed that the full-length *CP* gene comprised 900 nt and encoded 299 amino acid residues. The *CP* gene of the four isolates shared 78.3%-89.9% and 91.3%-95.7% identity at the nucleotide and amino acid level respectively. The third to 32$^{nd}$ amino acids of CP from different isolates were more variable compared with other region. The *CP* gene of Sichuan isolate was cloned into expression vector pET28a (+) for over expression in prokaryotic cells. The result of SDS-PAGE showed that a 36.5 kDa specific fusion protein was produced after induction by IPTG. The expressed protein was purified from SDS-PAGE and the antiserum against the protein was raised in rabbit. The ELISA titer of the antiserum in infected leaves supernatant was 1:128000. The antiserum was used for specific detection of SPCFV from field samples of sweet potato by ACP-ELISA.

### P22.057 Detection and characterization of an elm yellows (16SrV) group phytoplasma infecting *Sophora japonica* var *golden* in China

*Z.G. Ren, C.L. Lin, Y. Li, C.G. Piao, J.X. Hu, X.S. Geng, C.S. Song and G.Z. Tian*
*Key Laboratory of Forest Protection of State Forestry Administration, Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing 100091, P. R. China*
*Email: tiangz@caf.ac.cn*

*Sophora japonica* var *golden*, one variety of Chinese scholar tree (*Sophora japonica* L.) and characterized with its "golden stem", is an excellent green tree species for urban landscaping. The diseased branches of *S. japonica* var *golden* witches'-broom (SJGWB) were collected in Haidian district, Beijing, China, and the phytoplasma was detected and characterized in this study. Firstly, phytoplasma cells were revealed in phloem sieve elements of the symptomatic *S. japonica* var *golden* by transmission electron microscopy. Secondly, the total DNA was extracted from the leaf midribs and phloem stalk tissues using CTAB method, and the conserved genes 16S rDNA, *rp* (ribosomal protein) and *secY* were amplified by the primers 16mF2/16mR2, rp(V)F1/ rp(V)R1 and FD9f/FD9r, respectively. The PCR products were cloned into pMD18-T vector and sequenced. Sequence comparisons and phylogenetic analysis indicated that SJGWB phytoplasma had high sequence similarities (ranged from 98.0% to 99.9%) with elm yellows (EY) group (16SrV) phytoplasmas, and together with the representative strain of subgroup 16SrV-B, rpV-C and secYV-C, jujube witches'-broom phytoplasma strain JWB (GenBank Accession No. AY197661, AY197681 and AY197695 for 16S rDNA, *rp* and *secY* gene respectively) formed a monophyletic group in every phylogenetic tree based on 16S rDNA, *rp* and *secY* gene, respectively. In conclusion, the phytoplasma infecting *S. japonica* var *golden* belongs to the 16SrV group phytoplasmas, and it is most closely related to subgroup 16SrV-B, rpV-C and secYV-C JWB phytoplasma.

### P22.058 A molecular marker for distinguishing between A1 and A2 mating types of *Phytophthora capsici*

*P. Li, Z. Gao, Y. Dai, S. Cao, M. Guo, Y. Pan, F. Chen and H. Zhang*
*College of Plant Protection, Anhui Agricultural University, Hefei 230036, P. R. China*
*Email: gaozhimou@126.com*

The oomycete plant pathogen *Phytophthora capsici* Leonian is distributed worldwide and has become one of the most serious threats to vegetable production. *P. capsici* requires two opposite mating types, A1 and A2, for sexual reproduction, which plays an essential role in the biology and epidemiology of the pathogenic oomycete. In this paper, inter-simple sequence repeat (ISSR) was used to specifically detect different mating types of *P. capsici*. The ISSR random primer UBC821 (5'-GTG TGTGTGTGTGTGTT-3') detected a fragment that is specific in the A1 mating type of *P. capsici*. This fragment was cloned and sequenced. Based on the sequence data, Pcap-1 and Pcap-2 primer sets were designed to detect the A1 mating type of *P. capsici*. A 997 bp fragment was observed in the A1 mating type of *P. capsici*, but not in the A2 mating type of *P. capsici*. Similarly, a 508 bp fragment was observed in the A2 mating type of *P. capsici*, but not in the A1 mating type of *P. capsici*. Identification of mating type was performed with sexual reproduction and ISSR marker methods. The results of mating type test are the same for the two different methods, suggesting that this PCR assay could have the potential to be developed into a useful method for distinguishing mating type of *P. capsici* in field material.

### P22.059 Molecular diagnosis of blast pathogen in rice

*C.K. Yamini Varma [1], P. Santhakumari[2], M. Suharban[2], C.A. Mary[2], K.K. Sulochana[2] and P. Raji[3]*
*[1]Krishi Vigyan Kendra, Palakkad, Mele Pattambi P.O, Palakkad district, Kerala, India, PIN- 679 306; [2]Department of Plant Pathology, College of Agriculture,*

*Vellayani, Thiruvananthapuram, Kerala, India, PIN- 695 022; [3]Regional Agricultural Research Station, Pattambi, Palakkad Dist., Kerala, India, PIN- 679 306*
*Email: yamininavami@gmail.com*

In India blast disease of rice is a major constraint in rice production, causing total destruction of crop. The causal organism of rice blast disease is an ascomycete fungus, *Magnaporthe oryzae* B. Couch sp. Nov (anamorph-*Pyricularia oryzae* Cavara). A study was conducted at College of Agriculture, Vellayani and Regional Agricultural Research Station, Pattambi under Kerala Agricultural University, Kerala, India during 2011. Among the sixty isolates of blast pathogen, five virulent isolates were selected and the most virulent one, $P_4$ was identified as *P. oryzae* at IARI, New Delhi, India (accession number ITCC-7019). Molecular diagnosis of *M. oryzae* for confirmation of result was done. The variation studies of selected virulent isolates, *viz.*, $P_2$, $P_4$, $P_5$, $P_9$, $P_{12}$ was conducted. Genomic DNA was isolated, D1/D2 region of LSU (Large subunit 28S rDNA) gene was amplified by PCR from this isolated genomic DNA, using PCR universal primers (D1/D2 primers of LSU *viz.*, DR- 5'-GGT CCGTGTTTCAAGACGG-3' and DF- 5'-ACCCGC TGAACTTAAGC-3'). A single discrete band was observed when resolved on Agarose Gel. The gene sequence was used to carry out BLAST with the NR database of NCBI genbank database. The result showed 99% similarities for 4 cultures, $P_2$, $P_4$, $P_9$, $P_{12}$ and 97% similarities for one culture, $P_5$ with the *M. oryzae (P. oryzae).* In the field experiment Isoprothiolane (1.5 ml/l) had a significant effect by reducing the PDI up to 65 per cent. Therefore quick and accurate diagnosis of the pathogen and its variability studies, help in planning and implementing more effective and less costly control strategies.

**P22.060 PCR Template preparation for the detection of *Candidatus* Liberibacter asiaticus in citrus specimens**

*P. Bella[1], G. Licciardello[2], S. Dai[3], C. P. Strano[1], A. Sicilia[1], F. Li[3], M. Tessitori[1], A. Catara[2], X. Deng[4], Z. Deng[3], R. La Rosa[1] and V. Catara[1]*
*[1]Department of Agricultural and Food Science, University of Catania, Via Santa Sofia 100, 95123 Catania; [2]Science and Technology Park of Sicily, Catania, Italy; [3]National Center of Citrus Improvement, Hunan University of Agriculture, Changsha, P. R. China; [4]Laboratory of Citrus Huanglongbing Research, Department of Plant Pathology, South China Agricultural University, Guangzhou, Guangdong 510642, P. R. China*
*Email: vcatara@unict.it*

To prevent the risk of introduction or spread of Citrus Huanglongbing associated bacteria with propagation material or vectors in free areas but also to provide services to growers, nurserymen and third countries, the National Center for Citrus Improvement, Hunan Agriculture University, Changsha, China, the Department of Agricultural and Food Science, University of Catania, Italy and the Science and Technology Park of Sicily, Italy, work cooperatively on projects funded by P. R. China and Regione Siciliana, Italy, respectively. The aim is the standardization of safe laboratory procedures for the detection of HLB bacteria. DNA extraction methods were compared for routine real-time PCR detection of *Candidatus* Liberibacter asiaticus with primer pair HLBas/HLBr and HLBpr TaqMan probe in citrus samples collected in several districts of Guangdong province, P. R. China. Critical aspect of sample preparation and DNA extraction were analyzed. Extraction efficiency was indirectly evaluated by multiplexing the positive internal control targeting the host plant cytochrome oxidase gene. The highest amount of plant DNA was obtained from the bark. From midrib samples, highest CTs were obtained in increasing order from DNAs, FTA, and 3MM filters. *Ca.* L. asiaticus concentration was evaluated on the basis of the cloned PCR fragment and positive detection was correlated to the plant DNA extraction. From midribs and bark DNA samples it was detected in 66% and 100% of the tested samples respectively. 58% and 30 % of samples eluted from the FTA and 3MM membrane were positive, respectively. Proper method of tissue blotting on the membrane seems the more critical step in detection.

## Concurrent Session 23-Molecular Host-Pathogen Interaction

### O23.001 Co-adaptation of host and pathogen: TAL effector-mediated resistance and susceptibility in plants

*F. White*
*Department of Plant Plant Pathology, Kansas State University, Manhattan, KS 66502, USA*
*Email: fwhite@ksu.edu*

*Xanthomonas oryzae* pv. *oryzae* (Xoo) is the causal agent of bacterial blight of rice and harbors multiple members of the transcription activator-like (TAL) type III effector gene family. Extensive gene expression profiling of host responses to different strains and mutants of Xoo has revealed global changes in rice gene expression during the disease process. A number of TAL effectors have major effects on susceptibility, and the rice/Xoo disease complex has co-adapted to include an assortment of novel genetic controls for susceptibility and resistance. All strains of Xoo have a requirement for the induction of at least one member of the nodulin 3, or SWEET, gene family. Other host induced genes are predicted to function in inducing ecotopic host small RNA processing, enzyme induction, and gene regulation. Different strains of Xoo share common and strain specific effects on host transcription. On the host side, a variety of host genotypes exist that alter the effectiveness of TAL-mediated susceptibility, involving both dominant and recessive mechanisms. TAL effectors appear to be on the move in an evolutionary sense within members of the genus *Xanthomonas* and have been adapted to target genes in other disease-specific complexes. The function of TAL effectors in different diseases will be discussed and contrasted to the rice/Xoo system.

### O23.002 BIK1 mediates PAMP-induced stomatal closure and restricts bacterial entry

*M. Li, L. Li, Z. Zhou and J.M. Zhou*
*Center for Genome Biology, State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciecnes, P. R. China*
*Email: jmzhou@genetics.ac.cn*

Recognition of conserved signatures (Pathogen-Associated Molecular Patterns; PAMPs) of invading microbes by plant pattern recognition receptors (PRRs) is key to the rapid activation of plant immune system. We have previously shown that the receptor-like cytoplasmic kinase BIK1 acts directly downstream of multiple PRRs to regulate immune responses. BIK1 also acts downstream of PEPR1 and PEPR2 to mediate ethylene- and DAMP-induced immunity. The importance of BIK1 in plant immunity is further supported by the fact that *Pseudomonas syringae* and *Xanthomonas campestris* type III effectors AvrPphB and AvrAC directly target BIK1 to inhibit plant immunity. To elucidate mechanisms by which BIK1 regulates plant immune responses, we have performed immunoprecipitation coupled with mass spectrometry analysis to identify BIK1 interacting proteins. Genetic analyses of several BIK1-interacting proteins suggested a role of these proteins in regulating stomatal closure. Consistent with these findings, the *bik1* mutant is unable close stomata upon stimulation by PAMPs and displays enhanced susceptibility to certain *Pseudomonas syringae* strains when spray-inoculated. Our progress on how BIK1 regulates stomatal opening and restricts bacterial entry will be discussed.

### O23.003 Microbial pathogens trigger host DNA double-strand breaks whose abundance is reduced by plant defense responses

*A. Bent and J. Song*
*Dept. of Plant Pathology, University of Wisconsin–Madison, Madison, WI 53706, USA*
*Email: afbent@wisc.edu*

We have found that diverse microbial plant pathogens induce DNA double-strand breaks in host plant genomes. This is a relatively new topic in animal pathogenesis, and very little has been reported of this phenomenon in the plant pathology literature. We have observed phosphorylation of histone H2AX, the most standard marker for DNA double strand breaks, during infections by *Pseudomonas syringae* bacteria, *Phytophthora infestans* oomycetes and *Botrytis cinerea* fungi, but not after paraquat treatment that more generically causes cell death, and not after activation of defenses using the MAMP compounds flg22 and elf18. Comet assays directly confirm DNA damage after *P. syringae* pv. *tomato* infections. We do not yet know if the damage arises directly from pathogen compounds that damage host DNA, or from host responses that damage host DNA. Organisms are challenged to balance the health-promoting impacts of antimicrobial responses and the potential toxic effects of excessive or chronic inflammation. There is evidence that the host ROS burst can contribute to the genotoxicity of certain infections in animals. However, our work to date indicates that infection-associated *AtrbohD* and *AtrbohF*-dependent ROS production is not required for pathogen-induced elevation of γ-H2AX. Instead, we find that plant antimicrobial defense mechanisms contribute to suppressed formation and/or rapid repair of γ-H2AX-associated lesions. DNA double-strand break damage is apparently a common aspect of plant pathogenesis by virulent microbial pathogens, and protection against DNA damage an important feature of effective plant disease resistance.

### O23.004 Role of *Pseudomonas sax* genes in host colonization

*J. Fan, C. Crooks, G. Creissen, L. Hill, S. Fairhurs and P. Doerner*
*China Agricultural University, Beijing100193, P. R. China*
*Email: jfan@cau.edu.cn*

Pathogenic bacteria have to overcome a multitude of host barriers to be successful on a host plant. We found that following *Pseudomonas* infection Arabidopsis plant released sulforaphane (4-methylsulfinylbutyl isothiocyanate), a natural product derived from aliphatic glucosinolates, which restricted growth of non-host *Pseudomonas* bacteria *in planta*. Disruption of multiple *sax* genes in *Pseudomonas* species virulent on Arabidopsis inhibited bacterial survival both in rich medium supplemented with sulforaphane and in wild type Arabidopsis plant. In Arabidopsis mutant in which accumulation of aliphatic gulcosinolates was compromised the survival of the *sax* mutant was completely rescued to the levels of wild type bacterial strain. Introduction of *saxCAB* genes into non-host *Pseudomonas* strains enabled them to grow to higher levels on Arabidopsis. Our study shows that *sax* genes are essential for *Pseudomonas* bacteria to overwhelm aliphatic isothiocyanate-mediated defense during colonization of Arabidopsis.

**O23.005 Dynamic interplay between abscisic acid, salicylic acid and cytokinin molds innate immunity of rice against the leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae***

*J. Xu[1], K. Audenaert[1,2], M. Höfte[1] and D. De Vleesschauwer[1]*
*[1]Laboratory of Phytopathology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium; [2]Faculty of Applied Bioscience Engineering, Ghent University College, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium*
*Email: jing.xu@ugent.be*

The plant hormone abscisic acid (ABA) is involved in myriad plant processes, including the regulation of stomatal aperture and initiation of stress-adaptive responses to various environmental cues. In addition, ABA is also increasingly implicated in the regulation of plant immune responses, although little is known about the underlying mechanisms. Aiming to advance our understanding of ABA-modulated disease resistance, we have analyzed the impact, dynamics and interrelationship of ABA and other hormones during progression of rice infection by the leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). We found that exogenously administered ABA renders rice hypersusceptible to infection, whereas chemical and genetic disruption of ABA biosynthesis and signaling, respectively, led to enhanced *Xoo* resistance. In addition, we found successful *Xoo* infection to be associated with extensive reprogramming of ABA biosynthesis and response genes, suggesting that ABA functions as a virulence factor for *Xoo*. Several lines of evidence indicate that this immune-suppressive effect of ABA is due at least in part to suppression of salicylic acid (SA)-mediated defenses that normally serve to limit pathogen growth. In contrast, resistance induced by the ABA biosynthesis inhibitor fluridone is independent of SA and is likely due to induction of non-specific physiological stress. Finally, we also demonstrate that ABA negatively interacts with the plant hormone cytokinin (CK). Collectively, our findings favor a scenario whereby virulent *Xoo* hijacks the rice ABA machinery to cause disease and highlight the importance of ABA and its crosstalk with SA and CK in shaping the outcome of rice-*Xoo* interactions.

**O23.006 Disease resistance level of wheat cultivars differentially impacts production of biocontrol secondary metabolites in indigenous fluorescent *Pseudomonas* spp.**

*P. Broukanloui Madloo, Q. Marzani, G. Salehi Jouzani, M.Tohidfar and K. Behboudi*
*Department of Plant Protection, Faculty of Sciences and Agricultural Engineering, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran*
*Email: paribroukani@gmail.com*

New applications of resistant cultivars and biological control by antagonistic bacteria, such as *Pseudomonas* spp., have recently received much attention. The current study represents the first work regarding the impact of different resistance levels of wheat cultivars against *Fusarium culmorum* on production of biocontrol mediated metabolites in indigenous fluorescent *Pseudomonas* spp. Therefore, seven wheat cultivars with three level of resistance (resistant, tolerant, and susceptible) were selected. Then, fluorescent *Pseudomonas* spp. was collected from the rhizosphere of each cultivar. The isolated bacterial strains were screened for production of hydrogen cyanide (HCN), siderophore, phosphatase, lytic enzymes (protease, and lipase) and the presence of the two key biocontrol genes (*phlD* and *PCA loci)*. The results showed significant effect of cultivars on studied traits in *Pseudomonas* spp., excluding HCN production. The assay of production of lytic enzymes and siderophore in isolates indicated that the maximum quantity of these compounds was detected in indigenous strains of resistant cultivar. Interestingly, analysis of phosphate production indicated that the most susceptible cultivar supported higher strains with this trait. In the bases of the detection of *phlD* and *PCA loci*, strains contained the highest population of these genes (24 $phlD^{+}$ and *3* $PCA^{+)}$ among strains obtained from the resistant cultivar. From these results, it is evident that there is a positive correlation between the wheat resistance level to *F. culmorum* and biocontrol mediated traits of *Pseudomonas* spp. strains. These results indicate that disease resistance in a wheat cultivar positively influences the main biological traits in indigenous *Pseudomonas* spp.

**O23.007 Lysin motif–containing proteins LYP4 and LYP6 play dual roles in peptidoglycan and chitin perception in rice innate immunity**

*B. Liu, J.F. Li, Y. Ao, J. Wang and H.B. Wang*
*State Key Laboratory of Biocontrol and Guangdong Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, 510275 Guangzhou, P. R. China*
*Email: wanghb@mail.sysu.edu.cn*

Plant innate immunity relies on successful detection of trespassing pathogens through recognizing their microbe-associated molecular patterns (MAMPs) by pattern recognition receptors (PRRs) at the cell surface. We recently found that two homologous rice (*Oryza sativa*) lysin motif–containing proteins, LYP4 and LYP6, as dual functional PRRs sensing bacterial peptidoglycan (PGN) and fungal chitin. Transcription of these two genes could be induced rapidly upon exposure to bacterial pathogens or diverse MAMPs. Both proteins selectively bound PGN and chitin but not lipopolysaccharide (LPS) in vitro. Accordingly, silencing of either LYP specifically impaired PGN- or chitin- but not LPS-induced defense responses in rice, including reactive oxygen species generation, defense gene activation, and callose deposition, leading to compromised resistance against bacterial pathogen *Xanthomonas oryzae* and fungal pathogen *Magnaporthe oryzae*. Interestingly, pretreatment with excess PGN dramatically attenuated the alkalinization response of rice cells to chitin but not to flagellin; vice versa, pretreatment with chitin attenuated the response to PGN, suggesting that PGN and chitin engage overlapping perception components in rice. Collectively, our data support the notion that LYP4 and LYP6 are promiscuous PRRs for PGN and chitin in rice innate immunity. Moreover, we found that LYP4 and LYP6 could form homo- and hetero-dimers, and could interact with CEBiP, suggesting an unexpected complexity of chitin perception in rice.

**O23.008 The RpfG protein of *Xanthomonas oryzae* pv. *oryzicola* regulates synthesis of extracellular polysaccharides that contribute to biofilm formation and virulence on rice**

*Y.B. Zhang[1], C. Wei[2], W.D. Jiang[1], L. Wang[1], C.R. Li[1], Y.Y. Wang[2], J.M. Dow[3] and W.X. Sun[1]*
*[1]Department of Plant Pathology and the Ministry of Agriculture Key Laboratory for Plant Pathology, China Agricultural University, Beijing 100193, P. R. China; [2]Ministry of Education Key Laboratory for Agriculture Biodiversity and Plant Disease Management, Yunnan Agricultural University, Kunming, P. R. China; [3]BIOMERIT Research Centre, Department of Microbiology, BioSciences Institute, University College Cork, Cork, Ireland*
*Email: wxs@cau.edu.cn*

Bacterial leaf streak caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) is one of the most important diseases in rice. However, little is known about the pathogenicity mechanisms of *Xoc*. Here we have investigated the function of three HD-GYP domain regulatory proteins in biofilm formation, the synthesis of virulence factors and virulence of *Xoc*. Deletion of *rpfG* resulted in altered production of extracellular polysaccharides (EPS), abolished virulence on rice and enhanced biofilm formation, but had little effect on the secretion of proteases and motility. In contrast, the other two HD-GYP domain proteins had no effect on virulence factor synthesis and tested phenotypes. Mutation of *rpfG* led to up-regulation of the type III secretion system and altered expression of three putative glycosyltransferase genes *gumD*, *pgaC* and *xagB*, which are part of operons directing the synthesis of different extracellular polysaccharides. The *pgaABCD* and *xagABCD* operons were greatly up-regulated in the *Xoc ΔrpfG* mutant, whereas the expression of the *gum* genes was unaltered or slightly enhanced. The elevated biofilm formation of the *Xoc ΔrpfG* mutant was dramatically reduced upon deletion of *gumD*, *xagA* and *xagB*, but not when *pgaA* and *pgaC* were deleted. Interestingly, only the *ΔgumD* mutant, among these single gene mutants, exhibits multiple phenotype alterations including reduced biofilm and EPS production and attenuated virulence on rice. These data indicate that RpfG is a global regulator that controls biofilm formation, EPS production and bacterial virulence in *Xoc* and that both *gumD*- and *xagB*-dependent EPS contribute to biofilm formation under different conditions.

**O23.009 The effects of *Cucumber mosaic virus* and its silencing suppressor, the 2b protein, on plant-aphid interactions**

*J.P. Carr[1], J.H. Westwood[1], S.C. Groen[1], Z. Du[2], A.M. Murphy[1] and T. Tungadi[1]*
*[1]Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom; [2]Institute of Bioengineering, Zhejiang Sci-Tech University, Hangzhou, P. R. China*
*Email: jpc1005@hermes.cam.ac.uk*

The *Cucumber mosaic virus* (CMV) 2b protein not only inhibits anti-viral RNA silencing and microRNA-mediated host gene expression but also inhibits responses of plant genes to jasmonic acid, a key signaling molecule in defence against insects. This suggested that the 2b protein might affect interactions between infected plants and aphids, the insects that transmit CMV. Results in tobacco (*Nicotiana tabacum*) appeared consistent with this idea. Infection of tobacco with the 2b gene deletion mutant CMVΔ2b induced resistance to aphids (*Myzus persicae*) while CMV infection fostered aphid survival. Using electrical penetration graph (EPG) methodology we found that higher proportions of aphids showed sustained phloem ingestion on CMV-infected plants than on CMVΔ2b-infected or mock-inoculated plants. How-

ever, CMVΔ2b infection of Arabidopsis thaliana ecotype Col-0 did not induce resistance to *M. persicae*. Furthermore, in Arabidopsis wild-type CMV induced resistance to sustained phloem feeding (revealed by EPG) that was mediated by increased glucosinolate biosynthesis. The contrasting results in tobacco and Arabidopsis suggest that CMV and its 2b protein have host-specific effects on plant-aphid interactions. The findings may have implications for the spread and persistence of the virus and its vectors.

### O23.010 The roles of ABA genes in modulating *Bamboo mosaic virus*-host interaction

*M. Alazem[1,2], Y.L. Huang[1] and N.S. Lin[1,2]*
*[1]Institute of Plant and Microbial Biology, Academia Sinica, Taipei 115, Taiwan, China; [2]Molecular and Biological Agricultural Sciences Program, Taiwan International Graduate Program, National Chung-Hsing University and Academia Sinica, Taipei 115, Taiwan, China*
*Email: nslin@sinica.edu.tw*

Plant defense responses are controlled by hormone-regulated signaling pathways. Salicylic acid (SA) is considered the main defense pathway against virus infection. However, SA features antagonistic interrelations with other pathways such as abscisic acid (ABA), jasmonic acid (JA) and Ethylene (ET). To study the hormone-virus interaction, mutants impaired with SA, JA, ET and ABA were infected with *Bamboo mosaic virus* (BaMV). The results revealed that BaMV titers showed no difference in Et or JA mutants compared with wild type (WT) *Arabidopsis thaliana*, but increased in the SA mutants *eds1-2* and *sid2-1*. To test if ABA has any potential role in the increased susceptibility of SA mutants to BaMV, q-RT-PCR revealed that transcript levels of ABA biosynthesis genes *NCED3*, *ABA2* and *AAO3* were increased in either or both SA mutants. Interestingly, the mutants of these genes, *nced3*, *aba2-1* and *aao3* responded differentially; *nced3* and *aba2-1* with significantly reduced viral titers and *aao3* with increased titers. Mutants in ABA signaling pathway *abi-1*, *abi-3* and *abi-4* also increased BaMV titer. In addition, the low titers of BaMV was found in lines expressing lower levels of *ABA2*, like *nced3*, *aba2-1* and plants sprayed with an ABA inhibitor. The novel response by *aba2-1* was confirmed by an *ABA2*-overexpressing transgenic line, *ABA2-O/E*, with significantly increased viral titers. Moreover, results of virus-induced gene silencing of *EDS1* and *ABA2* in *Nicotiana benthamiana* were similar to that for *A. thaliana* mutants *eds1* and *aba2-1*. This study shows a novel role of *ABA2* which separates the ABA effect on BaMV accumulation and plant resistance.

### O23.011 Genetic analyses of incompatible interactions between bromoviruses and *Arabidopsis thaliana*

*K. Yazaki, T. Narabayashi, M. Kaido, T. Okuno and K. Mise*
*Laboratory of Plant Pathology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*
*Email: kmise@kais.kyoto-u.ac.jp*

Plant viruses encounter an array of defense mechanisms in infected plants and have evolved diverse strategies to encounter the plant defense. Virus-encoded suppressors of RNA silencing are well-studied examples of such counter-defenses. *Brome mosaic virus* (BMV), the type species of the genus *Bromovirus*, has three messenger-sense genomic RNAs 1, 2 and 3 and subgenomic RNA4 and encodes four proteins. BMV is a well-established model for dissecting virus-host interactions, whereas convincing suppressor activity has never been reported for BMV. We assume that BMV may use alternative mechanisms to escape the detrimental effects of RNA silencing. Therefore, we have studied BMV infection in *Arabidopsis thaliana* to gain insights into virus-plant interactions. BMV includes many strains, but any of the strains tested cannot infect any Arabidopsis ecotypes efficiently. We have tested systemic infectivity of 12 BMV strains in an Arabidopsis mutant harboring mutations in genes encoding dicer-like endoribonucleases 2, 3 and 4 that are involved in antiviral silencing. We found that three BMV strains efficiently infected the mutant plants. One of the nine non-infectious strains infected the mutant plants at low frequency due to the generation of mutants with single base substitutions in RNA3. These BMV mutants showed altered expression of 3a and coat proteins. Comparative analyses between an infectious strain and a non-infectious strain suggest that the 2a protein plays a role in BMV spread in the mutant plants. We also present comparative analyses of BMV and *Spring beauty latent virus*, another bromovirus that infects wild type Arabidopsis, and discuss bromovirus-Arabidopsis interactions.

### O23.012 Comparative analysis of phytoreoviral small RNA profiles in rice and leafhoppers reveals distinct patterns in hosts across kingdoms

*D. Qian[1], L. Jiang[1], H. Zheng[1], Y. Pu[1], J. Wu[1], S. Zhao[1], C. Wei[1], B. Ding[2] and Y. Li[1]*
*[1]Peking-Yale Joint Center for Plant Molecular Genetics and Agrobiotechnology, State Key Laboratory of Protein and Plant Gene Research, College of Life Sciences, Peking University, Beijing 100871, P. R. China; [2]Department of Molecular Genetics, Ohio State University, Columbus, Ohio 43210 USA*
*Email: liyi@pku.edu.cn*

RNA silencing/interference has evolved as a potent antiviral strategy in many eukaryotic organisms. The mechanisms of antiviral RNA silencing, mediated by

virus-derived small RNAs (vsiRNAs) produced from double-stranded (ds) viral RNA substrates by Dicer (Dcr) in animals or Dicer-like (DCL) in plants, have been well studies in a variety of model systems. However, little is known about how a virus infecting different hosts across kingdoms is recognized by their respective silencing machinery. We used *Rice dwarf phytoreovirus* (RDV), a dsRNA virus that infects both insect (leafhopper) and plant (rice) hosts, to begin to address this question. We deep-sequenced the RDV vsiRNAs in leafhoppers and rice. The vsiRNAs in infected rice were predominately 21- and 22-nt, suggesting involvement of OsDCL4 and OsDCL2 in their production. The dominance of 21-nt vsiRNAs in infected leafhoppers suggests involvement of a Dcr-2 homologue in their production. The relative vsiRNA reads in the total small RNA reads in rice were ~100-fold higher than in leafhoppers, which may at least be partially attributed to the activity of RNA-dependent RNA polymerase 6 (RDR6) in rice and the lack of RDR activities in leafhoppers. Our data establish a basis for further comparative studies on the evolution of RNA silencing-based interactions between a virus and its hosts across kingdoms.

**O23.013 Viroids may elicit down-regulation of host gene expression via RNA-silencing.**

*B. Navarro, A. Gisel, S. Delgado, R. Flores, F. Di Serio*
*Istituto di Virologia Vegetale, CNR, N 165/A Via Amendola 165/A, Bari, 70122, Italy*
*Email: f.diserio@ba.ivv.cnr.it*

Distinct molecular mechanisms have been proposed for explaining how viroids, small non-protein-coding RNAs that infect plants, interfere with their host gene expression and elicit disease symptoms. On the one hand, by mimicking structural features of cellular RNAs, viroids may impair the host transcriptional and RNA trafficking machineries, which are usurped and redirected to facilitate their own replication and movement. On the other hand, the identification in viroid-infected plants of viroid-derived small RNAs (vd-sRNAs) of 21-24 nt, structurally similar to the small interfering RNAs that mediate RNA silencing, suggests that this RNA-based regulatory network largely conserved in eukaryotes is used by viroids for modifying host gene expression. By deep sequencing and rapid amplification of cDNA ends (RACE) we have recently shown that a host mRNA, coding for a gene involved in chloroplast development, is actually targeted for cleavage (in a sequence specific manner as predicted by RNA silencing), by two vd-RNAs accumulating in peach tissues infected by a chloroplast replicating viroid (*Peach latent mosaic viroid*). These results thus support a direct involvement of RNA silencing in viroid pathogenesis, particularly considering that the two vd-sRNAs map at the viroid pathogenicity determinant. Several other potential targets of vd-sRNAs have been identified in the same experimental system by bioinformatics and degradome-based analyses, suggesting a wider role of RNA silencing in plant-viroid interactions. These results highlight the power of genome-wide approaches for further dissecting the host pathways targeted by viroids.

**O23.014 Nucleocapsid of *Tomato spotted wilt tospovirus* forms mobile particles that traffic on an Actin/ER network driven by myosin XI-K**

*Z. Feng[1], X. Chen[1], Y. Bao[2], J. Dong[3], Z. Zhang[3] and X. Tao[1]*
*[1]Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, P. R. China; [2]College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, P. R. China; [3]Yunnan Provincial Key Laboratory of Agri-Biotechnology, Institute of Biotechnology and Genetic Resources, Yunnan Academy of Agricultural Sciences, Kunming, Yunnan 650223, P. R. China*
*Email: taoxiaorong@njau.edu.cn*

The nucleocapsid (N) protein of *Tomato spotted wilt tospovirus* (TSWV) is a multifunctional protein known to play a pivotal role in the viral life cycle, including viral RNA synthesis and particle assembly. In this study, we discovered an entirely new property for the TSWV N protein: intracellular trafficking. The N protein of TSWV formed highly motile cytoplasmic inclusions that were closely associated with the endoplasmic reticulum/ actin network. Time-lapse confocal images demonstrated that these N inclusion bodies moved extensively along the cortical ER and actin filaments. Disruption of actin filaments by latrunculin B (LatB), an actin-depolymerizing agent, nearly stopped the intracellular movement of N inclusions whereas treatment with a microtubule-depolymerizing reagent, oryzalin, did not alter N inclusion movement. Furthermore, over-express- ion of a myosin XI-K tail, functioning in a dominant- negative manner, completely halted the movement of N inclusions. LatB treatment strongly inhibited the formation of TSWV local lesions in *Nicotiana tabacum* cv. Samsun NN leaves and delayed systemic viral infection in *N. benthamiana*. Collectively, these findings demonstrate a new function in intracellular trafficking for the N protein and the potential that this activity is associated with F-actin-mediate accumulation of the virus. An influence of the actin/ER network in trafficking a capsid protein from plant RNA viruses previously has not been observed and thus this protein represents a new functional class of protein that associates with and requires actin filaments for movement.

**O23.015 Endogenous florendoviral elements are major components of plant genomes and molecular fossils of reverse-transcribing viruses with unique**

**and variable genome organisations**

*A.D.W. Geering[1], F. Maumus[2], N. Choisne[2], M. Zytnicki[2], A.R. McTaggart[1], S. Scalabrin[3], H. Quesneville[2] and P.Y. Teycheney[4]*

*[1]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, GPO Box 267, Brisbane, Queensland 4001, Australia; [2]INRA, UR1164 URGI, INRA de Versailles-Grignon, Route de Saint-Cyr, Versailles, 78026, France; [3]Istituto di Genomica Applicata, Parco Scientifico e Tecnologico di Udine Luigi Danieli, Via J Linussio 51, 33100 Udine, Italy; [4]CIRAD UMR AGAP, Station de Neufchâteau, Sainte-Marie, 97130 Capesterre Belle-Eau, Guadeloupe-France*

*Email: Andrew.Geering@daff.qld.gov.au*

We have discovered a new genus of endogenous *Caulimoviridae*, for which we propose the name Florendovirus, and found that endogenous florendoviral elements (EFEs) are common in most flowering plants including members of the Monocotyledoneae, the Eudicotyledonae and the so-called ANITA grade angiosperms. The identification of EFEs in *Amborella trichopoda*, an ancient endemic species of New Caledonia, suggests a minimum age of 85 million years for the florendoviruses based on the timing of separation of this Pacific island from the Australian landmass. In *Ricinus communis*, *Jatropha curcas*, *Vitis vinifera* and *Citrus clementina*, EFEs constitute more than 0.5% of the total nuclear genome content, which is a level of abundance that is comparable to that of high copy number transposable elements. In *V. vinifera*, *c*. 9% of the EFEs are located within host gene introns and when combined with the detection of EFE-derived small RNAs, suggests a role in plant metabolism by modifying gene expression. Molecular evidence suggests that some EFEs could be replication competent and potentially infectious. By analyzing reconstructed florendovirus genomes, we demonstrate that the florendoviruses are most closely related to but distinct from *Petunia vein clearing virus* based on the presence of a second open reading frame. Some of the ancestral viruses appeared to have had a bipartite genome organization, a feature that has never been observed before for any viral retroelement and that provides insights into the evolution of the *Caulimoviridae*.

**O23.016 Investigation of the role of small RNA in plant-virus interactions in apple trees**

*M. Visser, H.J. Maree, D.J.G. Rees and J.T. Burger*

*Agricultural Research Council: Biotechnology Platform, Infruitec-Nietvoorbij, 1 Lelie Street, Stellenbosch, 7600, South Africa*

*Email: visserm@arc.agric.za*

Plant small RNA (sRNA) associated with virulent virus infections have been reported by previous studies, while the involvement of sRNA in latent virus infection, remains largely uncharacterized. Apple trees show a high degree of resistance and tolerance to viral infections. A next-generation sequencing approach was followed to identify sRNAs that are associated with latent virus infection in apple trees. The sRNA population of infected and healthy leaf material was sequenced and bioinformatically analyzed. Both known and novel miRNA were identified, along with other species of sRNA, which included vsiRNA, rasiRNA, phasiRNA and tRFs. Variation in the levels of different sRNA species was examined to identify sRNAs potentially involved in latent virus infection. Results from this study will expand our knowledge of plant disease resistance pathways and may aid in the development of artificial miRNA constructs for the introduction of resistance in susceptible varieties.

**O23.017 Effector biology and biotrophic invasion by the rice blast fungus, *Magnaportheoryzae***

*B. Valent[1], M.C. Giraldo[1], M. Yi[1], C.H. Khang[1,4], Y. Dagdas[2], Y. Gupta[2], T. Mentlak[2,5], M. Dalby[1], H. Saitoh[3], X. Wang[6], R. Terauchi[3], J.Y. Lee[6] and N.J. Talbot[2]*

*[1]Dept. Plant Pathology, Kansas State Univ., Manhattan, Kansas 66506-5502, USA; [2]School of Biosciences, Univ. of Exeter, Exeter, U.K; [3]Iwate Biotechnology Research Center, Kitakami, Iwate, Japan; [4]Dept. of Plant Biology, Univ. of Georgia, Athens, Georgia, USA; [5]Cambridge Consultants Ltd, Cambridge, U.K.; [6]Department of Plant and Soil Sciences, University of Delaware, Delaware, USA*

*Email: bvalent@k-state.edu*

During biotrophic invasion, *Magnaporthe oryzae* secretes cytoplasmic effectors, which preferentially accumulate in biotrophic interfacial complexes (BICs) and are translocated into the rice cytoplasm, and apoplastic effectors, which remain in the extracellular space between the fungal cell wall and the rice plasma membrane. BICs localize in front of the tips of filamentous hyphae that enter rice cells, and remain subapically beside the first bulbous invasive hyphal cells after hyphal differentiation. In contrast, secreted apoplastic effectors uniformly outline the entire bulbous invasive hypha. We have determined that cytoplasmic effector genes are highly expressed in BIC-associated cells at early invasion stages, and that effector promoters play a major role in determining this BIC localization. Disruption of the conventional ER-Golgi secretion pathway by Brefeldin A treatment blocks secretion of apoplastic effectors, but not secretion of cytoplasmic effectors. Pathogen mutants that fail to express exocyst components or a t-SNARE were impaired in secretion of cytoplasmic effectors and in pathogenicity. In contrast, secretion of apoplastic effectors was not impaired in these mutants. Our data suggest that *M. oryzae* possesses distinct secretory mechanisms for targeting cytoplasmic and apoplastic effectors during rice invasion. Initial studies on effector function have focused on six biotrophy-associated

secreted proteins that show unique localization patterns at the points where invasive hyphae have crossed the rice cell wall into neighboring cells. Correlative light and electron microscopy (CLEM) and live-cell imaging are being performed to address the hypothesis that the fungus manipulates rice pit fields containing plasmodesmata for effector trafficking and its own cell-to-cell movement.

### O23.018 *N*-glycosylation of effector proteins by an alpha-1,3-mannosyltransferase is required to evade host innate immunity by the rice blast fungus

*X.L. Chen, T. Shi, J. Yang, D. Chen, X. Xu, J.R. Xu, N.J. Talbot and Y.L. Peng*
*State Key Laboratory of Agrobiotechnology and Ministry of Agriculture Key Laboratory of Plant Pathology, China Agricultural University, Beijing, P. R. China*
*Email: pengyl@cau.edu.cn*

Plant pathogenic fungi deploy secreted effector proteins to suppress plant immunity responses. These effectors operate either in the apoplast or within host plant cells, but post-translational regulation of their activities has not been explored. In this study, Alg3-mediated *N*-glycosylation of the effector protein Slp1 was found to be essential for its activity in the rice blast fungus *Magnaporthe oryzae*. *ALG3* encodes an α-1,3-mannosyltransferase for *N*-glycosylation of proteins. Targeted deletion of *ALG3* resulted in a significant reduction in virulence and arrested development of infection hyphae. Similar to *Δslp1* mutants, *Δalg3* mutants induced massive production of reactive oxygen species (ROS) in host cells. Interestingly, invasive growth of the *Δalg3* mutant was recovered in plant cells pretreated with the NADPH oxidase inhibitor, diphenyleneiodonium. The Slp1 effector protein sequesters chitin oligosaccharides to avoid their recognition by the rice chitin elicitor binding protein (CEBiP) and the induction of innate immune responses including ROS production. We demonstrated that Slp1 is an *N*-glycosylated protein with three *N*-glycosylation sites. Simultaneous *N*-glycosylation of all three sites is required for Slp1 to be functional and *ALG3* is required for full *N*-glycosylation of Slp1. These results indicate that Alg3-mediated *N*-glycosylation of Slp1 is required to evade host innate immunity.

### O23.019 How oomycete and fungal effectors enter host cells and promote infection

*B.M. Tyler[1], Q.Q. Wang[1], S.D. Kale[2], K. Tao[1], H. Wise[1], F. Sun[2], J. Vega-Arreguin[2], Z. Shi[3], J. McDowell[2], S. Maximova[3], M. Guiltinan[3] and D. Capelluto[2]*
*[1]Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR97331; [2]Virginia Tech, Blacksburg, VA24061, USA; [3]Pennsylvania State University, University Park, PA 16802, USA*
*Email: brett.tyler@oregonstate.edu*

Effector proteins from diverse oomycetes and fungi can enter plant cells to facilitate infection. The genomes of oomycetes such as the soybean pathogen *Phytophthora sojae* encode hundreds of RxLR effectors. However only about 10% of the genes are actively transcribed during infection. Gene silencing experiments indicate that the majority of these highly transcribed effector genes are individually essential for full virulence. Current evidenceindicates that phosphatidylinositol 3-phosphate (PI3P) resident in the host plasma membrane mediates the entry of oomycete RxLR effectors and some fungal RxLR-like effectors, through the action of the RxLR domains of the proteins. We have used nuclear magnetic resonance (NMR) and surface plasmon resonance (SPR) to establish that *Phytophthora sojae* RxLR effectors Avr1b and Avh5 bind PI3P with high affinity through contacts in their RxLR and C-terminal domains. To confirm that PI3P binding mediates host cell entry during natural infection, we have used heterologous PI3P-binding proteins, such as the yeast VAM7p PX domain to replace the RxLR or C-terminal domains of Avr1b in *P. sojae* transformants. Our resultsreveal that the VAM7p PX domain can functionally replace the RxLR domain of Avr1b in carrying the C-terminal domain of Avr1b into soybean cells, to trigger an R gene-mediated defense response. Mutations which abolish the binding of VAM7p to PI3P substantially reduce delivery of Avr1b. Expression of secreted PI3P-binding proteins in cacao leaves protected the leaves against *Phytophthora* infection.

### O23.020 Broad-spectrum fungal resistance and cross-talk between immunity and yield traits in rice

*Z. He*
*Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, P. R. China*
*Email: zhhe@sibs.ac.cn*

Rice blast (*Magnaporthe oryzae*) is the most destructive disease of rice in China. Rice breeding for broad-spectrum blast resistance has been the most effective and economical approach to control the disease. The *Pigm* locus has been identified to confer broad-spectrum and durable resistance to rice blast. Genomic sequencing revealed that the *Pigm* locus contains an NB-LRR resistance protein cluster that has undergone evolution or domestication-selection. We recognize that the *Pigm* locus adopts a novel genetic and epigentic strategy to confer high resistance and balance yield traits. The Knocking down/out of the rice RNA silencing pathways that produce small interference RNA (siRNA) largely changed the locus function on blast resistance. We have used the *Pigm* locus to efficiently develop elite rice lines with broad-spectrum blast resistance using molecular marker-assisted selection through collaborating with rice

breeders, which exhibited no defense penalty under non-disease conditions, while greatly enhanced grain productivity under diseased conditions in different rice areas.

### O23.021 Identify soybean genes involved in resistance to *Phakopsora pachyrhizi* infection through molecular approaches

*Z.Y. Chen, S. Park, M. Ganiger, D.R. Walker and D. Hu*
*Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA*
*Email: zchen@agcenter.lsu.edu*

Asian soybean rust, caused by *Phakopsora pachyrhizi*, is a new disease in the continental U.S. since its discovery in late 2004. This disease has the potential to cause severe yield reduction and billions of dollars in economic losses due to the lack of rust-resistant commercial soybean varieties. In an effort to understand soybean-*P. pachyrhizi* interaction at the molecular level, a number of soybean accessions, including the recombinant inbred line (RIL) derived sister lines were evaluated for resistance to soybean rust spores collected in Louisiana. Two accessions and one RIL derived sister line showed consistent immune response in both detached leaf assay and greenhouse inoculations. These rust resistant soybean lines along with susceptible controls were compared for protein profile differences during the time course of *P. pachyrhizi* inoculation through proteomics. Based on the gel analysis, approximately 70 differentially expressed spots were identified in our comparisons. These protein spots were recovered and sequenced through LC-MS/MS. Some of the identified proteins have known functions in host responses to biotic and abiotic stresses, such as pathogenesis related protein 10, chalcone isomerase, and β-1,3-endoglucanase. The expression of these proteins at transcript level has also been evaluated using qRT-PCR. It was found that susceptible soybean lines can induce the same set of genes, but at a much slower pace, which may contribute to the differences in their susceptibility to rust. The importance of some of these proteins in soybean rust resistance is being evaluated through a virus induced gene silencing (VIGS) approach.

### O23.022 Autophagy vitalizes the pathogenicity of *Magnaporthe oryzae*

*X.Q. Zeng, H.B. Shi, X.H. Liu and F.C. Lin*
*State Key Laboratory for Rice Biology, Biotechnology Institute, Zhejiang University, Hangzhou, P. R. China*
*Email: fuchenglin@zju.edu.cn*

*Magnaporthe oryzae,* a filamentous fungus, is the casual agent of the rice blast disease which causes substantial cultured crop losses worldwide. Genomic sequence availability and genetic tractability of both *M. oryzae* and rice, coupled with multiple analytical tools, make them the model to study fungus-plant interaction. When infecting host, *M. oryzae* can accumulate numerous glycerol in the appressorium to generate as high as 8 MPa turgor pressures which allow the penetration peg of the appressorium to penetrate the leaf cuticle. Autophagy is a conserved process in eukaryotic cells and facilitates the bulk degradation of macromolecules and organelles to keep homeostasis. Autophagic processes have been widely studied from model yeast *Saccharomyces cerevisiae* to mammals. In pathogenic fungi such as the rice blast fungus *M. oryzae,* it is reported null mutants for the expression of autophagy gene homologs lose their pathogenicity for infection of host plants. Functional analysis of autophagy genes network in *M. oryzae* will lead to better understanding of the role of autophagy in fungal pathogenesis and supply some new insight for disease control.

### O23.023 Characterization of the role of the necrotrophic effector SnTox1 in disease

*Z.H. Liu, Y.M. Kim, Y. Gao, J.D. Faris and T.L. Friesen*
*Northern Crop Science Laboratory, Cereal Crops Research Unit USDA-ARS, Fargo ND 58102, USA*
*Email: timothy.friesen@ars.usda.gov*

Several fungal plant pathogens in the Dothideomycete class produce necrotrophic effectors (synonym: host selective toxins) that induce susceptibility. Necrotrophic pathogens often use a mechanism whereby effectors are secreted into the host environment to elicit programmed cell death (PCD) but rather than inhibiting the pathogen, the necrotrophic pathogen benefits from PCD by gaining nutrient from the dying cells. The *Stagonospora nodorum*-wheat interaction is a classic example of a necrotrophic interaction. Our research on the *S. nodorum*-wheat interaction indicates that the pathogen secretes as many as twenty necrotrophic effectors that interact specifically with host gene products to modulate host defense mechanisms to its advantage. Most recently we have characterized the SnTox1-*Snn1* interaction and have demonstrated that this effector-host gene interaction shows hallmarks of PCD including up regulation of defense response genes, induction of an early oxidative burst, and DNA laddering but the end result is susceptibility rather than resistance. SnTox1 has also been shown to bind cell wall components of both the fungus and the plant including chitin and cellulose. This indicates that SnTox1 has multiple roles in pathogenesis including the induction of host controlled PCD, protection from host-produced chitinases as well as potentially reducing the negative effects of PAMP/DAMP recognition. This research provides important insights into the molecular basis of the wheat-*S. nodorum* interaction, an emerging model for necrotrophic pathosystems.

**O23.024 Common mechanisms used by *Verticillium dahliae* and *Phytophthora sojae* for converting plant SA metabolism pathway**

*T. Liu, T. Song, H. Yuan, L. Liu, P. Liu, W. Li, Y. Zhu, M. Zhang, B. Zhang and D. Dou*
*Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095, P. R. China; Jiangsu Academy of Agricultural Sciences, Nanjing, P. R. China*
*Email: ddou@njau.edu.cn*

Salicylic acid (SA) is an important endogenous resistance signal in plant, indicating that pathogens might suppress host immunity by interfering with host SA accumulation and SA-mediated signal pathways. Here, we presented several lines of evidences to prove that both *Verticillium dahliae* and *Phytophthora sojae* could secrete isochorismate hydrolyses into host cells to degrade isochorismate that is a key precursor of SA in plant. The isochorismate hydrolyses were encoded by *VdIch1* and *PsIch1* respectively. Transcriptional analysis showed that both genes were up-regulated during infection process. Knocking out of the *VdIch1* in *V. dahliae* and silencing of *PsIch1* in *P. sojae* both reduce pathogenicity, and SA contents in host cells were consistently increased. Stable expression of the *VdIch1* in tobacco also decreased the resistance to hemibiotrophic pathogen and biotrophic pathogen and reduced the SA levels. Furthermore, we showed that both proteins were secreted enzymes. Fusion with the gene together with *P. sojae* Avr1b C-terminal regions could mediate Avr1b effector translocation into host cells, suggesting that these secreted proteins might enter into host cells.

**P23.001 Transport and fate of bacterial homoserine lactones in barley (*Hordeum vulgare* L.)**

*P. Schröder, T. Riedel, C. Götz, M. Matucha, T. Hartmann and P. Krämer*
*Research Unit Microbe Plant Interactions, Helmholtz Zentrum Muenchen, Ingolstädter Landstrasse 1, D-85764 Neuherber, Germany*
*Email: peter.schroeder@helmholtz-muenchen.de*

Bacterial intra- and interspecies communication 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 N-hexanoyl- (C6-HSL), N-octanoyl- (C8-HSL) and N-decanoyl-homoserine lactone (C10-HSL) were studied in axenic systems with barley (*Hordeum vulgare* L.). We used different methods including tritium labelling, sensor strain assays and monoclonal antibodies (mAb) to analyse the uptake and translocation of C8- and C10-homoserine lactone (HSL) into barley (*Hordeum vulgare* L. cv. "Barke"). Both AHLs were systemically transported into the shoot already 2h after application. AHL uptake could be inhibited by ortho-vanadate demonstrating that ABC transporters are involved in the uptake. Root transport occurs predominantly via the central cylinder which was shown by transport inhibition via KCl application and autoradiography of root cross sections. Furthermore a newly established detection method with mAb allowed the first detection of a systemic transport of long chain AHLs in plants. The coupled use of different AHL detection methods demonstrated, that the uptake and transport of AHLs into barley relies at least partially on active processes in the plant.

**P23.002 *Xylophilus ampelinus*: Characterisation of the Type III secretion system and its role in pathogenicity**

*Y. Petersen*
*Agricultural Research Council, Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa*
*Email: peterseny@arc.agric.za*

*Xylophilus ampelinus*, the causal agent of bacterial blight of grapevine, still causes extensive financial losses within the table grape industry in South Africa more than 70 years after the disease was first reported. As there are no totally resistant grape cultivars or entirely effective chemical control measures, the only recourse is to uproot infected vines to prevent the spread of the disease. Defining the bacterium's requirements for pathogenicity and virulence on grapevine would provide a valuable contribution to the pursuit for a durable solution for bacterial blight of grapevine. The Type III secretion system (T3SS) is a pathogenicity mechanism used by many, but not all, Gram negative phytopathogens to translocate bacterial type III effectors into the host cell for pathogenesis. To determine whether *X. ampelinus* encodes a T3SS and whether it is functional, genomics as well as functional analysis approaches were utilised. Sequencing results showed that *X. ampelinus* has the structural and regulatory genes required to form the T3SS apparatus, while individual gene knockouts resulted in the loss of pathogenicity of those *hrp*/*hrc* mutants when inoculated into the bacterial blight-sensitive cultivar, Red Globe. Complementation of the mutants with the corresponding wild type genes restored virulence. This emphasises the importance of the T3SS as a pathogenicity mechanism for *X. ampelinus* and has brought us one step closer to understanding the molecular basis of pathogenicity of this bacterium.

**P23.003 Jasmonate biosynthesis and signalling in cotton response to *Xanthomonas campestris* pv. *malvacearum***

*A. Champion[1,2], M. Pizot[2], A. Jalloul[3] and M. Nicole[4]*
*[1]Laboratoire Commun de Microbiologie IRD/ISRA/*

*UCAD, Bel-Air BP 1386 CP 18524 Dakar, Sénégal; [2]Institut de Recherche pour le Développement (IRD), UMR DIADE, Equipe Rhizogenèse, 911 av. Agropolis, 34000, Montpellier, France; [3]Faculty of Agronomy, Department of Plant Protection, Damsacus, Syria; [4]IRD UMR Résistance des plantes aux Bioagresseurs (IRD CIRAD UM2) 911 av. Agropolis, 34000, Montpellier, France*
*Email: michel.nicole@ird.fr*

Bacterial blight triggered by *Xanthomonas campestris* pv. *malvacearum* (*Xcm*) is one of the most damaging disease for cotton plants, affecting leaves and subsequently reducing cotton fiber yield. Cotton resistance to the bacterial pathogen *Xcm* is characterized by a hypersensitive response. Molecular mechanisms underlying resistance to *Xcm* are not fully understood, but strong direct links with the oxylipin pathway, including transcription of lipoxygenase genes and accumulation of methyljasmonates (MeJA). In the incompatible interaction between the cotton cultivat Reba B50 and the avirulent race 18 of *Xcm*, a sharp production of jasmonate (JA) and OPDA (precursor of JA) were observed few hours after cotyledon infection. JA is a plant signalling molecule that plays an important role in defence against certain pathogens and insects. JA induces the expression of a battery of genes encoding defense-related proteins and enzymes involved in biosynthesis of protective secondary metabolites. In this presentation, we also report the identification and characterization of potentials genes coding for i) JA-biosynthesis enzymes and ii) JA-responsive transcription factors specifically induced during bacterial blight resistance.

**P23.004 Use of transposon libraries to identify genes involved in *Pseudomonas syringae* plant colonisation**

*B. Manoharan[1], R.W. Jackson[2], J.T. Hancock[1] and D.L. Arnold[1]*
*[1]Centre for Research in Biosciences, University of the West of England, Frenchay Campus, Coldharbour Lane, Bristol BS16 1QY, UK, School of Biological Sciences; [2]University of Reading, Reading, United Kingdom*
*Email: dawn.arnold@uwe.ac.uk*

*Pseudomonas syringae* pv. *phaseolicola* (*Pph*) is the seed borne causative agent of halo blight in the common bean, *Phaseolus vulgaris*. Gene-for-gene interactions underpin varietal resistance and race structure in the *Pph*/bean interaction. *Pph* race 6 strain 1448A contains no known avirulence genes and can cause disease on all bean cultivars in the differential series. However, *Pph* race 4 strain 1302A contains the avirulence gene *avrPphB*, which matches resistance gene *R3* and causes a rapid hypersensitive response in bean cultivar Tendergreen. To investigate other genes responsible for the *Pph* interaction with plants, including potential elicitors of basal defence, a transposon mutant libraries were created for both 1448A and 1302A. The resulting mutants were screened for various bacterial phenotypic characteristics including colony morphology, growth rate in bean apoplastic fluid, motility and biofilm formation. Preliminary results have found knockouts in a number of genes involved in motility both in swimming and swarming assays. Mutants were also found that had reduced growth rates both *in vitro* and *in planta*. Other mutants exhibited increased growth rate in apoplastic fluid and these have been putatively identified as transporters, chemotaxis sensor proteins and transcriptional regulators. Biofilm formation was also reduced *in vitro* for several mutants and these knock outs showed disruption in genes for ABC transporters and transcriptional regulators. Characterisation of the effects of a number of these mutants on the pathogenicity towards plants is currently underway.

**P23.005 Identification of *Nicotiana benthamiana* signal chain components required for successful infection by *Pectobacterium carotovorum***

*O. Badalyan, Y. Nikolaichik, E. Kulik and A.N. Evtushenkov*
*Belarusian State University, Department of Molecular Biology, 4, Nezaliezhnasci ave., Minsk, 220030, Belarus*
*Email: oliagg@bk.ru*

*Pectobacterium carotovorum* (*Pca*) causes diseases of various plants including *Nicotiana benthamiana*. It's still not known how this necrotrophic pathogen overcomes plant defense. Our search for possible suppressors of defense mechanisms in potato host has so far identified only one protein, DspE, delivered into plant cells via the type III secretion system (CCTT). DspE and the CCTT of *Pca* are also required for the rapid hypersensitive response-like reaction in *N. benthamiana* leaves which is later followed by maceration of surrounding tissues. In this work we have tried to identify *N. benthamiana* proteins involved in recognition of the DspE protein and activation of the programmed cell death (PCD) pathway. First, we have used yeast two-hybrid system to check for proteins involved in interaction with DspE. This resulted in identification of two previously uncharacterized DspE-interacting receptor-like kinases (RLK2 and RLK5). Virus-induced gene silencing (VIGS) of the *RLK2* and *RLK5* genes showed that they are both required for PCD at the site of *Pca* infiltration and inactivation of either gene makes *N. benthamiana* plants more resistant to this pathogen. Further VIGS experiments have demonstrated that disease symptom development in plants infected by *Pca* also requires SGT1 (known as a positive cell death regulator), homologue of the tomato TPK1b cytoplasmic kinase, as well as the SIPK and WIPK MAP-kinases, but does not depend on on RAR1, EDS1, BAK1 and FLS2. These results suggest that *Pca* is capable of exploiting the standard plant immune system signal mechanisms, resulting in PCD which is favorable for plant infection by this necrotrophic pathogen.

**P23.006 Role of *Xanthomonas citri* subsp. *citri* type IV pilus in plant pathogen interaction**

*G. Dunger[1], C.R. Guzzo[1,2] and C.S. Farah[1]*
*[1]Departamento de Bioquímica, Instituto de Química; and [2]Departamento de Microbiologia, Instituto de Ciencias Biomedicas, Universidade de São Paulo, CEP 05508-000, SP, Brazil*
*Email: dunger@iq.usp.br*

*Xanthomonas citri* subsp. *citri* (Xac) is the causal agent of the citrus canker infecting plants worldwide. The pathogen enters host plant tissues through stomatal openings and wounds and then colonizes the apoplast. The infection is visualized as raised lesions on fruit, foliage and young stems. The genome of Xac has been completely sequenced and gives insights into the importance of genes and gene clusters governing strategic mechanisms of pathogenesis. Bacterial type IV pilus (T4P) are long, flexible surface filaments involved in a variety of important bacterial behaviors, including twitching motility, surface adhesion, pathogenicity, natural transformation, immune escape and biofilm formation. The filament of the T4P is composed of a helical polymer of mostly pilin subunits. Cycles of polymerization, attachment and depolymerization mediate several pilus-dependent phenomena. Xac has a functional T4P. The sequenced Xac genome codes for a large set of genes involved in T4P biogenesis and regulation, including several pilin homologs. We produced a Xac knockout strain in the gene coding for the major pilin subunit (*fimA*). We analyzed the production of biofilm on biotic and abiotic surfaces were we observed critical differences in the biofilm structure among Xac strains. Microscopy analyses were performed for compare patterns of bacterial migration. Additionally we performed inoculation to study the role of T4P in pathogenesis. The results of this study improve our understanding of how Xac T4P influences bacterial migration, adherence, biofilm and pathogenicity on plants.

**P23.007 Influence on gene expression and a direct R gene interaction by *OsVOZ1/2* in rice**

*J.M. Lang, A. Seck, R. Davidson, Q. Cheng, R. Corral, M. Bruce, V. Verdier, S.H. Hulbert, B. Zhao and J.E. Leach*
*1177 Campus Delivery, Colorado State University, Fort Collins, CO 80523-1177, USA*
*Email: jillian.lang@colostate.edu*

When expressed in rice, the maize *RXO1* gene, encoding a nucleotide-binding leucine-rich repeat (NB-LRR) resistance (R) protein, confers immunity against the rice pathogen *Xanthomonas oryzae* pv. *oryzicola* if the pathogen expresses the type III effector gene, *avrRXO1*. Here we show that the rice OsVOZ1 (Vascular plant One-Zinc-finger) transcription factor (TF) interacts with the NB domain of RXO1 in yeast-two-hybrid assays and is required for effector triggered immunity (ETI) mediated by *RXO1*. Two paralogs of this TF are present in the rice genome and computational analysis suggests that they may regulate the expression of more than 300 gene targets including diverse biotic and abiotic stress responsive genes. OsVOZ1 functions as a transcriptional activator in yeast and positively regulates the expression of two potential targets of OsVOZ1 as well as two defense response genes in rice. Transcriptome analysis of RXO1containing, OsVOZ-silenced lines challenged with *X. oryzae* pv. *oryzae* containing *avrRXO1* further elucidated the global impacts of OsVOZ. Our data suggest that OsVOZ1 sits at a key regulatory node in pathways that mediate immune responses in rice and may be a good candidate for plant improvement.

**P23.008 *Arabidopsis* WRKY, STP1, positively regulates defense signaling in interaction between *Pectobacterium* and *Arabidopsis***

*H.S. Kim, Y.H. Park, C. Choi and D.J. Hwang*
*National Academy of Agricultural Science, Rural development Administration, Suwon, 441-707, Republic of Korea*
*Email: djhwang@korea.kr*

To study the interaction between *Pectobacterium* and *Arabidopsis* we screened *Arabidopsis* T-DNA lines. We obtained several lines that did show difference in symptoms compared to wild type. Among them the *stp* (Susceptible to *Pectobacterium*) *1* mutant showed enhanced susceptibility to *Pectobacterium carotovorum* sp. *carotovorum* (*Pcc*). In contrast, the *stp1* mutant did not show difference in symptom upon infection of *Pseudomonas syringae* pv. *syringae* DC3000 compared to wild type. STP1 protein is a WRKY type transcription factor of group III known to be key regulators in defense-signaling pathway. However, the function of STP1 is not reported yet. Therefore, to determine the biological functions, we have generated transgenic plants for STP1 over-expression in *Arabidopsis*. The over-expressing line show enhanced resistance to *Pcc*, whereas *stp1* mutant resulted in enhanced susceptibility compared to wild type, while the transgenic and mutant lines didn't show altered response to the *Pst* DC3000. It appears that STP1 is a positive regulator in *Pcc* related defense signaling.

**P23.009 Characterization of several OsNAC genes related to bacterial leaf blight resistance**

*E.S. Kwak, G.H. Park, D.J. Hwang, I.P. Ahn, S.C. Bae and S.R. Park*
*Bio-crop Development Div., National Academy of Agricultural Science, Rural Development Administration, Suwon, 441-707, Republic of Korea*
*Email: srpark@korea.kr*

Plant specific gene family, NAC (NAM, ATAF, and

CUC) transcription factors have been characterized for their roles in plant growth, development, and stress tolerance. In this study, we isolated several *OsNAC* genes and analysed expression level by inoculation of bacterial leaf blight pathogen, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). NAC transcription factor family can be divided into five groups (I–V). NAC transcription factor family can be divided into five groups (I–V). On the basis of phylogenetic analysis, our OsNAC genes fell into group I-4, II, III and IV, respectively. OsNACs were strongly induced 1 hr after infected with *Xoo*. To investigate their biological function in the rice, we constructed vector for overexpression in rice, and then generated transgenic rices. Gene expression of OsNACs overexpressed transgenic rice lines were analyzed by northern blot. Analysis of disease resistance to pathogen *Xoo*, several OsNAC-overexpressed transgenic rice lines showing high expression level of OsNACs were shown more resistant than wild type. These results suggest that *OsNAC* genes may play regulatory role during pathogen infection.

**P23.010 Revealing the role of the Type Three effector protein HpaF in the infective process of the cassava pathogen *Xanthomonas axonopodis* pv. *manihotis***

*C.A. Trujillo, V. McCormick and A.J. Bernal*
*Universidad de los Andes, Bogotá, Colombia*
*Email: abernal@uniandes.edu.co*

Type three effector proteins are molecules that are involved in disease establishment of several pathogenic microorganisms, including bacteria, fungi and oomycetes. HpaF is an effector protein from the cassava pathogen *Xanthomonas axonopodis* pv. *manihotis* (*Xam*). Disruption of the HpaF coding gene results in a reduction of symptoms produced by the mutant in cassava, compared to the wilt type strain. To determine the role of HpaF in the establishment of disease, a heterologous system was implemented to determine if HpaF was able to suppress plant defenses. In addition, a yeast two-hybrid assay was carried out aiming at identifying the potential interactors of HpaF (IHFs) in the host plant. Three IHFs were identified and their relevance in plant defense processes was established in *Arabidopsis thaliana* mutants. The relevance of interactions between HpaF and IHFs were determined in *A. thaliana* using callose suppression assays. Finally, we could conclude that interactions between HpaF and its targets in cassava are important to suppress plant defenses elicited by recognition of MAMPs.

**P23.011 Transcript profiles of rice inoculated with different *Xanthomonas oryzae* pv. oryzicola strains reflect the geographic relationship of those strains**

*L. Wang, K.W. Wilkins, N.J. Booher and A.J. Bogdanove*
*Department of Plant Pathology and Plant-Microbe Interaction, Cornell University, 236 Tower Road, Ithaca, NY, 14850, USA*
*Email: lw523@cornell.edu*

Bacterial leaf streak of rice (*Oryza sativa*), caused by *Xanthomonas oryzae* pv. *oryzicola* (Xoc), can cause 15-25% rice yield loss and is widely distributed. Here, 67 Xoc strains were collected from Asia and Africa. Eleven strains were chosen to represent the diversity of transcription activator like effector (TAL) genes in the larger collection, based on Southern blot analyses. Rice transcriptomes following inoculation by these 11 strains or mock were deeply sequenced. 90% of the genes previously shown by microarray analysis to be upregulated by the Philippine strain BLS256 were shown to be up-regulated by this strain in this experiment as well. Each tested strain except the sole India strain induced about 1000 to 3000 rice genes. The India strain, BXORI, induced only 400 genes. 269 genes were up-regulated by all 11 strains compared to the mock. Each strain induced some genes that were not induced by any other strain. A relatedness tree based on the up-regulated genes grouped the strains in agreement with their grouping based on where they were isolated. Strains from Africa showed greater homogeneity than groups of strains within other regions. The pattern of rice gene induction by the India strain was the most distant in the tree. These transcriptome data offer insight into the complex adaptive relationship between Xoc and rice.

**P23.012 Degradation of hydroxycinnamic acids, a class of plant defense molecules, contributes to pathogenic success of *Ralstonia solanacearum***

*T. Lowe, R. Mitra, A.S. Milling and C. Allen*
*Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706 USA*
*Email: cza@plantpath.wisc.edu*

Plants defend themselves from pathogen attack by making hydroxycinnamic acids (HCAs), phenolic compounds which are directly antimicrobial and are also precursors to lignin, a key plant cell wall-reinforcing chemical. RNA-Seq analysis revealed that tomato plants infected by the xylem-colonizing wilt pathogen *Ralstonia solanacearum* up-regulate genes involved in HCA biosynthesis. In turn, *R. solanacearum* expresses genes encoding HCA-degrading enzymes during tomato pathogenesis. Total phenolic concentrations in xylem sap were 37% lower in infected tomatoes than in control plants, suggesting that the pathogen degrades plant HCAs when it colonizes xylem vessels. To determine if HCA degradation contributes to bacterial wilt virulence, we constructed a Δ*fcs* mutant strain of *R. solanacearum*, which lacks the first enzyme in the HCA degradation pathway. This Δ*fcs* mutant had reduced virulence on tomato. We tested several hypotheses that could explain how HCA degradation contributes to bacterial virulence:

(1) carbon acquisition, (2) breakdown of antimicrobial compounds, and (3) removal of lignin barriers that restrict pathogen movement in the plant. The Δ*fcs* mutant had no growth defect in xylem sap, indicating that HCAs are not a significant nutrient for the pathogen. However, the Δ*fcs* mutant had a 14-fold higher sensitivity to the HCA *p*-coumaric acid *in vitro*, suggesting that HCA degradation contributes to *R. solanacearum* virulence by purging plant antimicrobials from the infected tissues. We are currently investigating differences in plant colonization rates between wildtype and Δ*fcs* strains to test hypothesis.

**P23.013 Molecular crosstalk between disease resistance and low nitrogen tolerance in rice**

*C. Yu, H.M. Chen, F. Tian and C.Y. He*
*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No. 2, West Yuan Ming Yuan Road, Beijing 100193, P. R. China*
*Email: yudoxichao@yahoo.com*

Bacterial leaf blight, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is the most serious bacterial disease of rice in the world. Rice-Xoo interaction, as a model system, has been studied to understand the molecular mechanism of disease resistance in monocotyledonous plants. Nitrogen is an essential mineral nutrient element to help plant maintain normal growth and development and ensure the completion of life cycles. But excessive N use may cause problems. For example, rice plants become more susceptible to Xoo infection under high N conditions, while they are more resistant at low N conditions. In this study, we screened for miRNAs in rice at Xoo stress and low nitrogen stress by high- throughput sequencing, and found 61 miRNAs only responded to Xoo stress, 80 miRNAs only responded to low nirtogten stress. Interestingly, 72 miRNAs responded to both stresses, 12 out of which were confirmed by qRT-PCR experiments. Expression of miR5076, miR5072, miR156-5p, miR156-3p and miR1320-5p were induced, whereas miR812, miR169, miR396, and miR2869 were repressed at both stresses. Comparatively, expression of miR398 and miR399 were induced at Xoo stress, but repressed at low nitrogen stress, while the expression of miR5540 was repressed at Xoo stress but induced at low nitrogen stress. By analyzing the promoter sequence of these miRNAs, several known stress-responsive elements were identified, such as defense and stress-responsive element (TC-rich repeats), defense and wounding-responsive element (W box), and so on. It provides additional evidence that these miRNAs are very likely to be involved in the molecular crosstalk between Xoo stress and low nitrogen stress in rice. We also identified some target genes of the co-regulated miRNA, and the role of the miRNAs and their target genes will be further studied.

**P23.014 Rice OsFLS2 has the different recognition specificity and sensitivity to bacterial flagellins**

*S.Z. Wang, Z. Sun, H.Q. Wang, F. Lu, H. Zhang, J.Y. Wang and W.X. Sun*
*College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: 15210904930@126.com*

It was well known that FLS2 senses flagellins of phytopathgenic bacteria and thus activating PAMP-triggered immunity (PTI) in dicot plants. However, the perception of bacterial flagellins via OsFLS2 remains obscure in monocot rice. Here we demonstrated that no or little reactive oxygen species (ROS) burst was detected in rice cultured cells after the treatment of *in-vitro* purified flagellins of rice bacterial pathogens *Xanthomonas oryzae* pvs. *oryzae* (Xoo) and *oryzicola* (Xoc) while ROS burst occurred in response to purified *Acidovorax avenae* flagellin. Virulence of Xoo and Xoc to rice was not significantly altered when *fliC* genes were deleted in these bacteria. Additionally, OsFLS2-overexpressing transgenic *Arabidopsis fls2* plants (OsFLS2-OE) had no defense responses upon the treatment of *in-vitro* purified Xoo/Xoc flagellins and $flg22^{Xo}$, but were well responsive to flg22 and *A. avenae* flagellin. The data indicated that OsFLS2 have no ability to perceive Xoo/Xoc flagellins. Furthermore, it was demonstrated that the perception of flg22 by OsFLS2 is dependent on its expression level in transgenic Arabidopsis plants and that Col and OsFLS2-OE plants respond to flg22Xo(V-D) differently. Domain swaps between Xo and Aa flagellins revealed that OsFLS2 specifically recognizes the flg22 region in Aa flagellin. The results suggested that rice OsFLS2 has a different specificity and sensitivity to different bacterial flagellins.

**P23.015 The HpaR of *Xanthomonas campestris* pv. *campestris* negatively regulates the expression of extracellular protease genes via binding to their upstream regions**

*G.Y. Zhang, X. Wei, H.Z. Su, D.J. Tang, G.T. Lu, B.L. Jiang, Y.Q. He and J.L. Tang*
*College of Agriculture and College of Life Science and Technology, State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, The Key Laboratory of Ministry of Education for Microbial and Plant Genetic Engineering, Guangxi University, 100 Daxue Road, Nanning Guangxi 530004, P. R. China*
*Email: marge.zhang @163.com*

The MarR family transcriptional regulators of bacteria are involved in the regulation of many cellular processes, including pathogenesis. Our previous work demonstrated that HpaR is a MarR family regulator, which positively regulates the pathogenicity and hypersensitive response (HR), and negatively regulates the extracellular protease production via an unknown mechanism in

*Xanthomonas campestris* pv. *campestris* (*Xcc*). To understand how *Xcc* HpaR negatively regulates the extracellular protease production, we investigated the direct target of HpaR. The *Xcc* genome encodes ten extracellular proteases, all of which are secreted via the type II secretion system. The serine protease PrtA makes the largest contribution to *Xcc*'s total extracellular proteolytic activity. Electrophoretic mobility shift assay (EMSA) showed that HpaR could specifically bind to the upstream DNA sequences of the promoters of *XC3379* (encoding PrtA protease) and *XC3376* (encoding another protease), demonstrating that *XC3376* and *XC3379* are the direct target of HpaR. Further experiments exhibited that a 23 bp imperfect palindrome sequence located in the upstream region of *XC3379* promoter is indispensable for HpaR binding. Semiquantitative RT-PCR analysis showed that HpaR suppresses the expression of *XC3376* and *XC3379*. These results reveal that the *Xcc* HpaR functions as a repressor of the extracellular protease genes *XC3376* and *XC3379* via binding to their upstream regions.

**P23.016 Characterization of *Xa3/Xa26*-mediated rice-*Xoo* interaction**

*H. Zhang* and *S. Wang*
*National Key Laboratory of Crop Genetic Improvement, National Center of Plant Gene Research (Wuhan), Huazhong Agricultural University, Wuhan 430070, P. R. China*
*Email: bravetao@webmail.hzau.edu.cn*

Plant innate immunity system is composed of two types of process, PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). Transcription activator-like (TAL) effectors are important in interactions between *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and rice. *AvrXa3*, which was once reported to be avirulence gene of rice disease resistance gene *Xa3/Xa26*, encodes a TAL effector and were cloned from Japanese *Xoo* strain JXOIII. Our results showed that AvrXa3 could not bind to the promoter region of *Xa3/Xa26*, nor interact with Xa3/Xa26 protein in yeast. These results suggested that *AvrXa3* was not related to *Xa3/Xa26*-mediated rice-*Xoo* interaction. Pretreating plants that carry *Xa3/Xa26* gene with supernatant of incompatible *Xoo* strain PXO61 could sufficiently trigger the defense reaction and lead to resistant to compatible *Xoo* strain PXO99, while pretreating with supernatant of PXO99 could not trigger defense reaction. Similar results were obtained when using other *Xoo* strains. These results suggest that Xa3/Xa26 functions in a PTI-like process. The ligand of Xa3/Xa26 may exist in the supernatant of incompatible *Xoo* strains.

**P23.017 The type Ⅲ effector AvrXccB in *Xanthomonas campestris* pv. *campestris* suppresses plant innate immunity in *Arabidopsis thaliana***

*L.J. Liu, Q. Li, S. Li, Y.P. Wang, A.F. Fang and W.X. Sun*
*College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: melody_alpha@163.com*

*Xanthomonas campestris* pv. *campestris* (*Xcc*) is a Gram-negative bacterium that causes black rot, the most important disease of vegetable brassica crops worldwide. Here, we studied the virulence function of a putative the type III effector AvrXccB in Xcc. Secretion assays demonstrated that AvrXccB was secreted into the SMMXC minimal medium, which secretion is dependent on the Xcc type III secretion system. The GFP-labelled AvrXccB was localized onto the plasma membrane in transgenic Arabidopsis, probably through N-myristoylation; and the conserved glycine residue at amino-terminus of the protein was essential for the localization. We developed the transgenic *Arabidopsis thaliana* plants expressing AvrXccB under control of the dexamethasone(DEX)-inducible promoter. Chemical-induced expression of AvrXccB suppressed callose deposition and the burst of reactive oxygen species triggered by flg22 in Arabidopsis. AvrXccB can also promote *in-planta* bacterial growth of *Peudomomas syringae* pv. *tomato* DC3000 in the transgenic Arabidopsis. These results indicated that the type III effector AvrXccB of Xcc can suppress PAMP-triggered immunity in *A. thaliana*.

**P23.018 Genetic characterization of a two-component system that modulates the virulence factor production in *Dickeya zeae***

*J.N. Zhou, Y.Y. Cheng, Z.D. Jiang and L.H. Zhang*
*Department of Plant pathology, South China Agricultural University, Guangzhou 510642, P. R. China*
*Email: Jianuanzhou@scau.edu.cn*

Gram-negative bacteria pathogen *Dikeya zeae* is the casual agent of rice foot rot disease, which infects not only monocotyledons but also dicotyledons. Our recent study identified a novel gene designated as *zmsA* that encodes a polyketide synthase with 2,346 amino acids in length. Two polyamino compounds, i.e., zeamine and zeamine II, were isolated and purified from *D. zeae* strain EC1, accounting 60% and 40% of the total toxins, respectively. Gene knockout of *zmsA* abolished the production of these antimicrobial toxins and attenuated the virulence of *D. zeae*. These findings indicate that zeamines play a key role in the bacterial pathogenesis and in pathogen-host interactions. For understanding the molecular mechanisms regulating toxin biosynthesis, a Tn5 transposon mutant library was generated and screened for changed phenotypes in zeamine production. Bioassay results showed that mutation of a putative two component system (TCS) sensor gene resulted in decreased

production of zeamines and extracellular hydrolytic enzymes, suggesting its key role in modulation of the bacterial virulence and physiology. Detailed characterization of this sensor kinase and its cognate response regulator protein will be presented.

**P23.019 *Agrobacterium tumefaciens* represses *Xanthomonas oryzae* pv. *oryzae* -induced hypersensitive response in *Nicotiana benthamiana* by targeting its Type III secretion system and abolishing accumulation of reacitive oxygen species**

*W. Li and X.Z. Cai*
*Institute of Biotechnology, Zhejiang University, Hangzhou 310058, P. R. China*
*Email: liwen547249317@126.com*

*Xanthomonas oryzae* pv. *oryzae* (Xoo) rapidly triggers hypersensitive response (HR) and nonhost resistance in its nonhost plant *Nicotiana benthamiana*. Here, we report that *Agrobacteria tumefaciens* blocked the Xoo-induced HR in *N. benthamiana* when pre-infiltrated or co-infiltrated but not post-infiltrated 4 h after Xoo inoculation. This suppression by *A. tumefaciens* is local and specific to Xoo, since *A. tumefaciens* did not suppress the HR triggered by another bacterial pathogen *Psuedomonas syringae* pv. *tomato* DC3000 in *N. benthamiana*. The HR-inhibiting efficiency of *A. tumefaciens* was strain-dependent. Strain C58C1 had no effect on Xoo-induced HR; whereas strains EHA105, GV3101 and LBA4404 completely blocked this HR formation. Co-infiltration of *A. tumefaciens* and Xoo abolished Xoo-induced HR but did not alter Xoo bacterial number in comparison with inoculation of Xoo alone, revealing that *A. tumefaciens* blocks Xoo-induced HR but does not affect nonhost resistance to Xoo, and that HR is uncoupled from the nonhost resistance to Xoo in *N. benthamiana*. DAB staining analysis showed that *A. tumefaciens* dramatically reduced $H_2O_2$ accumulation in co-infiltrated leaves. Additionally, expression of an array of Xoo type III secretion system (T3SS) genes, especially *Hpa1* and *HrpD6*, was dramatically and simultaneously down-regulated in leaves that were co-infiltrated with *Agrobacterium* and Xoo than those were treated by Xoo alone. These results unveil that Xoo T3SS components trigger $H_2O_2$ burst thereby initiate HR in nonhost plants, and that *A. tumefaciens* targets the bacterial T3SS to avoid induction of oxidative burst in plants, thereby prevent Xoo from HR induction in nonhost plants.

**P23.020 Auxin modulates Harpin-induced defence signaling in *Vitis* cells**

*X.L. Chang[1, 2], M. Riemman[2], G.S. Gong[1] and P. Nick[2]*
*[1]Department of Plant Pathology, Agricultural College, Sichuan Agricultural University, Chengdu 611130, P. R. China; [2]Molecular Cell Biology, Botanical Institute 1, Karlsruhe Institute of Technology, Karlsruhe 76131, Germany*
*Email: xl.changkit@hotmail.com*

The phytohormone auxin has long been recognised as a regulator of plant growth and development ever since its discovery. Recent evidences show that auxin is also involved in plant defence and disease development. We have previously shown that the bacterial Harpin triggers a range of defence signals in the resistant *Vitis rupestris*, but weakly in the susceptible *Vitis vinifera* cultivar 'Pinot Noir'. To get insight into the role of auxin in Harpin-triggered defence, we investigated cellular responses upon auxin treatment (IAA, NAA, and 2, 4-D) in the two *Vitis* cell lines. We found that auxins accelerated Harpin-induced extracellular alkalinisation or just changed its amplitude in *V. rupestris*, whereas they delayed and then caused a stable pH increase in cv. 'Pinot Noir'. Application of auxins had no effect on Harpin-induced *StSy* and *PAL* expression, while 2, 4-D weakly induced pathogenesis-associated protein *PR*10 transcripts in both *Vitis* cells. All three auxins inhibited Harpin-induced cell death to different extent in *V. rupestris*, in contrast, there was almost little contribution in cv. 'Pinot Noir'. In addition, polymerisation of cytoskeletonal actin partly involved in auxin-dependent regulation of Harpin-induced cell death by pharmacological manipulation in *V. rupestris*, but did not function in cv. 'Pinot Noir'. Furthermore, IAA accumulation was repressed by Harpin inside the cells but elevated outside the cells. The data indicate that the phytohormone auxin modulate Harpin-triggered defence responses in *Vitis* cells, especially acting as a negative key player to inhibit hypersensitive cell death, which is not intimately correlated with defence gene transcription.

**P23.021 A role for inositol hexa*kis*phosphate in disease resistance in potato**

*R.E. Le Fevre, D.E. Hanke and J.P. Carr*
*Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK*
*Email: rel43@cam.ac.uk*

Inositol hexa*kis*phosphate (Ins$P_6$, the most abundant inositol phosphate in plants) complexes strongly with mineral cations and accumulates to high levels in seeds and tubers. This provides plants with a store of inositol, phosphate and mineral nutrients which are used for growth and development during germination. However, when consumed it can cause micronutrient malnutrition in humans and undigested Ins$P_6$ in animal waste can pollute aquatic ecosystems, therefore there has been interest in low-Ins$P_6$ crop development. Previously, we found mutant low-Ins$P_6$ *Arabidopsis thaliana* plants were more susceptible to several viral, bacterial and fungal plant pathogens, suggesting an additional role for Ins$P_6$ in basal resistance (Murphy *et al.*, 2008, Plant J., 56, 638-652). To explore this we used an RNAi approach

to knock-down expression of the gene encoding the last enzyme controlling Ins$P_6$ biosynthesis (inositol pentaki*s*phosphate 2-kinase, *IPK1*) in the crop plant potato (*Solanum tuberosum*). We then tested the pathogen resistance of these low-Ins$P_6$ lines. Initial experiments using the bacteria *Erwinia carotovora* ssp. *carotovora* indicated this pathogen accumulated to higher levels in low-Ins$P_6$ potato leaves, implying that basal resistance to this pathogen is compromised. We also found that the hypersensitive response mediated by the *Ny* resistance gene to the ordinary strain of potato virus Y ($PVY^o$) is impaired in low-Ins$P_6$ potato lines. These results suggest that decreasing Ins$P_6$ levels inhibits R protein-mediated recognition of $PVY^o$ - a normally avirulent pathogen.

**P23.022 *Ralstonia solanacearum* uses extracellular DNAases for root infection and biofilm modulation during bacterial wilt pathogenesis**

*T. Tran, M. Hawes and C. Allen*
*Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706 USA*
*Email: cza@plantpath.wisc.edu*

The genome of the root-infecting bacterial wilt pathogen *Ralstonia solanacearum* encodes two predicted extracellular nucleases, NucA and NucB. Both *nucA* and *nucB* are strongly expressed during tomato pathogenesis. Analysis of single and double *nuc* mutants of *R. solanacearum* confirmed that both these genes encode DNA degradation and revealed that a *nucAB* double mutant has reduced ability to infect both pea and tomato seedling roots and reduced virulence on tomato plants following soil-soak inoculation. Plant root cap border cells are an important physical defense barrier against soilborne pathogens. Border cells form the root cap slime, an extracellular matrix of protein, polysaccharide, and DNA that protects plant roots from pathogen invasion, like the well-characterized neutrophil extracellular traps (NET) in mammalian systems. We hypothesize that *R. solanacearum* uses its extracellular nucleases to degrade this matrix, thereby facilitating root infection. Consistent with this hypothesis, a *nucAB* double mutant was significantly less effective than wild-type at reducing host root growth, and microscopy with GFP-expressing bacteria revealed that the *nucAB* mutant colonized root tips more slowly than the wild-type strain. Further, *nuc* mutants also formed thicker biofilms *in vitro* and grew in tight, non-spreading colonies on agar plates, unlike the irregular wild-type colonies. These results suggest that *R. solanacearum*'s extracellular nucleases could participate in the formation of wild-type biofilms inside the plant host, thereby contributing to systemic translocation of the bacterium *in planta* as wilt disease develops.

**P23.023 XopN-T3SS effector of *Xanthomonas axonopodis* pv. *punicae* modulates cell-wall-associated immune response to induce bacterial blight in pomegranate**

*R. Kumar and K. K. Mondal*
*Division of Plant Pathology, Indian Agricultural Research Institute, Pusa Campus, New Delhi 110 012, India*
*Email: mondal_kk@rediffmail.com*

Bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae* (Xap) has emerged as a potential threat to pomegranate production in India, the biggest producer of pomegranate in the world. Most phytopathogenic strains of *Xanthomonas* secrete effector proteins by the type III secretion system (T3SS) to suppress pathogen-associated molecular pattern (PAMP)-triggered plant immunity (PTI). The T3SS effectors, referred to as *Xanthomonas* outer proteins (Xops), are known to be key factors required for bacterial growth and colonization in distinct eukaryotic hosts. We identified six Xops effectors (XopC2, XopE1, XopL, XopN, XopQ and XopZ) in Xap. We investigated the possible role of XopN, one of the conserved T3SS effectors across phytopathogenic *Xanthomonas*, using a *xopN* null mutant (Xap Δ*xopN*). We found that XopN was required for maximal Xap pathogenicity in its natural host pomegranate. Wild type Xap but not Xap Δ*xopN* produced intense watersoaking in infiltrated pomegranate leaves. Further, the mutant (Xap Δ*xopN*) showed 32-fold reduction in *in planta* colonization relative to the wild strain. Plant responds to bacterial infection by depositing callose, a β-1,3-glucan, around the site of inoculation and thus callose deposition is considered as a basal defense response associated with PTI. In our study, we examined that Xap Δ*xopN* mutant induced more callose deposition in infected pomegranate leaves. Taken together, the present study shows that XopN suppresses PTI and governs Xap growth and pathogenicity in pomegranate. Our understanding on XopN would provide insight into the disease susceptibility pathways and thereby strategies to exploit for the broad and durable resistance to blight.

**P23.024 The *KOD* gene modulates defence response in *Arabidopsis* against *Pseudomonas* infection**

*M.M. Kamal and A.H.M.M. Haque*
*Charles Sturt University, Australia; Pulses Research Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh*
*Email: mkamal@csu.edu.au*

The hypersensitive response (HR) is a plant defence mechanism which induces a localized cell death surrounding the pathogenic infection site. HR includes establishing a resistance against biotrophic pathogens which use living tissue to nourish it. HR is thought to be a strictly genetically controlled form of programmed cell death (PCD). Recently, a 25-amino-acid peptide has been identified, named *kiss of death* (*KOD*) that is part of the PCD pathway in *Arabidopsis thaliana*. We show

that the expression of *KOD* is inducible by plant pathogenic invasion in *Arabidopsis*. *KOD* exhibited a three folds transient increase in expression in Col-0 while it was not expressed in the mutant background *sag101* and *rar1* after *Pseudomonas* infection. In addition, chemical induction of *KOD* expression was found to induce death of *Arabidopsis* leaves and to stimulate caspase-3 like activities. A *KOD*::GFP fusion, however, was detectable as soon as 3 hours after chemical induction while cell death was only visible after 24 hours. In loss of function mutant lines *gabi2* and *p9s* infiltrated with $10^8$ cfu $ml^{-1}$ *Pseudomonas* strain *DC3000, RPM1* and *RPT2*, the conductivity was reduced at 18 hours post inoculation. Furthermore, quantification of lesions caused by *Pseudomonas* induced HR was found to be high in GabiWT plants while the number of lesions was comparatively lower in the mutant *p9s* plants. We propose that *KOD* is a component of the HR response in *Arabidopsis*.

**P23.025 Use of the green fluorescent protein variant GFPuv for analysis of *Pseudomonas syringae* pv. *actinidiae* infection, movement and colonization in Kiwifruit**

*X.N. Gao, Q.L. Huang, Z.B. Zhao, Q.M. Han and L.L. Huang*
*College of Plant Protection, Northwest A & F University, Yangling, Shaanxi, 712100, P. R. China*
*Email: huanglili@nwsuaf.edu.cn*

*Pseudomonas syringae* pv. *actinidiae* (*Psa*) is responsible for bacteria canker of kiwifruit that causes economically important diseases of plants. To investi- gate better bacterial infection process, we engineered a GFPuv-labeled strain to carry out inoculation experiments. The plate counting revealed all stems samples had isolated the target colonies at 3 h post different ino- culation ways, then the number of colonies increased gradually. The bacterium extensively colonized cortex cells, phloem cells, xylem vessel and intercellular spaces. At 5 day post smearing bacterium suspension on the leaves, a larger bacterium colonized mesophyll cell and intercellular spaces. The bacterium colonization resulted in marked alterations in stem and leaf tissues including plasmolysis and degeneration of protoplasts and cell walls. The bacterium could migrate up and down to lateral veins and the base of petiole; besides, the bacterium stopped expanding and growing in the leaf and vein when the culture temperature reached 24℃. Furthermore, the bacterium could colonize cortex cell and expanded into vascular bundle of root at 3 day post soil-drenching inoculation. The results indicated that the *Psa* can infect host plants by entering wounds, lesions, lenticels and stomas, and extensively colonize tissue cells and intercellular spaces, which damage to host cell walls and organelles. The temperature plays an important role in the movement and multiplication of the bacterium. These results presented evidences for infection ways and colonization sites of *Psa* in stem, leaf and root of kiwifruit, which provided useful information for further research of pathogenic mechanisms of *Psa*.

**P23.026 *Salmonella typhimurium* flagellin recognition leads to PAMP-triggered immunity in *Arabidopsis thaliana***

*A.V. Garcia, A. Charrier, A. Schikora, S. Pateyron, J. Bigeard, M.L. de Tauzia, A. Mithoefer and H. Hirt*
*URGV Plant Genomics Research (INRA/UEVE/CNRS), 2 rue Gaston Crémieux, 91057 Evry cedex, France*
*Email: garcia@evry.inra.fr*

We have previously shown that the bacterial enteropathogen *Salmonella enterica* serovar typhimu- rium is able to invade plant tissues and that the plant immune system has a role in containing the colonization. Nevertheless, the mechanisms through which plants detect and activate defenses against *S. typhimurium* are unclear. To further analyze the interaction between *S. typhimurium* and *Arabidopsis*, we performed a transcriptome analysis of *Arabidopsis* seedlings at 2, 4, 6, 12 and 24 hours after inoculation with *S. typhimurium* wild-type or the *S. typhimurium* type III secretion mutant *prgH*-. This analysis revealed that *Salmonella* triggers important reprogramming of defense-related transcripts, which showed stronger induction by the *prgH*- mutant at late time points. Accordingly, MAPK activation in response to *S. typhimurium* was also stronger after *prgH*- treatment as compared with wild-type bacteria. The *S. typhimurium*-induced transcriptional reprogramming and MAPK activation were reduced in *fls2 Arabidopsis* mutant plants, lacking the receptor for bacterial flagellin. We synthesized the *S. typhimurium* flg22 peptide (Flg22- ST), corresponding to the elicitor-active flagellin epitope, and showed that it induces various hallmarks of PAMP-triggered immunity such as ROS production, MAPK activation, gene induction and disease resistance to a similar extent as the canonical flg22 peptide. In sum, these studies show that *S. typhimurium* can be recognized in *Arabidopsis* through its flagellin and thereby induce PTI. Interestingly, we observed that other *S. enterica* serovar carry a more divergent and less active Flg22 peptide, indicating certain natural variation in the ability of *Arabidopsis* to recognize *Salmonella*.

**P23.027 Investigating the interactions between plant viruses and host stomata**

*R.R. Murray, A.M. Hetherington and G.D. Foster*
*Biological Sciences Building, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK*
*Email: rose.murray@bristol.ac.uk*

Stomata are microscopic pores situated in the epidermis of aerial parts of most higher plants. As well as being important gateways for gas exchange and water vapour,

they are also an integral part of the innate immune system and portals of entry for many pathogens. These pathogens are typically fungal, bacterial or nematodal, however stomata are not currently considered to be a viable entry point for viruses. Given the relative importance of stomata in the plant's physiology in controlling water loss and gas exchange there is very little information in the literature about the role of stomata in a plant virus infection. Work presented will include results from investigations into the relationship of a plant virus infection and host stomata in a molecular aspect containing work performed using candidate genes involved in stomatal development, also from an anatomical approach analysing cell types on the leaf epidermis. Another aspect of this research has been to consider whether plant viruses may utilise stomata as an entry portal to the host. Experiments have been performed to manipulate the apertures of stomata in a host, and purified virus has been applied to the leaf surface using an atomiser. Results from all of these investigations will be presented.

**P23.028 The effect of virus infection on polarisation reflection from plants**

*D.J. Maxwell, N.W. Roberts, N. Boonham, J.C. Partridge and G.D. Foster*
*School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG*
*Email: Daniel.Maxwell@bristol.ac.uk*

Plant surfaces are structured in such a way that light can become polarised following reflection from leaves or petals. Various insects are sensitive to polarisation; raising the possibility that polarisation may be a cue influencing viral vector behaviour and viral transmission. Therefore, viral symptoms may enhance the transmission of viruses by altering the polarisation properties of the host plant to attract vectors. Polarisation imaging has been used to compare the polarisation of light reflected from the leaves of *Nicotiana tabacum* infected with the aphid transmitted viruses *Potato virus Y* and *Cucumber mosaic virus*, as well as the mechanically vectored viruses *Tobacco mosaic virus* and *Pepino mosaic virus*. Additionally, quantitative real time PCR has been utilised to study virus-induced changes in the expression of genes involved in the formation of leaf surface structure, with scanning electron microscopy allowing visualisation of the surface morphologies of infected hosts. Loss of function and overexpressor mutants for cuticular wax and leaf hair synthesis in *Arabidopsis thaliana* are also being studied to gain further insights into interactions between viruses and plant surface structures. Results from each of these experiments will be presented.

**P23.029 Identification of HCRSV genome in the nucleus where viral microRNAs are produced**

*R.M. Gao[1], P. Liu[2] and S.M. Wong[1, 2]*
*[1]Department of Biological Sciences, National University of Singapore, 117543, Singapore; [2]Temasek Life Sciences Laboratory, 1 Research Link, 117604, Singapore*
*Email: g0901062@nus.edu.sg*

DNA and RNA are two types of genomes contained by viruses. Generally, DNA viruses replicate within nucleus, while most RNA viruses, especially (+)-sense single-stranded RNA, replicate and are present within cytoplasm. We hypothesized that (+)-sense single-stranded RNA viruses are not only present in the cytoplasm, but also can be present in the nucleus. The aim of this study is to investigate whether the genome of a plant RNA virus (non-retroviral) is present in the nucleus of infected cells and how does it enter nucleus? We showed that *Hibiscus chlorotic ringspot virus* (HCRSV) RNA was present in the nucleus of infected cells by fluorescent in situ hybridization. Western blot using anti-histone 3 and anti-phosphoenolpyruvate carboxylase showed that nuclei were highly purified from mock and HCRSV-infected kenaf (*Hibiscus cannabinus* L.) leaves, respectively. The p23 and HCRSV coat protein (CP) coding regions were both amplified from total RNA extracted from isolated nuclei. The reason for viral RNA present in the nucleus is that it may be used to generate viral microRNAs (vir-miRNAs). Five putative vir-miRNAs were predicted from HCRSV using the vir-miRNAs prediction database. The vir-miRNA (hcrsv-miR-H1-5p) was detected using TaqMan® stem-loop real-time PCR, and by northern blot using DIG-end labeled probe in HCRSV-infected kenaf leaves. In addition, a novel nuclear localization signal (NLS) was discovered in p23 of HCRSV. The NLS interacts with importin α and facilitates viral RNA genome to enter nucleus. In conclusion, we have demonstrated the presence of a (+)-sense single-stranded viral RNA within nucleus where viral miRNAs are produced.

**P23.030 *Cocksfoot mottle sobemovirus* establishes infection through the phloem**

*G. Uffert, M. Otsus, M. Sõmera, H. Paves, A. Olspert, B. Islamov and E. Truve*
*Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia*
*Email: gabriela.uffert@ttu.ee*

Whereas most plant viruses are transported from the initial site of infection to other parts of the plant through the phloem, sobemoviruses are thought to be an exception. This is well documented for *Rice yellow mottle virus* (RYMV). However, the fact that systemic spread of sobemoviruses has been associated with xylem does not necessarily mean that other sobemovirus use the same route. In the case of *Cocksfoot mottle virus* (CfMV), primary studies on virus identification have reported it's possible location in leaf mesophyll and phloem companion cells. Hereby, we describe the

dynamics of the tissue distribution of CfMV in oat plants during three weeks post inoculation (p.i.) by immunohistochemical staining of viral CP. CfMV localization in oat plants was analyzed to follow its spread through different tissues. In early stages of infection, the virus was first detectable in phloem parenchyma and bundle sheath cells of inoculated leaves, stems and systemic leaves of infected plants. In later stages of infection, CfMV spread also into the mesophyll surrounding vascular bundles and was seldom detected in xylem parenchyma of inoculated leaves. In systemic leaves, CfMV was not detected from xylem. Moreover, sometimes it was found from phloem only. In straw and roots, CfMV was detected both from phloem and xylem. According to our observations, CfMV predominantly moves through phloem, which makes the systemic movement of CfMV different from that of another monocot-infecting sobemovirus, RYMV.

**P23.031 Overexpression of tobacco calmodulin-like protein, rgs-CaM, elicits defense responses in tobacco**

*E.J. Jeon, T. Murakami, K. Tadamura, K.S. Nakahara, C. Masuta and I. Uyeda*
*Plant Breeding Science, Research Faculty of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan*
*Email: ej4865jh@gmail.com*

Calmodulin (CaM) and CaM-like (CML) proteins are primary sensors of calcium, a key second messenger, of various cellular processes in response to external stimuli in eukaryotes. We have previously revealed that a tobacco CML, rgs-CaM, reinforced anti-viral RNA silencing by binding to and sequestrating viral RNA silencing suppressors. Here, we found a link between rgs-CaM and other defense responses in tobacco. We made transgenic tobacco plants, which overexpressed rgs-CaM under control of the cauliflower mosaic virus 35S promoter. Among a dozen lines of the transgenic tobacco plants, two lines showed dwarf, yellowing and lesion mimic phenotype, accompanied with generation of reactive oxygen species, expression of the genes encoding pathogenesis-related proteins, and cell death. Microarray analysis confirmed the induction of defense-related gene expressions in these transgenic plants. Several CaM and CML proteins have been reported to participate in induction of defense responses, including those that involve salicylic acid production, in plants. Although how defense responses are induced in the transgenic tobacco overexpressing rgs-CaM remains to be elucidated, rgs-CaM may also play a role in plant defense responses besides RNA silencing.

**P23.032 Analysis of viroid-specific small RNAs derived from *Potato spindle tuber viroid* strains of different pathogenicity**

*C.R. Adkar-Purushothama and J. P. Perreault*
*RNA group, Department of Biochemistry, Faculty of medicine and health sciences, Applied Cancer Research Centre, Université de Sherbrooke, Sherbrooke, Quebec J1E 4K8, Canada*
*Email: charith.adkar@USherbrooke.ca*

Viroids are single-stranded, covalent closed circular RNAs with the size range of 246-400 nucleotides. They are the smallest known plant pathogens. Viroids do not encode any pathogen-specific proteins and therefore viroids propagation is fully dependent on the host biochemical machinery. They exclusively infect plants and autonomously replicates via RNA/RNA pathway using host machinery. As might be expected from their highly base-paired structure and RNA-RNA mode of replication, viroids have been shown to induce RNA silencing. Accumulation of viroid-specific small RNA (vd-sRNA) has been reported upon infection in host plant. Further, involvement of such vd-sRNA has been correlated with symptom production in host. *Potato spindle tuber viroid* (*PSTVd*) is a type member of the viroid family *Pospiviroidae*. Only a few nucleotide changes between *PSTVd* strains are sufficient to induce remarkably different symptoms in infected tomato plant, i.e. *Solanum lycopersicum* cv Rutgers. In this present study, tomato plants were infected with *PSTVd* intermediate and *PSTVd* severe strain, independently. After 30 days of post infection small RNA was extracted from the leaves. Large-scale small RNA sequence were carried out using Illumina Solexa sequencer viz., control tomato plants, *PSTVd* intermediate strain infected tomato plant and *PSTVd* severe strain infected tomato plants. By bioinformatics, vd-sRNA and plant miRNA were analyzed. A remarkable non-uniform ratio has been observed for specific miRNA in healthy and different *PSTVd* strain infected plants. Interestingly, for all the miRNAs analyzed, the ratio of miRNA derived from *PSTVd* intermediate infected plants were always distributed in between healthy and *PSTVd* severe infected plants.

**P23.033 Functions of the internal poly(A) tract in *Hibiscus latent Singapore virus* (HLSV) and upstream pseudoknots domain (UPD) in *Tobacco mosaic virus* (TMV)**

*S. Guo[1], S.N. Niu[1] and S.M. Wong[1,2]*
*[1]Department of Biological Sciences, National University of Singapore, 117543, Singapore; [2]Temasek Life Sciences Laboratory, 1 Research Link, 117604, Singapore*
*Email: a0082797@nus.edu.sg*

*Hibiscus latent Singapore virus* (HLSV) is a new plant virus discovered in Singapore. Different from most *Tobamoviruses*, HLSV possesses a variable length of internal poly(A) track which ranges from 77-96 nt in the 3' untranslated region (UTR). In *Tobacco mosaic virus* (TMV), the 3'-UTR is comprised of a tRNA-like struc-

ture (TLS), linked to an upstream pseudoknots domain (UPD). A chimeric virus TMV-43A, which contains an inserted internal poly(A) track of 43 adenines to replace the UPD of TMV, is able to cross protect *Nicotiana benthamiana* against TMV and induced mosaic symptom. However, TMV-43A is unable to infect *Arabidopsis shadahara*, a susceptible host of TMV. Here, we generated a series of chimeric viruses bearing different length of adenines upstream of the TMV-UPD to investigate functions of an added poly(A) tract and UPD upon TMV infection. We found that TMV(24A+UPD) showed a higher infectivity in *N. benthamiana* than TMV but did not kill the plants. In addition, the inserted poly(A) tract elongated more than 100 nt but with deletion of nucleotides at the 5'-end of UPD. The poly(A) tract also elongated in TMV(42A +UPD) on *N. benthamiana*. Therefore, our results demonstrated that the UPD is important for TMV infectivity, and the combination of internal poly(A) tract and UPD contributes to different symptoms during cross-protection.

**P23.034 A dual gene-silencing vector system for monocot and dicot plants**

*M.R. Liou, Y.W. Huang, N.S. Lin and Y.H. Hsu*
*Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan 40227, China*
*Email: yhhsu@nchu.edu.tw*

Plant virus-based gene-silencing vectors have been extensively and successfully used to elucidate functional genomics in plants. However, only limited virus-induced gene silencing (VIGS) vectors can be used in both monocot and dicot plants. Here, we established a dual gene-silencing vector system based on *Bamboo mosaic virus* (BaMV) and its satellite RNA (satBaMV). Both BaMV and satBaMV vectors could effectively silence endogenous genes in *Nicotiana benthamiana* and *Brachypodium distachyon*. The satBaMV vector could also silence the expression of the green fluorescent protein (GFP) transgene in *GFP* transgenic *N. benthamiana*. *GFP* transgenic plants co–agro-inoculated with BaMV and satBaMV vectors carrying sulfur and *GFP* genes, respectively, could simultaneously silence both genes. Moreover, the silenced plants could still survive with the silencing of genes essential for plant development such as *heat shock protein (Hsp) 90* and *Hsp70*. In addition, the satBaMV-based vector could enhance gene silencing efficiency in newly emerging leaves of *N. benthamiana* deficient in RNA-dependant RNA polymerase 6 whereas BaMV could not. The dual gene-silencing vector system of BaMV and satBaMV provides a novel tool for comparative functional studies in monocot and dicot plants.

**P23.035 Protein-protein interactions of *Citrus tristeza virus* revealed by yeast two hybrid**

*G.W. Wu, M. Tang, G.C. Nchongboh, G.P. Wang, F. Yang and N. Hong*
*National Key Lab. of Agromicrobiology, Huazhong Agricultural University, Wuhan 430070, P. R. China*
*Email: whni@mail.hzau.edu.cn*

*Citrus tristeza virus* (CTV) is the most serious viral pathogen of citrus. The virus has a highly complicated genome, and the ten open reading frames (ORFs) at the 3′-terminal of its genome have multiple biological functions. Specific interactions between proteins from a virus play important roles for its successful infection and replication. In this study, the interactions of three proteins (CPm, CP, and P20) among themselves and with other seven proteins were examined by yeast two- hybrid (Y2H), separately. Consistent and strong self- interactions of two proteins CP and P20 were identified in both directions, and the result was in consistent with a previously reported result. Further analysis revealed that the N-terminal conserved regions consisting of 13 amino acids ($^{1}$MRAYFSVNDYISL$^{13}$) of P20 and 10 amino acids ($^{29}$NLHIDPTLIA$^{41}$) of CP were essential for their self-interactions. Meanwhile, for the first time, our results showed that CPm and P20 also had relatively weak interactions with proteins P61 and P65 when CPm and P20 were used as baits and the C-terminal containing possible trans-membrane regions of preys (P61 and P65) were removed. The protein P20 encoded by CTV is a major component of inclusion bodies, and P61, P65 along with CP and CPm are the capsid components of CTV virons. Therefore, those results suggested that the interactions between those proteins might be involved in the assembly of CTV virions. However, the obtained results basing on Y2H assays should be confirmed by further in-vitro or in-vivo experiments.

**P23.036 Effects of increased phytic acid content in *Arabidopsis thaliana* on resistance against broad-spectrum pathogens**

*J.S. Poon, R.E. Le Fevre, A.M. Murphy, J.P. Carr and D.E. Hanke*
*Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK*
*Email: jacquelyne.poon@plantsci.cam.ac.uk*

Inositol hexa*kis*phosphate (Ins$P_6$) has been suggested as a signalling compound in basal defence responses against pathogen invasion (Murphy *et al.* 2008). Mutant Arabidopsis plants with reduced expression of *Atips2* (*inositol phosphate synthase 2*), but not *atips1* mutants, were previously shown to have enhanced susceptibility to a range of pathogens, including two RNA viruses (*Cucumber mosaic virus* and *Turnip mosaic virus*), a DNA virus (*Cauliflower mosaic virus*), a bacterium (*Pseudomonas syringae*), and a necrotrophic fungus (*Botrytis cinerea*). We are manipulating biochemical pathways leading to Ins$P_6$ production to further understand the role(s) of inositol polyphosphates in plant de-

fence. As T-DNA insertion mutants for *Ips3* were not previously tested, therefore *atips3* mutants are being challenged with pathogens. To test whether elevating Ins$P_6$ content enhances resistance to pathogens, we are decreasing expression of *myo*-inositol oxygenase (MIOX), which is encoded by a small gene family of four *Miox* genes in Arabidopsis. Biosynthesis of Ins$P_6$ relies primarily on the bioavailability of its precursors, *myo*-inositol and inorganic phosphate, the former of which is restricted by MIOX. MIOX converts inositol to glucuronic acid, directing biosynthesis towards polysaccharide production. Arabidopsis plants with reduced MIOX expression have increased Ins$P_6$ levels. We will test *miox* mutants (single or multiple null allele) for effects on pathogen susceptibility and for effects on signalling mediated by jasmonic acid and salicylic acid, phytohormones with key roles in defence.

**P23.037 Role of importin alpha proteins in targeting 2b protein of *Cucumber mosaic virus* into nuclei in *Arabidopsis thaliana***

*A.X. Xu[1], A.Z. Chen[1], H.M. Zhang[3], J.P. Carr[2] and Z.Y. Du[1]*

*[1]Institute of Bioengineering, Zhejiang Sci-Tech University, Hangzhou 310018, P. R. China; [2]Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, U.K.; [3]State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, P. R. China*

*Email: duzhiyou@yahoo.com.cn*

*Cucumber mosaic virus* (CMV) is an economically important pathogen that occurs globally and infects numerous plants. CMV encodes a nuclear-localized viral suppressor of host RNA silencing known as the 2b protein. Due to its nuclear localization signals (NLS), CMV 2b protein accumulates in the nucleus and nucleolus. However, the mechanism by which CMV2b is imported into nuclei remains unclear. We used bimolecular fluorescence complementation to explore interactions of CMV2b with all nine Arabidopsis importin alpha proteins (IMPα1-9). IMPα-2, IMPα-3 and IMPα-6 interacted with the 2b protein. To test the ability of these three importin alpha proteins to target CMV2b to the nucleus, we conducted agroinfiltration experiments in which 2b protein fused to green fluorescence protein (GFP) was co-expressed with each importin alpha protein. We found that over-expression of IMPα-2, but not IMPα-3 or IMPα-6, markedly enhanced nuclear accumulation of the 2b protein. Based on these findings, we propose that IMPα-2 plays a major role in nuclear targeting of CMV2b. We will report further experiments to confirm or refute our conjectures using Arabidopsis mutant lines for IMPα-2, IMPα-3 and IMPα-6, and transgenic lines over-expressing each of these importin alpha proteins.

**P23.038 SA and JA/ET signaling are required for *N*-mediated resistance against *Chilli veinal mottle virus***

*F. Zhu, D.H. Xi, X.G. Deng, X.J. Peng and H.H. Lin*

*Ministry of Education Key Laboratory for Bio-Resource and Eco-Environment, College of Life Science, Sichuan University, Chengdu 610064, P. R. China*

*Email: honghuilin@vip.sina.com*

The tobacco (*Nicotiana tabacum*) resistance gene *N* is a typical plant resistance (*R*) gene, introduced into tobacco from *Nicotiana glutinosa*. Genetic, physiological and molecular analyses have revealed that the stress-related phytohormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are known to participate in defense responses to mitigate biotic stress in plants. Recent evidence suggests that SA and JA signaling play an important role in the *N*-mediated resistance to *Tobacco mosaic virus* (TMV). *Chilli veinal mottle virus* (ChiVMV), a member of Potyvirus genus, family Potyviridae, infects mostly *Capsicum* sp. and is an aphid-transmitted in a non-persistent manner in the field. Leaf mottle and dark green vein-banding are the most characteristic symptoms. Such symptoms contribute to significant losses in yield and quality of produce. The *N*-gene resistance to ChiVMV has not been tested. Our recent study indicated that SA and JA/ET signaling are required for *N*-mediated resistance against ChiVMV. Quantitative real-time PCR analysis revealed that SA, JA/ET biosynthesis and signaling genes were up-regulated by ChiVMV infection. Furthermore, the expression of JA/ET and SA defense marker genes were induced by ChiVMV infection. Silencing of SA or JA/ET biosynthetic and signaling genes in *N. tabacum* impaired *N*-mediated resistance to ChiVMV. Taken together, SA and JA/ET signaling play an important role in the *N*-mediated resistance to ChiVMV.

**P23.039 Interaction between the P6 and P5-1 proteins of Southern rice black-streaked dwarf fijivirus is involved in formation of viroplasm in plant cells**

*J. Li[1], J. Xue[1,2], H.M. Zhang[1], J. Yang[1], M.F. Lv[1,3], L. Xie[1], Y. Meng[1,4], P.P. Li[1,4] and J.P. Chen[1]*

*[1]State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control; Key Laboratory of Plant Protection and Biotechnology, Ministry of Agriculture, China; Zhejiang Provincial Key Laboratory of Plant Virology, Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, P. R. China; [2]College of Bio-Safety Science and Technology, Hunan Agricultural University, Changsha 410128, P. R. China; [3]College of Agriculture and Biotechnology (CAB), Zhejiang University, Hangzhou 310058, P. R. China; [4] College of Chemistry and Life Science, Zhejiang Normal University, Jinhua 321004, P. R. China*

*Email: zhhengmu@tsinghua.org.cn*

Southern rice black-streaked dwarf virus (SRBSDV) is a recently-described member of the genus *Fijivirus*, family *Reoviridae*. The roles of the proteins encoded by the SRBSDV genome have rarely been studied. In a yeast two hybrid (YTH) assay using SRBSDV P6, a putatively multifunctional protein, as bait and a SRBSDV cDNA library as prey, an interaction between the P6 and P5-1 proteins was found. The strong interaction was confirmed by bimolecular fluorescence complement (BiFC) assay in plant cells. The reconstituted granular YFP fluorescence was similar to the viroplasm-like structures (VLS), randomly distributed in the cytoplasm and 0.5-10 μm in diameter. Immunogold labeling showed that both P6 and P5-1 localized within viroplasms in infected cells of rice plants, indicating that both proteins were components of viroplasms. YTH analyses using truncated mutants showed that the N-terminal region (amino acids 9-231) of P5-1 is necessary for its binding to P6 and that the N-terminal fragment (amino acids 1-93) of P6 is necessary for its interaction with P5-1. YTH and BiFC assays indicated that there was interaction between the minimal reactive mutants of P5-1 and P6 in yeast and plant cells, but these minimal truncated mutants did not aggregate into viroplasm-like structures, suggesting that something more than the interaction between the minimal interactive regions is necessary for VLS formation. This is the first report of interactions between P6 and P5-1 proteins of a fijivirus and of the involvement of the two proteins in viroplasm formation in SRBSDV.

**P23.040 Novel and conserved microRNAs in cucumber response to *Cucumber green mottle mosaic virus* infection**

*H.W. Liu[1,2], J.Q. Li[1,2] and L.X. Luo[1,2]*
*[1]Department of Plant Pathology, China Agricultural University; [2]Beijing Engineering Research Center of Seed and Plant Health (BERC-SPH)/Beijing Key Laboratory of Seed Disease Testing and Control (BKL-SDTC), Beijing, 100193, P. R. China*
*Email: luolaixin@cau.edu.cn*

The *Cucumber green mottle mosaic virus* (CGMMV) caused serious yield losses in family of *Cucurbitaceae* worldwide in recently years. MicroRNAs (miRNAs) are most approximately 23 nt, have similar secondary hairpin structures and conserved in many kinds of plants. It was demonstrated involved in gene expression at the post-transcriptional level in *vivo* of plants respond to abiotic stress or pathogen infection. In this study, the *Cucumis sativus* L. (cv. 'Zhongnong 16') was artificial inoculated by CGMMV at seedling stage. The leaf and flower samples were collected and the miRNAs were sequenced on 10, 30 and 50 dpi. The results showed that no mature miRNAs involved in cucumber response to CGMMV infection, 1 novel and 127 putative miRNAs were obtained according to the screening criterions and their predicated second structure. Eighty-eight conserved miRNAs were detected from mixture samples by chips expression profiles at p-value<0.01. Gene ontology (GO) analysis revealed that the target genes which screening by the Target Finder and miRanda algorithms were involved in molecular function (2265), cellular component (1362) and biological process (276). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that the target genes of these miRNAs were related to metabolism process (166), genetic information processing (40) and biosynthesis of secondary metabolites (12), respectively. The target genes could be mainly responsible for regulation of the metabolism and biosynthesis pathways. The results are helpful to understand the interaction between CGMMV and cucumber, and to screen the CGMMV-resistance genes from cucumber.

**P23.041 Identification and functional characterization for host factors interaction with *Soybean mosaic virus* encoded P3N-PiPo**

*P.W. Song[1,2], X. Chen[1], H.J. Zhi [2] and X.Y. Cui[1]*
*[1]Institute of Vegetable Crops, Jiangsu Academy of Agricultural Sciences, Nanjing, 210014, P. R. China; [2]College of Agriculture, Nanjing Agricultural University, Nanjing, 210095, P. R. China*
*Email: cxy@jaas.ac.cn*

A small open reading frame (ORF), pipo, overlaps with the P3 coding region of the potyviral polyprotein ORF, the PiPo protein exists in cells as a fusion to the N-terminal half of the P3 protein, giving a protein called P3N-PiPo. Previous evidence suggested that P3N-PiPo was a requirement for efficient viral cell-to-cell movement. To gain an understanding of P3N-PiPo's function, A yeast two-hybrid screen was performed using P3N-PiPo as bait and an soybean cDNA library infection by SMV as prey. As a result of sequential screening steps, 145 positive clones were isolated and sequenced. Protein function was predicted. The proteins included polypeptides involved in general metabolism, energy production, transcriptional regulation, signal transduction, tranport, defense, DNA binding and membrane-related. 19% of these polypeptides involved in tranport-related confirmed that P3N-PiPo was viral movement-associated protein, 25% of them involved in defense-related suggest that P3N-PiPo may be related to the pathogenesis of SMV-infected plants. Some interesting host proteins will be further test if these genes are required for SMV infection in soybean.

**P23.042 *Rice black-streaked dwarf virus* P7-2 interacts with SKP1, a core subunit of SCF ubiquitin ligase**

*Q. Wang[1,2], T. Tao[2], X.R. Chen[2], D.W. Li[2], J.L. Yu[2] and*

*C.G. Han[2]*
*[1]Key Laboratory for Tobacco Gene Resources, Tobacco Research Institute, Chinese Academy of Agricultural Sciences, No.11 Keyuanjingsi Road, Laoshan District, Qing dao, 266101, P. R. China; [2]State Key Laboratory for Agrobiotechnology and Department of Plant Pathology, China Agricultural University, No.2 Yuanmingyuan West Road, Haidian District, Beijing 100193, P. R. China*
*Email: wangqian_2000zb@163.com*

*Rice black-streaked dwarf virus* (RBSDV), a member of the genus *Fijivirus* within the family *Reoviridae*, causes rice black-streaked dwarf and maize rough dwarf disease in southeast Asian countries, and leads to severe yield losses in crops. Although several RBSDV proteins have been studied in detail, functions of the nonstructural protein P7-2 are still largely unknown. P7-2 gene is conserved among the genomes of most plant-infecting fijiviruses, except in that of *Nilaparvata lugens reovirus*, a non-phytopathogenic hopper-borne one, and is considered to be involved in the virus multiplication in plant hosts. In this study, P7-2 was found to interact with SKP1 proteins, a core subunit of the multicomponent SCF (SKP1/Cullin1/F-box/Rbx1) E3 ubiquitin ligase, by a two-hybrid screening of a cDNA library expressing *Zea mays* proteins. The interaction was further confirmed by yeast two-hybrid assay and bimolecular fluorescence complementation. Besides *Z. mays* SKP1 ($SKP1^{Maize}$), P7-2 can also interact with SKP1 proteins from other plants, including *Arabidopsis thaliana*, *Nicotiana benthamiana*, *Oryza sativa* and sugarcane. The $SKP1^{Maize}$ C-terminal region spanning residues 98 to 176 is crucial for P7-2-$SKP1^{Maize}$ interaction, while the interaction is more stable when the N-terminal region of BTB/POZ domain of SKP1 exists. Substituting two Ala for Leu79Pro80 in P7-2 doesn't affect the binding of P7-2 and $SKP1^{Maize}$. P7-2 might be a potential F-box protein encoded by RBSDV, and interfere with the process of plant resistance or symptoms development through ubiquitin pathway.

### P23.043 Global transcriptional response of apple in vitro shoots to *Apple stem grooving virus* infection

*S.Y. Chen, T. Ye, L. Hao, H. Chen, Z.F. Fan, L.Y. Guo and T. Zhou*
*Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: taozhoucau@cau.edu.cn*

Some viruses infecting apple (*Malus×demetsica*), such as Apple stem grooving virus (ASGV), usually induce no obvious symptom on apple tree and fruit, but the yield and quality of apple is reduced significantly, and the infection is permanent on apple tree. ASGV is known as one of the widest distributed latent pome fruit viruses in all apple growing areas. As ASGV is more tolerant to high temperatures, it is difficult to be eliminated by thermotherapy. Although ASGV causes severe losses on apple production and had been characterized well, little is known about the apple gene expression upon infection. Using cultured apple *in vitro* shoots, we investigated the apple global gene expression profile to ASGV by Illumina RNA-Seq technology. Totally, 320 genes showed differently expression in ASGV infected *in vitro* shoots. Through functional classification, the majority of up-regulated genes belong to four major biological changes, including transcription, defense, development and ripening related and translation, while the majority of down-regulated genes are related to seven major biological changes including defense, primary metabolism, transcription, stress, cell wall related, signal transduction and transport. Comparing other plant virus infection which induced a common set of genes mostly involved in pathogenesis and stress, ASGV infection specifically regulate transcription of defense- and stress-related genes may favor its persistence in apple as a result of long-term adaptation and coevolution.

### P23.044 S-adenosylmethionine synthetase, a host factor from rice interacted with *Rice gall dwarf virus* P6 protein

*X.L. Ruan, W.J. Shen and H.P. Li*
*Laboratory of Plant Virology, South China Agricultural University, Guangzhou 510642, P. R. China*
*Email: huaping@ scau.edu.cn*

*Rice gall dwarf virus* (RGDV), the causal agent of rice gall dwarf disease in China and South-East Asia, is classified in the genus *Phytoreovirus*. At present, the genome of several RGDV isolates from different countries has been sequenced, and function of partial viral genes has been determined, but the host factors interacted with viral proteins remains unknown. We recently performed to identify host proteins that interact with P6 protein of RGDV from a rice cDNA library by a yeast two-hybrid system and found that RGDV P6 could interact with S-adenosylmethionine synthetase (SAMS) from rice. Bimolecular fluorescence complementation (BiFC) analysis showed that P6 interacted with SAMS in living plant cells. Real-time quantitative PCR analysis indicated the expression levels of SAMS obviously reduced after the virus infection. SAMS, as a stress response protein, plays an important role in plants during salt stress, drought stress, cadmium stress and oxidative stress. Our results further showed that, during the virus infection, SAMS also acts as a response protein to launch the host resistance network. Some researchers found that knockdown of SAMS genes caused phenotype alterations in rice and tobacco, demonstrating SAMS also regulated plant phenotypes. Whether RGDV P6 is a pathogenicity determinant through interacting with SAMS in plants during RGDV infection, the conclusion should be determined by further studies.

**P23.045 Interaction of HC-Pro *Papaya ringspot virus* with various papaya (*Carica Papaya*) proteins**

*S. Roytrakul, N. Takaya, S. Chowpongpang and W. Siriwan*
*Center for Agricultural Biotechnology, Kasetsart University Kampaengsaen Campus, Nakhon Pathom 73140, Thailand*
*Email: g522900010@ku.ac.th*

Helper component proteinases (HC-Pro) is a multifunctional protein of potyvirus that displays self-interaction and interacts with other potyviral, polyprotein processing, aphid transmission, and suppression of antiviral RNA silencing. The display of HC-Pro interacted with plant proteins are presented in here. A pull down assay was used to study protein-protein interaction between HC-Pro of *Papaya ringspot virus* (PRSV) produced in *E. coli* that interacted with healthy and infected papaya proteins. The interacted protein complexes were identified by liquid chromatography-tandem mass spectrometry, which showed the novel finding of twenty-three different plant proteins interaction with HC-Pro and ten proteins were identified. These proteins are classified into eight groups of protein functions namely; plant defense (lipoxygenase (LOX) and TIR-NBS disease resistance-like protein), photosynthesis (bisphosphate carboxylase large chain and red chlorophyll catabolite reductase), membrane complex (membrane protein), transcription (reverse transcriptase), translation (chromosome associate protein subunit h), translocation (calcium-dependent lipid-binding-like protein), sucrose syntheses (sucrose-udp glucosyltransferase) and mannosyltransferase complex (beta-1,4-mannosyltransferase). In addition, the information of viral host proteins interaction with plant proteins is an important to understand the infection and development process of antiviral strategies in plant cells.

**P23.046 Soybean eukaryotic translation initiation factor 4e probably leads to resistant to *Soybean mosaic virus***

*X.Y. Cui [1], H.Y. Zhang [2], H.J. Zhi[2] and X. Chen[1]*
*[1]Institute of Vegetable Crops, Jiangsu Academy of Agricultural Sciences, Nanjing, 210014, P. R. China; [2]College of Agriculture, Nanjing Agricultural University, Nanjing, 210095, P. R. China*
*Email: cx@jaas.ac.cn*

Eukaryotic translation initiation factor 4E (eIF4E) was found to be essential determinants in the outcome of RNA virus infections. The interaction between soybean eIF4E gene and *Soybean mosaic virus* (SMV) encoded Vpg protein play a key role in the SMV infection. 17 soybean materials resistant to the two SMV strains(low virulence strain SC3 and high virulence SC15) were identified from 208 soybean materials. The eIF4E gene CDS sequences of susceptible variety Nannong 1138-2 as well as the 17 resistance materials were determined. The result showed that 8 eIF4E gene mutations in the 17 resistant materials, three of which were nonsense mutations and five of which were missense mutations. There are three amino acid changes in the missense mutations. By yeast two-hybrid system, SMV-VPg gene can interact with eIF4E gene from the soybean susceptible varieties 1138-2 and cannot interact with eIF4E gene in the amino acid change from asp (D) to asn (N) and arg (R) to lys (K) among three missense mutation materials. The results indicated that the change of eIF4E amino acid sequence affected the interactions between VPg and eIF4E gene and this is likely to be the reason that the materials are resistant to SC3 and SC15 strains.

**P23.047 The molecular identification and characterization of host proteins interacted with *Soybean mosaic virus* encoded P3 protein**

*X. Chen[1], H.X. Luan[2], H.J. Zhi[2] and X.Y. Cui [1]*
*[1]Institute of Vegetable Crops, Jiangsu Academy of Agricultural Sciences, Nanjing, 210014, P. R. China; [2]College of Agriculture, Nanjing Agricultural University, Nanjing, 210095, P. R. China*
*Email: cxy@jaas.ac.cn*

*Soybean mosaic virus* (SMV) belongs to the genus Potyvirus, family Potyviridae, which is the largest and most economically important plant virus family. Infection by SMV usually causes yield losses 35 to 50% under natural fields and up to 50 to 100% in severe outbreaks. Despite extensive research and study on SMV interaction with its host, its exact in infection, replication and movement mechanism remains unclear. SMV encodes a limited number of viral proteins in its genome, hence it has evolved to recruit host proteins to aid its replication and movement upon infection. In order to identify SMV host proteins involved in the viral infection, the soybean cDNA library was constructed and used in a yeast two-hybrid screen. SMV-encoded P3 protein was cloned as bait. Membrane-based yeast two- hybrid system screening have identified a total of 37 host candidates that interacted with P3. As reported, P3 was involved in virus replication and movement. Based on their predicted functions, some interesting of these candidate genes, with high homology to SNARE family, and related with host resistance were selected for further functional analysis. Interactions between the viral proteins and these selected host proteins are being verified by confirmation using the bimolecular fluorescence complementation assay (BiFC) *in planta*. Biochemical assays and reverse genomics tools will be used to elucidate the molecular mechanisms of the identified genes in the SMV infection process.

**P23.048 Comparative transcriptome of microRNAs in response to TuMV infection in non-heading cabbage**

*C.W. Zhang, Y.X. Li, Z. Wang and X.L. Hou*

*College of Horticulture, Nanjing Agricultural University, 1 Weigang, Nanjing, Jiangsu, 210095, P. R. China*
*Email: hxl@njau.edu.cn*

*Turnip mosaic virus* (TuMV) is the most prevalent viral pathogen infecting most cruciferous plants such as non-heading Chinese cabbage. MicroRNAs (miRNA) that are around 22 nucleotides long non-protein-coding RNAs, play key regulatory roles in plants. Recent research findings show that miRNAs are involved in plant-virus interaction. However we know little about plant defense and viral offense system networks throughout microRNA regulation pathway. In this study, two small RNA libraries were constructed based on on-heading Chinese cabbage leaves infected by TuMV and healthy leaves and sequenced using the Illumina-Solexa high-throughput sequencing technology. A total of 38 conserved and 214 novel miRNAs were identified in the Chinese cabbage. miRNAs differential transcriptome was calculated by IDEG6 software in two libraries. We found that 8 candidate miRNAs involved in plant growth are related to virus symptom were up-regulated in infected leaves. Six candidate miRNAs that may be involved in virus translation, replication and movement were down-regulated in infected leaves. The characterization of these miRNAs could contribute to a better understanding of plant-virus interaction throughout microRNA regulation pathway. This lead to find new approach to defend virus infection using miRNA in Chinese cabbage.

### P23.049 ZmRop1 involves in regulating *Sugarcane mosaic virus* viral multiplication and movement

*Y.Y. Cao, H.Y. Li, J.J. Li, L.F. Wang and H. Wang*
*Cereal Crops Institute, Henan Academy of Agricultural Sciences, No.116 Huayuan Road, Jinshui District, Zhengzhou, 450002, P. R. China*
*Email: caoyanyong@gmail.com*

The plants Rho-related GTPases (Rops) are versatile signalling regulators. Our previously study speculated that ZmRop1 involves in regulating maize resistance to *Sugarcane mosaic virus* (SCMV) infection. To further speculate the role of ZmRop1 in maize defense pathway to SCMV infection. *ZmRop1*$^{CA}$ (constitutively activated) over expression and *ZmRop1* RNA inference transgenic maize plants were obtained via by *Agrobacterium*-mediated transformation. The T3 *ZmRop1* transgenic plants samples were harvested for subsequent assay post SCMV inoculation. Real-time reverse transcription-polymerase chain reaction and Western blotting were employed for detecting SCMV mRNA level and viral multiplication in *ZmRop1* transgenic plants systemic leaves and protoplasts. The data indicated that the relative SCMV mRNA levels of *ZmRop1*-silenced plants was about 700% and 270% higher than that of *ZmRop1*$^{CA}$ over expression and wild type maize plants, respectively. Western-blotting also showed that SCMV accumulation in *ZmRop1* gene-silenced plants was at least three times higher than that in control plants. To investigate the influence of ZmRop1 on SCMV intercellular movement and long-distance movement, the cytology of SCMV infected *ZmRop1* transgenic and control maize plants was studied by using transmission electron microscopy coupled with gold-labeling SCMV CI (cytoplasmic protein) and HC (helper component protein) antibodies. The result indicated that the higher levels of CI labeling in *ZmRop1*-silenced maize plasmodesmata and very low levels of signals in control sections. Simultaneously, *ZmRop1*-silencing plants phloem sieve tube show higher levels of HC labeling compared to controls. All the above data demonstrated that ZmRop1 involves in regulating SCMV viral multiplication and movement.

### P23.050 *Cucumber mosaic virus* 2b protein acts as a RNA silencing suppressor and virulence determinant in a subcellular spatio-associated manner in *Arabidopsis thaliana*

*Z.Y. Du[1,2], A.Z. Chen[1], W.H. Chen[1], Y.M. Bao[4], M.J. Roossinck[3] and J.P. Carr[2]*
*[1]College of Life Sciences, Zhejiang Sci-Tech University, Hangzhou, Zhejiang Province, 310018, P. R. China; [2]Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, U.K.; [3]Center for Infectious Disease Dynamics, Pennsylvania State University, PA 16802, U.S.A; [4]National Center for Biotechnology Information, National Institutes of Health, MD 20892, U.S.A.*
*Email: duzhiyou@yahoo.com.cn*

*Cucumber mosaic virus* (CMV) is an important plant pathogen and important model for plant-virus interaction studies. CMV encodes the 2b protein, an RNA silencing suppressor and pathogenicity determinant. CMV2b accumulates in nuclei due to possession of either one (Subgroup II strains) or two (Subgroup I) nuclear localization signals (NLS). However, the biological significance of 2b nuclear localization is uncertain, as mutational studies showed that silencing suppression is independent of nuclear localization. Using GFP-2b fusions, we found that the 2b protein of LS-CMV (Subgroup II) is predominantly nuclear-targeted, while the Fny-CMV (Subgroup IA) 2b protein partitions between nucleus and cytoplasm. Further experiments showed that addition of the NLS sequence 22KRR RRR27 from LS2b to the C terminus of Fny2b produced a 2b variant that accumulated predominantly in the nucleus. Replacement of the Fny2b NLS 22KQRRRR27 with the LS2b NLS did not alter localization. Interestingly, increased enrichment of mutated Fny2b in the nucleus markedly impaired its ability to suppress siRNA- mediated gene silencing, and abolished its ability to disrupt miRNA-regulated silencing in transgenic Arabidopsis. Enhanced nuclear

localization mutated Fny2b decreased virus accumulation, but enhanced CMV pathogenicity by causing systemic necrosis. Systemic necrosis in mutant Arabidopsis mutant lines deficient in miRNA or siRNA biogenesis demonstrated that enhanced patho- genicity was unrelated to effects on miRNA- or siRNA- regulated functions. Thus, the distri- bution between different cellular compartments strongly influences the effects of CMV 2b on RNA silencing and virulence.

**P23.051 Methyl salicylate and jasmonate signaling are essential for systemic resistance in *Nicotiana benthamiana***

*F. Zhu, D.H. Xi and H.H. Lin*
*Ministry of Education Key Laboratory for Bio-Resource and Eco-Environment, College of Life Science, Sichuan University, Chengdu 610064, P. R. China*
*Email: honghuilin@vip.sina.com*

Systemic acquired resistance (SAR) is induced by pathogens and confers protection against a broad range of pathogens. Complex defense signaling pathways, controlled by different hormones, are involved in the reaction of plants to a wide range of biotic and abiotic stress factors. The nature of the mobile signal for establishing SAR remains elusive. Recent studies have indicated that the salicylic acid (SA) derivative methyl salicylate (MeSA) serves as a long-distance phloem- mobile SAR signal in tobacco, Arabidopsis, and potato. However, other experiments argue that jasmonic acid (JA) is a critical mobile SAR signal. Here, we present several lines of evidence that suggest MeSA and JA signaling are essential for systemic resistance, possibly acting as the initiating signal for SAR. Foliar hormone applications with JA followed by SA activate the expression of defense genes and trigger the strongest systemic resistance against *Tobacco mosaic virus* (TMV). Silencing of SA or JA biosynthetic and signaling genes in *Nicotiana benthamiana* increased susceptibility to TMV. Genetic experiments also proved the irreplaceable roles of MeSA and MeJA in SAR. JA and MeJA accumulated in phloem exudates of leaves at early stages, and SA and MeSA accumulated at later stages after TMV infection. Taken together, our results demonstrate that the sequential actions of MeJA and MeSA are required for SAR response against TMV.

**P23.052 *Citrus tristeza virus* p23: determinants for nucleolar localization and their influence on suppression of RNA silencing and pathogenesis**

*S. Ruiz-Ruiz[1,2], N. Soler[2], J. Sánchez-Navarro[1], C. Fagoaga[2], C. López[1,3], L. Navarro[2], P. Moreno[2], L. Peña[2] and R. Flores[1]*
*[1]Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Científicas-Universidad Politécnica de Valencia; [2]Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia; [3]Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universidad Politécnica de Valencia, Spain*
*Email: rflores@ibmcp.upv.es*

One of the three RNA silencing suppressors encoded by *Citrus tristeza virus* (CTV), p23 (209 amino acids), incites phenotypic aberrations when expressed ectopically in *Citrus* spp. To learn more about p23, the fusion p23-GFP was agroexpressed in *N. benthamiana.* Confocal laser-scanning microscopy revealed its accumulation in nucleolus, Cajal bodies and plasmodesmata, showing that p23 is the first closterovirus protein with a nucleolar localization signal (NoLS). To dissect this signal, typically associated with basic motifs, seven truncated and ten point-mutated versions of p23 were assayed. Deletion mutants showed that regions 50-86 and 100-157 (excluding fragment 106-114), both with basic motifs and the first with a Zn-finger, contain the (bipartite) NoLS. Alanine substitutions delimited this signal to three cysteines of the Zn-finger and some basic amino acids within and preceding it, and to fragment 143-155 with six basic amino acids. RSS activity of p23 (tested by its coagroexpression with GFP in *N. bentamiana*) was abolished by essentially all mutants, indicating that it involves most p23 regions. The necrotic-inducing ability of p23 when expressed in *N. benthamiana* as a subgenomic RNA of *Potato virus X,* was only retained by deletion mutant 158-209 and one substitution mutant, thus showing that the Zn-finger and flanking basic motifs form part of the pathogenic determinant. Ectopic expression of p23 and some deletion mutants in transgenic Mexican lime demarcated a similar determinant, suggesting that p23 affects related pathways in citrus and *N. benthamiana.* Both RSS activity and pathogenicity of p23 appear related to its nucleolar localization.

**P23.053 Variability in *Bipolaris oryzae* isolates for morphology, pathogenicity and genetic structure of population**

*S.P. Banu, B. Meah, D. Brar, H. Leung and C.M. VeraCruz*
*Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh*
*Email: salinapbanu@yahoo.com*

Variability in 22 isolates of *Bipolaris oryzae* was assessed on the basis of morphological features, pathogenicity with host-pathogen interaction and its DNA finger printing through VNTR-PCR method. The isolates exhibited distinct variation in colony characteristic like shape, elevation, margin and color on Potato dextrose agar. However, no association of these characteristics was observed with the isolates originating from different places and rice cultivars**.** The isolates were significantly different for growth rate (P<.0031), conidial concentration (P<.0001) and conidial germination (P<.0221) without any distinct group. There was differ-

ent pattern of germination in all 22 isolates, however dominant germination pattern was bipolar, from the two end cell of conidia which was significantly different (P<.0027) between isolates. Significant differences (P<.0001) were obtained in pathogenicity between 22 isolates, six rice germplasms of rice (IR 36, IR 64, UPLRi 7, 2 accessions of Dinorado and *O. minuta*), and isolate x cultivar interactions with evidence for the presence of physiological races, but not sufficient to designate into distinct races of the fungus. Analysis of VNTR-PCR DNA fingerprints identified six genetic groups at 72% similarity, but 91% of isolates was in one group at 50% similarity which supports the evidence of physiological races in pathogenicity. There was no association between genetic group, place and origin of cultivars of isolates. However, some association was observed between DNA finger printing and pathogenicity. This study suggested that morphology and genetic study are good support for pathogenicity to select virulent and consistent isolates for screening of rice germplasm and segregating populations. Differential isolate SM2 originating from IR 52 has consistent higher virulence level in morphology, pathogenecity, belongs to the group of 91% isolates in DNA finger printing and selected for further screening in genetic analysis. Dinorado (IRTP 12568) was confirmed to be highly resistant to all 22 isolates. IR 36, IR 64 and UPLRi 7 could serve as differentials for brown spot disease of rice.

**P23.054 How strawberry fights against the wilt pathogen *Fusarium oxysporum* f. sp. *fragariae***

*X.L. Fang[1], R. Jost[1], J. Kuo[2], M.P. You[1], P.M. Finnegan[1,3] and M.J. Barbetti[1,3]*
*[1]School of Plant Biology, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia; [2]The Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia; [3]The UWA Institute of Agriculture, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia*
*Email: fangx01@student.uwa.edu.au*

Strawberry (*Fragaria ananassa*) is a high-value berry crop. Fusarium wilt on strawberry caused by *Fusarium oxysporum* f. sp. *fragariae* (Fof) is a serious threat to commercial strawberry production worldwide. However, the infection process of Fof in strawberry and the resistance mechanisms of strawberry against Fof are largely unknown. Studies were conducted to determine how Fof infects strawberry and how strawberry responds to the infection upon either a compatible or incompatible host-pathogen interaction. The infection process of Fof in strawberry was examined in the root of one resistant cv. Festival and one susceptible cv. Camarosa using light and scanning electron microscopy. This study demonstrated that resistance of strawberry against Fof was due to the impeded pathogen growth and infection both on and within host tissue. In particular, cellular responses of strawberry in the hypodermal layer of the root played a key role. Comparative proteome analyses were conducted to determine temporal changes in the root proteome of the two cultivars during the early stages of infection by Fof. This study suggested that pathogenesis-related proteins, and proteins involved in ROS detoxification, ethylene/ jasmonic acid signaling pathways, secondary metabolite biosynthesis, glycolysis and/or ubiquitin-mediated protein degradation have great potential in mediating the resistance of strawberry against Fof. Protein modification may also make an important contribution. Overall, these studies provide first insights into the resistance mechanisms of strawberry against Fof, opening novel avenues to engineer new disease-resistant strawberry cultivars and to develop more effective and sustainable disease management strategies.

**P23.055 Characterization of soybean host resistance and Asian soybean rust (ASR) pathogen variability for durable resistance**

*P. Tian and S.M. Smith*
*Department of Plant Pathology, University of Georgia, Athens, GA 30602. USA*
*Email: pengtian@uga.edu*

*Phakopsora pachyrhizi* is the causal agent of Asian soybean rust (ASR) and is one of the most aggressive soybean diseases. Resistance to ASR is controlled by five resistance genes (*Rpp1* thru *Rpp5*) that have shown to be ineffective when challenged with different isolates of *P. pachyrhizi*. Significant efforts are being made to study the genetics of resistance to ASR, which can assist in the rapid and efficient development of new resistant varieties. Thus, knowledge of the genetics of resistance to ASR and the variability of ASR populations are essential in dissecting the relationship between the host and pathogen, and developing effective breeding strategies for resistance. This project has three major research components: 1) characterization of soybean host resistance, 2) analysis of Asian soybean rust pathogen variability, and 3) integration of host and pathogen datasets to establish pipelines for release of resistance cultivars. We propose to analyze collected ASR Georgia field isolates; characterize resistance phenotypes of released cultivars grown in Georgia in response to inoculation with ASR Georgia field isolates; identify candidate pathogenicity genes in ASR isolates; and characterize specific resistance genes that can be used to develop durable broad spectrum disease resistance strategies for soybean cultivars grown in Georgia and throughout the U.S.. By integration of the host and pathogen datasets, this work will assist in the release of resistant genotypes to diversify selection pressure on pathogens and increase the evolutionary barrier required

for the pathogens to overcome resistance, thereby increasing the durability of resistance.

### P23.056 Understanding the molecular mechanisms of apple rootstock resistance to soilborne *Pythium ultimum*

*S. Shin, J. Lv, F. Johnson, M. Mazzola and Y. Zhu*
*United States Department of Agriculture, Agricultural Research Service, Tree Fruit Research lab, Wenatchee, WA 98801, USA*
*Email: yanmin.zhu@ars.usda.gov*

A diversity of soil-borne fungal pathogens cause apple "replant diseases" with a range of symptoms from diminished productivity to tree death. The molecular mechanisms behind host resistance to specific necrotrophic pathogens, such as *Pythium ultimum*, in perennial root tissues are unknown. Biosynthesis and signal transduction pathways associated with the plant hormones ethylene (ET) and jasmonic acid (JA) have been indicated to play a role in resistance to non-host-specific necrotrophic pathogens, primarily based on *Arabidopsis* mutant analyses. Gene family members in apple ACS (1-aminocyclopropane-1-carboxylic acid synthase), ERF (ethylene response factor) and AOS (allene oxide synthase) gene families were analyzed by quantitative reverse transcription PCR (qRT-PCR) in apple root tissues during *P. ultimum* infection. Three out of 15 ACS genes and one out of 5 AOS genes exhibited a 10-60 fold up-regulated expression at 24-48 hours after *P. ultimum* inoculation. Two MdETR1 genes which are highly homologous to *Arabidopsis* ERF1 genes and function as ET/JA signal integrator, also showed up-regulated expression. These preliminary results are considered as the first experimental evidence for the proof of concept that ET/JA pathways, which were previously identified in aerial organs from other plant systems, are operational in the perennial root system when challenged by necrotrophic pathogens. RNA-Seq technology is also being used to identify potential novel pathways and genes specific to the root tissues of rosaceous tree crops that are activated in response to soilborne necrotrophic pathogens.

### P23.057 Genetic transformation of *Plasmodiophora brassicae*

*J. Feng, S.F. Hwang and S.E. Strelkov*
*Crop Diversification Centre North, Alberta Agriculture and Rural Development, Edmonton, AB, T5Y 6H3, Canada*
*Email: jie.feng@gov.ab.ca*

A protocol for genetic transformation of the obligate parasite *Plasmodiophora brassicae*, causal agent of clubroot of crucifers, was developed. In this protocol, protoplast preparation was superseded with lithium acetate treatment and the selection step was omitted. In two independent transformation experiments, germinating resting spores of *P. brassicae* were transformed by two fungal expression vectors containing either a green florescent protein gene or a hygromycin resistance gene. By the merit of high transformation efficiency, putative transformants were produced from both of the two transformations, with approximately 50% of the obtained galls containing resting spores from which transforming DNA could be identified by PCR. PCR, real-time PCR and genome walking were conducted on selected transformants and the results indicated that in these transformants the transforming DNA was intergraded into the *P. brassicae* genome. Verified transformants were used to inoculate canola plants and generate new galls for the next generation. PCR based on the next-generation galls indicated that transforming DNA was still resident in *P. brassicae*. From all obtained transformants, no GFP activity could be identified, indicating the malfunction of fungal promoters in *P. brassicae*. This is the first report on genetic transformation of *P. brassicae*. The information and data generated from this study will facilitate research in multiple areas of the clubroot pathosystem.

### P23.058 RAS GTPase activating protein gene CoIRA1 involved in infection-related morphogenesis and cAMP signal through Coras2 in *Colletotrichum orbiculare*

*K. Harata and Y. Kubo*
*Laboratory of Plant Pathology, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, 606-8522 Japan*
*Email: spnp4er9@mint.ocn.ne.jp*

Through *Agrobacterium tumefaciens*-mediated transformation (AtMT) of *Colletotrichum orbiculare* strain 104-T (MAFF240422), an anthracnose disease fungus of cucumber, a mutant named AA4510, which showed abnormal infection-related morphogenesis and attenuated pathogenicity was previously isolated. Analysis of the mutation confirmed an insertion into a gene which putatively encodes 2255-amino acid protein with a predicted RAS GTPase-activating protein (RASGAP) domain. And we named this gene as *CoIRA1*. Targeted gene deletion mutants of *CoIRA1* indicated that *CoIRA1* is involved in proper appressorium development and penetration hyphae development. Appressorium produced by the *coira1* mutants showed irregular shape of appressorium on glass slides. And the *coira1* mutants formed bulb shape penetration hyphae on cellulose membranes unlike tubular form of the wild type. Accordingly, the *coira1* mutants showed reduced pathogenicity on the cucumber leaves compared with the wild type. We analyzed whether *CoIRA1* is involved in cAMP signal through Coras1 and Coras2 in *C. orbiculare*, since in *Saccharomyces cerevisiae*, Ira1 and 2 inactivate Ras1

and 2 which in turn activate adenylate cyclase Cyr1 and systhesizes cAMP form ATP. The *coira1* mutant, the wild-type carrying dominant active form *CoRAS2* allele strains and the *coira1* mutant carrying dominant negative form *CoRAS2* allele strains showed increased responsivity to exogeneous cAMP. We further found that intracellular cAMP level in the *coira1* mutant was high compared with the wild type. These data indicated that *CoIRA1* is involved in cAMP signal transduction through Coras2 in *C. orbiculare*. In conculusion, *CoIRA1* is involved in infection-related morphogenesis and cAMP signal through Coras2 in *C. orbiculare*.

**P23.059 *Colletotrichum orbiculare CoPAG1*, a *Saccharomyces cerevisiae PAG1* homologue, is involved in appressorium development triggered by plant-derived signals**

*S. Kodama, A. Sakaguchi and Y. Kubo*
*Laboratory of Plant Pathology, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, 1-5 Hangi-cho, Shimogamo, Sakyo-ku, Kyoto, 606-8522, Japan*
*Email: ze14skodama@gmail.com*

We previously reported that cucumber anthracnose fungus *Colletotrichum orbiculare CoKEL2*, a *Schizosaccharomyces pombe tea1* homologue is essential for proper morphogenesis of appressoria on artificial substrates but is dispensable for appressorium formation on the host plant surfaces. Our results suggested that there is a bypass pathway that transduces plant-derived signals for appressorium formation independent of *CoKEL2*. To determine specific components of the plant-derived signaling pathway that leads to appressorium formation, we obtained six *cokel2* double mutants that formed abnormal appressoria not only on artificial substrates but also on the host plant surfaces. Expectedly, reintroduction of *CoKEL2* into those *cokel2* double mutants restored normal appressorium formation on artificial substrates. We identified candidate-mutated genes by whole genome sequencing of the six *cokel2* double mutants. By blastp search, we characterized candidate- mutated gene of *cokel2* double mutant kanI-9 as *CoPAG1*, a *Saccharomyces cerevisiae PAG1* (*TAO3*) homologue. It was reported that *Saccharomyces cerevisiae PAG1* is involved in cell polarity and morphogenesis. To define the involvement of *CoPAG1* in appressorium formation, we observed the phenotypes of *copag1Δ* mutants and *copag1Δcokel2Δ* double mutants. As expected, *copag1Δcokel2Δ* showed same phenotypes as kanI-9, indicating that existence of signaling pathway for appressorium morphogenesis triggered by plant-derived signals and that *CoPAG1* is one of specific components of this signaling pathway.

**P23.061 Biosynthetic pathway for host-specific AAL-toxin in the tomato pathotype of *Alternaria alternata***

*Y. Akagi, T. Tsuge, E. Nanba and M. Kodama*
*Faculty of Agriculture, Tottori University, Koyama-Minami 4-101, Tottori, Japan*
*Email: royaltouch111jp@yahoo.co.jp*

The phytopathogenic fungus *Alternaria alternata* tomato pathotype (Synonym *A. alternata* f. sp. *lycopersici, A. arborescens*) produces host-specific AAL-toxin. AAL-toxin production is essential for pathogenicity of the pathogen on host plants. The chemical structure of the AAL-toxin resembles to that of a mycotoxin fumonisin produced by *Gibberella* spp., and both toxins are classified as sphinganine-analog mycotoxins (SAMT) due to their structural similarity to sphinganine (dihydrosphingosine, DHS), which is the backbone precursor of sphingolipids. Fumonisin biosynthetic pathway has been elucidated by the identification and functional analysis of the genes involved in fumonisin biosynthesis. While, there are few information of the biosynthetic pathway and the genes involved in the AAL-toxin biosynthesis. *ALT1*, a gene encoded polyketide synthase, was found as an AAL-toxin biosynthetic gene (*ALT* gene). *ALT1* and *FUM1* encode polyketide synthase, a key enzyme for biosynthesis of the toxins, and share high homology at amino acid level. In this study, we performed shotgun sequencing of the genome of the tomato pathotype and identified AAL-toxin biosynthetic (*ALT*) gene cluster consisting of at least 13 genes homologous to the fumonisin biosynthetic (*FUM*) genes in *F. verticillioides*. The *ALT* cluster includes genes for P450 monooxygenase (*ALT2*), dehydrogenase (*ALT3*) and others in addition to *ALT1*. Functional analysis of the *ALT* genes showed that some of them are involved in AAL-toxin biosynthesis by the pathogen and pathogenicity/virulence of the pathogen against susceptible tomatoes.

**P23.062 Roles of lignin metabolism and several disease defense-related genes in *Brassica napus*-*Leptosphaeria maculans* pathosystem**

*X. Zhang[1], W.G. Dilantha Fernando[1] and M. Belmonte*
*[1]Department of Plant Science, University of Manitoba, 222 Agriculture Building, 66 Dafoe Road, Winnipeg, MB R3T 2N2, Canada*
*Email: zhangx39@cc.umanitoba.ca*

It has been believed that lignin metabolism plays an important role in plant defense against pathogen invasion and has a central role in some host-pathogen pathosystems. The fungal pathogen *Leptosphaeria maculans* is the causal agent of blackleg in *Brassica napus* (canola, oilseed rape). The interaction between *B. napus* and *L. maculans* follows a typical gene for gene theory at the seedling stage. In this study, three *B. napus* accessions showed resistance (R- carry major blackleg resistance gene *Rlm5*), intermediate resistance (IR- carry other major blackleg resistance genes), and susceptible reaction (S- no resistance genes) respectively to a *L. maculans* isolate that carries *AvrLm5* were used to investigate

the role of lignin metabolism. Histochemical analysis of lignin revealed differences of lignin levels in R, IR and S accessions. All three accessions showed an increased level of lignin accumulation as well as expression of lignin synthesis-related genes after infection of *L. maculans*, but the increase in resistant and intermediate resistant cultivars/lines was greater and rapid, where thick multi-layer of lignified materials around the infection site were observed. Quantitative real-time PCR (qPCR) was performed to compare expression of lignin synthesis-related genes and pathogenesis related genes (PR genes), WRKY transcription factor genes in different accessions after the infection with *L. maculans*. To further confirm the role of lignin metabolism in *B. napus*-*L. maculans* pathosystem, transcriptome changes in mesophyll cells collected via Laser capture microdissection from infected cotyledons of *B. napus* after *L. maculans* infection will be profiled by RNA-seq.

**P23.063 Histone lysine methyltransferases in *Magnaporthe oryzae* are involved in various aspects of infection and pathogenesis**

*K.T.M. Pham, B.V. Vu, Q.B. Nguyen, K. Ikeda and H. Nakayashiki*
*Laboratory of Plant Pathology, Graduate School of Agricultural Science, Kobe University, Nada Kobe 657-8501, Japan*
*Email: hnakaya@kobe-u.ac.jp*

The rice blast fungus *Magnaporthe oryzae* shows global transcriptional changes during infection processes possibly resulted from genome-wide chromatin remodeling. Here we report genetic dissection of seven putative histone lysine methyltransferase (HMT) genes, which we named MoHMT1 to 7, in *M. oryzae*. Western blotting analysis of histone protein in knock-out (KO) mutants of the seven MoHMT genes revealed that MoHMT1, MoHMT3, and MoHMT4 were associated with methylation of histone H3 lysine 9 (H3K9me), hitone H4 lysine 20 (H4K20me), and histone H3 lysine 4 (H3K4me), respectively. Four of the seven HMT-KO mutants, MoHMT1-KO, MoHMT2-KO, MoHMT4-KO, and MoHMT5-KO showed defects in vegetative growth, conidiation, appressorium formation, and/or pathogenicity at variable levels. Remarkably, MoHMT4-KO mutants were severely impaired in appressorium formation and completely lost pathogenicity on the original host wheat, indicating that H3K4me is an important epigenetic mark for infection-related gene expression in *M. oryzae*. Appressorium formation was greatly restored in the MoHMT4-KO mutants by exogenous addition of cAMP or the cutin monomer 16-hydroxypalmitic acid, suggesting that MoHMT4 might be involved in signal perception leading to appressorium formation. However, the MoHMT4-KO mutants were not infectious to wheat even with the cAMP or cutin monomer treatments. Interestingly, the MoHMT4-KO mutants were still infectious on the susceptible barley cultivar Nigrate, suggesting its role in overcoming some host-specific resistance. Chromatin immunoprecipitation (ChIP) and ChIP-seq analyses revealed dynamic changes in distribution patterns of H3K4me in the *M. oryzae* genome including several pathogenicity gene loci during infection-related morphogenesis.

**P23.064 Bub2-Bfa1 complex, a SPOC component in *Saccharomyces cerevisiae*, is involved in proper progression of cell cycle in *Colletotrichum orbiculare***

*F. Fukada, A. Sakaguchi and Y. Kubo*
*Laboratory of Plant Pathology, Graduate School of Kyoto Prefectural University, Life and Environmental Sciences, Shimogamo, Kyoto 606-8522, Japan*
*Email: ddswb383@yahoo.co.jp*

The orientation of the mitotic spindle with respect to mother-daughter polarity axis is important for the accuracy of asymmetric cell division in *Saccharomyces cerevisiae*. In response to spindle orientation defects, a surveillance mechanism named spindle position checkpoint (SPOC) prevents from exiting mitosis. *BUB2* is a component of SPOC, and we generated gene disruption mutants of *BUB2* homolog in *Colletotrichum orbiculare*. The *cobub2* mutants formed abnormal appressoria and the pathogenesis to cucumber showed much less than that of the wild type. The mitotic behavior of the *cobub2* mutants during a process of appressorium development was different from the wild type. In the wild type, mitosis occurred in appressorium developing conidia after 4h incubation, whereas interestingly, in the *cobub2* mutants, mitosis occurred in pre-germinated conidia after 2h incubation. Then, the timing of S phase and M phase was evaluated by cell cycle specific inhibitors. It was shown that the transition from G1 phase to S phase of the *cobub2* mutants accelerated about 2h than that of the wild type. In *S. cerevisiae*, Bub2 forms GTPase activating protein (GAP) complex with Bfa1, and Bub2-Bfa1 GAP complex constitutes SPOC. We generated *bfa1* homolog mutants in *C. orbiculare*. The *cobfa1* mutants showed similar mitotic behavior to the *cobub2* mutants, showing mitosis in pre-germinated conidia. Therefore, it is assumed that Cobub2 functions as GAP complex with Cobfa1, however, the GAP complex has different roles in cell cycle progression from that in *S. cerevisiae*, maintaining G1 phase duration or setting up the proper time of S phase.

**P23.065 Deciphering the role of lectin receptor kinases in disease resistance to *Phytophthora* pathogens**

*Y. Wang[1,2], K. Bouwmeester[1], W. Shan[2] and F. Govers[1]*
*[1]Laboratory of Phytopathology, Plant Sciences Group, Wageningen University, Wageningen, The Netherlands; [2]College of Plant Protection, Northwest A&F University,*

*Yangling, Shaanxi, P. R. China*
*Email: yan.wang@wur.nl*

In agriculture, plant diseases caused by *Phytophthora* species are widespread and cause enormous yield losses in a large variety of crops. Disease control is costly and often depends on preventive fungicide treatments that can have harmful side effects. Development of durable resistant cultivars to control plant diseases is therefore under a high demand. We are studying on a novel type of immune receptors that could be employed as genetically determined *Phytophthora* disease resistance components. Previously, Arabidopsis lectin receptor kinase I.9 (LecRK-I.9) was found to be essential for Phytophthora resistance (Bouwmeester et al. 2011 PloS Pathogens). LecRK-I.9 is a member of a family of 45 LecRKs in Arabidopsis. The functions of these LecRKs were determined by screening the phenotypes of a genome-wide collection of T-DNA insertion mutants upon infection with various *Phytophthora* spp. and with bacterial and fungal pathogens. One candidate was selected for more detailed analysis. In addition, homologs of Arabidopsis LecRKs in potato and tomato are being investigated for a function in *Phytophthora* disease resistance.

**P23.066 Functional analysis of native strains of *Trichoderma harzianum* for mycoparatisim and chitinase gene expression during interaction with *Fusarium oxysporum* f. sp. *lycopersici***

*L. Prasad, S. Sagar, S. Chaudhary and A. Tomar*
*Plant Health Clinic, Department of Plant Pathology, Department of Recombinant Techniques, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250110, India*
*Email: laxmanprasad25@yahoo.com*

*Trichoderma* spp. has been widely used as bio-control agents under greenhouse and field conditions, due to their ability to prey on a diverse range of phytopathogens. *Trichoderma* parasitizes the hyphae of phytopathogenic fungi using prehensile coils and hooks that penetrate the Fusaria mycelia, aided by the hydrolytic activity of chitinases and glucanases. An experiment was conducted to study the functional analysis of chitinase gene expression in native strains of *T. harzianum* during mycoparasitism with *F. oxysporum* f. sp. *lycopersici*. *T. harzianum* and *F. oxysporum* f. sp. *lycopersici* were cocultivated on potato dextrose agar (PDA) medium and incubate at 25℃ in BOD. Mycoparasitic activities of *Trichoderma* were done by using optical microscope at 72 hours after inoculation. Chitinase gene expression study of *T. harzianum* was done by isolation of RNA, preparation of cDNA and its amplification using chitinases gene specific primers. Results indicate that the native strain of *T. harzianum* parasitized the mycelia of *F. oxysporum* f. sp. *lycopersici* and penetrates the *Fusarium* mycelia. It produces coils and hooks along with lysis due to hydrolytic enzyme. RT-PCR amplification indicates that during interaction of both fungi chitinase gene in *T. harzianum* was expressed leading to chitinase enzyme production.

**P23.067 A new insight of race emergence in tomato wilt fungus *Fusarium oxysporum* f. sp. *lycopersici***

*T. Kashiwa, K. Inami, A. Sato, T. Suzuki, Y. Morita, M. Yamasaki, T. Teraoka and T. Arie*
*United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo, Japan*
*Email: tkashiwa712@gmail.com*

Race emergence in plant pathogenic fungi is caused by the mutation of avirulence genes. Takken (2010) reported that in the tomato wilt fungus *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), deletion of *AVR1*, an avirulence gene unique in *Fol* and corresponding to the tomato resistance gene *I*, resulted in the emergence of race 2; point mutations in *AVR2*, corresponding to *I2*, generated race 3. However, we found that a race 3 isolate, KoChi-1 from Kochi, Japan, possessed the *AVR1* truncated by a transposon *Hormin* and the *AVR2* carrying a point mutation, and we proposed a new insight of emergence of races in *Fol* (Inami 2012). No race 2 isolate carrying the *AVR1* truncated by a *Hormin* had been reported, so far. This time we report a new type of race 2, Chiba6, that possesses *AVR1* truncated by a *Hormin* and *AVR2* with no point mutation isolated in Chiba, Japan. *Hormin*-insertion occurs at the different position of *AVR1* between Chiba-6 and KoChi-1, and both of the truncated *AVR1* code defective Avr1 proteins. The isolates Chiba-6 and KoChi-1 belong to different phylogenetic clades A1 and A2 in the tree based on rDNA-IGS (Kawabe 2005), respectively. These suggest that insertion of *Hormin* in *AVR1* in Chiba-6 and KoChi-1 occurred as distinctive events at different positions in the phylogeny, both of which generated the homogeneous race differentiation in *Fol* in different clades.

**P23.068 Genomic studies on resistance of rice to blast, *Magnaporthe oryzae*, at rising temperature**

*G. Onaga[1], K. Wydra[2], B. Koopmann[1], Y. Sere[3]* and *A. von Tiedemann[1]*
*[1]Division of Plant Pathology and Crop Protection, Department of Crop Sciences, Georg-August- University Göttingen, Germany; [2]Georg-August-University Göttingen, CLB-Tropical and Subtropical Agriculture and Forestry, Germany; [3]Africa Rice Center, Cotonou, Benin*
*Email: onafrey@yahoo.com*

Elevated global temperatures are a putative key factor of expected climate change. Temperature also plays a prime role in plant disease development and resistance

responses of crop plants. Rice blast, caused by *Magnaporthe oryzae*, infects rice and other cereal crops in more than 85 tropical and sub-tropical countries globally. Yield losses ranging from 50–70% have been reported, sometimes reaching 100% when predisposing factors favor epidemic development. *M. oryzae* infection is dependent on temperature, humidity and the resistance response determined by the host genetic background. Our studies focus on identifying host genetic factors from different genetic backgrounds that contribute to resistance as affected by rising temperature. We investigated host phenotypic reaction and genome-wide gene expression profiles of two genetic backgrounds (Co39 and LTH), carrying the same R gene, exposed to *M. oryzea* infection, at two temperatures (28℃ and 35℃). Our results show a delay in disease progression in both genetic backgrounds at 35℃ compared to 28℃, demonstrating that high temperature enhances resistance and increases the incubation period of the pathogen. Gene expression data show that there are only small differences between the two genetic backgrounds, with temperature playing a significant role in influencing the plant response to *M. oryzae* infection. The small changes due to the genetic background and the influence of temperature may be significant in determining the resistance potential of the host. Further analysis and gene ontology on the various transcripts identified is ongoing, and is expected to provide important information to design more efficient breeding programs in order to produce rice varieties better adapted to both high temperature and rice blast conditions.

**P23.069 Nitrogen fertilization of rice plants affects *Magnaporthe oryzae* infection process and rice resistance**

*E. Ballini[1], T. N'guyen[1], H. Huang[2], X. He[2], J.L. Notteghem[1] and J.B. Morel[3]*
*[1]SupAgro, UMR BGPI INRA/CIRAD/SupAgro, Montpellier, France; [2]College of Plant Protection, Yunnan Agriculture University, Kunming, Yunnan, P.R. China; [3]INRA, UMR BGPI INRA/CIRAD/SupAgro, Montpellier, France*
*Email: ballini@supagro.inra.fr*

Nitrogen-Induced Susceptibility (NIS) of plant diseases is a widespread phenomenon. We set an experimental system in which nitrogen supply strongly affects rice blast susceptibility whereas it is only slightly perturbing plant growth. We show that fungal growth is affected before and after penetration in the plant but that the final penetration rate is not affected. Differences in total nitrogen amount and defense gene expression before and after infection are unlikely to be responsible for the observed increase in penetration. On the other hand, the fungus seems to perceive small differences in nitrogen amount after penetration and this may explain enhanced growth under high nitrogen regime. Indeed, exogenous treatment with some free amino acids after inoculation mimicked NIS, further arguing that this phenomenon is mostly due to a trophic relation between the plant and the fungus. We also used our experimental system to show that the capacity of rice to display NIS is highly polymorphic and does not correlate with difference related to indica/japonica sub-groups. However, nitrogen partially breaks down resistance triggered by the *Pi1* gene. Using the CSSL mapping population between Nipponbare and Kasalath, we identified a Kasalath locus on chromosome 1, called *NIS1*, which dominantly increases susceptibility under high nitrogen. We discuss the possible relationships between Nitrogen Use Efficiency (NUE), disease resistance regulation and NIS. This work provides evidences that robust forms of partial resistance exist across diversity and can be genetically mapped. This work also suggests that under certain environmental circumstances, complete resistance may breakdown.

**P23.070 Transcriptome profiling of *Heterobasidion annosum* during saprotrophic growth in wood and under abiotic stress**

*T. Raffaello, H. Chen and F. O. Asiegbu*
*Department of Forest Sciences, University of Helsinki, Finland, Latokartanonkaari 7, P.O. Box 27, 00014, Helsinki, Finland*
*Email: tommaso.raffaello@helsinki.fi*

The basidiomycete *Heterobasidion annosum* is a filamentous fungus which is considered to be one of the most destructive conifer pathogen in temperate forests of the northern hemisphere. This study investigates the changes in the transcript profiles when the fungus is exposed to general abiotic stress compared to the growth in natural pine wood material. *H. annosum* was grown under different abiotic stresses (osmotic, temperature, nutrient starvation, and oxidative stresses) and saprotrophic conditions (pine bark, heartwood, and sapwood wood material) and microarray experiments were performed. The data analysis reveals that the transcriptome profiles during saprotrophic growth (pine wood material) differs clearly from the profile relative to abiotic stresses (osmotic, oxidative, temperature, and nutrient starvation) indicating the activation of genes that are specifically involved during saprotrophic growth. On the other hand, the strong activation of basic intracellular pathways and up-regulation of protein kinases suggest a rapid adaptation response of *H. annosum* during changes in environmental conditions (simulated by the abiotic stresses). Interestingly, different enzymes involved in wood degradation (laccases, glycosyl hydrolases, carbohydrate esterases, polysaccharide lyases, and cytochrome P450) were selectively up-regulated in different wood compartments (bark, sapwood or heartwood) suggesting preferential adaptation of *H. annosum* to these wood components. We concluded that changes in the environmental conditions (abiotic stress) leads to preferential

activation of signalling proteins (i.e. Kinases) to facilitate rapid fungal colonization. Consequently, prolonged growth in the wood tissues requires specialized battery of enzymes to be able to degrade and penetrate the specific substrate.

**P23.071 Identification of small secreted proteins from *Hemileia vastatrix*, candidate effectors of coffee leaf rust disease**

*C. Gonçalves[1,2], S. Tavares[1], H.G. Azinheira[1], H. Oliveira[1], M.C. Silva[1] and P. Talhinhas[1]*
*[1]Centro de Investigação das Ferrugens do Cafeeiro/ BioTrop/Instituto de Investigação Cientfica Tropical. Quinta do Marquês. 2780-505 Oeiras, Portugal; [2]Departamento de Ciencias e Engenharia de Biossistemas/Instituto Superior de Agronomia/Universidade Tecnica de Lisboa, Lisboa, Portugal*
*Email: sagtavares@gmail.com*

*Hemileia vastatrix* is an obligate biotrophic rust fungus responsible for one of the most destructive and widespread disease of coffee plants. In the last decade an increasing number of studies attributed to small-secreted proteins (SSP) from plant pathogens a role as effectors in host–pathogen interactions. ESTs from three mRNA samples, germinated urediniospores, appressoria and 21-day rust-infected leaves were sequenced by 454 pyrosequencing technology. A bioinformatics pipeline allowed identifying sequences encoding putative fungal SSPs. A search for new motifs in the pool of SSPs was conducted using the MEME algorithm, since typical effectors do not share common features or conserved domains. Four sequences were selected on the basis of sharing motifs, constituting a group named HC49 (one of the elements has homology to HESP-C49 of *Melampsora lini*). A second group, comprising two sequences, was named H178 (by homology with HESP-178 of *M. lini*). All sequences were rich in the amino acid cysteine, and the group H178 also in proline, and only expressed *in planta*, with the exception of Hv04352 (group HC49). Transcript profiling of the selected SSP showed distinct pattern between the sequences of group HC49 and H178 during the infection process. The group HC49 showed high induction of gene expression in coffee leaves seven days after inoculation with *H. vastatrix* when the presence of haustoria is elevated. The imnunolocalization of selected candidates in the two groups will be discussed. The identification of candidate effectors is central to the recognition of resistance genes in coffee genotypes.

**P23.072 Chitin deacetylase genes from *Hemileia vastatrix* (causal agent of coffee leaf rust), identification and gene expression during the infection cycle**

*L. Azevedo, P. Talhinhas, H.G. Azinheira, M.C. Silva and S. Tavares*
*Centro de Investigação das Ferrugens do Cafeeiro/ BioTrop/Instituto de Investigação Cientfica Tropical. Quinta do Marquês. 2780-505 Oeiras, Portugal*
*Email: sagtavares@gmail.com*

Chitin is a polysaccharide of fungi cell walls. Chitin deacetylases (CDAs) are extracellular chitin-modifying enzymes, which deacetylate chitin to form chitosan, a polymer of -1,4-linked D-glucosamine residues. Chitosan has been described as a minor component of the fungal cell wall. CDA genes identified from various fungal species belong to the carbohydrate esterase family 4 (CE-4). A EST sequencing project of the biotrophic fungus *Hemileia vastatrix*, the causal agent of coffee leaf rust, allowed us the identification of a gene family encoding CDA-like proteins. ESTs from germinated urediniospores, appressoria and 21-day rust-infected leaves disclosed ten CDA-like genes. All translated ESTs, except one, exhibited the catalytic domain of CDAs (polysaccharide deacetylase catalytic domain, pfam 01522) found in CE-4 family. All CDA-like genes were expressed in coffee leaves and germinated urediniospores, although only seven in appressoria. A distinct pattern of expression was observed for different CDA-like genes on coffee leaves infected with *H. vastatrix* and along the infection process. Several biological roles have been assigned to fungal chitin deacetylases. Our results confirm that, during the infection process, various CDA-like genes are expressed and each gene probably assumes unique roles, linked with the activity of particular proteins. The deacetylase activity of recombinant proteins produced in *Pichia pastoris* in conjunction with the transcript profiling will allow us to discuss the role of CDA-like proteins.

**P23.073 Describing uredospores *in vitro* germination and differentiation of early infection structures of the eucalyptus rust, *Puccinia psidii***

*A.P. Bini , M.C. Quecine and C.A. Labate*
*Laboratory Max Feffer of Plant Genetics, Department of Genetics, Escola Superior de Agricultura "Luiz de Queiroz" – University of São Paulo (ESALQ-USP). 11, Padua Dias Av., 13418-900, Piracicaba-SP, Brazil*
*Email: andressabini@usp.br*

Rust fungi are obligate biotrophic plant pathogens closely associated with their hosts. *Puccinia psidii* is the causal agent of eucalyptus rust, being responsible for economic and yield losses in Brazil and around the world. Due to the biotrophic life style, several tools are being developed to improve *in vitro* germination and differentiation of *Puccinia* uredospores aiming to facilitate molecular studies. Thus, the main goal of this work was to develop a feasible *in vitro* method to induce *P. psidii* uredosporos germination and their differentiation, triggered by chemical and physical signals. *P. psidii*, uredospores were inoculated in both agar medium and in

agar medium supplemented with 0,5% of different lipids, mimicking the leaf wax layer composition as the chemical stimulus. All plates were incubated at 20℃ and microscopy observations were performed 24 and 48 hours after inoculation. The uredospores germination upon agar medium supplemented with olive oil was the most effective (approximately 80%, of germination rate) but, early infection structures were not observed. Defined the best chemical stimulus source, two different membranes (cellophane and dialysis sacks) placed over olive oil medium, were tested as a physical stimulus. Surprisingly, uredospores inoculated over dialysis membranes, germinated and formed appressorium followed by penetration hyphae at high rate (75% of observed uredospores). The described method will be an important approach to facilitate future studies of *in vitro* gene expression at the first stages of fungal host infection, aiming a better comprehension of *P. psidii* biology as well as its interaction with eucalyptus.

**P23.074 *Agrobacterium tumefaciens*-mediated transformation of *Sporisorium scitamineum*, the causal agent of smut in sugarcane**

*G. Carvalho[1], M.C. Quecine-Verdi[1], J. Almeida[1], T. Shyton[1], M. Marrafon[1], S.A.C. Souza[2], A.A. Pizzirani-Kleiner[1] and C.B. Monteiro-Vitorello[1]*
*[1]Escola Superior de Agricultura "Luiz de Queiroz" – ESALQ/USP, Av. Pádua Dias, 11, CEP: 13.418-900, Piracicaba/São Paulo, Brazil; [2]Centro de Cana – IAC, Rodovia Antonio Duarte Nogueira, km 321, CEP: 14.001-970, Ribeirão Preto/São Paulo, Brazil*
*Email: gisellecarvalho05@gmail.com*

The fungus *Sporisorium scitamineum* is the causal agent of smut in sugarcane. Yeast-like haploid cells of sporidial line Ssc39 of opposite mating-types were transformed with the pFAT-gfp plasmid, harbouring the hygromycin B resistance gene *hph* of *Escherichia coli* under the control of glyceraldehyde-3-phosphate dehydrogenase gene promoter region, *gpd*, of *Glomerella cingulata* and *gfp*, the gene encoding green fluorescence protein. Transformation was confirmed by fluorescent cells growing on 50 μg $mL^{-1}$ of hygromycin and PCR amplification of *gfp* and *hph* genes. Southern blot analysis was used to confirm single insertion of the T-DNA region. Agrotransformation was successfully achieved by Southern blot analysis, revealing random insertion of the T-DNA region only one site of insertion per mutant. Transformed haploid cells of opposite mating type were grown together in mating plate experiments confirming hyphal fusion and dikaryotic white mycelial colony establishment. The results proved that the *S. scitamineum* agrobacterium-mediated transformation is an efficient and quick tool to random insertional-muta- genesis aimed at the development of *S. scitamineum* GFP- expressing mutants that may be used for plant colonization monitoring.

**P23.076 Suppression of *lipoxygenase*-, *allene oxide synthase*-, *allene oxide cyclase* and *12-oxo-phytodienoic acid reductase*-mRNA in pea reduces disease development by *Mycosphaerella pinodes***

*K. Toyoda[1], Y. Kawanishi[1], Y. Kawamoto[1], C. Kurihara[1], N. Yamagishi[2], A. Tamura[2], N. Yoshikawa[2], Y. Inagaki[1], Y. Ichinose[1] and T. Shiraishi[1]*
*[1]Laboratory of Plant Pathology and Genetic Engineering, Graduate School of Environmental and Life Science, Okayama University, 1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan; [2]Plant Pathology Laboratory, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka 020-8550, Japan*
*Email: pisatin@cc.okayama-u.ac.jp*

Using a recently developed model pathosystem involving *Medicago truncatula* and *Mycosphaerella pinodes*, the causal agent of Mycosphaerella blight on pea, we identified nonredundant 151 cDNA fragments as newly expressed genes in *M. truncatula* leaves after application of the fungal suppressor. These include genes encoding lipoxygenase (LOX) and enoyl-CoA hydratase, which are presumably involved in jasmonic acid (JA) synthesis. To understand the molecular response to the fungal suppressor, potential genes encoding plastidic enzymes including allene oxide synthase (AOS) and allene oxide cyclase (AOC), and other peroxisomal enzymes involved in *β*-oxidation were predicted from a Medicago Gene Index EST database and tested for altered expression by semi-quantitative RT-PCR. The coordinated expression of genes encoding both plastidic and peroxisomal enzymes showed that the suppressor likely conditions certain cellular process(es) through the JA synthesis in *M. truncatula*. To explore the role of JA or JA-regulated cellular process (es) in conditioning susceptibility, pea genes including *LOX*, *AOS*, *AOS* and *12-oxo-phytodienoic acid reductase* (*OPR*) were silenced using an *Apple latent spherical virus* (ALSV)-based virus-induced gene silencing (VIGS) technology. In *LOX*-, *AOS*-, *AOC*- or *OPR*-silenced pea plants, disease development induced by *M. pinodes* was remarkably reduced. Similarly, silencing of *LOX*, *AOS*, *AOC* or *OPR*-mRNA reduced the sensitivity to a phytotoxin, coronatine, which is believed to act through the JA-dependent process. On the basis of these results, it is conceivable that *M. pinodes* has evolved a strategy to condition susceptibility by manipulating the physiology of host cells, in particular JA-regulated cellular process (es), to promote disease development in pea.

**P23.077 Ecto-apyrase regulates the peroxidase-catalyzed apoplastic oxidative burst in cowpea**

*K. Tanaka[1], K. Toyoda[1], N. Yamagishi[2], N. Yoshikawa[2], Y. Inagaki[1], Y. Ichinose[1] and T. Shiraishi[1]*
*[1]Laboratory of Plant Pathology and Genetic Engineering,*

*Graduate School of Environmental and Life Science, Okayama University, 1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan; [2]Laboratory of Plant Pathology, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550, Japan*
*Email: gag422131@s.okayama-u.ac.jp*

The supprescins A and B from *Mycosphaerella pinodes* are glycopeptide suppressors for defenses, but they act as common elicitors on non-host plants. Recently, one target for the supprescins is proposed to be ecto-apyrase (ecto-ATPase) in the cell wall. Indeed, they can inhibit the ATP-hydrolyzing activity in cell walls of pea, but stimulates the activity of non-host plants such as cowpea, soybean and kidney bean. In this study, cowpea was used to analyze the role of ATP hydrolysis in non-host responses. Pure supprescins induced biphasic generation of SOD-sensitive superoxides ($O_2^{\cdot-}$). Pharmacological studies with inhibitors and antioxidant enzymes showed that the $O_2^{\cdot-}$ generation largely depends on an extracellular peroxidase(s) rather than a membrane-bound NADPH oxidase, because it was sensitive to salicylhydroxamic acid (SHAM). Since NADH inhibitor I-1 completely reduced the $O_2^{\cdot-}$ generation, the oxidation of apoplast NADH (as an electron donor) is likely involved in the peroxidase-catalyzed $O_2^{\cdot-}$ generation. Interestingly, the $O_2^{\cdot-}$ generation was accompanied by a production of a low molecular weight anti-fungal (yet-unidentified) compound(s), which suppresses fungal penetration from appressoria. Silencing of *VsNTPase1* encoding the cowpea ecto-apyrase attenuated the $O_2^{\cdot-}$ generation, allowing to be susceptible to infection by a non-pathogenic fungus. Experiments with adenine nucleotide analogues revealed that ADP enhanced $O_2^{\cdot-}$ generation induced by the supprescins. Moreover, a non-hydrolysable ADP[ ]S alone evoked SHAM-sensitive $O_2^{\cdot-}$ generation. On the basis of these results, it seems likely that ecto-apyrase spatially regulates the peroxidase-catalyzed apoplastic oxidative burst through the hydrolysis of adenine nucleotides, substantially sustaining non-host resistance in cowpea.

**P23.078 Dual roles of a pea infection-inhibitor, dihydromaleimide, in induced resistance in pea**

K. Iio, C. Kamada, T. Watanabe, M. Izumi, Y. Inagaki, Y. Ichinose, K. Toyoda and T. Shiraishi
*Graduate School of Environmental and Life Science, Okayama University, 1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan*
*Email: ag20011@s.okayama-u.ac.jp*

A glycoprotein elicitor from *Mycosphaerella pinodes* induces resistance in pea through the generation of infection-inhibiting compound(s) within 1 h after treatment (Yamamoto et al. 1986). Moreover the infection-inhibiting activity was also generated in isolated cell walls of pea upon treatment with the elicitor. Thus the generation of infection-inhibitor is one of rapid defense responses against invading pathogens. By purification with TLC and HPLC, at least two active compounds were identified in the elicitor-treated cell walls, one of which was identified as dihydromaleimide (DHM). HPLC analysis revealed that DHM accumulated in pea epicotyl tissues (approximately 2.7 nmol/gFW) within 1 h after treatment with the elicitor or 1 mM $CuSO_4$. Dihydromaleimide (above 50 μM) inhibited penetration from appressoria of *M. pinodes*, although it scarcely affects germination and appressorial formation. Penetration by several pathogenic fungi such as *Colletotrichum orbiculare*, *C. destructivum* and *Alternaria alternata* were also inhibited by treatment with above 5 μM DHM. Interestingly, 0.5~5 μM DHM alone, which could not block the penetration, induced rejection reaction at 24 h after treatment and stimulated the transcriptional activation of several defense-related genes at 3 h after treatment in *Medicago truncatula* and *Arabidopsis thaliana* Col-0. Such induced resistance was abolished in Col-0 mutants *eds5*, *npr1*, *jar1* and *pad3*, indicating that DHM-induced resistance is likely associated with phytoalexin production and/or several signal transduction pathways including salicylic acid and jasmonic acid. On the basis of these results, we discuss the role of DHM in induced defense response.

**P23.079 Production of an anti-fungal compound(s) in the extracellular space of cowpea leaves challenged with the fungal elicitor**

M. Uchioki, K. Toyoda, K. Tanaka, Y. Inagaki, Y. Ichinose and T. Shiraishi
*Laboratory of Plant Pathology and Genetic Engineering, Graduate School of Environmental and Life Science, Okayama University, 1-1-1 Tsushima-naka, Kita-ku, Okayama 700-8530, Japan*
*Email: ag421021@s.okayama-u.ac.jp*

In cowpea leaves challenged with the fungal elicitor from a pea pathogen *Mycosphaerella pinodes*, the apoplastic oxidative burst; i.e. superoxide production is induced through the oxidation of NADH (as an electron donor) by an extracellular peroxidase(s), substantially contributing to non-host-resistance of cowpea (see a poster by Tanaka, K. *et al.*). In this study, to clarify the role of inducible defense(s) in the extracellular space of cowpea, an *in vitro* assay with ethanol-killed onion epidermis and phytopathogenic fungus including *Ascochyta punctata* and *Colletotrichum orbiculare* was carried out to examine whether an anti-fungal compound(s) is newly generated in cowpea leaves. When leaf discs from epidermis-peel-off cowpea leaves were floated on the elicitor solution, $O_2^{\cdot-}$ was abundantly released into the test solution within 15 min, accompanied by a production of anti-fungal (yet-unidentified) compound(s) suppressing penetration from appressoria but scarcely affects spore germination. Dilution-end-point analysis for the extra-

cellular solution showed that the putative compound(s) was effective even at one hundred-fold dilution. The compound(s) was a hydrophilic and heat-stable ($95^0$C for 10 min). Separation with ultrafiltration and subsequent HPLC analysis revealed that compounds less than 500 Da were responsible for the penetration inhibition. On the basis of these results, it is more likely that cowpea leaves respond to produce an infection-inhibitor(s) extracellularly upon the fungal elicitor-treatment. Further purification and characterization of the compound(s) are now underway to understand the role of the extracellular defense in non-host resistance of cowpea.

**P23.080 Isolation of pathogenesis-related (PR) genes and resistance (R) gene candidates in bananas and their expression during host-pathogen interaction**

*M.Y. Wong, C.Y. Yang, M. Sariah and Y.H. Tan*
*Department of Plant Protection, Faculty of Agriculture and Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
*Email: muiyun@putra.upm.edu.my*

Two pathogenesis-related (PR) genes, chitinase and beta-1,3-glucanase, and four resistance (R) gene candidates (RGCs) were isolated from four local banana genotypes ['Rastali wild type' (AAB), 'Rastali Mutiara' (AAB), 'Jari Buaya' ('Lady Finger', AA) and 'Rastali Transgenic' (AAB)]. PCR-amplified chitinase sequence showed 99% homologous to *Musa acuminata* class III acidic chitinase (Accession AY525367) and beta-1,3-glucanase sequence was 100% homologous to *Musa acuminata* beta-1,3-glucanase (Accession EF051254). RGC1 has 99% similarity to *Musa acuminate* subsp. *malaccensis* NBS-type protein (RGC3) (Accession EU239821), RGC2 and 3 were 95% similar to *Musa* ABB group NBS resistance protein (RGA-H) (Accession EU855838) and RGC5 showed 84% identity to *Musa acuminata* clone PTO-06 Pto-like serine/threonine kinase gene (Accession AF529042). The former three cultivars were inoculated with *Fusarium oxysporum* f. sp. *cubense* Race 4 (FocR4) and the transcript levels of the mentioned genes were determined semi-quantitatively. Differential gene expression was observed in each genotype tested over time. Chitinase was expressed constantly in all genotypes at all sampling intervals [0, 2 and 4 weeks after inoculation (wai)]. Beta-1,3-glucanase was expressed at 2 wai only in 'Rastali Mutiara' and RGC2 in all genotypes tested but both were down-regulated at 4 wai. Two RGCs were not expressed in all genotype tested. In 'Rastali Mutiara' which is known locally to be tolerant to Fusarium wilt, three out of five target genes were expressed while 'Rastali wild type' and 'Jari Buaya' demonstrated the expression of only two target genes. Chitinase may play an important role in disease resistance against FocR4 as it was expressed in all three genotypes tested.

**P23.081 Differential defense response towards *Pythium myriotylum* in resistant and susceptible *Zingiber* spp. revealed by comparative whole genome RNA-Seq analysis**

*L. Augustine, A.G. Kiran and G. Thomas*
*Plant Molecular Biology, Rajiv Gandhi Centre for Biotechnology, Trivandrum, Kerala, 695014, India*
*Email: lesly@rgcb.res.in*

Ginger (*Zingiber officinale* Rosc.), a valued spice used worldwide, is susceptible to the necrotrophic oomycete *Pythium.* The complete sterility prevents conventional breeding methods to improve *Pythium* resistance in ginger. *Zingiber zerumbet* (L.) Smith, a wild congener of ginger is resistant to *Pythium*. This study aims at understanding the molecular basis of resistance in *Z. zerumbet* following a comparative transcriptome analysis before and 24 hours post inoculation with *Pythium myriotylum*. Sequencing was done on Illumina GA-IIx Platform and the high quality reads were assembled using Velvet 1.1.05. The transcripts were generated using Oases and CAP3 to obtain singletons. *Z. zerumbet* yielded 76302 and 73936 singletons in control and treated samples respectively, whereas 77281 singletons were obtained from untreated ginger and 78317 singletons in treated. The fold changes (digital gene expression) of transcripts were determined by BOWTIE and SAM tool. The gene ontology categorization and pathway mapping was performed using the transcripts. RT-qPCR analysis of a set of transcripts confirmed digital gene expression. RNA-Seq data reveals significant upregulation of genes mediating hormone signalling, hypersensitive response (HR) and pathogen recognition in *Z. zerumbet*, whereas the inductions of genes involved in oxidative stress and general metabolism were found in ginger. Robust induction of the defence related genes seem to play a key role in *Pythuim* resistance in *Z. zerumbet*. In ginger, the transcriptome reprogramming was targeted mainly to protect vital physiological activities. The study highlights the importance of RNA-Seq analysis in drawing inference about the basis of host resistance in a non-model plant.

**P23.082 Incompatible response of *Zingiber zerumbet* (L.) Smith. to the necrotrophic pathogen *Pythium sp.* involves hypersensitive response and salicylic acid signalling**

*A.G. Kiran, L. Augustine, V. Smini and G. Thomas*
*Plant Molecular Biology, Rajiv Gandhi Centre for Biotechnology, Trivandrum, Kerala, 695014, India*
*Email: kiran@rgcb.res.in*

*Zingiber zerumbet,* a wild relative of ginger (*Zingiber officinale* Rosc.) shows incompatible response to the necrotrophic pathogen *Pythium* sp. which causes soft rot in the crop species. To understand gene modulation

leading to the incompatible response, a total of 60 key genes belonging to major defense pathways were analysed using quantitative real time PCR in both the species. Our study revealed transcript induction of genes involved in the biosynthesis of major plant defense hormones; jasmonic acid, ethylene and salicylic acid in post inoculated Z. *zerumbet* whereas in *Z. officinale* only jasmonic acid and ethylene pathway genes were found upregulated. Salicylic acid responsive gene, *PR1* (*Pathogenesis related 1*) was also found to be induced to significantly higher levels in the resistant wild species. Genes involved in phenolic compound production like phenylalanine ammonia lyase, cinnamoyl CoA reductase, caffeic acid o-methyltransferase, flavone 3 hydroxylase and leucoanthocyanidin dioxygenase also had a higher transcript accumulation in *Z. zerumbet.* Reactive oxygen species (ROS) generating genes studied showed similar levels of induction in both the resistant and susceptible pathosystems. However genes responsible for ROS scavenging showed more robust induction only in the resistant wild species. Hypersensitive response (HR) related gene, HSR203J and MAP Kinase involved in HR induction, MAPK3 also showed a higher fold induction in *Z. zerumbet*. The study strongly suggests the incompatible response of the wild species to be HR mediated involving salicylic acid signalling and also phenolic compound production.

**P23.083 Signalling events in *Brassica napus* activated by *Leptosphaeria maculans* effectors**

*L. Burketov á[1], M. Novakova[1,2], V. Sasek[1], O. Valentova[2], I. Fudal[3] and M.H. Balesdent[3]*
*[1]Institute of Experimental Botany, AS CR, Rozvojova 313, Prague, 165 02 Czech Republic; [2]Institute of Chemical Technology Prague, Technicka 5, Prague, 160 00 Czech Republic; [3]INRA, UR 1290 Bioger, Campus AgroParisTech, Thiverval Grignon, France*
*Email: burketova@ueb.cas.cz*

*Leptosphaeria maculans* is a hemibiotroph ascomycete causing a black leg disease of oiseed rape (*Brassica napus*) worldwide. Crop protection against this serious pathogen mostly relies on the exploitation of resistant cultivars, however this resistance is relatively quickly overcome by mutations in formerly recognized avirulence genes. For deep insight into the manipulation of *B. napus* defence by *L. maculans* the knowledge of the effector functions and responding plant signalling is essential. We show that AvrLm1 activates concurrently both salicylic acid and ethylene signalling pathways in a resistant cultivar, which probably correlates with a hemibiotrophic lifestyle of the fungus. AvrLm4-7 is the last cloned effector, which confers resistance in *B. napus* plants harbouring either Rlm4 or Rlm7. Expression study by means of RT-qPCR of the main signalling pathways, regulated by salicylic acid, jasmonic acid and ethylene revealed no suppression of any of the marker genes, however we hypothesize involvement other possible events such as manipulation by reactive oxygen species in *B. napus* leaves by AvrLm4-7.

**P23.084 Bacterial-fungal biodiversity during Ganoderma disease establishment in oil palm (*Elaeis guineensis*)**

*H.J. Tung[1, 2], Y.K. Goh[1], Y.K. Goh[1], K.J. Goh[1] and W.C. Wong[1, 2]*
*[1]Applied Agricultural Resources Sdn. Bhd, No.11, Jalan Teknologi 3/6, Taman Sains Selangor 1, Kota Damansara, 47810 Petaling Jaya, Selangor, Malaysia; [2]Advanced Agriecological Research Sdn Bhd, AAR-UNMC Biotechnology Research Centre, Jalan Broga, 43500 Semenyih, Selangor, Malaysia*
*Email: tunghj@aarsb.com.my*

The biodiversity of microbes in different habitats can bring great impact to disease suppression and assistance yet little is known or studied when it comes to the environment of basal stem rot in oil palm caused by a fungal pathogen *Ganoderma boninense*. In this experiment, we have employed different molecular approaches in an attempt to reveal the microbial diversity in both soil and oil palm trunk tissue via Denaturing Gradient Gel Electrophoresis (DGGE) and Next Generation Sequencing (NGS). The 16S rRNA and 18S rRNA are the key regions used for the microbial identification. From the preliminary investigation, it is found that the different samples tested showed different distribution of the microbes. A conceptual framework of the microbe community distribution towards the disease progression and suppression are proposed. Based on the framework, potential biocontrol which is natural to the agricultural practices and environment can be incorporated in the disease management strategies.

**P23.085 Use of polymerase chain reaction to assess the colonization of *Trichoderma* strains on muskmelon roots in competition with *Monosporascus cannonballus***

*S. Keshavarzi, K. Behboudi and A. Sarpeleh*
*Department of Plant Protection, College of Agriculture & Natural Resources, University of Tehran, Karaj*
*Email: keshavarzi.s@ut.ac.ir*

Root rot and vine decline disease of melon plants induced by *Monosporascus cannonballus*, causes economic losses in many arid and semi-arid production regions worldwide. In this study, the potentials of six different strains of *Trichoderma* antagonistic agent including *T. atroviridae* G1T1 (Ta-1), *T. harzianum* IRAN 1103 (Th-1), *T. harzianum* IRAN 1482 (Th-2), *T. virens* IRAN 1101 (Tv-1), *T. virens* TH 18 (Tv-2), and the commercially available strain, *T. harzianum* T22 (Trianum-P) were assessed on colonization and competition

with *M. cannonballus in situ* conditions. Spore suspensions of all antagonists were used to treat the roots of muskmelon seedling both before (as root dip) and after (as soil drench) transplanting to the soil already inoculated with *M. cannonballus*. The presence and relative quantity of *M. cannonballus* in treated roots was then determined using polymerase chain reaction (PCR) molecular tool 30 days after treatment. Our results revealed a unique 112-bp band belonging to *M. cannonballus* in all samples however; the density of the bands was less in the samples treated with antagonists compared to the control. Comparison of means was obtained by valuation of band density and expanse using UVIgeltec software. The minimum densities of visualized bands were observed in samples treated with Tv-1 and Th-1 strains in both root dip and soil drench tests. The two strains, Tv-2 and Ta-1, showed fewer efficacies than other strains in colonization and competition with *M. cannonballus* in root dip and soil drench method, respectively. Among *Trichoderma* spp. Tv-1 was best in colonization of muskmelon roots against *M. cannonballus*. The strain is capable to decrease pathogen colonization nearly up to control and so, can be used for management of root rot and vine decline disease of muskmelon.

**P23.086 Function characterization of the grapevine *MrRPV1* downy mildew resistance gene**

*L. Yin[1,2], A. Feechan[2], J. Lu[1] and I. B. Dry[2]*
*[1]College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, 100083, P. R. China; [2]CSIRO Plant Industry, PO Box 350, Glen Osmond SA 5064, Australia*
*Email: yin.ling.1985@gmail.com*

*MrRPV1* encodes a TIR-NB-LRR resistance gene from the wild grape species *Muscadinia rotundifolia* that confers resistance to downy mildew (*Plasmopara viticola*). It is co-located with a closely related gene *MrRUN1* (86% aa identity) that confers resistance to powdery mildew (*Erysiphe necator*). These are the first mildew resistance gene to be cloned from a grapevine species. We are characterizing the role of the different protein domains in MrRPV1 in signal transduction, protein targeting and effector recognition. Transient expression of truncated fragments of *MrRPV1* in tobacco leaves demonstrated that the TIR domain is the minimum functional region required for cell death induction (defense signalling). Site-directed mutagenesis of highly conserved amino acids within the MrRPV1-TIR domain, predicted to be involved in self-dimerisation, had no effect on fusion protein expression, but abolished cell-death induction, indicating that TIR dimerisation is essential for MrRPV1 signalling. Recent studies have shown that nuclear localisation of certain NB-LRR proteins is essential for disease resistance. MrRPV1 contains a predicted nuclear localisation signal (RKRRR) in the C-terminal domain. A MrRPV1::GFP fusion protein was shown to localise predominantly to the nucleus in onion epidermal cells and mutation of the RKRR motif blocked nuclear localization, demonstrating this NLS to be functional. To analyse the role of the LRR domain in effector recognition, we transformed *V. vinifera* cv. Shiraz with LRR domain swap constructs of MrRUN1 and MrRPV1. Preliminary results indicate that swapping the LRR domain has changed the specificity of pathogen recognition indicating that the LRR domain is involved in effector recognition.

**P23.087 Functional analysis of Rho GTPases in the wheat scab pathogen *Fusarium graminearum***

*C. Zhang, Z. Zhai, Y. Wang, J. Wang, G. Lu and Z. Wang*
*Key Laboratory of Biopesticide and Chemistry Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, Fujian, 350002, P. R. China*
*Email: Zonghuaw@163.com*

Rho GTPases, as signaling molecules, have multiple cellular functions including regulating metabolism and vesicular trafficking, and pathogenesis. Wheat scab, caused by *Fusarium graminearum*, is one of the most important diseases on wheat. There are six Rho GTPases in *F. graminearum*. To investigate their roles in development and pathogenesis, we functionally characterized all six Rho GTPases in *F. graminearum*. Loss of *FgRHO1* is lethal, suggesting that FgRho1 is essential for fungus survived. While FgRho3 only involved in vegetative growth of fungus, other four Rho GTPases, FgRho2, FgRho4, FgCdc42 and FgRac1, were multi-functional, they were all required for sexual development and pathogenesis. Besides played roles in cell wall integrity as FgRho2, FgRho4 also involved in nuclear division and was essential for septum formation. FgRho4, FgCdc42 and FgRac1 are important in hyphal growth and conidiation. The Δ*Fgrho4*, Δ*Fgcdc42* and Δ*Fgrac1* mutants all grew much slowly and their conidia morphology were abnormal while conidiogenesis. The Δ*Fgrac1* mutant displayed a hyperbranching phenotype and multiple sites germinating, indicating FgRac1 negative controlled hyphal branching and germination. We finally identified FgCla4 as an effector of FgRac1, which however, is not important for sexual development but for other functions as governed by FgRac1. In general, our data show that Rho GTPases contribute diversely to growth, conidiogenesis, sexual reproduction and pathogenesis in *F. graminearum*.

**P23.088 *Phytophthora sojae* effector CRN63 induces plant cell death by interfering with plant catalases**

*M. Zhang, L. Liu, Q. Li, T. Liu, P. Liu, Y. Zhu and D. Dou*
*Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095, P. R. China*
*Email: ddou@njau.edu.cn*

Phytopathogenic oomycetes, such as *Phytophthora sojae*, secrete effector proteins that translocate into host cells to promote infection. However, virulence targets and modes of action of these effectors are largely unknown. *P. sojae* effector CRN63 that is essential for its full virulence can induce cell death in soybean and *Nicotiana benthamiana*. We found that CRN63 associated with catalases (CATs) from both soybean and *N. benthamiana*. Functional analysis of CAT encoding genes indicated they were responsible for degradation of hydrogen peroxide and important for plant immunity. Furthermore, we showed that CATs, normally localized in plant cytoplasm (peroxisome), could be recruited to the nucleus by interacting with effector protein CRN63. Overexpression of *CAT* gene also abolished cell death and hydrogen peroxide accumulation triggered by CRN63. These results together indicated that *P. sojae* CRN63 might interfere with host immunity and hydrogen peroxide metabolism by interacting with and relocalizing host CATs.

**P23.089 Demonstration of the 1, 8-dihydroxynaphthalene melanin pathway in *Ascochyta anemones***

*D. Su and J.F. Fu*
*Department of Plant Pathology, College of Plant Protection, Shenyang Agricultural University, Shenyang, Liaoning, 110866, P. R. China*
*Email: sdsyau@163.com*

Ascochyta leaf spot is one of the major diseases of windflower in China, causing severe yield losses and significantly reducing seed quality. It is caused by *Ascochyta anemones* and largely spread by wind and rain-borne pycnidiospores. Symptoms occur on infected leaves and stems, round spots with brown margin, where pycnidia are arranged in concentric rings. The fungi produce the dark brown to black pigment melanin, which accumulates in the cell walls of hyphae and Pycnidia. Melanin has been implicated as a pathogenicity factor in some phytopathogenis fungi. Three lines of evidence were obtained to suggest that *A. anemones* use the 1, 8-dihydroxynaphthalene melanin (DHN-melanin) biosynthesis pathway: (1) Tricycazole, a specific inhibitor of DHN melanin synthesis, can prevent the accumulation of the melanin in *A. anemones*. Colonies were black on the control medium but had red brown colour on the tricycazole containing media at low concentration. (2) The same specific DHN melanin inhibitor when applied to windflowers reduced disease severity caused by *A. anemones.* (3) Degenerate PCR primers were used to isolate a *PKS* gene of *A. anemones* encoding a polypeptide with high similarity to polyketide synthase (*PKS)* involved in biosynthesis of DHN-melanin in other ascomycetous fungi. A fragment of 724 bp was acquired by PCR amplified of two wild isolates. The *PKS* gene of *A. anemones* possesses 87% and 84% percentage of identity with the *PKS* involved in DHN-melanin biosynthesis in *A.rabiei* and *A. pinodes*, respectively. The results in this study provided powerful evidences that *A. anemones* use DHN biosynthesis pathway.

**P23.090 A multiple-KH domain protein is important for infection-related morphogenesis in the rice blast fungus**

*W. Zhou, J. Yang, Z. Li, J. Peng, D. Chen, X. Chen and Y.L. Peng*
*State Key Laboratory of Agrobiotechnology and Ministry of Agriculture Key Laboratory of Plant Pathology, China Agricultural University, Beijing, P. R. China*
*Email: pengyl@cau.edu.cn*

KH domain-containing proteins are widely conserved and play diverse roles in post-transcriptional gene control. However, few studies deal with their roles in pathogenesis of plant pathogenic fungi. Here, we identified a novel virulence gene *PCG6* in the rice blast fungus *Magnaporthe oryzae* that encodes a protein with multiple KH domains. Deletion of *PCG6* led to severely attenuated virulence toward host plants. Microscopic observation showed that the deletion mutant was reduced in appressorium formation, penetration peg formation, and invasive growth of infection hyphae during infection process. In addition, the deletion mutant was also significantly reduced in vegetative hyphal growth and conidiation. Pcg6 encodes a cytoplasmic protein that is expressed in mycelia, conidia, appressoria, and infection hyphae. Interestingly, the expression level of *GLT1*, a gene for glutamate synthase, was markedly increased in the *pcg6* null mutant as compared with the wild-type strain. Consistently, over-expression of *GLT1* in the wild-type strain exhibited retarded growth of vegetative hyphae. Taken together, *PCG6* for a multiple KH domain protein in *M. oryzae* is involved in the infection-related morphogenesis and asexual developments possibly by regulating mRNA level of *GLT1*.

**P23.091 *N*-glycosylation of effector proteins by an alpha-1,3-mannosyltransferase is required to evade host innate immunity by the rice blast fungus**

*X.L. Chen, T. Shi, J. Yang, D. Chen, X. Xu, J.R. Xu, N.J. Talbot and Y.L. Peng*
*State Key Laboratory of Agrobiotechnology and Ministry of Agriculture Key Laboratory of Plant Pathology, China Agricultural University, Beijing, P. R. China*
*Email: pengyl@cau.edu.cn*

Plant pathogenic fungi deploy secreted effector proteins to suppress plant immunity responses. These effectors operate either in the apoplast or within host plant cells, but post-translational regulation of their activities has not been explored. In this study, Alg3-mediated *N*-glycosylation of the effector protein Slp1 was found to be essential for its activity in the rice blast fungus *Magnaporthe oryzae*. *ALG3* encodes an α-1,3-manno-

syltransferase for *N*-glycosylation of proteins. Targeted deletion of *ALG3* resulted in a significant reduction in virulence and arrested development of infection hyphae. Similar to *Δslp1* mutants, *Δalg3* mutants induced massive production of reactive oxygen species (ROS) in host cells. Interestingly, invasive growth of the *Δalg3* mutant was recovered in plant cells pretreated with the NADPH oxidase inhibitor, diphenyleneiodonium. The Slp1 effector protein sequesters chitin oligosaccharides to avoid their recognition by the rice chitin elicitor binding protein (CEBiP) and the induction of innate immune responses including ROS production. We demonstrated that Slp1 is an *N*-glycosylated protein with three *N*-glycosylation sites. Simultaneous *N*-glycosylation of all three sites is required for Slp1 to be functional and *ALG3* is required for full *N*-glycosylation of Slp1. These results indicate that Alg3-mediated *N*-glycosylation of Slp1 is required to evade host innate immunity.

**P23.092 The γ-subunit of SNF1 complex is essential for pathogenicity by responding to carbon source and pH signals in the rice blast fungus**

*X. Xu, X. Chen, J. Yang, W. Zhao and Y.L. Peng*
*State Key Laboratory of Agrobiotechnology and Ministry of Agriculture Key Laboratory of Plant Pathology, China Agricultural University, Beijing, P. R. China*
*Email: pengyl@cau.edu.cn*

The SNF1 protein kinase plays important roles in cellular responses and is required for energy homeostasis in eukaryotes. The SNF1 is a heterotrimer complex, which contains a catalytic α subunit and two noncatalytic regulatory subunits (β and γ). Here, we address the roles of the γ-subunit SNF4 in *Magnaporthe oryzae*. Deletion of *MoSNF4* led to significant reduction of hyphal growth, conidiation, and lost the whole virulence to barley and rice. During infection, only half conidia of *Δmosnf4* formed appressoria and the intracellular extension was dramatically blocked in the barley epidemis cells. When growth in different carbon sources, we found that *Δmosnf4* cannot grow in the galactose and the growth rates were sharply reduced in the medium with NaAc, glycerol and ethanol as sole carbon source, which indicated that the *MoSNF4* is involved in alternative carbon source assimilation. In addition, *Δmosnf4* was sensitive to acidic pH. Next observation found that the peroxisome distribution of *Δmosnf4* was abnormal compared to the wild type, suggesting *MoSNF4* could take part in the regulation of peroxisome formation. Taken together, the MoSNF4 is function as a regulator subunit of SNF1 by responding to alternative carbon source and acidic pH, and is important for the virulence of the rice blast fungus.

**P23.093 Characterization and functional analysis of a U-Box protein VaPUB from *Vitis amurensis* Rupr**

*L. Jiao, Y.L. Zhang and J. Lu*
*College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, 100083, P. R. China*
*Email: lijiao022@gmail.com*

U-box domain proteins, the most recently identified family of E3 ligases, play important roles in plant development, plant defence and plant stress responses. In this study, the gene *VaPUB* (*Vitis amurensis* Rupr. Plant U-box protein) was isolated from the *Vitis amurensis* Rupr. Zuoshan1. The cDNA sequence of *VaPUB* was 1,329 bp. The open reading frame encoding a protein of 442 amino acid residues, with protein molecular weight of 48.6 kD and isoelectric point of 8.0. The predicted protein showed the U-Box domain from amino acid residues 35 to 98. According to the sequence published on GENESCOPE website, the *VaPUB* have the high homology (99.25%) with a CMPG1 protein GSVIVT01011616001 in Pinot Noir. Besides, the result of real-time quantitative PCR indicated that *VaPUB* was triggered rapidly and transiently by *Plasmopara viticola* infection. Taken together, these data suggested that *VaPUB* was a particularly fast-responding gene which possessing a U box domain and might play an important role in host plant defense against *P. viticola* infection in grapevine.

**P23.094 Prediction and functional analysis of protein kinase CK2 in *Magnaporthe oryzae***

*L.H. Zhang, J. Lin, G.D. Lu and Z.H. Wang*
*Plant Protection of science, Fujian Agriculture and Forestry University Fuzhou, Fuzhou Fujian, 350002, P. R. China*
*Email: guodonglu@yahoo.com*

Rice blast fungus, *Magnaporthe oryzae*, is an important model organism for the study of fungal growth, development, pathogenic mechanism and interaction between host plant and pathogenic fungus. Protein kinase CK2 that has been found in all eukaryotic organisms is a conservative serine/threonine kinase that is typically consisted in tetrameric complexes consisting of two catalytic subunits and two regulatory subunits. In *M. oryzae*, the catalytic subunit of CK2 is encoded by gene MGG_03696; the two regulatory subunits are encoded by genes MGG_00446 and MGG_05651. The two regulatory subunits deletion mutants has a defect phenotype in the vegetative growth, sporulation and pathogenicity; but the catalytic subunit deletion mutant is lethal. Predictive analysis of bio-informatics, there is an obvious interaction phenomenon between CK2 and MoRho3 and the experiment of yeast two hybrid has verified the conclusion. The experiments is needed to be done to test whether the interaction is also existed in the CK2 and other small G proteins Rac1, cdc42, Rho1, Rho2 and Rho4. The interaction between CK2 and MoRho3 reveals the complex signal transduction and provides the

theoretical basis for the research the pathogenic mechanism in the *M. oryzae*.

### P23.095 Laser capture microdissection of Sclerotinia-infected leaf tissue

*M. Belmonte, X. Mao and D. Fernando*
*Department of Biological Sciences, 50 Sifton Road, University of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2*
*Email: belmonte@cc.umanitoba.ca*

Host pathogen interactions are controlled by complex gene regulatory networks and are specified in a cell and tissue-specific manner in both space and time. The interaction between the fungal pathogen *Sclerotinia sclerotiorum* and the host plant *Brassica napus* (canola) has devastating effects around the globe and is particularly hard hit in the western provinces of Canada. Thus, understanding the plant's response to this aggressive pathogen is paramount in the identification of genes and gene products responsible for resistance. While our understanding of the host pathogen interaction is becoming clearer, there is remarkably little information available for Sclerotinia, especially its pathogenicity against canola. Moreover, we know nothing about how this interaction is specified at the genetic or cellular level. Therefore, we have taken an initiative to identify all of the genes expressed in every cell and tissue of the canola leaf when subjected to the Sclerotinia fungus. We used a combination of laser capture microdissection and RNA sequencing technology to provide the first and only profile of gene activity in cells of the canola leaf before during and after the infection process in infected and un-infected cells. We have complemented the genetic data with a complete histological analysis of the interaction between Sclerotinia and canola from the earliest stages of infection. Taken together, patterns of gene activity and histological observations reveal putative candidate genes in the transcriptional regulation of disease susceptibility and resistance.

### P23.096 Analysis of cell death mediated by the coiled-coil domain of rice blast resistance proteins

*P.Q. Liu, Y. Shi, Y. Yang, Y. Lei, Y.Y. Huang, Q. Li, Y. Li, J.M. Deng, P. Li and W.M. Wang*
*Rice Research Institute, Sichuan Agricultural University, Chengdu 611130, P. R. China*
*Email: rlpi2012@163.com*

Most characterized rice blast resistance genes encode coiled-coil-nucleotide-binding-site-leucine-rich repeat (CC-NBS-LRR) proteins conferring race-specific immune responses that are culminated in cell death. Because some CC domains of CC-NBS-LRR proteins from dicots are sufficient to initiate defense responses including cell death, it is intriguing to know whether the CC domain of rice blast resistance proteins has similar function. Thus, as the first step to dissect the signaling transduction mediated by the CC-NBS-LRR types of rice blast resistance proteins, we undertook a subcellular localization test by fusion of a fluorescence protein to the CC and NB domains of a few rice CC-NBS-LRR proteins and transiently expressed in leaves of *Nicotiana benthamiana*. As expected, the tested NBS domains were all mainly located in nucleus; whereas, we observed two localization patterns among the tested CC domains, which were specifically located in cytoplasm and in nucleus, respectively. Interestingly, ectopic expression of the nucleus-located CC domain led to nucleolus disintegration and subsequently cell death, while the cytoplasm-located CC domains did not cause any obvious phenotypes. Our data imply that there is presumably distinct mechanism in signaling transduction of initiating cell death for different rice blast resistance proteins and certain CC domain is sufficient to initiate immune response.

### P23.097 RasGEF regulates mycelial growth, sporulation and pathogenesis of *Colletotrichum higginsianum*

*Q.N. Gu, L. Zheng, Y.D. Wei and J.B. Huang*
*The Key Lab of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan, Hubei, 430070, P. R. China*
*Email: qngu@webmail.hzau.edu.cn*

To discover pathogenicity genes of the crucifer anthracnose fungus, *Colletotrichum higginsianum*, a collection of approximately 2000 random-insertional trans-formants were generated with *Agrobacterium tumefaciens*-mediated transformation (ATMT) protocol. Pathogenicity tests of all ATMT transformants from this library were carried out by inoculating conidial suspensions ($1\times10^6$ conidia/ml) onto detached and attached *Arabidopsis thaliana* (*Arabidopsis*) leaves, and then five mutants impaired at different stages of their development on *Arabidopsis* was obtained. Among these, one mutant Ch-1-G281 did not cause any visible disease symptoms in detached or attached *Arabidopsis* leaves. Southern blot analysis indicated that Ch-1-G281 had a single T-DNA insertion. The T-DNA tagged gene RasGEF was identified by recovering flanking sequences with inverse PCR. To determine whether RasGEF is linked to phenotypes of mutant Ch-1-G281, a gene replacement vector p3300neoRasGEF was constructed for gene knockout experiment. The mutant Ch-1-G281 and the knockout mutant Δ*rasgef* showing slow mycelial growth and less sporulation, were significantly different from the wild-type on potato dextrose agar (PDA). By microscopic analysis, the abnormal dark hyphae grown from appresorium of Ch-1-G281 and Δ*rasgef* at 4 dpi were observed on the surface of *Arabidopsis* leaves and failed to infect into the *Arabidopsis* leaves. Hence, RasGEF

should be involved in mycelial growth, sporulation and pathogenesis of *C. higginsianum*. In order to further confirm the function of the gene, studies of complementation and localization of RasGEF will be conducted.

**P23.098 Proteomics identification of differentially expressed leaf proteins in response to *Setosphaeria turcica* infection in resistant maize**

*X.L. Zhang[1], B.W. Si[2], C.M. Fan[1], H.J. Li[1], S.L. Sun[1] and X.M. Wang[1]*
*[1]Institute of Crop Sciences/National Key Facility for Crop Gene Resources and Genetic Improvement, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Haidian District, Beijing 100081,P.R. China; [2]Institute of Feed Research, Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Haidian District, Beijing 100081, P. R. China*
*Email: wangxiaoming@caas.cn*

Northern corn leaf blight (NCLB), caused by the heterothallic ascomycete fungus *Setosphaeria turcica*, is a destructive foliar disease of maize and represents a serious threat to maize production worldwide. A comparative proteomic study was conducted to explore the molecular mechanisms underlying the defense responses of the maize resistant line A619 *Ht2* to *S. turcica* race 13. Leaf proteins were extracted from mock and *S. turcica* infected leaves after inoculated for 72 h and analyzed for differential protein expression using two-dimensional electrophoresis and mass spectrometry identification. One hundred and thirty-seven proteins showed reproducible differences in abundance by more than 2-fold at least, including 50 up-regulated proteins and 87 down-regulated proteins. Forty-eight protein spots were successfully identified by MS analysis, which included 10 unique, 6 up-regulated, 20 down-regulated and 12 disappeared protein spots. These identified proteins were classified into 9 functional groups and involved in multiple functions, particularly in energy metabolism (46%), protein destination and storage (12%), and disease defense (18%). Some defense-related proteins were upregulated such as β-glucosidase, SOD, polyamines oxidase, HSC 70 and PPIases; while the expressions of photosynthesis- and metabolism-related proteins were downregulated, by inoculation with *S. turcica*. The results indicated that a complex regulatory network was functioned in interaction between the resistant line A619 *Ht2* and *S. turcica*. The resistance processes of A619 *Ht2* mainly resided on directly releasing defense proteins, modulation of primary metabolism, affecting photosyntesis and carbohydrate metabolism.

**P23.099 Function, evolution, and interaction of the coupled genes responsible for the *Pik-h* encoded blast resistance of rice**

*C. Zhai[1], Y. Zhang[1], L. Hua[1], N. Yao[2], F. Lin[1], Z. Liu[2], Z. Dong[1], L. Wang[1], L. Wang[1] and Q. Pan[1]*
*[1]National Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Rice Blast Research Center, South China Agricultural University, Guangzhou, P. R. China; [2]National Key Laboratory of Biocontrol, College of Life Sciences, Sun Yat-sen University, Guangzhou, P. R. China*
*Email: panqh@scau.edu.cn*

*Pik-h*, which is an allele of *Pik*, confers resistance against certain races of rice blast. Its positional cloning showed that it comprises a pair of NBS-LRR genes, *Pikh-1* and *Pikh-2*. The allele is distinguishable from other known blast resistance genes on the basis of key variable nucleotides, and SNP diagnosis among the five rice populations implies that it appears to be the most recently evolved of the set of *Pik* alleles. Comparisons between the sequences of *Pik-h* and other *Pik* alleles showed that the functional K haplotype exists as two sub-haplotypes, which both evolved prior to the domestication of rice. While *Pikh-1* appears to be constitutively transcribed, the transcript abundance of *Pikh-2* responds to pathogen challenge, suggesting that while Pikh-1 may well be involved in elicitor recognition, Pikh-2 is more likely to be responsible for downstream signalling. *In vitro*, the CC domain of Pikh-1 was shown interact directly with both AvrPik-h and Pikh-2. Transient expression assays demonstrated that Pikh-2 mediates the initiation of the defence response. In the proposed *Pik-h* resistance pathway, it is suggested that Pikh-1 acts as an adaptor between AvrPik-h and Pikh-2, while Pikh-2 transduces the signal to trigger *Pik-h*-specific resistance.

**P23.100 A novel removing PCR method for isolation of *Blumeria* specialized genes**

*J. Huan, K. Wan and W. Dong*
*Department of Plant Pathology, College of Plant Science and Technology and the Key Lab of Crop Disease Monitoring & Safety Control in Hubei Province, Huazhong Agricultural University, Wuhan 430070, Hubei Province, P. R. China*
*Email: dwb@mail.hzau.edu.cn*

Researchers always need to know which genes are present or absent and which genes are induced or repressed. Although the next generation high-throughput sequencing technology is available, it is still expensive, and post-sequencing data analysis is complicated. PCR is a fast, inexpensive, and widely used technique to amplify desired genes. However, how undesired genes are removed from a gene pool, which includes both desired and undesired genes, is still a challenging question. A novel Removing PCR, which is a Reverse process of PCR or Restriction PCR (R-PCR), has been developed for the elimination of undesired DNA fragments. Compared with PCR, major components of the R-PCR include removing primers (R-primers), a thermostable

restriction enzyme-*Ape*KI, and an adapter. The R-primers were generated from undesired genes. In each cycle of R-PCR, R-primers are annealed to complementary sequences in a mixed gene pool, which includes desired and undesired genes. In this gene pool, only the undesired ones can match R-primers and allow extension by Taq DNA polymerase. Thus, *Apek*I restriction sites of the undesired genes were recovered, and adapters of these undesired fragments were removed. The R-PCR method can be applied to remove undesired genes, whereas the PCR can be applied to amplify desired genes. *Blumeria graminis* f. sp. *hordei* (Bgh) and f. sp. *tritici* (Bgt) are serious pathogens in barley and wheat, respectively. By using the R-PCR, in total, we isolated 121 *Blumeria* specialized DNA fragments, including 10 pathogenesis effector related genes. In Bgh and Bgt speciation, most effectors represent species- specific adaptations.

**P23.101 Functional diversification between the two broad-spectrum resistance proteins RPW8.1 and RPW8.2 in *Arabidopsis***

*Y. Li[1], X.F. Ma[1,2], Y. Lei[1], Z.J. Yang[3], Y.G. Lu[1], Y.J. Xu[1], Y.Y. Huang[1], Y. Shi[1], T.T. Wang[1], J.Q. Zhao[1], J.L. Sun[1], S. Xiao[2] and W.M. Wang[1,2]*
*[1]Rice Research Institute, Sichuan Agricultural University, Chengdu 611130, P. R. China; [2]Institute for Biosciences and Biotechnology Research and Department of Plant Sciences & Landscape Architecture, University of Maryland, College Park, MD 20742, USA; [3]College of Life Science, University of Electronic Science and Technology, Chengdu, P. R. China*
*Email: j316wenmingwang@163.com*

The Arabidopsis resistance locus *RPW8* contains two adjacent broad-spectrum resistance genes, *RPW8.1* and *RPW8.2*. RPW8.2 is specifically targeted to the extrahaustorial membrane (EHM) that encases the feeding organ (haustorium) of powdery mildew, whereby RPW8.2 activates haustorium-targeted defense. RPW8.1 shares only 45% identity and 65% similarity in amino acid sequences with RPW8.2, raising a question whether there is functional diversification between these two resistance proteins. Using fluorescent protein-tagged constructs, we show that while expression of RPW8.2 from its native promoter is induced by powdery mildew and restricted to mildew-infected epidermal cells, expression of RPW8.1 from its native promoter is constitutive albeit discretely accumulated in clusters of mesophyll cells and further inducible upon mildew infection in mesophyll cells beneath the infected epidermal cell. In contrast to RPW8.2's EHM-specific subcellular localization, RPW8.1 is surrounding chloroplasts in mesophyll cells. Interestingly, when expressed by the *RPW8.2* promoter, RPW8.1 is also specifically targeted to the EHM in the infected epidermal cells, while retains a certain level of expression surrounding the chloroplasts in the mesophyll cells underneath the invaded epidermal cells. By contrast, RPW8.2 is rarely detectable in either type of cells when expressed by the *RPW8.1* promoter. Importantly, Ectopic expression of RPW8.1-YFP from its native promoter and the *RPW8.2* promoter in mildew-susceptible Arabidopsis plants confers enhanced resistance to both powdery mildew and downy mildew. Taken together, our results demonstrate that while RPW8.1 also possesses the EHM-targeting capacity, there is functional diversification between RPW8.1 and RPW8.2 with regard to spatiotemporal expression and subcellular localization, implying a concerted action from both RPW8.1 and RPW8.2 in conferring broad-spectrum mildew resistance in Arabidopsis.

**P23.102 Phylogenetic and transcriptional study of an expanded bZIP transcription factor family in *Phytophthora sojae***

*W.W. Ye, Y. Wang, S.M. Dong and Y.C. Wang*
*Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095, P. R. China*
*Email: wenwuye@gmail.com*

The basic leucine zipper transcription factor family, one of the largest and most diverse families and presents exclusively in eukaryotes, is responsible for central developmental and physiological processes of plants and additionally affects the host pathogenicity of pathogens. But little is known in *Phytophthora sojae*, one of the most studied fungus-like oomycete plant pathogens. In this study, a total of 71 bZIP TF candidates were bioinformatically identified in *P. sojae*, this number was larger than previous known. For comparison, bZIPs in other four oomycetes, two diatoms, and two fungi were also predicted and revealed the *Phytophthora* genomes encoded a larger bZIP TF family. That was found because of a set of novel members containing bZIP domains with substitutions at the conserved DNA binding sites. The major novel types bZIPs could not find expansion even presence in any other species. Phylogenetic analyses revealed that they located in distinct evolution clades and expanded by extensive gene duplication. Gene expression profiling of the *P. sojae* bZIP family revealed diverse transcription patterns but with greater relation to transcription shifts during either zoospores-cysts or host infection stages. Many bZIPs were discovered with regard to the transcription response of oxidative stress at one or more time point(s) post $H_2O_2$ treatment. The identification of novel types bZIP domains with substitutions at the DNA binding region implied novel targets in *Phytophthora* that would be associated with their specific stages in life cycle such as the zoospores and oospores stages and their phylogeny.

**P23.104 RNA-Seq reveals infection-related global gene changes in *Phytophthora capsici***

*X.R. Chen, Y.P. Xing, Y.P Li, Y.H. Tong and J.Y. Xu*

*College of Horticulture and Plant Protection, Yangzhou University, Wenhui Eastern Road 48, Yangzhou, Jiangsu Province, 225009, P. R. China*
*Email: xrchen@yzu.edu.cn*

*Phytophthora capsici* is a soilborne oomycete pathogen capable of infecting a wide range of plants, including many solanaceous crops. However, genetic resistance and fungicides often fail to manage *P. capsici* due to limited knowledge on the molecular biology and basis of *P. capsici* pathogenicity. To begin to rectify this situation, we used Illumina RNA-Seq to perform massively parallel sequencing of three cDNA samples derived from *P. capsici* mycelia (MY), zoospores (ZO) and germinating cysts with germ tubes (GC). Approximately 11 million sequence reads for each cDNA library were sequenced. After read mapping to the gene models of the *P. capsici* reference genome, 13901, 14633 and 14695 genes were identified from the sequenced tags for the MY, ZO and GC libraries, respectively. Comparative analysis between two of samples showed major differences between the expressed gene content of MY, ZO and GC stages. A large number of genes associate with specific stages and pathogenicity were identified with gene description, gene ontology and clusters of orthologous group terms. The transcriptional levels of 50 effector genes during the developmental and infection stages of *P. capsici* were monitored by RT-PCR. Expression analyses in *Nicotiana benthamiana* showed that *P. capsici* RXLRs and CRNs can suppress host cell death triggered by elicitins and other elicitors. This study provides a first look at the transcriptome of *P. capsici* during important pre-infection stages and its encoded effector arsenal.

### P23.105 Identification of target proteins associated with PsGPA1 involved in chemotaxis to soybean isoflavones

*X. Zhang, Y.J. Hao, P.P. Nie, S.M. Dong and Y.C. Wang*
*Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, P. R. China*
*Email: 2009202014@njau.edu.cn*

*Phytophthora sojae* has a narrow host range and is one of the most important pathogens which causes the root and stem rot of soybean. Zoospore is a key stage in the life cycle of *P. sojae*. Whose chemotaxis to isoflavones is believed to be critical for host recognition and initiating infection. To investigate the role of G-protein in chemotaxis, we analyzed the expression of several genes known to be involved in these pathways. The G-protein alpha subunit *PsGPA1* was identified and characterized, which was specifically expressed in sporangia and zoospores but not in mycelium. *PsGPA1*-deficient stable transformants were obtained by gene silencing strategies. Our results showed that zoospore behavior including chemotaxis to the soybean isoflavone daidzein was severely impaired in the silenced transformants. *PsGPA1* silencing also affected zoospore encystment and cyst germination and led to the infectious inability of the *PsGPA1*-silenced mutants to soybean. To better understand the G-protein signaling in chemotaxis, we used yeast two-hybrid screens to identify *PsGPA1*-associaing proteins. We found that *PsGPA1* physically interacted with the receptor for activated C-kinase 1 homologue gene *PsRACK1*. *PsRACK1* silencing reduced distinctly the number of oospores but did not impair chemotaxis of zoospore, which suggested that *PsRACK1* may modulate downstream pathways independent on the chemotactical signal controlled by *PsGPA1*.

### P23.106 Variation of pathogenicity of *Plasmopara vicola* on different resistant grape varieties

*X.L. Li, Y.L. Zhang and J. Lu*
*College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, 100083, P. R. China*
*Email: L.Xinlong123@gmail.com*

Seven isolates of *Plasmopara viticola*, the causal agent of downy mildew on grape, were collected from seven different resistant grape varieties of China. The isolates were tested for their pathogenicity by sporulation density on leaf discs of the highly susceptible variety Thompson seedless. Significant differences were observed between all seven isolates. The highest sporulation density was isolate JL-7-2 found on the highly resistance variety Beta($9.1\times10^4$ sporangia/ml), the next were isolates found on the resistant (or partially resistant) varieties ($7.5\times10^4$-$4.3\times10^4$ sporangia/ml), the lowest was isolate PL-3-1 found on the susceptible variety Cabernet Sauvignon($2.6\times10^4$ sporangia/ml). Therefor, we can conclude that stronger the grape varieties resistance to *P. viticola* are, higher the pathogenicity of isolates found on these grape varieties are.

### P23.107 Comparative functional analysis of *R*-genes for rice blast and the durable non-race-specific blast resistance gene *Pb1*

*X.Q. Liu[1,2], H. Inoue[1], C.J. Jiang[1], N. Hayashi[1] and H. Takatsuji[1]*
*[1]Disease Resistant Crops Research Unit, National Institute of Agrobiological Sciences, Kannondai 2-1-2, Tsukuba, 305-8602 Japan; [2]Key Biotechnology of State Ethnic Affairs Commission, College of Life Science, South-Central for Nationalities, Wuhan, 430074, P. R. China*
*Email: takatsuh@affrc.go.jp*

Rice blast resistance by *Resistance* (*R*) genes for elicitor-triggered immunity (ETI) is race specific and prone to break down. Most of the *R*-genes encode the proteins containing an N-terminal coiled-coil (CC), a central

nucleotide-binding (NB), and a C-terminal leucine-rich-repeat (LRR) domain. *Pb1* is a panicle blast resistance gene that confers a durable non-race-specific blast resistance to rice, and therefore it is non-R by definition. *Pb1*, in spite of being non-R, also encodes a CC-NB-LRR protein. To explore molecular basis underlying the differences in the nature of blast resistance between the R-genes and non-R *Pb1* gene, we generated chimeric genes, in which the 3 functional domains of *Pb1* and an R-gene *Pita* were swapped, and overexpressed them in transgenic rice. Blast resistance test showed that the lines overexpressing either chimeric gene showed blast resistances at largely similar levels to those of *Pb1*-overexpressing lines without clear race specificity, suggesting that the domains in the two proteins function in largely equivalent manners in the chimeric genes. We previously found that Pb1 interacts with WRKY45, a central transcription factor in the rice salicylic acid signaling pathway, through its CC domain, and that this interaction is important for the blast resistance by Pb1. Targeted yeast-2-hybrid assays showed that all the five rice R-proteins of CC-NB-LRR type tested, Pi36, Pib, Pita, Pit and Pizt, interacted with WRKY45 (and WRKY66) through their coiled-coil domains. However, blast resistance by these *R*-genes was not affected by the knockdown of *WRKY45* unlike that by *Pb1*. We will discuss the significance of these observations.

**P23.108 The application of real-time PCR to the detection and quantification of *Rhizoctonia solani* AG-3, the tobacco target spot pathogen using TaqMan Real-time PCR**

*Y.Q. Zhao[1, 2], Y.H. Wu [1], X.X. Zhao[1], M.N. An[1] and J.G. Chen[1]*
*[1]College Plant Protection, Shenyang Agricultural University, No. 120 Dongling Road, Shenhe District, Shenyang, 110866, P. R. China; [2]College Agriculture, Inner Mongolia for Nationality Universities, No.536 Huolinhe Street, Tongliao, 028000, P. R. China*
*Email: wuyh09@vip.sina.com*

*Rhizoctonia solani* Kühn is the causal pathogen of tobacco target spot, a serious fungal disease of tobacco that severely impairs yield and quality in many countries. It is difficult to detect and identify the pathogen in the early phase of the infection based on visual inspection. Therefore, reliable and fast procedures are important for the detection of the pathogen. In this study, DNA-based real-time polymerase chain reaction (QPCR) was evaluated to detect and quantify *R. solani* AG-3 DNA from infected tobacco tissue. A specific primer pair and probe were designed and based on the internal transcribed spacer region (ITS) of the fungal ribosomal DNA, and primer specificity was examined using convention PCR and real-time PCR, respectively. Quantification showed a linear association between the log of DNA concentration and the Ct value over the range of DNA concentration. Purified *R. solani* DNA was successfully detect at quantities as low as 100 fg. In this study, the method was used for measuring the relative growth and absolute biomass of *R. solani*, quantifying *R. solani* aggressiveness. The result showed that lesions produced by *R. solani* YC-9 strain were bigger than those produced by LF-2 on all hours post inoculation, while concentrations of *R. solani* strain YC-9 DNA were also higher than that of LF-2 strain.

**P23.109 The differential expression profiling of resistant and susceptible rice varieties following inoculation with *Ustilaginoidea virens***

*Y.Q. Han[1], Y. Zhang[1], J. Yang[1], L. Wang[1], N. Zhang[1], Y.F. Liu[2], Z.Y. Chen[2] and W.X. Sun[1]*
*[1]College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, P. R. China; [2]Institute of Plant Protection, Jiangsu Academy of Agricultural Science, Nanjing 210014, P. R. China*
*Email: yanqinghan125@126.com*

Rice false smut caused by *Ustilaginoidea virens* has recently become one of the most devastating grain diseases in the majority of the rice-growing areas of the world. To investigate molecular resistance mechanisms to *U. virens* in rice, differential gene expression in the resistant (IR28) and susceptible (LYP9) rice varieties in response to *U. virens* inoculation was analyzed by RNA-seq. Gene expression profiling analyses of both resistance and susceptible cultivars at 6 h, 24 h and 48 h after *U. virens* inoculation discovered that 3,249 and 2,720 genes were up-regulated and 3361 and 3079 genes were down-regulated in IR28 and LYP9 in response to infection, respectively. Gene ontology (GO) analyses revealed that differentially expressed genes in a set of resistance-related GO terms were enriched in both varieties. Despite this commonality, the gene sets contributing to common GO enriched terms were dissimilar. Some genes involved in the recognition of pathogen-associated molecular patterns (PAMPs), activation of effector-triggered immunity (ETI), hormone and phytoalexin biosynthesis and cell wall modification were identified to be associated with rice resistance against *U. virens*. The results indicated that molecular mechanisms under rice resistance to *U. virens* are involved in the recognition of PAMPs, the activation and regulation of pathogenesis-related genes and members of gene families involved in plant resistance signaling transduction pathways.

**P23.110 Functional identification of nucleocytoplasmic trafficking signals in the broad-spectrum resistance protein RPW8.2**

*Y.Y. Huang[1], Y. Shi[1], Y. Lei[1], Y. Li[1], S. Xiao[2]* and *W.W. Wang[1]*
*[1]Rice Research Institute, Sichuan Agricultural Univer-*

*sity, Chengdu 611130, P. R. China; [2]Institute for Biosciences and Biotechnology Research and Department of Plant Sciences & Landscape Architecture, University of Maryland, College Park, MD 20740, USA*
*Email: h1985yy@163.com*

Nuclear localization signals (NLSs) and nuclear export signals (NESs) are important intramolecular regulatory elements for protein nucleocytoplasmic trafficking. The *Arabidopsis thaliana* gene *RPW8.2* encodes a 174-amino acids protein that specifically localizes at the host-pathogen interfacial membrane–the extrahaustorial membrane (EHM) induced by powdery mildew, to render broad-spectrum resistance. Intriguingly, RPW8.2 is predicted to contain both NESs and NLSs. To assess the activity of the predicted NESs and NLSs in RPW8.2, we developed a simple and rapid method in which the activity of putative NLSs or NESs is reported by subcellular localization of two tandem fluorescent proteins in fusion with the respective NLSs or NESs after transient expression in leaves of *Nicotiana benthamiana*. By using this method, we tested the activity of eight overlapping fragments of *RPW8.2* each of which encodes 40 amino acids including a predicted NLS or NES. Subsequently, we discovered that RPW8.2 possesses one NES in the N-terminus, two NLSs in the middle and at the least three NESs in the C-terminus. We then investigated the function of these NESs and NLSs in RPW8.2-mediatied cell death and disease resistance by site-directed mutagenesis. Our data suggest that RPW8.2 may be subject to regulation by opposing trafficking forces for initiation of defense response and raise a possibility of partial localization of RPW8.2 in the nucleus in addition to its EHM-localization. How these NESs and NLSs might functionally contribute to RPW8.2-mediated cell death and disease resistance will be discussed.

**P23.111 Isolation of *vfLRR* gene conferring resistance to Tung *Fusarium* wilt**

*Y.C. Chen, S.S. Yang and Y.D. Wang*
*Institute of Subtropical Forestry, Chinese Academy of Forestry, No 73 Daqiao Road, Fuyang 311400, Zhejiang Province, P. R. China*
*Email: yicun_chen@163.com*

Tung Fusarium Wilt, caused by *Fusarium oxysporum* Schelecht, is the most crushing disease to *Vernicia fordii* (tung tree, tung oil tree). The disease is widely distributed in tung tree planting areas. Here, the pathogen was isolated from the susceptible tung tree plant and the test showed that *F. oxysporum* has special and strong pathogenecity on tung tree. *vfLRR* gene was first identified from tung tree Subtractive Library. Bioinformatics analysis indicated *vfLRR* encodes a protein containing Ser-Thrkinase domain (STK) and leucine-richrepeat (LRR) domains, a class of proteins commonly involved in the recognition of effectors from pathogens. *vfLRR* expression in various tissues of tung tree was analyzed using qRT-PCR method and it was found mainly expressed in the vascular tissues of stem, leaf and stipe. *vfLRR* gene expression and protein structure analysis laid foundations for further study of *vfLRR* role in Tung Fusarium wilt mechanism.

**P23.112 Functional conservation of *MoCRZ1* in *Magnaporthe oryzae* and its orthologs in *Fusarium graminearum* and *Neurospora crassa***

*D.Y. Lee, J. Choi, J. Jeon, S.Y. Park and Y.H. Lee*
*Department of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea*
*Email: dy88lee@gmail.com*

Calcium signaling is one of the most common and important signal transduction cascades present in any living organism and is said to be highly conserved in throughout evolution. In *Magnaporthe oryzae*, a rice blast pathogen, calcium signaling pathway is required for conidiation and infection-related development, which contributes to the pathogenicity of the fungus. *M. oryzae* gene (*MoCRZ1*) encoding calcineurin-responsive zinc finger 1 is involved in the calcium-dependent signaling pathway of *M. oryzae*, acting as a transcription factor. Deletion mutants of MoCRZ1 (Δ*Mocrz1*) showed drastic reduction in conidiation, as well as loss of pathogenicity. In this study, we planned to identify and phylogenetically analyze the orthologs of this gene in *Fusarium graminearum* (necrotrophic cereal pathogen) and *Neurospora crassa* (saprophyte). Orthologs of MoCRZ1 in *F. graminearum* (FgCRZ1) and *N. crassa* (NcCRZ1) were identified using BLASTP. Domain analyses showed that MoCRZ1 and its orthologs possess DNA binding-$C_2H_2$ zinc finger domains and conserved PxIxIT motifs, suggesting that the orthologs may be transcription factors involved in calcium signaling. In addition, when *FgCRZ1* and *NcCRZ1* were introduced into Δ*Mocrz1* mutants, these transformants restored conidiation and pathogenicity on rice. Further analysis on conservation and/or evolution of gene function among different species will be presented.

**P23.113 Functional characterization of histone demethylases in the rice blast fungus, *Magnaporthe oryzae***

*A. Huh, J. Jeon, S.Y. Park, J. Choi, S. Kim, J. Jeon and Y.H. Lee*
*Department of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea*
*Email: huharam@snu.ac.kr*

The post-translational modification of histones plays important roles in regulating chromatin dynamics and transcription. It has been shown that disruption of proper modifications can lead to developmental defects and cancer in plants and mammals, respectively. Despite the generality of histone modifications as epigenetic mechanisms in eukaryotes, implication of histone modifications in fungal pathogenesis is beginning to emerge. Here research plan was set out to identify and characterize putative histone demethylases in the model plant pathogenic fungus, *Magnaporthe oryzae*. To date, two classes of histone demethylases have been identified: LSD and JmjC domain-containing family proteins. Combining BLAST and HMMER, we identified 8 genes encoding putative JmjC domain-containing histone demethylases and named them as *MoJMJ1* to *MoJMJ8*. Phylogenetic analysis showed that six of them belong to JARID, JHDM2, JMJD2, and JmjC-only domain families, while two proteins are orphans. Deletion of *MoJMJ1*, which is the orthologue of *AtREF6* in *Arabidopsis thaliana*, resulted in defects in vegetative growth, asexual reproduction, appressorium formation and pathogenicity. This indicates the importance of regulating the steady-state level of histone methylation during fungal development and pathogenesis. We are currently undertaking deletion of remaining putative histone demethylase genes. These genetic approaches will be followed by biochemical approaches to examine the demethylase activity and identify the genes whose expression is regulated by histone methylation. We anticipated that this work would provide not only the insight into epigenetic regulation of fungal pathogenesis but also the knowledge that can be used in devising new control strategies against rice blast.

### P23.114 MYSTery HAT in the rice blast fungus is essential for pathogenic development

*S. Kwon, J. Jeon, A. Huh, S. Kim, S.Y. Park and Y.H. Lee*
*Department of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Republic of Korea*
*Email: kwon.seomun@gmail.com*

Histone acetylation emerged as one of the major epigenetic mechanisms controlling gene expression and various cellular processes in eukaryotes. Filamentous fungi, however, remain a relatively unexplored territory in epigenetics. One of the idiosyncrasies in fungi that have not been examined in the model organisms is a fungal-specific group of MYST family histone acetyltransferases (HATs) with a unique domain organization. Here we set out investigate the role of such HAT, *MoHAT10*, in a model plant pathogenic fungus, *Magnaporthe oryzae*. Identified among the 10 putative *M. oryzae* HATs predicted by the Profile hidden Markov model, targeted deletion of *MoHAT10* rendered the fungus completely non-pathogenic due to defects in radial growth and appressorium formation. This is in stark contrast to the lack of obvious defective phenotypes in the mutant for *SAS3* gene, an orthologue in *Saccharomyces cerevisiae*, and suggests functional divergence associated with differences in domain organization. Detailed biochemical and genetic analysis are underway to reveal how MoHAT10-mediated histone modifications are implicated in the regulation of fungal development and pathogenesis.

### P23.115 PsSRP54 is involved in sexual development and soybean infection of oomycete plant pathogen *Phytophthora sojae*

*D.L. Li, Z.J. Zhao, M. Yao, C.H. Zhai, S.M. Dong, X.B. Zheng and Y.C. Wang*
*Department of Plant Pathology, Nanjing Agricultural University, Nanjing, 210095, P. R. China*
*Email: wangyc@njau.edu.cn*

To successfully infect hosts, plant pathogens secrete effector proteins to suppress plant immunity. These secreted proteins should be translocated into the ER, which is the first step of the eukaryotic secretory pathway. Translocation of the polypeptide into the ER lumen can occur either cotranslationally or posttranslationally. Essential to cotranslational translocation is the presence of the signal recognition particle (SRP), that binds signal sequences by SRP54. However, the mechanism by which oomycete pathogens deliver effector proteins during plant infection remains unknown. PsSRP54 is a multidomain protein with ahelical bundle, a GTPase domain and a methionine-rich domain bound the signal sequence. *PsSRP54* was up-regulation in five infection stages (1.5, 3, 6, 12, and 24 h post inoculation) of *P. sojae*, that indicated it may play an important role during host infection. *PsSRP54* was not indispensable in growth, formation of sporangium and release of zoospores. Interestingly, the silenced mutants not only affected oospores development, but also reduced activities of extracellular enzymes. Silenced mutants were impaired in invasion of susceptible soybean, however these mutants were found an inability to elicit the hypersensitive reaction (HR) on soybean differential hosts. To further determine whether silenced *PsSRP54* affects secretion of effector proteins, we silenced *PsSRP54* by dsRNA in *Avr1b*-overexpressed strain and found transformants of *PsSRP54* greatly impaired *Avr1b*-mediated resistance to susceptible soybean, presumably due to a defect in secretion of the Avr1b protein. These results demonstrate the importance of *PsSRP54* to the virulence of *P. sojae* and extracellular protein secretion.

### P23.116 Molecular cloning and expression analysis of the Polygalacturonase *Rspg1* from *Rhizoctonia solani* causing tobacco target spot

*Y.Q. Zhao[1, 2], Y.H. Wu [1], X.X. Zhao[1], M.N. An[1] and J.G. Chen[1]*
*[1]College Plant Protection, Shenyang Agricultural University, No. 120 Dongling Road, Shenhe District, Shenyang, 110866, P. R. China; [2]College Agriculture, Inner Mongolia for Nationality Universities, No.536 Huolinhe Street, Tongliao, 028000, P. R. China*
*Email: wuyh09@vip.sina.com*

Tobacco target spot (*Rhizoctonia solani*) is a newly reported and an important tobacco disease which impacted on the yield and quality of flue-cured tobacco in China. In order to study its pathogenic molecular role a polygalacturonase gene *Rspg1* was cloned from *R. solani* by using PCR and the phylogenetic tree of *Rspg1* was constructed with MEGE 4.0 software, the expression of *Rspg1* in tobacco was also analyzed by real-time RT-PCR. The results showed that the open reading frame of *Rspg1* with a Poly(A) tail was 1080 bp, which encoded a protein of 361 amino acid residues. The result of evolutionary analysis indicated that *Rspg1* was gathered with other endoPG genes of *R. solani*, and all endoPG genes of these *R. solani* formed an independent branch. Results of real-time RT-PCR demonstrated that *Rspg1* expressed abundantly in leaf of inoculated tobacco. Expression of *Rspg1* of the strong pathogenic strain YC-9 and the weak pathogenic strain LF-2 has a coincident tendency. The expression quantity of the strain YC-9 was faster and higher than that of the strain LF-2 in early period of inoculated time, while the disease lesions caused by strain YC-9 was bigger than by strain LF-2. In conclusion, polygalacturonase gene *Rspg1* plays an important role in the pathogenic process of *R. solani*.

**P23.117 *In planta* expression screening identifies PpE2 from *Phytophthora parasitica* that induces cell death in *Nicotiana benthamiana***

*Y.L. Meng, G.Y. Huang, Q. Zhang, M. Zhang, Q.H. Wang, M.X. Zhang, B. Gu, J.L. Quan and W.X. Shan*
*State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China*
*Email: mengyuling@nwsuaf.edu.cn*

*Phytophthora* species, some of which can cause enormous economic losses, secrete large number of effector proteins that facilitate plant infection and colonization. Also plants respond to infection using different immune systems, usually, a hypersensitive cell death response at the infection site. Since most effector proteins of oomycetes fulfill their functions *in planta*, some assays have been developed such as agroinfiltration which allows rapid functional expression of pathogen genes in plants. In this research, we aimed at identification of effector proteins from *P. parasitica* that induce the plant cell death. A high quality cDNA library was constructed using mRNAs derived from tobacco leaves infected with three *P. parasitica* strains which have great difference in virulence spectrum on a set of 12 tobacco lines. The library was transformed into *Agrobacterium tumefaciens* AGL1 via triparental mating. We employed a high-throughput *Agrobacterium*-mediated transient expression assay on tobacco leaves to identify effectors. A screen of 10000 cDNAs led to the identification of 12 candidate effectors that have hypersensitive response (HR)-inducing activities. This screening identified PpE2, a putative secreted protein that induces cell death in *Nicotiana benthamiana*. PpE2 was localized in the plant endoplasmic reticulum. Infection assays of *P. parasitica* transformants expressing PpE2:GFP on *N. benthamiana* leaves, showed that GFP fluorescence was enriched in haustoria which thought to play an important role during infection process.

**P23.118 Potato leucine-rich repeat receptor-like kinase gene contributes to late blight resistance**

*Z.D. Tian, W. Liu, F. Shao and H.L. Chen*
*Key Laboratory of Horticultural Plant Biology (Huazhong Agricultural University), Ministry of Education; Huazhong Agricultural University, Wuhan, Hubei 430070, P. R. China*
*Email: tianzhd@mail.hzau.edu.cn*

Plants possess the specific mechanism to monitor the infection of diverse pathogens. In the plant cell surface, PAMPs are recognized by PRR, which initiate PAMP-triggered immunity (PTI), or by recognition by intracellular receptors of pathogen effectors to induce effector-triggered immunity (ETI). PTI is the first line of defense for a broad range of pathogens. The oomycete pathogen *Phytophthora infestans*, the causal regent of late blight on potato and tomato, always successfully overcome PTI by means of secreted effectors that suppress PTI responses. We found that overexpressing two potato leucine-rich repeat receptor-like kinase (LRR-RLK) genes *StLRK1* and *StBL/SGL-STPK* could enhance late blight resistance in transgenic potato against several *P. infestans* isolates. The increased resistance coordinated with the activation of defense related genes, active oxygen species and callose deposition. Our results suggest that it could be possible to reinforce potato late blight resistance through manipulating PTI.

**P23.119 *MoPAX1* and *MoLRG1* are essential for infection-related morphogenesis and pathogenicity by *Magnaporthe oryzae***

*Y. Li, X.F. Yue and Z.Y. Wang*
*State Key Laboratory for Rice Biology, Biotechnology Institute, Zhejiang University, Hangzhou, 310058, P. R. China*
*Email: zhywang@zju.edu.cn*

*Magnaporthe oryzae* is an important model fungal pathogen for understanding the molecular basis of plant-fungus interactions. Here, four genes encoding putative LIM proteins in *M. oryzae* genome, including *MoPAX1*, *MoLRG1*, *MoLRG2* and *MoLDP1*, were functionally characterized. Targeted gene deletion of either *MoPAX1* or *MoLRG1* led to a significant reduction of vegetative growth. *Molrg1* mutants could produce very few morpholigically abnormal conidia on CM medium, while *Mopax1* was unable to produce conidia. Both *Mopax1* and *Molrg1* mutants lost the ability to form appressoria on inductive surfaces and failed to cuase rice disease. Deletion of *MoLRG2* resulted in the production of longer conidia compared with these of wild type stain and reduced in formation of appressoria on artificial hydrophobic surface but not on plant surface. However, no morphological alterations were observed for the deletion of *MoLDP1*. Meanwhile, double gene deletion mutants *Moldp1Molrg2* were full pathogenic to susceptible hosts. Analysis of peotein cellular localization showed that both MoLrg1 and MoLrg2 localized to septum center (septal pores) and MoPax1 distributed to whole cytoplasm of cells. Functional characterization of domains of MoLrg1 discovered that both the 2nd LIM domain and RhoGAP domain were essential to protein function and localization, whereas the first LIM domain was only necessary for its localization. Taken together, *MoPAX1* and *MoLRG1* are essential for infection-related morphogenesis and pathogenicity of the rice blast fungus *M. oryzae.*

### P23.120 Genetic diversity and pathogenic variability of *Fusarium oxysporum* f. sp. *cubense*

*A. Bhattacharyya, P. Saikia[1], P. Sen[2] and K.H. Begum[3]*
*[1]AICPR on Tropical Fruits,[1]Dept. of Plant Pathology; [2]Dept .of Agril Biotechnology; [3]Horticultural Mission for NE India and Himalayan states; Assam Agricultural University, Jorhat-785013, Assam, India*
*Email: ashok-yya@yahoo.com.in*

Fusarium wilt of banana (*Musa* spp.) caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) has been considered as a very important disease in Assam, India with a record of 100% disease incidence. The result of a recent study revealed pathogenic variability amongst the isolates collected from 4(four) agro-climatic zones of Assam on the banana cultivar "Malbhog" (AAB). The isolates further studied in respect of genetic diversity of total soluble protein using 12 percent SDS-PAGE revealed polymorphism with molecular weight of polypeptides ranging from 14.30 KDa to 97.40 KDa. Overall six soluble protein bands of different molecular weight were detected, of which a major polypeptide band of molecular weight of 20.10 KDa was common to all the Foc isolates. On the other hand, a major polypeptide band with molecular weight of 66 KDa was present in 9 (nine) out of 10 (ten) isolates, whereas another band with molecular weight of 14.30 KDa was found in other set of 9 (nine) isolates out of 10 (ten). Jaccards similarity coefficient value ranged from 0.200 to 1.000 indicating high level of genetic diversity amongst the isolates. The details of the aspects have been discussed in the paper.

### P23.121 Elucidating the response of wheat to the exposure of *Stagonospora nodorum* effectors

*L. Du Fall and P.S. Solomon*
*Plant Sciences, The Australian National University, Canberra ACT, Australia 0200*
*Email: lauren.dufall@anu.edu.au*

The dothideomycete *Stagonospora nodorum* is a necrotrophic fungal pathogen of wheat and is the causal agent of Stagonospora nodorum blotch (SNB)[1]. This disease is responsible for over $100 million of yield losses in Australia annually. Recent studies have shown that this fungus produces a number of effector proteins that are internalised into host cells of susceptible wheat cultivars. The mechanism by which these effectors induce tissue necrosis in susceptible hosts is yet to be fully elucidated. We have applied a metabolomics approach to elucidate the cellular processes leading to disease and provide insight into the mode-of-action of these effectors. Gas chromatography-mass spectrometry analysis of primary polar metabolites has been undertaken on tissue extracts and apoplastic fluid from SnToxA infiltrated wheat. Results illustrate widespread perturbations in primary metabolism and reveal the first direct evidence of an increase in energy production in response to a pathogen effector. To further understand the host response to SnToxA at the secondary metabolism level, samples were also analysed using liquid chromatography-mass spectrometry. Our data indicate SnToxA causes an increase in defence-related secondary metabolites. These metabolites have significant effects on *Stagonospora nodorum* growth and sporulation *in vitro* and *in planta*. These complementary approaches have provided novel insight into the contribution of the SnToxA effector protein to SNB in wheat.

### P23.122 Pathogenicity differentiation and genetic analysis of *Curvularia lunata* in northern China

*S.Q. Xiao, Y. Gao, M.L. Zhang and C.S. Xue*
*Department of plant protection, Shenyang Agricultural University, No.120 Dongling Road Shenyang 110161, P. R. China*
*Email: chunshengxue@hotmail.com*

Thirty-eight isolates of *Curvularia lunata*, the causal agent of Curvularia Leaf Spot, were collected in 9 corn-growing locations throughout four provinces of Northern China during 2003 to 2004, to determine their pathogenicity on host differentials (Shen135, CN165, Mo17, Luyuan92, 78599-1, Ye478, B73, E28 and

Huangzaosi). The isolates were grouped into 3 different pathogenic types (I, II and III) based on their infection types on the host differentials and disease index. Genetic analysis of within and between pathogenic type by universally primed PCR (UP-PCR) revealed high genetic diversity in *C. lunata* population. A phylogenetic dendrogram was generated by the UPGMA method based on the average similarity coefficient between pathogenic type groups using program of the NTSYSpc 2.10s. Six clusters (A, B, C, D, E and F) were resolved at the 0.64 similarity level. UP-PCR results suggested that pathogenic type groups were genetically similar within one geographic locations, but genetic migration could possibly happen between some locations which might lead to relative high genetic diversity within one geographic location.

**P23.123 Efficient gene knockout in the maize pathogen *Curvularia lunata* using *Agrobacterium tumefaciens*-mediated transformation (ATMT)**

*L. Zhang, M.L. Zhang, S.Q. Xiao and C.S. Xue*
*Department of plant protection, Shenyang Agricultural University, No.120 Dongling Road Shenyang 110161, P. R. China*
*Email: chunshengxue@hotmail.com*

*Curvularia lunata* is the causal agent of Curvularia leaf spot of maize which periodically causes significant yield losses in China. To explore molecular mechanisms of fungal pathogenicity and virulence to the host, an efficient targeted gene knockout transformation system using *Agrobacterium tumefaciens* was established with field collected strains CX-3. The starting materials, incubation time, induction medium type, *Agrobacterium* cell density, and method of co-incubation were optimized for deletion of hydroxynaphthalene reductase (*Brn1*), a gene in the melanin biosynthesis pathway, as a test case. Transformation efficiency was about 24±3 transformants per $1\times10^6$ germlings and homologous recombination efficiency ranged from 25-50%.

**P23.124 A novel gene control virulence in *Fusarium oxysporum* causing cabbage yellow wilt**

*J.H. Feng, W. Zhang, J.G.Huang and J.Y. Yan*
*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, No. 9 of ShuGuangHuaYuan Middle Road, Haidian District, Beijing, 100097, P. R. China*
*Email: jiyeyan@gmail.com*

Cabbage yellow wilt caused by *Fusarium oxysporum* f. sp. *conglutinans* occurred in several provinces of China in the past decades. A wild-type strain A6 isolated from Yanqing County was used for REMI (Restriction Enzyme Mediate Integration) transformation, and up to now, 1050 transformants were constructed with *Hin*d III. A mutant PDM003 showed lower virulence was isolated by pathogenicity test. The growth evaluation of PDM003 showed that it is slower than wild-type strain. The Southern blot analysis of PDM003 suggested that it was a single insertion locus mutant with the probe of Hygmycine B. The insertion flank sequences of PDM003 were obtained by plasmid rescue. Bioinformatics analysis showed that a predicted gene named *Foc*1 was disrupted by the insertion of REMI transformation plasmid. Loss of function of *Foc*1 by knock out showed that the growth rate of mutant was slower 16% on PDA medium, natural sporulation decreased 32% on PDA medium, and virulence on cabbage cultivar Zhonggan21 decreased 35% than wild type, respectively. There was no significant characterization variation on conidial morphological. Colony morphology showed that mutant was denser than wild type, and mycelia stained with CFW (Calcofulor white) also showed that the mutant has much more branches, which is consistent with the morphological observations.

**P23.125 Characterization and function analysis of the genes involved in melanin biosynthesis pathway of *Setosphaeria turcica***

*Z.Y. Cao, H. Jia, Q.J. Meng, L.J. Guo, Z.M. Hao, S.Q. Gu and J.G. Dong*
*Mycotoxin and Molecular Plant Pathology Laboratory, Agricultural University of Hebei, Baoding, 071001, P.R. China*
*Email: dongjingao@126.com*

DHN melanin is common in ascomycetous and imperfect fungi, which can protect microorganisms against UV light and microbial lytic enzymes. Melanin is considered as an important virulence factor in regard to the melanization of the appressorial cell wall and the penetration process in phytopathogenic fungi. It was reported that a polyketide synthase, two hydroxynaphthalene reductases, one scytalone dehydratase and an oxidative dehydratase were involved in the DHN melanin biosynthesis. *Setosphaeria turcica*, which causes northern corn leaf blight (NCLB), penetrates host with the melanization appressoria. Six genes of *S. turcica* were cloned and named as *StPKS*, *StSCD*, *St4HNR*, *St3HNR*, *StLAC1* and *StMR*, which encoded polyketide synthase, scytalone dehydratase, naphthalene reductase, laccase and a transcription factor respectively. All of them shared high amino acid homology with the corresponding proteins in other phytopathogenic fungi. The *StPKS* gene was involved in DHN melanin biosynthesis, conidia and appressorium formation, and the ability of penetration. Carpropamid, a specific inhibitor of scytalone dehydratase, could inhibit the conidial germination and appressorium production of *S. turcica*. *St3HNR* and *St4HNR* belonged to two groups of reductase and catalyzed different substrates. The *St3HNR* gene-disruption mutants exhibited brownish-red colony, weakened turgor pres-

sure and penetration of appressorium, and low pathogenicity on health host. The mutant ∆*StLAC1* displayed a gray-white colony, irregular-shaped mycelium, slower vegetative growth rate and decreased laccase activity. *StMR* RNAi transformants exhibited less-pigmented colony. It was concluded that all genes were confirmed to be involved in melanin biosynthesis and some of them related to penetration of pathogens.

### P23.126 The research progress of mitogen-activated protein kinase cascade pathway in *Setosphaeria turcica*

*X.Y. Zhang, L. Tian, P. Zhang, Y. Wang, S. Gu, J. Han and J.G. Dong*
*Mycotoxin and Molecular Plant Pathology Laboratory, Agricultural University of Hebei, Baoding 071000, P. R. China*
*Email: shmdjg@hebau.edu.cn*

*Setosphaeria turcica*, the pathogenic agent of Northern Corn Leaf Blight, is the primary cause of severe corn disease in most corn-producing areas worldwide. Mitogen-activated protein kinases (MAPK) comprise a family of serine/threonine kinases that are activated in specialized cells in response to a variety of stimuli. This set of three functionally interlinked protein kinases has been identified and conserved in modular form in all known eukaryotes. In filamentous fungi, MAPKs are classified into three subgroups: Hog1-homologue MAPK cascade, Fus3/Kss1-homologue MAPK cascade and Slt2-homologue MAPK cascade. In our research, three MAPK cascade pathways, which were highly homologous to other fungi, were found in *S. turcica*. Three RNA interference transformants of StPBS2, which was a MAP kinase and belonged to Hog1-homologue MAPK cascade pathway, were obtained and showed hypersensitive to osmotic stress conditions, suggesting that Hog1-MAPK cascade pathway may control osmotic stress response in *S. turcica*. Moreover, a gene knockout mutant of StBCK1, which was a MAP kinase kinase kinase gene and fell into Slt2-homologue MAPK cascade pathway, was highly susceptible to cell wall degrading enzymes. Furthermore, not only StPBS2 RNAi transformants but StBCK1 knockout mutants lost their pathogenicity, suggesting that these two cascades may regulate the pathogenicity, however, the regulation mechanism should be further studied.

### P23.127 Structural features and biological functions of microconidia of rice blast fungus

*H.L. Zhang[1], C.F. Wang[1], H.C. Zhang[1], S.J. Zhang[1] and J.R. Xu[1, 2]*
*[1]Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, P. R. China; [2]Department of Botany and Plant Pathology, Purdue University, USA*
*Email: 17108085@nwsuaf.edu.cn*

Rice blast caused by *Magnaporthe oryzae* is a devastating disease of rice throughout the world. Although sexual fruiting bodies have not been observed in nature, microconidia produced by *M. oryzae*, are assumed to function as spermatia. However, the actual biological function of microconidia in the life cycle of the rice blast fungus is not clear and germination of microconidia has not been reported. In this study, we developed a culture condition that is suitable for producing massive amount of microconidia. For two different wild-type strains tested, we were able to obtain approximately $10^7$ microconidia/ml in PDB cultures. DAPI staining revealed that majority of microconidia have one nucleus although a few of them have two nuclei. When stained with MitoTracker® Green FM, mitochondria were observed in macroconidia but not in most of the microconidia. Transmission electron microscopic (TEM) examination confirmed the lack of mitochondria in most of microconidia examined. On the surface of 1% water agar, approximately 5% of freshly harvested microconidia germinated after incubation for 48h-72 h at 25$^o$C. When germinated microconidia were transferred onto nutrient medium, they could develop into colonies. We are in progress of conducting spray and inject infection assays with microconidia harvested from liquid PDB cultures. Data from these experiments and RNA-seq analysis will be presented to determine the possible role of microconidia in plant infection and genes expressed in microconidia of *M. oryzae*. If microconidia are found to be infectious, the infection cycle of the rice blast or wheat blast fungus may need to be revisited.

### P23.128 The *FgPRP4* kinase is important for RNA processing, growth, and pathogenesis in *Fusarium graminearum*

*X.L. Gao[1], Q.J. Jin[1], Q.J. Xu[1], C.H. Li[1], H.Q. Liu[1], C.F. Wang[1] and J.R. Xu[1,2]*
*[1]Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, P. R. China; [2]Department of Botany and Plant Pathology, Purdue University, USA*
*Email: gaoxuli518@163.com*

Pre-mRNA splicing is an important gene expression regulation mechanism in eukaryotic cells. The PRP4 gene encode the only protein kinase involved in pre-mRNA plicing. In the fission yeast and mammals, it is an essential gene but the budding yeast lacks a distince Prp4 ortholog. In a previous study of systematic characterization of protein kinases in the wheat scab fungus *Fusarium graminearum*, we found that the *Fgprp4* mutant was viable although it was significantly reduced in growth rate. The goal of this study is to determine the

biological and biochemical functions of *FgPRP4* in *F. graminearum*. We characterized the defects of the *Fgprp4* mutants in details, including phenotypes associated with hyphal growth, conidiogenesis, pathogenesis, and sexual reproduction. RNA-sequence analysis showed that pre-mRNA splicing of more than 50% gene was affected in the *Fgprp4* mutant. Reduced RNA splicing efficiency was verified by RT-PCR analysis. Interestingly, the *Fgprp4* mutant was unstable and we have isolated a total of 49 spontaneous fast-growing suppressors. These suppressors could be divided into three major classes based on their growth rate. The majority of them could grow as fast as the wild type but they were still defective in sexual reproduction or plant infection. Sequencing analysis identified suppressor mutations in four genes orthologous to *PRP1*, *PRP31*, *BRR2* and *PRP8*. The effects of these mutations on the interactions among components of the spliceosome and spliceosome function are being investigated. Further characterization of the *Fgprp4* mutant and related suppressor mutations may lead to better understanding of the regulation of splicing efficiency and alternative splicing in eukaryotic organisms.

**P23.129 A novel protein kinase gene involved in sexual production in *Fusarium graminearum***

*Y.F. Dai[1], S.J. Zhang[1], Q. Zheng[1], X.Y. Gu[1], C.F. Wang[1] and J.R. Xu[1,2]*
*[1]Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, P.R.China; [2]Department of Botany and Plant Pathology, Purdue University, USA*
Email: *daiyafenghappy@163.com*

Head blight, caused by *Fusarium graminearum* is one of the most important diseases on wheat and barley. Plant infection is initiated by ascospores as the primary inoculum in wheat florets from anthesis through the soft stage of kernel development. In this study, we identified a novel protein kinase gene that is required for normal sexual production. This gene was well conserved in filamentous fungus but lacks a distinct ortholog in *Saccharomyces cerevisiae*. It was named *PUK2* for protein kinase gene unique to filamentous fungi 2. Deletion of the *PUK2* gene had no effect on growth, conidiation, or colony morphology. The *puk2* mutant also was normal in stress responses. However, it was defective in sexual reproduction. Ascospores produced by the *puk2* mutant often fragmented two-celled and fragmented in the middle. The number of ascospores per ascus was reduced in the mutant and ascospores were rarely discharged from perithecia. In wheat head infection assays, the *puk2* mutant was reduced in virulence. The results indicate that *PUK2* must play a specific role in ascosporogenesis and ascospore releasing in filamentous fungi. *PUK2* may be also involved in plant colonization. Because of the important of ascospores in the infection cycle of *F. graminearum*, *PUK2* is a suitable fungicide target to control FHB.

**P23.130 Functional analysis of the *FgCDC14* gene in the wheat scab fungus *Fusarium graminearum***

*C.H. Li[1], S.J. Zhang[1], M. Hall[2] and J.R. Xu[1,3]*
*[1]Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, P. R. China; [2]Department of Biochemistry, Purdue University, USA; [3]Department of Botany and Plant Pathology, Purdue University, USA*
*Email: chaohuili@yeah.net*

Mitosis and cytokinesis are essential cellular processes for cell division and proliferation in eukaryotic organisms. Many genes important for cell cycle have been well characterized in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, including *CDC14* that encodes a member of the dual specificity phosphatase family highly selective for serine-proline phosphorylation sites generated by cyclin-dependent kinase. Interestingly, whereas Cdc14 is an essential gene for regulating multiple events during anaphase such as mitotic exit in *S. cerevisiae*, it ortholog is not essential in *S. pombe* although it is important for coordinating nuclear division with cytokinesis. Cdc14 orthologs are well conserved in filamentous fungi, but none of them have been functionally characterized. In this study, we aimed to determine the functions of Cdc14 in growth, development, and pathogenesis in *Fusarium graminearum*. Deletion of *FgCDC14 (Fg00543)* is not lethal in *F. graminearum*. The *Fgcdc14* deletion mutant was significantly reduced in vegetative growth and conidiation. In self-cross and plant infection assays, the *Fgcdc14* mutant was sterile and non-pathogenic. The mutant conidia had abnormal morphology and fewer septa than the wild-type ones. Septation in hyphae also was reduced and irregular. Some conidium and hyphal compartments contained multiple nuclei, indicating that the *Fgcdc14* mutant was defective in the coordination between nuclear division and cytokinesis. Interestingly, foot cells of macroconidia were able to exit G1-arrest and extend and function as conidiogenous cells in the *Fgcdc14* mutant, resulting in the production of inter-connected conidia. These results indicate that *FgCDC14* plays a role in cell division and septum formation in *F. graminearum*. We are in progress in determining the subcellular localization of FgCdc14 and further characterizing its functions in nuclear division and septation. Data from these experiments will be presented.

**P23.131 RNA interference of the leucine rich repeat gene *Os74* increases susceptibility to *Magnaporthe oryzae* in transgenic rice plants**

*Q.K. Xing, X.J. Chen* and *Z.J. Guo*
*Key Laboratory of Plant Pathology, Ministry of Agriculture; Department of Plant Pathology, China Agricultural University, Beijing 100193, P. R. China*
*Email: guozj@cau.edu.cn*

Rice blast caused by *Magnaporthe oryzae* is a widely-spread disease in the world's primary rice production areas. Investigating the genes that play roles in rice–*M. oryzae* interactions helps us to understand the defense mechanism in rice. In this study, we report a nucleotide-binding site (NBS)-leucine rich repeat (LRR) gene *Os74*, which confers resistance to *M. oryzae* at seedling stages of the rice. Phylogenetic analysis of Os74 and the 42 characterized NBS-LRR proteins revealed that Os74 is more closely related to OsPi36. Quantitative real time PCR assays showed that *Os74* is preferentially expressed in leaf blades and leaf sheaths. Based on β-glucuronidase (GUS) activity staining in transgenic plants expressing the *Os74* promoter fused to the GUS gene, expression of *Os74* was detected in node, immature panicles and pollens. Os74 protein is located to the cytoplasm and nucleus based on transient expression of Os74-GFP (green fluorescent protein) fusion construct in *Nicotiana benthamiana* epidermal cells. Additionally, *Os74* is rapidly induced by flg22 and chitin treatments, as well as by both virulent and avirulent pathovars of *M. oryzae*. Knockdown of *Os74* by means of RNA interference (RNAi) exhibited enhanced susceptibility against *M. oryzae* compared to the wild type controls. Moreover, the enhanced susceptibility against *M. oryzae* of the RNAi plants may be mediated by inhibition of the expression of some defense-related genes. Taken together, these results suggest that *Os74* plays roles in the interactions between rice and *M. oryzae*.

### P23.132 The role of a kinase gene unique to filamentous fungi in pathogenesis

*X.Y. Gu*[1], *Y.F. Dai*[1], *S.J. Zhang*[1], *C.F. Wang*[1] *and J.R. Xu*[1,2]
[1]*Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, P. R. China;* [2]*Department of Botany and Plant Pathology, Purdue University, USA*
*Email: gxyjiayou@126.com*

*Fusarium graminearum* is the causal agent of Fusarium head blight (FHB), which is a destructive disease on wheat and barley. The pathogen not only causes severe yield losses but also contaminates infested grains with mycotoxins. In 2012, there was a nationwide outbreak of FHB on wheat in China. To better understand pathogenesis of this important pathogen, we functionally characterized the *PUK1* (protein kinase unique to filamentous fungi 1, FGSG_03146) kinase gene in *F. graminearum*. The *puk1* mutant had no obvious defects in vegetative growth but was significantly reduced in virulence in wheat head infection assays. And the production of conidium was reduced to some extent in comparison with PH-1 but it had no effect on conidium morphology. When we took the complementation assays with GFP markers, its biological functions and phenotype were resumed totally. In addition, we generate the knockout mutant of the *PUK1* ortholog in the rice blast fungus *Magnaporthe oryzae*. The Mopuk1 mutant has no distinct changes in phenotype compared with Guy11. Similarly, its pathogenicity was reduced seriously just as we have predicted before. These results indicate that the *PUK1* kinase gene may have a conserved function in plant infection and other biological functions.

### P23.133 Developmental stage-specific accumulation of tRNA-derived small RNAs in the oomycete pathogen *Phytophthora sojae*

*Q.H. Wang, K. Xu, W. Zhang, X.L. Wang, M.X. Zhang, G.J. Fan, W.B. Jin, J.L. Quan, M.B. Wang and W.X. Shan*
*State Key Laboratory of Crop Stress Biology in Arid Areas and College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China*
*Email: wangqinhu@nwsuaf.edu.cn*

Small non-coding RNAs are critical for the regulation of gene expression in eukaryotes. Oomycetes are fungus-like eukaryotic microorganisms that cause severe losses to agricultural production and damages to ecosystems. However, little is known on the presence and diversity of small RNAs in the oomycete pathogens. In this study, we report the identification of tRNA-derived small RNAs (tsRNAs) in the soybean pathogen *Phytophthora sojae*, by deep sequencing and classical small RNA cloning. The presence of oomycete tsRNA was confirmed by Northern. The tsRNAs were selectivity cleaved and conserved within *Phytophthora*. We consistently showed a negative correlation between the accumulation of tsRNAs and their target transcripts by profiling tsRNAs and quantitative analyses of their target transcripts. Furthermore, we showed the associated mechanisms of the tsRNAs in regulating expression of their targets, by 5' RLM-RACE. The results demonstrated the presence of functional endogenous small noncoding RNAs in oomycetes and further expanded the small RNA families, shedding light on the small RNA biology and epigenetic mechanisms in the eukaryotic pathogens.

### P23.134 *Ta*MYB1 interacts with *Ta*WRKY1 to regulate disease resistance against powdery mildew fungus in wheat

*S. Jing, C. Chang, D. Yu, X. Han and Q.H. Shen*
*Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, P. R. China*
*Email: sjjing@genetics.ac.cn*

Plant immune responses are triggered by immune receptors that are either membrane-bound PAMP-recognition receptors or intracellular localized nucleotide binding (NB) and leucine-rich repeat (LRR) domain containing NLR receptors. The immune receptors activate defense responses involving massive transcription reprogramming. We previously showed that barley MLA NLRs sequester the *Hv*WRKY1 repressor and stimulate the DNA-binding activity of *Hv*MYB6 and gene expressions thus trigger immune responses against *B. graminis* fungus. We identify the corresponding wheat transcription factor homologs, *Ta*MYB1 and *Ta*WRKY1. Transient gene expression analysis show that *Ta*MYB1 and *Ta*WRKY1 act as positive and negative regulator in wheat disease resistance against *B. graminis* fungus, respectively. *Ta*MYB1 acts as a transcriptional activator while *Ta*WRKY1 acts as a transcription repressor in Arabidopsis protoplast transfection assay. We show that *Ta*WRKY1 can specifically interacts with *Ta*MYB1 and suppress *Ta*MYB1-dependent DNA-binding and transcriptional activation activity. Transgenic wheat lines overexpressing C-terminal epitope-tagged *Ta*MYB1 and/or *Ta*WRKY1 have been generated for further ChIP-seq analysis. We aim to dissect *Ta*MYB1 and/or *Ta*WRKY1-regulated defence transcription networks in wheat and compare to that in barley.

### P23.135 A ring-type E3 ubiquitin ligase regulates the barley MLA-mediated immune responses

*T. Wang, C. Chang and Q.H. Shen*
*Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, No.1 West Beichen Road, Chaoyang District, Beijing, 100101, P. R. China*
*Email: wt2915@genetics.ac.cn*

Plant intracellular immune receptors are members of a superfamily containing a central nucleotide-binding (NB) and C-terminal leucine-rich repeat (LRR) domain, designated NLRs. The barley mildew A (*MLA*) locus is highly polymorphic (*Hordeum vulgare*) harboring allelic genes each encoding CC-subtype NLRs containing a N-terminal coiled-coil structure. Each MLA receptor triggers race-specific disease resistance against barley powdery mildew fungus. Previous studies showed that activated MLA may trigger disease resistance by derepressing WRKY1/2-mediated repression on basal resistance, and that MLA specifically sequesters the WRKY1 repressor and stimulates the DNA-binding activity of MYB6 to initiate disease resistance signaling in the nucleus. Here we report the identification of an E3 ubiquitin ligase, MIR1 (MLA-Interacting RING-type E3 ligase 1) that directly interacts with MLA and may regulate the steady-state level of MLA. We showed that the C-terminal Tetratricopeptide repeat (TPR) domain of MIR1 is required for interaction in yeast with the N-terminal CC-NB(1-225) fragment of MLA1 and MLA6, but not MLA10. MIR1 displayed E3 ubiquitin ligase activity in an *in vitro* ubiquitination assay. Furthermore, virus-induced gene silencing of *MIR1* could increase MLA1 level in barley leaf tissue. Transient overexpression of MIR1 could compromise MLA-mediated disease resistance. Future experiments will reveal whether MLA is a direct MIR1 substrate in vivo and the biological significance of the MIR-MLA association.

### P23.136 Target of tae-miR164, a NAC family protein gene (*TaNAC21/22*), negatively regulates resistance of wheat to stripe rust

*H. Feng, X.Y. Duan, Q. Zhang, X.J. Wang, B. Wang, L.L. Huang and Z.S. Kang*
*State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, 712100, P. R. China*
*Email: kangzs@nwsuaf.edu.cn*

The conserved miRNA miR164 participates in various physiological and biochemical processes in plants by regulating corresponding target genes. Many NAC transcription factors, as targets of miR164, play important roles in regulation of plant development and responses to abiotic and biotic stresses. In this study, a novel target gene, designated *TaNAC21/*22, of tae-miR164 was identified using degradome sequencing and co-transformation technology. We isolated full-length cDNA clones, and showed the biological function of *TaNAC21/22* when induced by abiotic and biotic stress. TaNAC21/22 was located in the nucleus and could function as a transcriptional activator. The expression of *TaNAC21/22* in wheat in response to *Puccinia striiformis* f. sp. *tritici* (*Pst*) infection was associated with a similarly diverse tae-miR164 expression pattern. *TaNAC21/22* was induced by abiotic stresses and ABA. Silencing of individual cDNA clones showed that *TaNAC21/22* negatively regulates resistance to stripe rust. These results indicate that a novel NAC transcription factor from the NAM subfamily plays an important role in regulating resistance of host plants to stripe rust through a tae-miR164 regulation pathway.

### P23.137 Isocitrate lyase is essential for urediniospore germination of *Puccinia striiformis* f. sp. *tritici*

*J. Liu[1], Q.L. Wang[1], Q. Chang[2], L.N. Han[1], G.L. Pei[1], Y.Q. Xue[1], L.M. Jia[1], K. Zhang[1], Y.Y. Duan[1] and Z.S. Kang[2]*
*[1]College of Life Sciences, State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling 712100, Shaanxi, P. R. China; [2]College of Plant Protection, State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling 712100, Shaanxi, P. R. China*
*Email: liujie2003@hotmail.com*

The *PsICL1* gene, encoding isocitrate lyase, which is a key enzyme in the glyoxylate cycle, was cloned and characterized in the biotrophic wheat pathogen *Puccinia striiformis* f. sp. *tritici* (*Pst*). Expression analyses of *PsICL1* showed high levels of transcripts in ungerminated uredinospores, whereas isocitrate lyase enzyme activity was low. In planta, *PsICL1* expression was continuously down-regulated upon germination. During the later stages of the infection of wheat, the level of *PsICL1* expression was extremely low. The function of *PsICL1* was identified via mutant complementation. The expression of *PsICL1* in *Saccharomyces cerevisiae* can complement the defects of the Δ*ICL* mutant. Using 3-nitropropionate, we observed that inactivation of isocitrate lyase greatly reduced the germination rate of urediniospores, indicating that *PsICL1* plays a key role during *Pst* germination. Furthermore, analysis of lipid bodies revealed that lipid components continuously entered the germ tube from the urediniospore cell during germ tube elongation. Moreover, the lipid contents continued to decrease, and total carbohydrates markedly increased in this period, demonstrating that the conversion of lipids into carbohydrates was in progress. These results suggest that *PsICL1* is essential for *Pst* germination. This work elucidates the function of *PsICL1* in *Pst* and may provide a theoretical basis for controlling strip rust.

**P23.138 Wheat CBL-CIPK signaling system is involved in wheat and stripe rust fungus interaction**

*J. Guo, Y.H. Duan, X.X. Shi, C.M. Huang, L.L. Huang and Z.S. Kang*
*State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China*
*Email: guojunwgq@nwsuaf.edu.cn; kangzs@nwsuaf.edu.cn*

Perturbations in the cytosolic free $Ca^{2+}$ are essential in early pathogen perception and subsequent plant innate immune response. Calcineurin B-like proteins (CBL) are a new family of $Ca^{2+}$ sensors and interact with a specific group of serine-threonine protein kinases, CBL-interacting protein kinases (CIPK). In this study, seven wheat CBL and 11 wheat CIPK genes were isolated and designated as *TaCBL1*, *2*, *3*, *4*, *6*, *7*, 9 and *TaCIPK02*, *05*, *07*, *09*, *10*, *14*, *15*, *17*, *23*, *31*, *32*. Subcellular location assays indicated that the wheat CBL proteins were localized in the membrane of onion epidermal cells. However, TaCIPKs were observed to be differentially localized in the cells. Yeast two hybrid revealed a complicated interaction network between wheat CBL and CIPK proteins, except that TaCIPK02 and TaCIPK15 did not interacted with all wheat CBL proteins *in vitro*. Real-time RT-PCR assays indicated that *TaCIPK15* was induced in the incompatible interaction of wheat-stripe rust, while the expression of *TaCBL4* and *TaCIPK05* was up-regulated in both compatible and incompatible interaction. Silencing of *TaCBL4*, *TaCIPK05* and *TaCIPK15* enhanced susceptibility in wheat against avirulent stripe rust pathotype CYR23, and silencing of *TaCIPK05* and *TaCIPK15* reduced necrotic area of cells neighboring the infection sites and the expression levels of wheat PR genes. These results suggested that *TaCBL4*, *TaCIPK05* and *TaCIPK15* played positive roles in wheat defense against stripe rust fungus, and *TaCBL4* might play a regulator by interacting with TaCIPK05 to transduce calcium signal in response to stripe rust infection.

**P23.139 A new actin-depolymerizing factor, TaADF7, contributes to wheat resistance against *Puccinia striiformis* f. sp. *tritici***

*Y.P. Fu, X.J. Wang, X.Y. Duan, L. Deng, G.R. Wei, Z.S. Kang*
*State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, 712100, P. R. China*
*Email: kangzs@nwsuaf.edu.cn*

Actin-depolymerizing factors (ADFs) are important actin-binding proteins, which regulate the dynamics of cytoskeleton. Although the cytoskeleton is reportedly involved in plant defense responses, the function of the ADF family in plant disease resistance is largely unknown. Here, a new actin-depolymerizing factor, designated *TaADF7*, was cloned from wheat cv. Suwon11. Subcellular localization, biochemical analyses and overexpression in yeast demonstrated that *TaADF7* is an actin depolymerizing factor locating on actin cytoskeleton and possessing actin binding/severing activities. The expression level of *TaADF7* was more sharply induced at different infection stages during the incompatible interaction than during the compatible interaction between wheat and *Puccinia striiformis* f. sp. *tritici* (*Pst*). The expression of *TaADF7* was highly induced by exogenous salicylic acid (SA) treatment, suggesting that *TaADF7* may participate in the wheat-*Pst* interaction via the SA-mediated signaling pathway. Furthermore, when *TaADF7* was silenced by virus-induced gene silencing (VIGS), hyphal growth increased and defense reactions were weakened in the interaction between wheat and avirulent *Pst* race. Moreover, Cytochalasin B partially rescued the hypersensitive response in TaADF7-knockdown plant. Together with its F-actin binding/severing function, we show that *TaADF7* contributes to wheat resistance against the stripe rust pathogen probably by modulating the dynamic cytoskeleton.

**P23.140 Understanding the pathogenesis of *Valsa mali* var. *mali* through transcriptome analysis of infection in apple twigs**

*X.W. Ke, Z.Y. Yin, Y.Y. Liu, D.X. Huang, Q.Q. Dai, N. Song, Y. Hu, Z.P. Li, X.N. Gao, Z.S. Kang and L.L. Huang*
*College of Plant Protection, Northwest A & F University, Yangling, Shaanxi, 712100, P. R. China*
*Email: huanglili@nwsuaf.edu.cn*

The apple valsa canker, caused by the fungus *Valsa mali* var. *mali* (*Vmm*), is one of the destructive diseases for the apple production in China. More understanding of apple/pathogen interaction is helpfule for making proper management strategies. Thus, in the current study, we sequenced the transcriptome of the *Vmm* mycelium and infected apple bark using an effective short read sequencing by Hig-Seq2000 (Illumina). All of the contigs from the two transcriptomes were annotated and mapped to the GO terms and the KEGG pathways. Comparing to the mycelia transcriptome, 8,615 genes are common expressed in infected tissues. In which, there were 5,306 genes with differential expressed, and 58 genes were up-regulated expressed while the others were down-regulated expressed. Statistical analysis of the GO terms found that the category of 'pectin catabolic process' and 'hydrolase activity' were enriched. The largest numbers of up-regulated genes were mapped with the KEGG pathways of 'metabolic pathways' and 'biosynthesis of secondary metabolites', in which the genes are either annotated as cell wall hydrolases or associated with mycotoxin biosynthesis. The results indicated that the cell wall associated hydrolases are responsible to the tissue maceration during the fungal infection. The mycotoxin might play an important role in host cell death too. In conclusion, the cell wall hydrolysis associated enzymes are crucial for *Vmm* infection and the mycotoxins are synergistic. These results provided a valuable resource for the future studies in the pathogenesis of *Vmm* on apple and laid the foundation for sustainable disease management strategy development.

### P23.141 TaGLY1 and TaGLI1 contribute to wheat resistance to *Puccinia striiformis* f. sp. *tritici*

*Y.H. Yang, J. Zhao, H.J. Xing, P. Liu, C.C. Li and Z.S. Kang*
*State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P.R. China*
*Email: kangzs@nwsuaf.edu.cn*

Glycerol-3-phosphate (G3P) is proposed to be a potent regulator of plant defense signaling involved in basal resistance and systemic acquired resistance (SAR). GLY1-encoded glycerol-3-phosphate dehydrogenase (G3Pdh) and GLI1-encoded glycerol kinase (GK) are two key enzymes involved in glycerol-3-phosphate (G3P) biosynthesis in plants, but their physiological importance in wheat defense against *Puccinia striiformis* f. sp. *tritici* (*Pst*) remains unclear. In this study, *TaGLY1* and *TaGLI1* were identified and characterized from wheat leaves challenged with *Pst*. Quantification assays revealed that these two genes differentially induced by avirulent *Pst* infection. Moreover, knocking down *TaGLY1* and *TaGLI1* individually or simultaneously by barley stripe mosaic virus-induced gene silence (BSMV-VIGS) compromised the resistance in wheat cv. Suwon 11, while the accumulation of SA and the expression of SA-induced marker gene *TaPR1* in silencing plant leaves were altered significantly. These results supported that *TaGLY1* and *TaGLI1* contribute to wheat resistance to stripe rust. Meanwhile, co-silencing of multiple genes simultaneously by the VIGS system proved to be a powerful tool for multi-gene function analyses in plants.

### P23.142 Identification and characterization of effectors in *Puccinia striiformis* f. sp. *tritici*

*Y.L. Cheng, X.J. Wang, C.L. Tang, M.J. Liu, L.L. Huang and Z.S. Kang*
*State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, 712100, P. R. China*
*Email: kangzs@nwsuaf.edu.cn*

*Puccinia striiformis* f. sp. *tritici* (*Pst*), the causal agent of wheat stripe rust, has strong deleterious impacts on wheat production worldwide. Filamentous pathogens such as rust fungi secrete molecules called effectors that are delivered into host cells and play important roles in both disease development and disease resistance response. However, the understanding of effectors from rust fungi, especially wheat rust pathogens with economic importance, remains relatively unexplored compared with oomycetes and other fungi. Here, we report a screening of *Pst* candidate effectors and a survey of transcription and variation of these candidate effectors. We bioinformatically identified 198 candidate effectors, with small amino acids (< 300) and rich cysteine (≥4), from the *Pst* genome sequence, of which 19 candidate effectors could suppress programmed cell death triggered by BAX using transient expression in *Nicotiana benthamiana*. Of these 19 effector candidates, most do not contain PFAM domains, except one gene containing a domain of unknown function protein that could be involved in cell-cell recognition or cell-surface receptor signaling. Single nucleotide polymerphisms (SNPs) and insertion/deletion were detected in the coding region of some genes among eight different *Pst* isolates. Quantitative real-time PCR analyses revealed that transcripts of most genes were highly abundant in planta compared to urediniospores and germ tubes, but quite the opposite for some other genes. These results will provide useful information for further deciphering functions of rust effectors and pathogenic mechanism for rust fungi.

## Concurrent Session 24-Mycotoxins

### O24.001 Regulatory mechanisms of pH signaling and nitrogen metabolism on TRI6 expression and DON production in *Fusarium graminearum*

*R. Hou[1], Q. Zheng[1], C.F. Wang[1] and J.R. Xu[1, 2]*
*[1]Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, P. R. China; Department of Botany and Plant Pathology, Purdue University, USA*
*Email: wangchenfang@nwsuaf.edu.cn*

Deoxynivalenol (DON) produced by *Fusarium graminearum* is harmful to human and animals. Although the trichothecene biosynthetic gene clusters, including two pathway specific transcription factor *TRI6* and *TRI10*, have been studied extensively, the global regulation of DON synthesis are not well characterized. It has been reported that acidic pH and agmatine could induce the mycotoxins production. In this study, we aim to further characterize the roles of the *ARE1* and *PAC1* genes, two key regulators of nitrogen metabolisms and pH signal in filamentous fungi, in the regulation of *TRI6*/*TRI10* and DON biosynthesis. The *are1* mutant was significant reduced in vegetative growth, DON production, and virulence. The expression of *TRI5*, *TRI6*, and *TRI10* genes was reduced in the *are1* mutant. Expression of *ARE1*-GFP fully complemented the mutant phenotypes and the localization of Are1-GFP to the nucleus was enhanced by nitrogen starvation. A putative nuclear localization signal of Are1 was identified by site-directed mutagenesis. The promoters of *TRI6* and *TRI10* have 6 and 3 putative AreA-binding sites, and 1 and 2 putative PacC-biding sites, respectively. The functions of these promoter elements in the regulation of *TRI6* and/or *TRI10* expression are being characterized. We are also in progress in characterizing possible direct interactions of Pac1 or Are1 with Tri6 and/or Tri10. The activation of Pac1 under different pH conditions and its regulation of *TRI* genes are being characterized. The GFP-Pac1 fusion protein appeared to be activated and enter the nucleus under alkaline conditions. The possible negative regulation of *TRI* gene expression by Pac1 and its relationship with Are1 in DON biosynthesis will be presented.

### O24.002 *Fusarium* mycotoxins HT-2 and T-2: issues and control strategies in small grain cereals

*S.G. Edwards and T. Stancic*
*Crop and Environment Sciences, Harper Adams University, Newport, Shropshire TF10 8NB, UK*
*Email: tstancic@harper-adams.ac.uk*

HT-2 and T-2 toxins are two of the most potent trichothecenes mycotoxins produced by several *Fusarium* species. They have a group (HT-2+T-2) Tolerable Daily Intake of 0.1 μg/kg body weight /day. The European Commission recently published a recommendation to monitor HT-2 and T-2 in cereals and cereal products and if indicative limits are found to be exceeded, then to identify why they were exceeded and what control mechanism can be used to avoid high concentrations of these mycotoxins occurring again. Legislative limits will be considered in 2015. The distribution of HT-2 and T-2 appears to be largely restricted to Europe. Of the cereal species, HT-2 and T-2 usually have higher incidences and concentrations on oats followed by barley and then wheat; however, this can vary between countries. A recently identified species, *Fusarium langsethiae*, has been implicated as an important producer of HT-2 and T-2 in European cereals. There is limited data available regarding this species' pathogenicity and mycotoxin production. The impact of agronomy on the concentration of HT-2 and T-2 in cereals has not been clearly identified but it is evident that it is different to the impact of agronomy on deoxynivalenol in wheat. Lower concentrations of HT-2 and T-2 were identified on organic compared to conventional oat crops and fungicides appear to give inconsistent control. Studies on oat varieties in the UK have identified that significantly higher HT-2 and T-2 occurs in some varieties of winter oats compared to spring oats.

### O24.003 The multifaceted fumonisins and *Fusarium verticillioides*-maize interaction

*P. Battilani[1], I. Lazzaro[1], C. Falavigna[2], G. Galaverna[2] and C. Dall'Asta[2]*
*[1]Institute of Entomology and Plant Pathology, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italia; [2]Department of Food Science, University of Parma, Viale delle Scienze 17/A, 43124 Parma (Italy)*
*Email: paola.battilani@unicatt.it*

*Fusarium verticillioides* was described for a long time as an endophyte and the interest towards its interaction with maize plant was negligible before the discovery of fumonisin in 1989. The detection of hidden fumonisins in 2009, and their occurrence in raw maize confirmed in 2010, increased health concerns. Besides, fumonisin derivatives obtained by the esterification of $FB_1$ with fatty acids were recently described. Fumonisin masking was confirmed in raw maize. The chemical composition of hybrids and the main role of fatty acids were highlighted. Hidden fumonisins were never observed in *Fusarium* cultures grown on synthetic media (malt extract) but were detected in maize-based medium, suggesting that the masking phenomenon can occur only in a complex matrix. Fumonisin production was studied using cornmeal or single components of kernels, like starch, and it was higher in cornmeal than in starch based medium. As regard fumonisin hidden forms, they may occur in cornmeal medium, whereas they have never been

found at significant levels in corn starch medium. Fumonisin esters were detected in cornmeal medium, while they were never observed in *Fusarium* cultures grown on a malt extract medium, suggesting that also the esterification of $FB_1$ can occur only in a complex matrix such as maize. Finally, their occurrence in mouldy maize was also demonstrated under natural conditions. Therefore, we do not know the role of fumonisin in plant-pathogen interaction, but it is confirmed that there is a lot of interaction.

**O24.004 Systems approach to understand climate change impacts on mycotoxin contamination of food**

*N. Magan, A. Medina and D. Aldred*
*Applied Mycology Group, Cranfield Health, Cranfield University, Cranfield Beds. MK43 0AL, U.K.*
*Email:n.magan@cranfield.ac.uk*

Food security issues have become important as prices of staple grains have soared in the last few years. Climate change is expected to increase pressure on food supply/quality/sustainability worldwide. Changes in rainfall patterns, drought, temperature and $CO_2$ all impact on staple food production systems. It has been suggested that there will be "hotspots" in different regions of the world where the temperature may increase by +2-4$^oC$, where rainfall patters and drought events may increase, resulting in more rapid desertification and significantly impacting on staple food crop yields. Thus for staple cereals a doubling of $CO_2$ (350 to 700 μl/l) and an increase in temperature will have a penalty on yield and nutritional quality while for rice this could increase crop biomass although effects on yield and quality are less clear. Plant stress inevitably leads to increased susceptibility to fungal infection, pre- and post-harvest, and potential for increased contamination by mycotoxins. There has been significant interest in the impact that climate change environmental factors have on the ecology of mycotoxigenic moulds pre- and post-harvest in staple cereals. Using a mycotoxin microarray with sub-arrays for trichothecene B, aflatoxin and fumonisins we have examined the interaction between toxin gene clusters, growth and toxin production under different climate change environmental factors (water availability, temperature, $CO_2$) using strains of *Fusarium graminearum*, *Aspergillus flavus* and *F. verticillioides* respectively. These data sets have been integrated for the first time using a mixed growth model and linking this to expression of key biosynthetic structural and regulatory genes in the biosynthetic pathways for mycotoxin production (6 *TRI*, 10 *Afl* and 9 *FUM* genes) to develop predictive models. This approach also allows the relationship between different key genes in to be determined and the importance of individual genes under different environmental conditions to be evaluated. The models can also be used to predict the relative risk of production of these mycotoxins under different climate change scenarios. This approach could have implications and benefits for the development of novel control systems as well as a better understanding of the impacts that climate change may have on mycotoxin production by taking into accounts the influence of a range of key parameters.

**O24.005 Evaluation of deoxynivalenol biosynthesis related gene expression during wheat-*Fusarium graminearum* colonization**

*C.C. Amarasinghe and W.G. Dilantha Fernando*
*Department of Plant Science, University of Manitoba, Winnipeg, Canada, R3T2N2*
*Email: D_fernando@umanitoba.ca*

Fusarium head blight (FHB) is a devastating disease of wheat and barley, mainly caused by *Fusarium graminearum*. During infection, the pathogen produces the trichothecene mycotoxin deoxynivalenol (DON), which increases fungal virulence. To date 15 DON biosynthetic genes have been characterized. The objective of this study was to examine the level of expression of *TRI4*, *TRI5*, *TRI9* and *TRI14* in resistant and susceptible wheat cultivars after inoculating with *F. graminearum* 3ADON and 15ADON isolates. Two wheat cultivars Glenn (MR) and Roblin (S) were grown in the greenhouse and inoculated with 3ADON and 15ADON isolates. The level of expression of DON biosynthesis related genes were evaluated at 0, 6, 12, 24, 48, 72 hrs and 7 days after inoculation. The relative expression of the above genes were analysed in comparison with the *GAPDH* control gene using qPCR. The expression of these genes was initiated at 72 hrs after inoculation and expression was greater at 7 dai when compared to 72 hai. The relative expression of *TRI5* was higher in the cv. Roblin as compared to cv. Glenn in most of the treatments. In several treatments, *TRI5* gene expression differed among the two isolates within the same chemotype group confirming the isolate variation during plant-pathogen interaction. Our results indicate that DON biosynthesis genes continue to express 7 dai. Consequently, wheat samples collected at 10 dai, 14 dai and 21 dai are now being analysed to examine the role of DON biosynthesis genes during wheat senescence.

**O24.006 Effect of cultivars and storage temperature on Trichothecene production in potato tubers inoculated with three *Fusarium* strains**

*H.L. Xue[1,2], Y. Bi[1], Y.M. Tang[1], Y. Zhao[1] and Y. Wang[1]*
*[1]College of Food Science and Engineering, Gansu Agricultural University, Lanzhou,730070, P. R. China; [2]College of Science, Gansu Agricultural University, Lanzhou, 730070, P. R. China*
*Email: biyang@gsau.edu.cn*

Four kinds of trichothecenes (Fus-X, 3ADON, DAS and T-2) were detected in susceptible and resistance potato

tubers inoculated with *F. sulphureum, F. graminearum* and *F. solani*. Potato cultivars, *Fusarium* strains and storage temperature affected significantly the toxin production. The concentration of Fus-X, 3ADON, DAS and T-2 in the susceptible cultivar were higher than in the resistant one. Susceptible tubers infected with *F. sulphureum* had maximum concentration of Fus-X, 3ADON and DAS, and *F. solani* had a stronger ability to produce T-2 toxin than *F. sulphureum* and *F. graminearum* in susceptible and resistant cultivars. Room temperature storage were more likely to accumulate trichothecenes than low temperature storage. Meanwhile, the trichothecenes could not only be found in the lesion, but also in distant healthy looking parts. And there was a gradient in trichothecenes concentration showing a strong decline with an increasing distance from the infection point. It is suggested that consumers should pay attention that the common practice of removing the lesion from disease tubers may be not effective enough to prevent the intake of trichothecenes.

**O24.007 Novel approach to enhance maize resistance to *Aspergillus flavus* infection and aflatoxin contamination**

*Y. Raruang, Q. Wei, Y. Ramegowda, B. Bluhm, R.L. Brown, D. Bhatnagar and Z.Y. Chen*
*Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, U.S.A.*
*Email: zchen@agcenter.lsu.edu*

Maize (*Zea mays* L.) is one of the major crops susceptible to *Aspergillus flavus* infection and subsequent accumulation of the toxic and highly carcinogenic secondary metabolites, aflatoxins. Although maize genotypes resistant to *A. flavus* infection and /or aflatoxin contamination have been identified in the past through field evaluations, the poor agronomic quality of these lines, renders them of little direct commercial value. The lack of identified markers also has slowed the incorporation of resistance into lines with commercially-acceptable genetic backgrounds. Several recent studies demonstrated that small RNAs expressed in the host plants can interfere with pathogen growth and reduce disease development. In the present study, this novel "host induced gene silencing" strategy is explored to see whether it can reduce *A. flavus* infection of maize and aflatoxin contamination. Several *A. flavus* genes important for its growth or toxin production have been introduced into maize through *Agrobacterium*-mediated transformation, and the changes of these transgenic lines in resistance to *A. flavus* infection and aflatoxin contamination are currently being evaluated for the effectiveness of this novel strategy in controlling *A. flavus* infection in maize, which could have significant potential impact in reducing aflatoxin contamination in other susceptible crops, such as cotton and peanuts, as well as plant fungal disease control in general. To the best of our knowledge, this is the first report of such study in maize.

**O24.008 Natural and synthetic fungicides from fungal cells – regulated transport or transported regulators?**

*A. Dawidziuk, D. Popiel, G. Koczyk and J. Kaczmarek*
*Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland*
*Email: adaw@igr.poznan.pl*

Traditionally toxic molecules are viewed as harmful compounds, detrimental to cell adaptation. One of the main coping strategies is efflux or compartmentalisation, however a widespread but less toxic compound can also serve as a potential signal enabling adaptive reaction. We examine a distinct effect of natural and artificial toxins on morphology, growth patterns and transporter gene expression after stimulation in both mycotoxin producing and non-producer isolates of divergent *Fusarium* species. Toxigenic capability of examined strains was confirmed by sequencing based on cross-species specific degenerate primers and HPLC. We demonstrate emergence of a distinct dosage-dependent "producer effect" (producing species being more resistant to their own toxin, relic resistance in species that lost the biosynthetic capacity), as well as possible explanation for chemotype divergences due to adaptation of some toxins, as signalling molecules. In particular deoxynivalenol, a mycotoxin commonly produced by the widespread and aggressive cereal pathogen *Fusarium graminearum* shows distinct patterns of influence on both morphology and gene expression of both producer and non-producer species, above and beyond the (potential) toxicity.

**P24.001 *Fusarium graminearum* mycotoxins associated with grain mold of maize and sorghum in South Africa**

*M. Mavhunga, S. Koch, B.C. Flett and N.W. McLaren*
*Department of Plant Sciences, University of the Free State, P.O. Box 339, Bloemfontein, South Africa, 9300*
*Email: mclarenN@ufs.ac.za*

Members of the *Fusarium graminearum* species complex (FgSC) result in reduced grain quality and mycotoxin contamination. Colonization of local maize and sorghum grain by *F. graminearum* (*sensu lato)* from 2006 to 2009, using quantitative real-time polymerase chain reaction (qPCR), ranged from 1-3920 and 4-3790 pg/mg respectively, with highly significant season and locality effects. Highly significant cultivar effects were also recorded indicating clear differences in the susceptibility of grains to colonization. Analysis of the translation elongation factor-1α gene revealed that *F. boothii* was the predominant species infecting maize kernels while *F. meridionale, F. acacia-mearnsii* and *F. corta-*

*deriae* were associated with sorghum grain. Based on the *Tri*12 and *Tri*6 portion of the trichothecene gene, all maize FgSC isolates were 15-Acetyldeoxynivalenol (15-ADON) chemotype while the sorghum isolates where all nivalenol (NIV) chemotype. No 3- Acetyldeoxynivalenol chemotypes were detected. Deoxynivalenol (DON), NIV and zearalenone (ZEA), determined using LC-MS/MS, showed that DON, NIV and ZEA co-occurred in both maize and sorghum grain. Significant differences in all toxin levels, due to cultivar effects, were recorded in maize while significant differences in ZEA concentrations between sorghum cultivars were recorded but not in NIV and DON. The occurrence of NIV in maize and DON in sorghum suggests that toxins may originate in tissues other than grain. Although incidences of high colonization of grains by members of the FgSC and associated mycotoxins were recorded, indications are that cultivars differ significantly in susceptibility and that selection for resistance may be possible.

**P24.002 The role of *Fusarium torulosum* in Kikuyu poisoning of cattle in Western Australia**

*K.L. Cosgrove-Wilke[1], M.P. You[1], G.R. Flematti[2], S.E. Shaw[3], D. Wright[4], W. MacLeod[4], J. Allen[4] and M.J. Barbetti[1,5]*

*[1]School of Plant Biology, Faculty of Science, The University of Western Australia, Nedlands WA 6009 Australia; [2]School of Biomedical, Biomolecular and Chemical Sciences, Faculty of Science, The University of Western Australia, Nedlands WA 6009 Australia; [3]Department of Agriculture and Food Western Australia, 444 Albany Hwy, Albany WA 6330 Australia; [4]Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth WA 6151 Australia; [5]The UWA Institute of Agriculture, Faculty of Science, The University of Western Australia, Nedlands WA 6009 Australia*

*Email: 21230926@student.uwa.edu.au*

Kikuyu poisoning of grazing animals was first reported in the early 1960's in New Zealand, Kenya, Zimbabwe and South Africa. Outbreaks began occurring in Australia in the 1970's, first in Western Australia and then in 1980 in New South Wales. While the occurrence of incidences is increasing, especially in the past decade, the causative agent of kikuyu poisoning still remains unconfirmed. During an outbreak in 2007 near Albany, Western Australia, the endophyte, *Fusarium torulosum*, was readily isolated from samples taken from toxic fields where a high prevalence of cattle deaths occurred. In contrast, no *F. torulosum* was detected in samples taken from surrounding fields where no cattle deaths occurred. Previous studies indicated that *F. torulosum* produces the mycotoxins (secondary metabolites) wortmannin, butenolide, moniliformin and enniatins. Several of these mycotoxins can produce similar lethal symptoms in rats and such symptoms have been observed in the cattle suffering from kikuyu poisoning. However, the role played by *F. torulosum* in this disorder has not been definitively determined and the toxins produced by *F. torulosum* have not been characterised in the context of the Western Australian kikuyu poisoning events. Studies are being undertaken to determine the survival, carryover, infection and colonisation processes of *F. torulosum* on kikuyu; identify the range of mycotoxins produced by this fungus; and finally, investigate the relative toxicity of the mycotoxins produced using the established brine-shrimp toxicity bioassay.

**P24.003 The fragmented Dothistromin gene cluster and its regulation by AflR**

*P. Chettri, K.C. Ehrlich, J.W. Cary, J. Collemare, M.P. Cox, S. Griffiths, M.A. Olson, P.J.G.M. de Wit and R.E. Bradshaw*

*Bio-Protection Research Center, Institute of Fundamental Sciences, Massey University, Palmerston North 4442 New Zealand*

*Email: p.chettri@massey.ac.nz*

Red band disease, caused by the fungus *Dothistroma septosporum*, is a devastating disease of pine plantations globally. The red banding is associated with the polyketide toxin dothistromin which is structurally similar to versicolorin B, a precusor of aflatoxin. Unlike most other secondary metabolites, dothistromin is produced mainly during the early exponential growth phase in culture. The *D. septosporum* genome was fully sequenced by the DOE Joint Genome Institute; this enabled the identification of all putative dothistromin genes, including an ortholog of the aflatoxin regulatory gene *AflR*, and also revealed that most of the genes are spread over six separate loci on chromosome 12 (1.3 Mb) leading to the question of how such a fragmented cluster is regulated. To address this question, AflR function was analyzed in *D. septosporum.* Inactivation of the *DsAflR* gene (*ΔDsAflR*) resulted in a drastic reduction in dothistromin. This contrasts with orthologous Δ*AflR* mutants in *Aspergillus* species that produce no aflatoxin. Expression patterns in Δ*DsAflR* mutants helped to predict the complete set of genes involved in dothistromin production and also showed no correlation between dothistromin gene expression and gene distance from the telomere. An orthologous set of dothistromin genes with similar arrangement was found in the closely related biotrophic tomato pathogen *Cladosporium fulvum*, even though this species does not produce dothistromin. In *C. fulvum*, pseudogenization of key biosynthetic genes explains the lack of dothistromin production. The fragmented arrangement of dothistromin genes provides an example of coordinated control of a dispersed set of secondary metabolite genes.

**P24.004 Can seed systems contribute to mycotoxin control?**

*P.C. Biemond [1, 2], T.J. Stomph [2], P. Lava Kumar [3] and P.C. Struik [2]*
*[1]International Institute of Tropical Agriculture (IITA), Sabo Bakin Zuwo Road, PMB 3112, Kano, Nigeria; [2]Centre for Crop Systems Analysis, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; [3] International Institute of Tropical Agriculture (IITA), PMB 5320, Ibadan, Oyo state, Nigeria*
*Email: christiaan.biemond@wur.nl*

Mycotoxins, toxins produced by fungi, are threatening millions of people in developing countries. Consumption of mycotoxin-contaminated maize can cause cancer and even sudden death. Infection with mycotoxigenic fungi is required for mycotoxin production to occur. Infection can take place by seed-to-seedling transmission when seeds are infected. Seed samples are often heavily infected with mycotoxigenic fungi. We carried out a risk analysis of infection with mycotoxigenic fungi in formal and informal seed systems. A schematic overview of the maize value chain is presented to identify key risks and opportunities in the formal and informal seed system. A range of potential control measurements is discussed based on criteria for effective and sustainable control. Finally, an integrated approach is recommended based on control measures in the seed system.

**P24.005 Development of microcantilever based immunosensors to detect aflatoxins and ochratoxin A**

*C. Ricciardi[1], I. Ferrante[2], F. Frascella[3], S.L. Marasso[1], K. Santoro[1], A. Lorè[2], A. Prelle[2], M.L. Gullino[2] and D. Spadaro[2]*
*[1]DISAT-LATEMAR, Polytechnics of Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy; [2]AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*
*Email: davide.spadaro@unito.it*

Among the known mycotoxins, ochratoxin A (OTA) and aflatoxins (AFs) are of greatest concern due to their frequent occurrence in foods and their severe effects on animal and human health. The availability of reliable methods for the determination of AF and OTA in foodstuffs is highly desirable in order to fulfil the need to protect consumer health from the risk of exposure to the toxin. An innovative immunosensing method for mycotoxin detection, based on antibody-immobilized microcantilever resonators, was developed. The effect on microcantilever resonance frequency of the incubation buffer composition and of the washing and drying procedure were evaluated. Microcantilever resonator arrays could be used to effectively identify total aflatoxins and ochratoxin A, at low concentrations (3 ng/mL and less than 6 ng/mL, respectively), with relatively low uncertainty (about 10%) and good reproducibility for the same target concentration. The developed immunosensing method showed limited cross-reactivity to different mycotoxins, paving the way to a highly specific technique, able to identify different mycotoxins in the sample.

**P24.006 Determining the risk of fumonisin producing *Fusarium* spp. and fumonisin synthesis in commercial South African maize**

*B. Janse van Rensburg, N.W. McLaren, B.C. Flett and A. Schoeman*
*Agricultural Research Council-Grain Crops Institute, Private Bag X1251, Potchefstroom, 2520, South Africa*
*Email: BelindaJ@arc.agric.za*

Fumonisins are mycotoxins produced primarily by *Fusarium verticillioides* and *F. proliferatum* on maize. These mycotoxins are secondary, carcinogenic metabolites and have been reported on maize worldwide. The natural occurrence of fumonisin producing *Fusarium* spp. and fumonisin contamination of maize grain were quantified in selected maize cultivars from principal production areas of South Africa. Grain colonization by *Fusarium* spp. was determined using quantitative real-time PCR and contamination with fumonisins using HPLC analysis. Results indicated high natural infection by fumonisin-producing *Fusarium* spp. and fumonisin concentrations in warmer production areas such as Northern Cape, North West and Free State Provinces. Site-specific weather data, including temperature, radiation, humidity, rainfall and evapo-transpiration were provided by the ARC-Institute for Soil Water and Climate's meteorology office. *Fusarium* colonization of grain and fumonisin concentrations were related to prevailing weather conditions during early post-flowering and dough stage of grain development, respectively. Both colonization and fumonisin production were significantly inversely correlated with mean maximum temperature (r=-0.77 and r=-0.60, respectively) and minimum relative humidity (r=-0.83 and r=-0.79, respectively) during these critical growth periods. A preliminary model based on the non-linear, 3-dimentional Lorentzian equation (Sigmaplot 10.0) was developed and evaluated.

**P24.007 Promising new approaches for inhibition of aflatoxin B1 biosynthesis and its biological degradation**

*V.G. Dzhavakhiya[1], L.A. Shcherbakova[1], R.M. Khomutov[2], E.N. Khurs[2], T.N. Nazarova[1], O.D. Mikityuk[1], T.I. Osipova[2], N.S. Zhemchuzhina[1] and B.C. Campbell[3]*
*[1]Russian Research Institute of Phytopathology, VNIIF, B. Vyazemy, Odintsovo distr., Moscow reg., 143050, Russia; [2]Engelgard Institute of Molecular Biology RAS, 32 Vavilova str. Moscow 119991, Russia; [3]Plant Mycotoxin Research, Western Regional Research Institute, USDA, Albany, CA 94710, USA*

*Email: larisa@vniif.ru*

Contamination of agricultural feedstock and food products with aflatoxin B1, a highly durable hepatotoxic and carcinogenic mycotoxin of *Aspergillus flavus*, is a serious international problem. Development of methods to inhibit B1 biosynthesis or its decontamination is of paramount importance to public health and agricultural. Aflatoxigenesis is stimulated by oxidative stress, a presumed fungal defense. To inhibit aflatoxigenesis, we considered targeting stress-activated fungal antioxidative systems. We found a number of antioxidants and organophosphorous analogues of amino acids that effectively repress the B1 biosynthetic pathway. For instance, the antioxidants, ascorbic, caffeic, chlorogenic, and gallic acids, as well as phosphoanalogues of aspartic acid, alanine and leucine, considerably decreased B1 production. Treatment of the fungus with caffeic acid resulted in the complete shutdown of the aflatoxin biosynthetic gene cluster. With regard to decontamination of B1, past efforts involved environmentally unsuitable methods, such as ammoniation under high pressure and temperature. To develop safer methods, we screened fungi, co-colonizing natural substrates with toxigenic *A. flavus*, for B1-degradative properties. Using culture liquid (CL), we found the most active B1 degradation in isolates of *Phoma glomerata*, *Cladosporium* sp., *Gliocladium roseum* and *Ulocladium* sp. The highest level of B1-catabolizing activity was detected in protein fractions of the CL of *P. glomerata*. This activity was thermolabile, pH-dependent and significantly reduced by enzymatic proteolysis. Additionally, we found that *A. flavus* can regulate not only the biosynthesis of B1, but also its catabolism. The promise for the use of these new methods for the control of aflatoxin is discussed.

### P24.008 Potential use of RNAi technology to control mycotoxin production

*N. Magan, A. Abdel-Hadi, G. Moreire Leite and A. Medina*
*Applied Mycology Group, Cranfield Health, Cranfield University, Beds. MK43 0AL, U.K.*
*Email: n.magan@cranfield.ac.uk*

*Aspergillus flavus*, *Fusarium graminearum* and *Penicillium verrucosum* are important pathogens of food crops producing aflatoxins, trichothecenes and ochratoxins both in the field and during storage. We have designed siRNA sequences to target mRNA sequence of key genes (*aflD* and *aflR*, *Tri5*, *OTApks*Pv) gene to examine the potential for using RNA silencing technology to control toxin production by these species. Results showed that the effect of siRNAs targeting of two key genes in the aflatoxin biosynthetic pathway, *aflD* (structural) and *aflR* (regulatory gene) and on aflatoxin B1 (AFB1) was to significantly reduce the activity of these genes and reduced toxin production significantly. The results with RNAi sequences for *Tri5* and *OTApks*Pv showed that inhibition of the latter gene was more effective. In all cases the concentration of the RNAi influenced the level of control of key regulatory/structural gene expression and phenotypic toxin production. These studies suggest that potential does exist for harnessing RNAi approaches as an intervention strategy to try and minimise production of mycotoxins in staple foodstuffs.

### P24.009 Oat resistance to HT2 and T2-producing *Fusarium langsethiae*

*T. Stancic[1], A.A. Cowan[2] and S.G. Edwards[1]*
*[1]Harper Adams University College, Newport, Shropshire, TF10 8NB, UK; [2]IBERS, Aberystwyth University, Plas Gogerddan, Aberystwyth, SY23 3EB, UK*
*Email: tstancic@harper-adams.ac.uk*

*Fusarium langsethiae* is a newly identified species from the *Fusarium* genus which was first described in 2004. It is known that *F. langsethiae* is the predominant HT2 and T2-producing species on oats in the UK. The European Commission has proposed guideline limits for the combined concentration of HT2 and T2 (HT2+T2) in food and feed. In observational studies across the UK between 2002 and 2008 around 16% samples collected at harvest exceeded the proposed guideline limit of 1000 μg/kg HT2+T2 for unprocessed oats intended for human consumption. From 2005 to 2011, oats from over 20 winter and more than 14 spring national Recommended List variety trials were analysed for the presence of HT2+T2 mycotoxins. All winter variety trials had higher levels of HT2+T2 compared to the spring variety trials. Spring varieties had lower levels of HT2+T2 than winter varieties and in most trials there was no significant difference between levels of toxins in spring varieties. It is not clear whether the difference observed between winter and spring varieties is due to agronomic (ie drilling date) or genetic difference. To test the hypothesis that the difference observed were not due to agronomy, six spring and six winter varieties were drilled together in randomised block experiments at three sites in the UK in autumn 2011 and in spring 2012. Samples were collected before and after harvest and are currently being quantified for level of HT2 and T2.

### P24.010 Food condiment series 1: fungal contaminant of *Detarium microcarpum* sold in Etche and Bori local markets in rivers state of Nigeria

*I. Nwogu[1], C. Gloria[2], E. Edith Nkem[2] and C. Happiness[3]*
*Department of Plant Science and Biotechnology, University of Port Harcourt, Department of Plant Science & Biotechnology Faculty of Science, University of Port Harcourt. Nigeria*
*Email: Chinyerum.nwogu@yahoo.com*

Fungal contaminants of food condiments; *Detarium macrocarpum* seeds was studied. Whole and grind samples of *D. macrocarpum* were obtained from two different markets in Rivers State namely; Etche and Bori. A survey was conducted and the following fungi; *Aspergillus flavus, A. niger, A.* spp., *Botyriodiplodia theobromae, Fusarium solani, Rhizopus stolonifer, Penicillium* spp., *P. chrysogenum and F. moniliiforme* were found growing on the *D. macrocarpum*. These organisms were isolated using the Blotter Method and pure cultures were obtained on Potatoes Dextrose Agar (PDA). From the results obtained, it showed that the *D. macrocarpum* obtained from Etche market have the highest percentage incidence of (81.83%) as compared to those obtained from Bori market, that has a percentage incidence of (79.16%). The aim of this work is to identify toxins associated with the fungal contaminants *D. microcarpum*. *D. microcarpum* is a good substrate, which pathogenic fungi such as *Aspergillus* spp. grow on. These pathogenic fungi produce toxins that are infectious and detrimental to human because of the diseases they cause such as: damage and weakening of the immune system, to tumors in the human urinary tract, liver cancer, fever, cough, chest pain and breathlessness.

### P24.012 Dynamic of free and hidden fumonisins in maize during the growing season and related lipidomic

*P. Giorni[1], R. Gregori[1], C. Dall'Asta[2], M. Cirlini[2], G. Galaverna[2], V. Scala[3], M. Reverberi[3], E. Camera[4], C. Fanelli[3] and P. Battilani[1]*
*[1]Institute of Entomology and Plant Pathology, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy; [2]Department of Organic and Industrial Chemistry, Università di Parma, 43100 Parma, Italy; [3] Department of Environmental Biology, Università Sapienza, 00165 Roma, Italy*
*Email: paola.battilani@unicatt.it*

Recent studies showed fumonisin contaminations of maize mainly influenced by the different hybrids and the year of cultivation. A correlation between fumonisins and fatty acids content (in particular linoleic acid) and a correlation between the ratios oleic acid/linoleic acid and free/hidden fumonisins have been shown. A study has been planned to sample different maize hybrids cultivated in Italy to monitor toxigenic fungi, free and hidden mycotoxins and lipids composition of maize in order to confirm/explain previous data. Ten ears of different maize hybrids were collected starting from the early dough to harvest. Kernels obtained were used to quantify fungal incidence (focus on *Fusarium* spp.) and fumonisin content and for lipidomic analysis. Results obtained show that the presence of *Fusarium* species is directly correlated to an higher presence of both free and hidden fumonisins; however, differences exist along the growing season regarding both fungal incidence and fumonisin between hybrids. The lipidomic approach supported by the Principal Coordinate Analysis clearly indicates that the maize hybrids can be sorted into high- (HFC) and low-fumonisin conductive (LFC) by specific lipid compounds. Notably, 5 lipid compounds can sort HFC apart from LFC in all the phenological stage of maize kernel development. Moreover, at ripening up to 44 lipid compounds can differentiate HFC from LFC. Further studies, aimed to characterize these distinctive lipid compounds are ongoing.

### P24.013 Maize components, and the production and masking of fumonisin B, A, C, in *Fusarium verticillioides* and *F. proliferatum*

*I. Lazzaro, C. Falavigna, C. Dall'Asta, G. Galaverna and P. Battilani*
*University Cattolica del Sacro Cuore, Piacenza, 29122, Italy*
*Email: paola.battilani@unicatt.it*

*Fusarium verticillioides* and *F. proliferatum* are the two main fungi causing pink ear rot on maize, able to produce fumonisins. Fumonisins B and fumonisin C (FC) were recovered on naturally contaminated maize, while fumonisin A (FA), and fumonisin P (FP) only *in vitro*; hidden fumonisin were recovered in raw and processed maize, and in maize-based synthetic media. There is lack of information about which maize component could affect fumonisin pattern production and the masking phenomenon, for this reason we analysed the production of FB, FA, FC and FP and the masking phenomenon on *F. verticillioides* and *F. proliferatum* cultured on cornmeal and corn-starch based media for 7-14-21-30-45 days. Fumonisin B, FA, FC were produced by both species ($P \leq 0.01$), mainly *F. verticillioides* in cornmeal; in corn-starch only FB were recovered, with *F. proliferatum* as the main producer; FP were never detected. In cornmeal, fumonisin series followed a similar trend in all *F. verticillioides* and *F. proliferatum* strains, with FB and FA significantly increased up to 21 days of incubation, while the peak of production for FC was registered at 30 days. In corn-starch FB production resulted delayed, with maximum production at 30 days for ITEM1744, and at 45 days for the other strains. In this study, the occurrence of hidden fumonisins has also been addressed. Although hidden fumonisins amount found under the applied conditions was quite low, data suggested that the masking effect may occur in cornmeal media. On the contrary, hidden fumonisins have never been found at significant levels in corn-starch media. The occurrence of low amount of hidden fumonisins in cornmeal media seems to suggest that the masking phenomenon is mainly matrix-driven and could be described as a physical interaction between mycotoxins and matrix constituents. Further studies should be performed in order to better understand the role played by the substrate in hidden fumonisins accumulation.

**P24.014 Generation of targeted gene deletion mutants in *Ustilaginoidea virens***

*D.W. Zheng*[1], *C.F. Wang*[1], *H.Q Liu*[1], *Z. Tang*[1], *Z. Bian*[1] *and J.R. Xu*[1, 2]
[1]*Purdue-NWAFU Joint Research Center and State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shanxi, P. R. China;* [2]*Department of Botany and Plant Pathology, Purdue University, USA*
*Email: dabingzh@gmail.com*

Rice false smut caused by *Ustiaginoidae viren*s has become a major disease in China, particularly on high-yield rice cultivars. In addition to yield losses, the pathogen produces ustiloxins that are toxic to high eukaryotes by interfering with microtubule functions. Although its genome was sequenced recently, functional characterization of pathogenesis- or toxin production-related genes has not been reported in *U. virens*. In this study, we aimed to establish targeted gene deletion approaches and characterize the function of candidate non-ribosomal peptide synthase (NRPS) genes in ustilioxin biosynthesis. Five NRPS genes named *NRPS1, 2, 3, 4* and *5* were identified in the genome sequence of *U. virens*. For each NRPS gene, the upstream and downstream flanking sequences were amplified and fused with the hygromycin phosphodiesterase gene (*hph*) by double-joint PCR. The resulting PCR product was cloned into vector pCB1302. Similar approaches were used to generate gene replacement vector for the *CvPMK1* and *CvMPS1* MAP kinase genes. All the resulting plasmids were transformed into *Agrobacterium tumefaciens* strain AGL-1. The *A. tumefaciens*-mediated transformation protocol was optimized for transformation of *C. virens*. Hygromycin-resistant transformants were screened by PCR for knockout mutants. To date, the *NRPS1* and *CvPMK1* mutant have been confirmed by Southern blot analysis. Phenotypes of these mutants are being characterized and will be presented. We are also in process of identifying knock out mutants of the other NRPS genes although the gene replacement efficiency appeared to be relatively low (approximately 1/300) approximately.

**N024.001 Biological control of isolates aflatoxigenic *Aspergillus flavus* on pistachio by *Pichia guilliermondii* under modified atmospheres**

*N. Khaledi and H. Aminian*
*Department of Entomology and Plant pathology, Aboureyhan Campus, University of Tehran, Tehran, 3391653755, Iran*
*Email: nkhaledi@ut.ac.ir*

Aflatoxin is one of the most potent naturally occurring carcinogens and mutagens and is produced primarily by *Aspergillus flavus*. In section study of biological control, effect three isolates of *Pichia guilliermondii* on growth and aflatoxin production three isolates aflatoxigenic *A.flavus* were evaluated. Study antagonist effect on fungal growth includes tests, test dual culture, test volatile metabolites, test non-volatile metabolites and combining tests of non-volatile and volatile compounds in PDA medium. Effect of antagonists in control of mycelia growth of isolates *A. flavus* in dual culture was 72.35 to 74.41 percent, in volatile metabolites test was 58.63 to 63.85 percent, in non-volatile metabolites test was 74.99 to 77.37 percent and in combining tests non-volatile and volatile compounds were 79.29 to 88.11 percent. Also in section study modified atmosphere, maximum fungal growth in the treatment of concentration 100% $O_2$ gas and maximum inhibition of growth isolates *A. flavus* in the concentration 100% $CO_2$ gas was observed the amount of 83.29 to 84.09 percent. The review effect concentration 100% $CO_2$ on growth three isolates of *P. guilliermondii* inhibitory effect was not observed. Then, the produced aflatoxin was extracted and subjected to qualitative determination Thin Layer Chromatography. Pistachio kernels in Akbari cultivar inoculated with *A. flavus*, then they under treatments: concentration 100% $CO_2$, isolate M47 of *P. guilliermondii*, combination concentration 100% $CO_2$ and isolate M47. After 60 days of inoculation pistachios, from the qualitative determination, no aflatoxin B1 any of treatments were produced. Thus integrating biological control and modified atmosphere can be used as a new method in control of postharvest diseases.

## Concurrent Session 25-Nanotechnology for Plant Health

### O25.001 *Red clover necrotic mosaic virus*, a plant viral nanoparticle platform for the tunable delivery of agricultural compounds

*S.A. Lommel, R.H. Guenther, J. Cao, T.L. Sit, C.H. Opperman and J.A. Willoughby*
*Department of Plant Pathology, North Carolina State University, Campus Box 7342, Raleigh, NC 27695-7342, USA*
*Email: steve_lommel@ncsu.edu*

*Red clover necrotic mosaic virus* is a robust, soil-borne icosahedral plant virus that is being evaluated for use as a Plant Viral Nanoparticle (PVN). EDTA treatment produces holes in the virion (while retaining its structural integrity) allowing infusion of neutral or positively charged small molecules. To optimize the active delivery and release performance of PVNs, the uptake/release behavior of doxorubicin (DOX) was determined as a function of molecule binding affinity to the virion. By varying the level of EDTA utilized during the infusion process for PVN formulation, we shifted the electrostatic interactions between DOX and the virion. Use of lower EDTA concentrations produced weak virion-DOX surface associations while higher EDTA concentrations led to the majority of DOX molecules being infused into the virion. Functional testing of the PVN for crop protection purposes involved formulations with abamectin (ABA) for the control of plant parasitic nematodes. ABA could be loaded into PVNs and when dosed directly to nematodes, this formulation proved active against both *Caenorhabditis elegans* and *Meloidogyne* spp. in culture. When applied to columns of various soil types, the PVN ABA formulations demonstrated increased mobility as compared to the free ABA. However, enhanced mobility alone is insufficient to afford lasting protection. To impart controlled release, PVNs were integrated within a tunable polymeric matrix to provide an optimal ABA release profile in the soil. This hybrid crop protection system with tunable release profiles will lead to highly efficacious and optimized seed treatments.

### O25.002 Potential application of *Hibiscus chlorotic ringspot virus* as a nano-protein cage for delivery of anti-cancer drugs

*S.M. Wong*[1, 2, 3]
[1]*Department of Biological Sciences, National University of Singapore, 117543;* [2]*Temasek Life Sciences Laboratory, Singapore 117604;* [3]*National University of Singapore Suzhou Research Institute, Jiangsu, China 215123*
*Email: dbswsm@nus.edu.sg*

The coat protein (CP) of certain plant viruses may reassemble into empty virus-like particles (VLPs) and these protein cages may serve as potential drug delivery platforms. We have produced VLPs from purified virions of *Hibiscus chlorotic ringspot virus* (HCRSV) by destabilizing the virions in 8 M urea of Tris buffer, pH 8 in the absence of calcium ions, followed by removal of viral RNA by ultrahigh speed centrifugation, and reassembled the CP in sodium acetate buffer, pH 5. The loading of foreign materials into the VLPs were dependent on electrostatic interactions. Anionic polyacids, such as polystyrene sulfonic acids and polyacrylic acids, but not neutrally-charged dextran molecules, can be successfully loaded into the VLPs. The molecular mass (Mm) threshold for the loaded polyacids cargo was about 13 kDa. Smaller molecules can be diffused out through the gaps between the S domains present on the surface of the VLPs. The polyacids-loaded VLPs had comparable size, morphology and surface charge density, when compared to the virions. The HCRSV-derived VLPs may provide a promising nano-sized protein cage for delivery of anionic drug molecules.

### O25.003 Carbon-dot to ferry drugs against plant pathogens: A trans-phloem delivery and controlled release study

*M. Sharon*
*N.S.N. Research Centre for Nanotechnology & Bionanotechnology, Jambhul Phata, Kalyan-Badlapur Road, Ambernath (West), 421505, Maharashtra, India*
*Email: sharonmadhuri@gmail.com*

Our efforts are in developing a small water-soluble nanoparticles that can facilitate uptake and uniform distribution of micronutrients (e.g., Cu/Zn) and naturally occurring biocides (essential oils) or synthetic drug into phloem of the bacteria infected mango, date-palm or citrus trees that will potentially help in combating the disease. Photoluminescent, <10nm Carbon dots have been envisaged by us as delivery vehicle because of their high chemical stability, resistance to photodegradation, biocompatibility, low toxicity, easily tuned optical properties and good water solubility. We have explored use of natural precursors (Gum Arabic, Neem Gum and Sugar-cane juice) for synthesis of crystalline C-dots using microwave assisted heating in alkaline environment. Sucrose density gradient centrifugation was used to separate C- dots, which separated them into three fractions showing green fluorescence of different intensities in UV light. Optical and morphological properties of the C-dots were confirmed by UV-Vis spectroscopy, Fluorescence spectroscopy, Raman, XRD and FE-SEM. UV-Vis spectra recorded in the spectral window 200-600nm displayed typical signature absorption of C-dots. FE-SEM showed presence of spherical 5-10nm C-dots. Micronutrient/C-dots/drug complex was synthesized & dialyzed against nanopure water to remove unattached drug and characterized by FTIR and TGA. Drug loading efficiency of C-dot was calculated.

For successful delivery of micronutrients along with antibiotics in diseased trees following strategies are being planned: coating C-dots with materials that can be transported across phloem; grafting molecular probes such as Si RNA to switch of the expression of functional genes responsible for proliferation of pathogens and also try C-dots protected gold nanoparticles to deliver drugs.

**O25.004 Development of nanodevices for fighting against plant pathogens**

*A. Pérez-de-Luque*
*IFAPA, Centro Alameda del Obispo, Área de Mejora y Biotecnología, Avda. Menéndez Pidal s/n, PO Box 3092, 14080 Córdoba, Spain*
*Email: alejandro.perez.luque@juntadeandalucia.es*

The emergence of nanotechnology and the development of new nanodevices and nanomaterials open up potential novel applications for defence against plant pathogens in agriculture. New tools for genetic manipulation and the rapid detection and treatment of diseases or for delivering specific substances into specific sites are some of the examples being developed. The possibility of controlled release of the agrochemical, either regulated by time, location, or triggered under certain circumstances, makes this approach very attractive for future developments. Organic polymers (such as nanocapsules), inorganic nanocarriers (such as nanoparticles), and clays and organoclays (such as nanocomposites) are already being tested as nanocarriers. Nanosensors developed for agricultural use should allow a farmer to use and interpret them easily without any specialized knowledge, being cheap, small and portable, and should not need any complex sample preparation. Recent research aims to use nanoparticles or nanovesicles as carriers of DNA into the plant cell nucleus for genetic manipulation, which is highly interesting from a genetic resistance point of view. The potential of nanotechnology in plant pathology is huge; however, most of the research projects are still under development and awaiting field assays and extensive testing. In addition, people must be kept informed about the risks and benefits of this technology and how to understand it. If certain nanodevices entail risks that can be assumed for a medical treatment against cancer but not for eating an apple, the consumer must be told that. That is the only way to obtain a safe application in the future.

**O25.005 Synthesis and characterization of chitosan nanoparticles and their antifungal and plant growth promoting properties in oilseed crops**

*R.D. Prasad, S. Kamandula and M. Venkatesham*
*Directorate of Oilseeds Research, Rajendranagar, Hyderabad-500 030, (A.P), India*
*Email: ravulapalliprasad@gmail.com*

Chitosan nanomaterials in safety and health management improved the productivity and development of various innovative applications. Here, Chitosan Nano Particles (CSNP) were synthesized using chitosan and tripolyphosphate to exploit the antifungal and Plant Growth Promoter properties in oilseed crops. The method of synthesis was a single step ionic gelation process involving high speed homogenization under the influence of a magnetic field. The synthesized CSNP were characterized for the surface morphology and ionic conductivity using Photon Correlation Spectroscopy (PCS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM).The mean particle distribution of the peaks was identified as 92.6 nm and 621.5 nm respectively with a critical variance of 10.22 and 12.95 respectively. This was considered identical to the fact that the nanoparticles in the solution have been in the size of 92.6 nm with a diameter of 85.9 nm. The antifungal property of CSNPs in combination with bioagents *Trichoderma* was exploited over economically important pathogens like *Macrophomina phaseolina*, *Fusarium oxysporum* and *Sclerotium rolfsii*. Chitosan antimicrobial activity depends on the MW (Molecular weight), % of DA (Deacetylation), pH of the solution and, of course, the target organism, the same phenomena is applicable to the CSNP. The *in vitro* spore germination assay revealed that the interaction with CSNP-Bioagent induces slow germination of the spores. The *in vitro* anti-fungal assay on the contrary has revealed an extensive growth of the bioagent over the pathogenic. The *in vitro* anti-fungal assay of all the fungi with CSNP-bioagent and chitosan-bioagent against the control, have proved that CSNP-bioagent controlled the development of the plant pathogens. One of the reasons for this might be the cationic charge and competence of the bioagent. Based on their performance in the *in vitro* spore germination test, CSNP-Bioagent were included in the seed treatment tests. Control plants treated with 0.2% Tween 20 and deionized water, exhibited disease symptoms early. Biocontrol strains, fungicide and chitosan showed significant increase in germination percentage as well as growth, but the seeds treated with CSNP showed 100% seed germination with effective disease control as well as better root and shoot development. Treatment with CSNP and spore suspensions of biocontrol agent ameliorated the effect on seedling germination and they were transferred to pots with infested soil. It has been observed that the plants continued the growth without showing any disease symptoms. The results reported herein suggest, application of CSNP-Bioagent as a conjugant organic nanocomposite for better productivity of crops and against the pathogens.

**P25.001 Evaluation of nano-materials on improving efficacy of inhibition to fungicide-resistant isolates**

*Y.J. Huang[1], J.W. Huang[1], J.J. Lin[2], P.F. Chang[1]and W.H. Chung[1]*
*[1]Department of Plant Pathology, National Chung Hsing University, No. 250, Kuo Kuang Rd., Taichung, 402 Taiwan, China; [2]Department of Prospective Chemical Materials and Nano-polymer, No. 1, Sec. 4, Roosevelt Road, Taipei, 10617 Taiwan, China*
*Email: wenchung@nchu.edu.tw*

Agricultural chemicals are commonly used in controlling plant diseases. However, the fungicide resistance, residue and its environmental impact are serious problems in agriculture. The nano-materials are one of the new alternatives used for controlling plant diseases. Previous studies demonstrated that the nano-materials of Ag and ZnO showed activity against microbes and used to control plant diseases. In this study, several nano-materials, nanosilicate platelets, nano-silvers and carbon nanotubes, were tested for their ability on the inhibition of the growth of plant pathogens. Results demonstrated that the nanosilicate platelets NSS1450 and NSS3150 efficiently inhibited the growth of plant pathogens. Of these two nano-materials, NSS1450 could inhibit spore germination of *Botrytis cinerea* and *Colletotrichum gloeosporioides* at greater than 99% and 84-100% respectively in 50 mg/L, while inhibited mycelial growth of *B. cinerea* and *C. gloeosporioides* at 60.2-100% and 26.2-42.6% respectively in 500 mg/L. Moreover, when NSS1450 at 100mg/L was combined with azoxystrobin at 100mg/L the efficacy on inhibiting growth of fungicide-resistant *B. cinerea* and *C. gloeosporioides* isolates was improved to 44.4-58.6% and 26.35-33.37%, respectively. Based on scanning electron microscopic observation, the mycelia of *B. cinerea* and *C. gloeosporioides* showed shrinkage and distortion after being treated with NSS1450. Besides, when observed under a transmission electron microscope both cytoplasms and cells of *B. cinerea* and *C. gloeosporioides* spores and mycelia showed depredation after being treated with NSS1450. Results demonstrated that NSS1450 may be adsorbed to the cell surface and destroyed the cells, even caused cell leakage or interrupted cellular processes such as metabolism and respiration.

**P25.002 Element analysis of cassava leaves induced resistance by *Bacillus subtilis* CaSU007 against *Colletotrichum gloeosporioides* f.sp. *manihotis* using Micro-beam Synchrotron X-ray Fluorescence (μ-SXRF)**

*R. Sungpueak[1], M. Sompong[1], K. Thumanu[2], W. Tantanuch[2], D. Athinuwat[3], S. Prathuangwong[4] and N. Buensanteai[1]*
*[1]School of Crop Production Technology, Institute of Agriculture Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand; [2]Synchrotron Light Research Institute (Public Organization), Ministry of Science and Technology, Nakhon Ratchasima, 30000 Thailand; [3]Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, Pathumthani, 12121 Thailand; [4]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900 Thailand*
*Email: Fongfangfang_M5430222@hotmail.com*

*Bacillus subtilis* CaSU007 is a PGPR that induces systemic protection in cassava against various diseases and enhances cassava growth. In this recent study, treatment of cassava stakes cv. Rayong72 with CaSU007 provided anthracnose disease protection, caused by *Colletotrichum gloeosporioides* f.sp. *manihotis*. CaSU007 treatment also increased phenolic content and salicylic acid levels in leaves over non-treated plants. Differential expression of these biochemical traits was more rapid and pronounced when CaSU007 treated plants were infected by *C. gloeosporioides* f.sp. *manihotis*, this pattern indicated systemic inducing. Chemical element analysis using μ-SXRF indicated that Calcium (Ca), chromium (Cr) and potassium (K) content were increase in CaSUT007-treated plants, compared with non-treated plants. These element changes might be related to the cassava growth promotion and resistance to pathogen defense function, induced by positive effect of CaSUT007.

**P25.003 Energy dispersive X-ray fluorescence spectroscopy analysis of Chinese cabbage elements infected by *Erwinia carotovora* pv. *carotovora***

*C. Seangchan[1], W. Kaewchanuan[1], N. Boonkerd[2], K. Thumanu[3], W. Tantanuch[3], D. Athinuwat[4], S. Prathuangwong[5] and N. Buensanteai[1]*
*[1]School of Crop Production Technology, Institute of Agriculture Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand; [2]School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand; [3]Synchrotron Light Research Institute (Public Organization), Ministry of Science and Technology, Nakhon Ratchasima, 30000 Thailand; [4]Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, Pathumthani, 12121 Thailand; [5]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900 Thailand*
*Email: kunnai_khaikem@hotmail.com*

Micro-beam Synchrotron X-ray Fluorescence (μ-SXRF) was employed to determine the chemical element profile during infection process of Chinese cabbage cv. max108 leaves infected by *Erwinia carotovora* pv. *carotovora*, the causal agent soft rot disease. Our results indicated that significant alteration in K, Ca, Mn and Fe contents were observed in the infected leaves when compared with the healthy leaves. The relationship between K/Ca, K/Fe ratios found to reduce in infected leaves while the ratios of Ca/Fe, K/S, K/Cl was increased. This finding

might lead to more an understanding of chemical element profile and essential cofactors in the soft rot infection processes and Chinese cabbage defense mechanism.

**P25.004 Application of colloidal metal nanoparticles in diagnosis of quarantine bacteria – *Clavibacter michiganensis* subsp. *sepedonicus***

*W. Przewodowski, A. Przewodowska and K. Treder*
*Plant Breeding and Acclimatization Institute - National Research Institute, Radzików; Department of Potato Protection and Seed Science in Bonin, 76-009 Bonin, Poland*
*Email: wlodzimierz.przewodowski@tu.koszalin.pl*

*Clavibacter michiganensis* subsp. *sepedonicus* (Cms) - cause of the ring rot disease, is one of the most important pathogens of potato. As a quarantine organism, Cms is subjected to official control. The occurrence of the ring rot is associated with serious consequences for potato producers. The most effective way to control this disease is application of healthy seed potatoes and rapid elimination of the disease outbreaks. Both cases need sensitive and specific diagnostic tools for detection of the cause pathogen. Identification of Cms is very difficult, mostly because currently used diagnostics methods have many limitations. Therefore, it is necessary to search for innovative solutions solving known methodological difficulties and allowing for fast, specific and sensitive detection of Cms. Thus the purpose of reported research was to develop diagnostic test for Cms. This test is based on nanocolloids applied as a useful material in diagnosis and control of the cause of the ring rot of potato.

## Concurrent Session 26-Natural Compounds and Disease Control

### O26.001 Tryptophan-derived metabolites in the immunity of model Brassicaceae species

*M. Piślewska-Bednarek[1], E.V.L. van Themaat[2], K. Kułak[1], P. Schulze-Lefert[2] and P. Bednarek[1]*
*[1]Institute of Bioorganic Chemistry, Polish Academy of Science, Noskowskiego 12/14, 61-704 Poznań Poland; [2]Max Planck Institute for Plant Breeding Research, Carl-von-Linne-Weg 10, 50829 Köln, Germany*
*Email: bednarek@ibch.pozna.pl*

One of the evolutionary conserved responses of flowering plants to pathogen attack involves biosynthesis and secretion of secondary metabolites. Model plant *Arabidopsis thaliana* accumulates upon infection Trp-derived metabolites, including indole-3-carboxylic acids (I3CAs) and phytoalexin camalexin. Our recent study revealed in this plant species a pathogen triggered pathway for metabolism of indole glucosinolates (IGs), which is critical for defence against a number of fungal and oomycete pathogens. Currently we investigate conservation of pathogen-inducible Trp metabolism in *A. thaliana* relatives. Our metabolic survey reveals a surprising conservation of the pathogen-triggered IG metabolic and secretory pathway between the tested plant species, suggesting an ancient and important function of this metabolic branch in Brassicaceae pre-invasive defence responses. In contrast, I3CA and camalexin biosynthesis appear to be clade-specific innovations within the conserved framework of pathogen-inducible Trp metabolism and represent relatively recent manifestations of the plant-pathogen arms race.

### O26.002 Mechanism and application of chitooligosaccharides in plant disease resistance

*H. Yin, X.M. Zhao and Y.G. Du*
*Liaoning Provincial Key laboratory of Carbohydrates, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, No. 457 Zhongshan Road, Shahekou District, Dalian, 116023, P. R. China*
*Email: duyg@dicp.ac.cn*

Chitosan is one of the most abundant carbohydrate biopolymers in the world. Chitooligosaccharides (buildup with β-1,4 glucosamines, degree of polymers 2-20) prepared from chitosan is a potent plant elicitor. Numerous papers report that chitooligosaccharides can elicit resistant to more than 60 diseases on several plants. The control efficacy of chitooligosaccharides on several plant diseases such as TMV, *Sclerotinia sclerotiorum* was validated in our lab. Based on recent reports and our results, we deduce that chitooligosaccharides control plant disease via activated plant innate immunity. This mode of chitooligosaccharides act on plant is similar with general vaccines act on human and animals. So we suggest maybe chitooligosaccharides can be considered as a "plant disease vaccine". Some important steps in chitooligosaccharides induced resistance process are chitooligosaccharides signal perception; signal transduction; chitooligosaccharides response genes and proteins activation; chitooligosaccharides induced defense-related secondary metabolites accumulation. We recently found that chitooligosaccharides elicited different signal pathways (SA or JA) aim at different pathogens. Besides the fundamental research, chitooligosaccharides bio-pesticides were developed and widely used in some province of China. These bio-pesticides have good effect on food crops, economic crops, vegetable crops, fruits and horticultural plants.

### O26.003 Spirobisnaphthalenes from the endophytic fungus Dzf12 of *Dioscorea zingiberensis*

*L. Zhou, T. Shan, J. Zhao, X. Cai, Y. Li, P. Li, Y. Mou, R. Zhang, C. Yin, L. Xu, X. Wang and M. Wang*
*Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: lgzhou@cau.edu.cn*

Spirobisnaphthalenes are a group of fungal secondary metabolites, consisting of 1,8-dihydroxynaphthalene-derived spiroketal unit linked to a second oxidized naphthalene moiety, that show a great variety of biological activities such as antibacterial, antifungal, antitumor, allelochemical and anti-leishmanial properties. More than 90 spirobisnaphthalenes have been obtained from nature. The endophytic fungus Dzf12 was isolated from the healthy rhizomes of a medicinal plant *Dioscorea zingiberensis* C. H. Wright (Dioscoreaceae). More than ten spirobisnaphthenes have been isolated and identified from Dzf12. Palmarumycin $C_{12}$ and palmarumycin $C_{13}$ (diepoxin ζ) were found as the two main spirobisnaphthalenes. Palmarumycin $C_{13}$ was examined to enhance diosgenin production in seedling and cell suspension cultures of *D. zingiberensis*. In order to increase palmarumycin $C_{13}$ yield in Dzf12 mycelial liquid culture, a few methods such as using yeast extracts and its fractions, in situ resin adsorption, addition of polysaccharides and oligosaccharides from its host plant *D. zingiberensis* have been studied in detail. Endophytic fungus Dzf12 should be a candidate for producing spirobisnaphthalenes in large scale. Investigations of the biosynthesis, metabolic regulation, biotransformation of spirobisnaphthalenes in this fungus are in progress.

### O26.004 Regulator-based genome mining for bioactive compounds in rhizosphere bacteria

*X. Cheng[1], I. de Bruijn[1], M. van der Voort[1], J.E. Loper[2] and J.M. Raaijmakers[1]*
*[1]Laboratory of Phytopathology, Wageningen University,*

*Droevendaalsesteeg 1, 6708 PB, Wageningen, the Netherlands; [2]USDA-ARS Horticultural Crops Research Laboratory and Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, United States of America*
*Email: xu.cheng@wur.nl*

*Pseudomonas* species are ubiquitous in plant-associated environments and produce an array of antimicrobial compounds. The biosynthesis of many of these metabolites is regulated by the GacS/GacA two-component regulatory system. Whole-genome transcriptome analysis of *Pseudomonas fluorescens* SBW25 showed that 702 genes were differentially regulated (fold change>4, P<0.0001) in a *gacS*::Tn5 mutant, with 300 and 402 genes up- and down-regulated, respectively. Similar to the Gac-regulon of four other *Pseudomonas* species, genes involved in motility, biofilm formation, siderophore biosynthesis and oxidative stress were differentially regulated in the *gacS* mutant of SBW25. Our analysis also revealed, for the first time, that transcription of nineteen rhizosphere-induced genes and of genes involved in type II secretion, (exo)polysaccharide, pectate lyase biosynthesis, twitching motility and an orphan nonribosomal peptide synthetase (NRPS) were significantly affected in the *gacS* mutant. Furthermore, the *gacS* mutant inhibited growth of oomycete, fungal and bacterial pathogens significantly more than wild type SBW25. Site-directed mutagenesis of the orphan NRPS gene cluster showed that the encoded peptide may act as a siderophore, but does not contribute to the enhanced antimicrobial activity of the *gacS* mutant. Subsequent random plasposon mutagenesis followed by activity bioassays revealed that genes involved in pyrroloquinoline quinone biosynthesis play an important role in the enhanced antimicrobial activity of the *gacS* mutant. Bioassays further showed that acidification via gluconic acid production are the underlying mechanism of the enhanced and broad-spectrum antimicrobial activity. Collectively these results showed that global two-component regulatory systems can be exploited to discover new mechanisms and bioactive compounds in rhizosphere bacteria.

### O26.005 Effect of plant secondary metabolites against *Fusarium oxysporum* f. sp. *lycopersici in vitro*

H.E. Flores-Moctezuma
*Centro Desarrollo de Productos Bióticos IPN. Carr. Yautepec-Jojutla Km.6, calle CEPROBI No.8, San Isidro, Yautepec, Morelos, México. CP. 62731*
*Email: hfloresm@ipn.mx*

An important role of plant secondary metabolism is the defense against herbivores, pests and pathogens. When farmers use some chemicals in agriculture, they can have negative effects in nature. One of the objectives of this study was to test *in vitro* twelve different plant metabolites as alternative fungicides against *Fusarium oxysporum* f. sp. *lycopersici*, the etiological agent of vascular tomato wilt. Doses of 5000, 2500, 1200, 600, 300 ppm were mixed with PDA medium. The metabolite and PDA medium mixture was poured into Petri dishes. One disc (5 mm diameter) of PDA previously colonized by the fungus was placed per Petri dish and incubated at 25 ℃ for 8 days. There were 10 replicates of each treatment and PDA was used as control. The diameter of the mycelial growth of the fungus was measured and data used in statistics analyses of variance. Mycelial growth at 600 ppm and 300 ppm doses of thymol, carvacrol and geraniol was not observed. To confirm the potential fungicidal effect of these metabolites will also require glasshouse experiments.

### O26.006 *Gypsophila paniculata* root saponins as an environmentally safe treatment against two nematodes, natural vectors of grapevine fanleaf degeneration

F. Pensec, A. Marmonier, A. Marchal, S. Gersch, N. Nassr, J. Chong, M. Henry, G. Demangeat and C. Bertsch
*Laboratoire Vigne Biotechnologie et Environnement EA3991, Université de Haute-Alsace, 33 rue de Herrlisheim, F-68000 Colmar, France*
*Email: flora.pensec@uha.fr*

*Xiphinema index* and *Xiphinema diversicaudatum* are nematodes that transmit the *Grapevine fanleaf virus* (GFLV) and the *Arabis mosaic virus* (ArMV), respectively. These viruses are the two major agents responsible for the disease that causes the most economic damage to grapevines worldwide. The infectious degeneration of grapevines affects vine performance and grape quality. The control of *Xiphinema* populations by soil disinfection is now impossible because of the removal from the market of the last available chemical treatments. In this study, saponins are assessed as an alternative treatment to control nematode populations. The nematicidal effect of a saponin-rich extract from *Gypsophila paniculata* roots was tested in *X. index* and *X. diversicaudatum*. In aqueous media, a concentration of 1 $mg.mL^{-1}$ was associated with a mortality of greater than 95% in both nematodes, while in rearing-soil, 73% of *X. index* and 85% of *X. diversicaudatum* were killed by 150 µg of saponin per gram of soil. In addition, an ecotoxicological study was undertaken on two soil bio-indicators (the mycorrhizal fungus *Glomus mosseae* and soil nitrification) and revealed that they were not affected by *G. paniculata* saponins at nematicidal concentrations. In the soil, investigations of the major *G. paniculata* root saponins revealed that these molecules were completely degraded in the soil within four days. We show that *G. paniculata* saponins are environmentally friendly and an efficient treatment against two nematodes that transmit

GFLV. This saponin-based alternative to chemical treatments could provide an environmentally safe and efficient solution for vine growers to use against grapevine fanleaf vector nematodes.

### O26.007 The rice *OsTPS7* gene regulates resistance to *Magnaporthe oryzae*

*X.J. Chen*[1], *H. Chen*[2], *Y.F. Guo*[1], *Y.Y. Chen*[1], *F. Chen*[2] and *Z.J. Guo*[1]
[1]*Key Laboratory of Plant Pathology, Ministry of Agriculture; Department of Plant Pathology, China Agricultural University, Beijing 100193, P. R. China;* [2]*Department of Plant Sciences, University of Tennessee, Knoxville, TN 37996, USA*

*Email: chenxj@cau.edu.cn*

Terpenes are the largest group of plant natural products and terpene synthases (TPS) are the pivotal enzyme for terpene biosynthesis. However, our understanding of the biological/ecological functions of TPS remains limited. Here we report the functional characterization of a rice monoterpene synthase gene, *OsTPS7*. Protein sequence analysis using TargetP revealed that OsTPS7 contains a transit peptide targeting to plastids, which was confirmed by the transient expression analysis of the OsTPS7-eGFP reporter in rice cells. Blast analysis showed that OsTPS7 showed homology to some known limonene synthases. The terpene synthase enzyme assay with geranyl diphosphate (GPP) as the substrate demonstrated that OsTPS7 produced multiple monoterpene products with (*S*)-limonene was the dominant one. qRT-PCR analysis indicated that the expression of *OsTPS7* was strongly induced by treatments of wounding and methyl jasmonate. Interestingly, overexpression of *OsTPS7* emitted high quantities of limonene and enhanced resistance to the rice blast fungus. These results suggest that *OsTPS7* plays an important role in response to biotic stresses.

### O26.008 The formula efficacy of the novel natural compounds of Bio-LC4 in control of plant diseases

*L.I. Sudirman, S. Fatimah and Hamim*
*Department of Biology, Bogor Agricultural University, Jl. Agatis, Kampus IPB Darmaga, Bogor 16680, Indonesia*
*Email: lsd@indo.net.id*

The research relates to a novel antibiotic-producing *Lentinus cladopus* LC4 that exhibits antifungal and antibacterial activity. Bio-LC4 was obtained from mycelial extract of this tropical mushroom. In order to implement this biochemical fungicide effectively, it is important to study the way it is formulated and applied. The previous results showed that the mycelial extract application on the healthy soybean seeds followed by inoculation of *Xanthomonas campestris* pv. *glycines* gave a high inhibition of pustule formation. The further Bio-LC4 application in the area of soil amendment was tested. Caisim plants were grown in soil media with different formula and or compost contents respectively. Seedlings grown on soil media that contains the formula and or compost were less infected by damping off disease than those grown without formula. All media treatment gave significant effect on plant height, leaf number, leaf width and plant dry weight but the good plant growth obtained from soil media without formula; 25% of formula or 25% of compost respectively. The soil media with more than 25% of formula or the soil media with the mixture of 25% of formula and 25% of compost caused plant growth inhibition. Considering of these results, the research on Bio-LC4 formulation is still in progress, as well as for controlling *Ganoderma boninense* in oil palm plantation.

### P26.001 Extract of botanicals and cow urine for the management of post-harvest fungal rots of apple

*H. Raj*
*Department of Plant Pathology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-173230, Solan, Himachal Pradesh, India*
*Email: hrg_mpp@yahoo.com*

Apple (*Malus domestica* Borkh.) is the most important fruit crop in India which is grown in about 2.82 lakh hectare area with a production of 17.7 lakh metric tones. Post-harvest losses in fruits are to the tune of 30 per cent of the total yield which are valued at approximately Rs. 13, 600 crores annually. In apple, post-harvest losses ranges from 10 to 25 per cent. Seven important post-harvest pathogens like *Alternaria alternata, Botrytis cinerea, Glomerella cingulata, Botryosphaeria, Penicillium expansum, Rhizopus stolonifer* and *Tricothecium roseum* have been found associated with post-harvest rot of apples. Water-based (Field Formulation-1) and cow urine-based (Field Formulation-2) formulation comprising of leaves of *Bougainvillea glabra, Eucalyptus globulus, Mentha piperita, Roylea elegans, Dedonia viscosa* and seed of *Melia azedarach,* were evaluated in comparison with Neemazal (*Azadiracta indica* oil based formulation), chemical treatment with Saaf (mancozeb + carbendazim) and surface coating with edible wax to find the effect on the post-harvest rotting of apple fruits and also on surface micro-flora of the apple fruits. Among two Field Formulations, cow urine based Field Formulation-2 was found more effective. FF-2 impregnated papers were found at par in efficacy with fruit dip in Saaf in reducing the apple rot by 90.2 per cent after 75 days of storage at 4$^{o}$C. Though, bacterial and fungal surface micro-flora was reduced by 88.1 per cent in fruits treated with Saaf yet fruits wrapped in FF-1 and FF-2 also resulted in 80.9 and 86.6 per cent reduction in surface micro-flora.

**P26.002 Antifungal activity of *Myroxylon balsamum* extract against *Fusarium guttiforme* in pineapple**

*M. Diana, C. Sales, J.A. Ventura and P.M.B. Fernandes*
*Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural – Incaper Universidade Federal do Espírito Santo; Núcleo de Biotecnologia; Brazil; South America*
*Email: ventura@incaper.es.gov.br*

The intensive and indiscriminate use of chemicals in agriculture has caused several problems such as negative environmental impact, food contamination, and human exposure to chemical residues. In this context, there is increasing interest to search for new alternatives that are less harmful to the environment and to the health of consumers to protect plants against diseases. In Brazil, the yield of pineapple, *Ananas comosus* var. *comosus* is still considered low particularly due to fusariosis, a disease caused by the fungus *Fusarium guttiforme*, responsible for important losses in the fruit production and propagative material. Antifungal activities of different extracts from botanical species was assessed on inhibiting mycelial growth of the fungi *Fusarium guttiforme* and evaluated the effectiveness in disease control of pineapples. Plant extract samples representing 28 plant species were tested and showed antifungal activity against *F. guttiforme*. The fractions of *Erythroxylum ovalifolium, Kielmeyera membranacea* and a leaf fraction of *Passiflora mucronata* showed representative values of antifungal activity for *F. guttiforme. Myroxylon balsamum* mother tincture showed strong antifungal activity against *Fusarium guttiforme* in pineapple under both *in vitro* and *in vivo* conditions. The 0.2% extract delayed the mycelial growth of the fungus and showed significant antifungal activity against *F. guttiforme* on pineapple inoculated leaves with an efficacy comparable to that of the synthetic fungicide.

**P26.003 Screening of mango polyphenols and sorghum grain biorefinery by-products for growth inhibition of two common postharvest pathogens of fruit**

*T.K. Do[1], R. Stanley[2] and E.K. Dann[1]*
*[1]Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Ecosciences Precinct, Dutton Park, QLD 4012, Australia; [2]Centre for Food Innovation, University of Tasmania, Launceston, TAS 7250 , Australia*
*Email: trung.do1@uqconnect.edu.au*

Plants have protective phytochemicals and may also produce phytoalexins in response to fungal attack. Phytochemicals from food waste sources may have potential application as crop protectants. Mangiferin, quercetin and quercetin 3-o-galactoside are the main phenolic compounds in mango peel, while Condensed Distillers Solubles (CDS) and Dried Distillers Grains (DDG) are by-products of ethanol production from sorghum grain. This study tested the inhibitory effects of these compounds on growth of two postharvest fungal pathogens of mango, *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* using Petri dish growth inhibition assays. Mangiferin, quercetin and quercetin 3-o-galactoside did not significantly affect growth of the fungi at maximum concentrations tested of 10, 50 or 1 mg/ml, respectively. CDS and DDG significantly inhibited growth of *C. gloeosporioides*, but only DDG inhibited growth of *L. theobromae* after 6 days of incubation. The $EC_{50}$, (Effective Concentration at which mycelial growth is inhibited by 50%), for *C. gloeosporioides* inhibition by CDS was 10.4% v/v of the crude extract. The $EC_{50}$ of DDG for *C. gloeosporioides* and *L. theobromae* was 8.4 mg/ml and 19.1 mg/ml, respectively. Mycelial growth of *L. theobromae* was significantly increased by CDS at 25, 10 and 1% v/v after 4 and 5 days of incubation, but after 6 days of incubation there was no significant difference from controls and lower CDS concentrations. We are now testing the sorghum grain biofuel by-products as postharvest treatments and field sprays, to assess their potential in reducing losses by postharvest diseases in commercial horticultural production.

**P26.004 Eco-friendly management of jute seed-borne fungal flora and diseases through seed cleaning and using of botanicals**

*S.M.A. Islam, Z. Pervez and M.S. Islam*
*Department of Plant Pathology, Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh*
*Email: litonpath@yahoo.com*

Seed borne fungal flora of jute (*Corchorus capsularis* L.) seed were investigated and controlled by seed cleaning and garlic extract (1 g garlic clove in 2 ml water), where Vitavax 200 (0.4% of seed weight) was used as positive control. Seed health test of jute seed reveled that farmers' saved seed (negative control) yielded all together 13 different fungi of 11 genera. Prevalence of *Colletorichum corchori*, *Macrophomina phaseolina*, *Botryodiplodia theobromae*, *Fusarium* spp., *Penicillum* spp., *Aspergillus niger*, and *A. flavus* were by 4.25, 10.75, 2.00, 4.25, 27.20, 8.00, and 22.50%, respectively. The highest reduction of seed borne fungal flora were observed by Vitavax 200 followed by garlic extract and seed cleaning. Germination was negatively correlated with the prevalence of fungal flora on jute seeds. Incidence of different seed borne diseases viz., anthracnose, stem rot, and black band caused by *C. corchori*, *M. phaseolina*, *B. theobromae*, respectively were observed. Incidence of these diseases including number of diseased plants and number of lesions plant$^{-1}$ were higher in farmers' saved seed and those seed borne diseases had been found to be significantly reduced by Vitavax 200 followed by garlic extract and seed cleaning. Garlic extract and Vitavax 200 increased

the yield of jute by 47.38% and 46.05%, respectively over control. Use of garlic extract was all most similar to use of Vitavax 200. So garlic extract can be used to control jute seed borne fungal flora and diseases instead of fungicide (Vitavax 200) for eco-friendly management.

**P26.005 Control of *Podosphaera aphanis* in strawberry plants by using potassium salts and essential oils from uruguayan's native plants**

*E. Pérez, L. Rubio, O. Blanco, N. Umpierrez, E. Vicente and E. Dellacassa*
*Cátedra de Farmacognosia y productos naturals, Universidad de la República, Montevideo, Uruguay; Programa Nacional de Producción Hortícola, Instituto Nacional de Investigación Agropecuaria, Salto, Uruguay, South America*
*Email: elenaperez@inia.org.uy*

The feasibility of controlling powdery mildew of strawberry caused by *Podosphaera aphanis* by using molecules as an alternative to synthetic fungicides has been widely reported. Our aim is to develop a control strategy based on the use of safe molecules for the human consumption. In view of this, we performed an in vitro screening of potassium salts ($KHCO_3$ and $C_6H_7KO_2$) and three essential oil obtained from Uruguayan native plants (*Baccharis trimera, Ocinum selloi* and *Schinus molle*). The inhibition of the spores germination was evaluated through a bioassay test using foliar disc of a susceptible cultivar. We tested the protective effect, through inoculating the pathogens following the treatments. All mentioned molecules were able to reduce significantly the spore germination between 11 and 63% in relation to untreated control ($p<0.05$ based on Tukey's test). Nevertheless, neither of them were more effective than the synthetic fungicide Bellys (12.8% pyraclostrobin + 25.2% boscalid) with 100 % of spores germination control. Based in this result, we concluded that it is feasible to have a set of innocuous molecules to continue with the challenge to elaborate new formulations and control strategies, which may be as effective as the synthetic fungicides.

**P26.006 Effect of essential oils on germination of *Tilletia caries* and *T. controversa* teliospores**

*P. Rysanek, J. Mazakova, M. Zouhar and R. Pavela*
*Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague, Czech Republic*
*Email: rysanek@af.czu.cz*

Bunts caused by *Tilletia caries* and *T. controversa* are important diseases of wheat. Causal fungi are seed- and/or soilborne. The protection fully depends on seed treatment and/or crop rotation. Several fungicides may be used in conventional agriculture but their use is excluded in ecological agriculture. In the past, some essential oils were proved to be efficient in the control of some pests and pathogens. So, we also tested their effect onto *T. caries* and *T. controversa* teliospores germination *in vitro*. A range of concentrations from 0.05 µl to 0.8 µl/10 ml of water agar was tested. Fungicides Dividend and Celest and also not treated agar were used as controls. Teliospores were sown onto treated agar surface. Petri dishes were placed into the thermostat at 16 °C and 5 °C + light for *T. caries* and *T. controversa*, respectively. Germinated spores were counted 3$^{rd}$, 5$^{th}$, 7$^{th}$ and 10$^{th}$ day in the case of *T. caries* and 35$^{th}$, 42$^{nd}$ and 49$^{th}$ day in the case of *T. controversa*. Altogether 34 essential oils were tested. The highest concentration of all of them had effectiveness comparable to commercial fungicides and some of them were still quite well effective even at the lowest concentration. The most effective oils were also tested for wheat seed treatment in pot experiments. Presence of *T. caries* in plants was checked by PCR 2 months after sowing. Some formulations completely prevented plant infection. Thus, the use of certain essential oils is promising relatively safe way of bunt control.

**P26.007 Separation and antiviral activity of chitooligomers with different degree of polymerization**

*W.X. Wang, S.G. Li, H. Yin, X.M. Zhao and Y.G. Du*
*Liaoning Provincial Key laboratory of Carbohydrates, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, No. 457 Zhongshan Road, Shahekou District, Dalian, 116023, P. R. China*
*Email: duyg@dicp.ac.cn*

Plant virus disease is a major limiting factor in plant production. Oligosaccharides from natural product have promising bioactive ability in plant virus diseases control. The antiviral activity of oligosaccharide is significantly related to their degree of polymerization. In order to further reveal the relationship between oligosaccharide antiviral activity and their degree of polymerization, screen more effective oligosaccharide for plant virus diseases control, a fast and efficient antiviral activity screening system was establishment by detecting salicylic acid and virus coat protein. Meantime, single degree of polymerization of chitosan oligosaccharide was isolated through hydrophobic interaction preparative high performance liquid chromatography. Obtained chitomonomer was further explored for their activity in tobacco against *Tobacco mosaic virus* and the effect of different degree of polymerization was discussed.

**P26.008 The efficiency of a novel green manures in controlling replant disease on Mountain Ash (*Sorbus aucuparia*)**

*J. Lock*

*The University of Northampton, Park Campus, Boughton Green Road, Kingsthorpe, Northampton, Northamptonshire, NN2 7AL, United Kingdom*
*Email: julia.lock@moulton.ac.uk*

Specific Replant Disease (SRD) is a persistent problem that affects the propagation and cultivation of broadleaf trees, especially those being cultivated in nurseries across the UK. This is also a global problem with unknown aetiology, although studies have indicated that it is caused by a build-up of an array of pathogens, such as nematodes, fungi, bacteria, and viruses. The financial implications of SRD impact on green industries, fruit trees and ornamental trees suffer losses when yields are low and trees fail to grow to their full potential. Plant species in the Rosaceae family are particularly prone to SRD. On nurseries SRD was previously treated by the broad spectrum fumigant methyl bromide. In 1993 methyl bromide was revoked, with the pursuit for an alternative control measure currently being undertaken. It is proposed that the biocidal properties of novel green manures have the potential to treat SRD on *Sorbus aucuparia*. The green manure has been applied with a view to influence populations of micro-organisms within the rhizosphere surrounding the saplings. Additionally, microbial studies will be undertaken to determine Microbial Inhibitory Concentrations of the green manure being investigated. Comparisons will be made to standard green manures, with varying antimicrobial properties that are being used as control treatment, such as mustard (*Brassica juncea*), and plant material not normally considered as a green manure, garlic (*Allium sativum*). The changes in the microbial populations as a result of the addition of treatments will be quantified and analysed.

**P26.009 An antifungal protein, MCha-Pr, from the intercellular fluid of bitter gourd (*Momordica charantia*) leaves**

*C. Xie[1], B. Zhang[2], C. Wang[1], Q. Li[1], X. Wang[1] and X. Yang[1]*
*[1]The College of Life Science, Chongqing Normal University, 401331 Chongqing, P. R. China; [2]Sichuan Academy of Food and Fermentation Industries, Sichuan, P. R. China*
*Email: yangxy94@swu.edu.cn*

An antifungal protein, designated MCha-Pr, was isolated from the intercellular fluid of bitter gourd (*Momordica charantia*) leaves during a screen for potent antimicrobial proteins from plants. The isolation procedure involved a combination of extraction, ammonium sulphate precipitation, gel filtration on Bio-Gel P-6, ion exchange chromatography on CM-Sephadex, an additional gel filtration on HiLoad 16/60 Superdex 30, and finally, HPLC on a SOURCE 5RPC column. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry indicated that the protein had a molecular mass of 25733.46 Da. Automated Edman degradation was used to determine the N-terminal sequence of MCha-Pr, and the amino acid sequence was identified as V-E-Y-T-I-T-G-N-A-G-N-T-P-G-. The MCha-Pr protein had some similarity to the sequences of pathogenesis-related proteins from *Atropa belladonna* (deadly nightshade), *Solanum tuberosum* (potato), *Ricinus communis* (castor bean), and *Nicotiana tabacum* (tobacco). Analysis of the circular dichroism spectra indicated that MCha-Pr predominantly contains α-helix and β-sheet structures. MCha-Pr had inhibitory effects towards a variety of fungal species, including *Aspergillus niger*, *Alternaria brassicae*, *Cercospora personata*, *Fusarium oxysporum*, *Mucor* spp., *Rhizoctonia solani* and *Verticillium dahliae*. In addition, this novel antifungal protein can inhibit the germination of fungal spores. These results suggest that MCha-Pr in bitter gourd leaves plays a protective role against phytopathogens and has a wide antimicrobial spectrum.

**P26.010 Structure determination and antifungal mechanism of cytosinpeptidemycin**

*L.X. Mu, J.G. Chen, Y.H. Wu and X.X. Zhao*
*College Plant Protection, Shenyang Agricultural University, No. 120 Dongling Road, Shenhe District, Shenyang 110866, P. R. China*
*Email: wuyh09@vip.sina.com*

Cytosinpeptidemycin is a high efficient, low-toxic and low-residue bio-pesticide used to control multiple crop fungi and virus diseases. Under the guidance of anti-*Alternaria alternata* activity, the crude fermentation broth was purified to eliminate protein and other mess, absorbed by Diaion HP20 and eluted by 50% methanol. The bio-active fractions were further purified through TOSOH SP650M ion exchange chromatography. Finally, the pure compound was obtained by Daisogel ODS-B reverse phase chromatography. The molecular formula was elucidated as $C_{12}H_{13}N_5O_4$ by interpretation of UV, IR, MS and NMR data. The HPLC method mainly uses DIKMA Diamonsil Ⅱ C18 column and UV detector by using methanol / water (20:80, by vol) as mobile phase with 1 mL/min flow rate and UV detection at 278 nm. The column temperature was 30℃, the retention time was 10.2 min. The liner correlation of this method was 0.9998. Antimicrobial spectrum test showed that compound was against 20 phytopathogenic fungi such as *Sclerotinia sclerotiorum* and *A. alternate*. The compound had a obvious inhibition effect on mycelial growth and spore germination. It also can cause the hypha and the tube to grow in abnormal shapes. Studies of the compound active mechanism were conducted, the results showed that it can cause the protoplasm leaked out from hypha. At the same time, the ergosterol content in mycelia of *A. alternata* decreased remarkably and MDA content in mycelia and cultural filtrate increased significantly and it changed the content of protein in mycelia.

**P26.011 Antifungal activity of essential oil from cumin against four postharvest fungi**

*Z. Zhang, Y. Bi, D.Q. Li, Y. Wang and K.P. Shen*
*College of Food Science and Engineering, Gansu Agricultural University, NO.1 Yingmen Village, Anning District, Lanzhou 730070, P.R. China*
*Email:zhangzhong@gsau.edu.cn*

Cumin essential oil was hydrodistilly extracted via a traditional Clevenger apparatus from the cumin seeds (*Cuminum cyminum*) with an oil yield of 2.56% and its predominant components were identified as 2-carene-10-aldehyde, cumin aldehyde and 3-carene-10-aldehyde. The essential oil demonstrated strong bioactivitiy against four popular postharvest fungi *Fusarium semitectum*, *Alternaria alternata*, *Penicillium expansum* and *Trichothecium roseum* with MICs from 400 to 500 μL/L *in vitro*, and among them *T. roseum* was the most sensitive to the oil. The antifungal mechanisms of the essential oil include the disruption of membrane integrity and modified cellular structures of assayed cells. The observations of *A. alternate* and *P. expansum* under SEM and TEM showed that the mycelium morphology and cellular ultrastructure were modified after treated with the oil. Cellular surface deformities and fractures, organelle indistinct or irregular shape, frequent higher dense electron bodies and large empty vacuoles were also observed on treated cells. Moreover, the fumigation treatment with the essential oil significantly inhibited development of pink rot (caused by *T. roseum*) on intact or inoculated muskmelon (*Cucumis melo* L. cv. Yindi). On intact fruit, the decay index and good fruit rate of treatment at 10 μl/ L were 18.5% and 70% with counterparts of 41.5% and 32% on the untreated control. On inoculated fruit, treating the wounds with oil at 4000 μl/L decreased the incidence of rot disease and lesion diameter to 5.8% and 3.1 mm while the corresponding data were 96.7% and 28.6 mm in control without oil treatment. It is suggested that cumin essential oil demonstrate an excellent antifungal activity, and could be used for control of postharvest diseases. However, the fumigation with the oil could change the flavor of fruit and vegetables.

**P26.012 Evaluation of some botanicals for the control of seedborne fungi of maize (*Zea mays* L.)**

*E.N.K. Sowley, A.H. Abubakari and P.A. Seglah*
*Department of Agronomy, Faculty of Agriculture, University for Development Studies, P. O. Box TL1882, Tamale*
*Email: esowley@gmail.com*

Maize is a staple in many African countries including Ghana, but its production is hampered by diseases, some of which are seedborne. Since there is an outcry against the excessive use of synthetic chemicals, this study sought to determine the effectiveness of aqueous extracts of acacia (*Cassia sophera*), neem (*Azadirachta indica*) and garlic (*Allium sativum*) in controlling seedborne fungi of maize (*Zea mays* L.). Isolations were made from farmer-saved and certified seed samples with Potato dextrose agar (PDA) as the growth medium. Growth inhibition of the isolates was determined by growing pure cultures on PDA plates amended with 10% of aqueous extracts of acacia leaf, garlic and neem seed. Three fungi namely, *Aspergillus flavus*, *A. niger* and *Botryodiploidia theobromae* were isolated. *A. flavus* had a significantly higher ($P<0.05$) occurrence (59.3%) than the other isolates. There were significant differences ($P<0.05$) between the treatments with carbendazim being the highest, followed by neem seed, garlic, acacia leaf and the control (water). For instance, percentage growth inhibition of *A. flavus* by carbendazim, neem seed, garlic, acacia leaf and the control (water) in farmer-saved seeds were 100, 76.5, 47.0, 23.5 and 0, respectively. Since neem seed extract was most effective after carbendazim, it can be used as a surface protectant of maize seed. However in the absence of neem seed, acacia leaf and garlic extracts may be used since they were more effective than the control.

**P26.013 Effect of plant extracts and an essential oil on the control of brown spot disease, germination, stem and root length, tillering and number of panicles increase in rice**

*J. Nguefack[1], E.G. Wulff[2], J.B. Lekagne Dongmo[1], F.R. Fouelefack[2], P.H. Amvam Zollo[1], J. Mbo[3] and J. Torp[2]*
*[1]University of Yaoundé-I, Faculty of Science, Department of Biochemistry, Box 812 Yaoundé Cameroon; [2]Danish Seed Health Centre for Developing Countries (DSHC), Department of Agriculture and Ecology, Faculty of Life Science, University of Copenhagen, Hoejbakkegaard Alle 9, DK 2630, Taastrup, Denmark; [3]Institute of Agricultural Research and Development, Box 2123, Yaoundé-Cameroon*
*Email: jnguefack@gmail.com*

The effects of ethanol and aqueous extracts of *Callistemon citrinus* and *Cymbopogon citratus* and an essential oil of *C. citrinus* on the control of brown spot (caused by *Bipolaris oryzae* [Breda de Haan] Shoemaker), the stem and root length, the tillering and number of panicles increase in rice were evaluated under laboratory and field conditions. Under laboratory conditions, seed treatment with the essential oil of *C. citrinus* reduced the incidence of *B. oryzae* in seeds by 85-100% compared to the non-treated controls. Similarly, the seed treatment increased the germination of an irrigated rice cultivar by 10.6%, whereas the percentage of germinated seeds of upland rice was not significantly affected. The highest germination (85-94%) was found in the non-treated and treated samples with a low incidence (0-4%) of *B. oryzae*. Seed treatments with 2% ethanol extract of *C. citratus* and *C. citrinus* increased the stem length of irrigated

rice by 58% and 23%, respectively. The same treatments increased the root length of rice cultivar Tox by 79% and 37%, respectively. Under field conditions, the combined use of the essential oil of *C. citrinus* as a seed treatment and spraying the plants with 2% ethanol followed by 2% (w/v) aqueous extracts of *C. citrinus* or *C. citratus* increased the emergence, tillering, and panicles/plant of the irrigated rice. In addition, the brown spot severity was reduced by 42%. For the upland rice, the same treatments increased the tillering and number of panicles/plant and resulted in a 20-80% reduction in the brown spot severity. From our results, we concluded that the solvent extracts of *C. citrinus* and *C. citratus* and essential oil of *C. citrinus* have potential as control agents against brown spot and other seed-borne fungal diseases in rice under both conventional and organic farming.

**P26.014 Incidence and preliminary control of brown spot disease of rice in southwest Nigeria**

*D.B. Olufolaji*
*Dept. of Crop, Soil and Pest Management, The Federal University of Technology, Akure, Nigeria*
*Email: tundefolaji022@gmail.com*

Rice (*Oryza sativa* L.), being an economic crop in Nigeria is the readily available grain which assisted in food sufficiency for most developing countries. Studies were carried out on the occurrence of Brown spot disease of rice caused by the fungal pathogen *Cochliobolus miyabeanus* (*Helminthosporium oryzae*) in southwest Nigeria. While 32-55% incidence was obtained on the farm land, the severity ranges from 3.1-4.5. These are very devastating in rice production. From the studies conducted, 30 to 50 % of yield loss in rice was recorded and this has caused untold hardship to rice farmers in southwest up-land agro-ecological zone of Nigeria. Preliminary control measure involved, aqueous extracts of three botanicals namely; *Tectona grandis, Gmelina arborea,* and *Chromolaena odorata*, applied *in vitro* at concentrations 20, 40, 60 and 80% on *C. miyabeanus.* Also, aqueous extracts in concentrations 60 and 80% were applied to rice (NERICA) seedlings, artificially inoculated with the rice pathogen in screen house studies. *G. arborea* at 60% and *C. odorata* at 80% showed some promise in reducing mycelial growth and sporulation of the causal organism. It was observed that *G. arborea* at 80% concentration significantly ($P \leq 0.05$) controlled the brown spot disease better than 60% concentration with 2.5 and 4.2 disease severity, respectively. This served as preliminary control strategy for brown spot disease since the local rice farmers benefited from the usage of the aqueous extract of the botanicals. Molecular studies will determine the variations in the fungal isolates.

**P26.015 Evaluation of marjoram essential oil (*Origanum majorana* L.) as antimicrobial against some microorganisms**

*H.A.Y. Gibriel*
*Ain Shams University, P.O.Box 259,Maadi,Cairo 11728, Egypt*
*Email: heshamgibriel@gmail.com*

The essential oil of marjoram (*Origanum majorana* L.) grown in Egypt was extracted by steam distillation. Gas chromatography (GC) analysis for its constituents was carried out. The essential oil was examined for its antimicrobial activities against four strains of fungi, two strains of yeasts and two strains of bacteria. In addition, the effect of storage conditions on physico-chemical properties of the essential oil was investigated. The data showed that, the marjoram essential oil was rich in linalool (20.98 %), limonene (16.78 %), and β-pinene (12.49 %) *P*-cymene (10.88 %), α-pinene (9.69 %) and 1, 8- cineol (6.84 %). The identified compounds are representing 83.42 % of the total essential oil. Marjoram essential oil totally inhibited *Aspergillus niger, A. flavus, Fusarium moniliform* and *Penicillium expansum* at concentrations of 300-400 µl/100 medium, while it inhibited *Pichia anomala* and *Rhodotorula minuta* at concentrations of 100 and 120 µl/disc, respectively. The minimum inhibitory concentrations were 100 and 160 µl/disc against *Bacillus cereus* and *Escherichia coli*, respectively. The data also showed that, the oil samples stored at room temperature about 30 ℃ had a remarkable effect on the properties of the oil as compared with those samples stored in refrigerator temperature.

## Concurrent Session 27-Nematology and Plant Diseases

### O27.001 Challenges in developing phytochemicals for use as nematode management agents

*D.J. Chitwood and S.L.F. Meyer*
*Nematology Laboratory, United States Department of Agriculture, Agricultural Research Service, Building 010A, Room 109, BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705, USA*
*Email: david.chitwood@ars.usda.gov*

Plants and fungi produce diverse classes of chemicals toxic or otherwise antagonistic to nematodes. Because phytochemicals are often safer than synthetic compounds and consequently receive less regulation, researchers are actively engaged in development of these materials as components of management systems for plant-parasitic nematodes. Unfortunately, actual utilization in agriculture has remained problematic for several reasons. Often the cost of purifying or synthesizing rather complex molecules has impeded utilization; low toxicity toward nematodes has sometimes resulted in impractical quantities required for application. Moreover, many of the same hurdles facing the development of new synthetic chemical nematicides confront nematicidal phytochemicals. In addition to acceptable target specificity, the ideal compound should migrate in soil sufficiently to provide control in the root zone yet not move into groundwater. Persistence in soil should be for an agriculturally effective yet environmentally safe period. Experimental approach and design are critical to achieving targets for expense, persistence and soil mobility. For example, the plant material should be inexpensively produced if not widely available. Bioassay-guided fractionation of compounds from known nematode-antagonistic plants may lead to the identification of compounds more interesting than those obtained by inferential deduction about the chemical nature of the phytochemical. Purification schemes should generally not focus on lipophilic compounds because of their poor ability to move in soil. The ideal bioassay organism should be a phytoparasitic nematode, not a microbivorous species which ideally would be unaffected. High minimally effective concentrations are not usually appropriate for future greenhouse or field experimentation, except in unusual circumstances.

### O27.002 Unraveling the rice-*Meloidogyne graminicola* interaction

*G. Gheysen, T. Kyndt, A. Haegeman, K. Nahar, H. Ji and M. Höfte*
*Department of Molecular Biotechnology, Ghent University, Coupure links 653, 9000 Gent, Belgium*
*Email: Godelieve.Gheysen@UGent.be*

Our research focuses on rice as model plant to analyse the interaction with nematodes at the cellular and molecular level. To get a comprehensive overview of the compatible plant response to nematode infection, mRNA sequencing was performed on rice after nematode infection. Local infected tissue was compared with systemic tissue after infection by the root knot nematode *Meloidogyne graminicola* or the migratory nematode *Hirschmanniella oryzae* and with control tissue of the same developmental stage. One of the results is the downregulation of plant defense genes locally and systemically after root knot nematode infection. We are also studying the role of several plant hormones in the plant's basal defense. For a functional analysis of plant genes that are differentially expressed upon nematode infection, we perform infection experiments on mutants or transgenics with lower or higher expression of that specific plant gene. To get insight in the proteins that are secreted by nematodes into the plant in order to establish a succesful infection, a transcriptome analysis was performed on *Meloidogyne graminicola* preparasitic juveniles. One of the strategies is to identify nematode proteins that are capable of suppressing plant defense. In the future we want to extend our analyses to other types of rice nematodes (cyst nematode, stem nematode, white tip nematode).

### O27.003 Biological control of *Meloidogyne incognita* by *Streptomyces rubrogriseus* HDZ-9-47

*N. Jin, X.Y. Wang, W.J. Li, H. Xue and H. Jian*
*Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: hengjian@cau.edu.cn*

HDZ-9-47 was isolated from the egg mass of *Meloidogyne hapla* in China and it was identified as *Streptomyces rubrogriseus*. To explore its potential of biological control of nematode, we investigated nutritional requirements for mycelial growth of *S. rubrogriseus* in liquid media. The optimized combination for mycelial growth is that carbon to nitrogen ration at 40:1, 0.5mol/L carbon concentration, D(+)-cellobiose, potassium nitrate, manganese and boron. And the optimized industrial medium has also been developed by using Orthogonal experimental design and Plackett-Burman method. The culture filtrate could reduce egg hatching rate of *M. inconigta* by 97.1% and also caused 97.8% J2 mortality within 12h exposure. Field application of the fermented filtrate of HDZ-9-47 have been conducted for control *M. incognita* in successive four growing season's trials. Control efficacy of the fermented filtrate contained $10^{12}$ spores of *S. rubrogriseus* could reach to 51.5-70.0% on tomato or 38.5-66.0% on cucumber, which were equal to those of Fosthiazate treatment, respectively. Effects of spores and fermented filtrate of *S. rubrogriseus* on soil nematode community was evaluated. Indexes of maturity, plant parasite, diversity and evenness of soil

nematodes were calculated and analyzed, respectively. The result showed that each tested index between HDZ-9-47 treatment and blank control had no obvious difference, respectively. The results mean that *S. rubrogriseus* had no or very limited interference to other nematodes. In conclusion, our results indicate that HDZ-9-47 is a promising agent for biological control of root knot nematode, and future researches should focus on the identification of nematicidal compounds and commercial formulation of HDZ-9-47.

**O27.004 Gene silencing in root lesion nematodes significantly reduces reproduction in host plants**

*M.G.K. Jones, J.A.C.W. Tan and J. Fosu-Nyarko*
*Plant Biotechnology Research Group, School of Veterinary and Life Sciences, Western Australian State Agricultural Biotechnology Centre, Murdoch University, Perth WA 6150, Australia*
*Email: m.jones@murdoch.edu.au*

Root lesion nematodes (RLNs, *Pratylenchus* species) are major root pests of many crop plants such as wheat and sugarcane. In Australia *P. thornei, P. penetrans* and *P. neglectus* can reduce wheat yields by 7-15%, and *P. zeae* reduces sugarcane yields by a similar amount in fine textured soils. To study the potential of applying gene silencing technology to RLNs, the transcriptome of *P. thornei* has been sequenced using Roche 454FLX technology and annotated (Nicol, P. et al 2012, *Int. J. Parasitol*. 42, 255-237). These data provided information on potential gene targets for silencing. After optimising protocols to deliver dsRNA to *P. thornei* by 'soaking', the results showed that *P. thornei* and *P. zeae* are both highly amenable to gene silencing. Following soaking of RLNs in solutions containing dsRNA to two genes involved in nematode movement, and culture for five week on' mini' carrot discs, there was a 77-81% reduction in nematode replication (Tan, J-A.C.W. et al 2013, Experimental Parasitology 133, 166–178). These results were confirmed and extended by challenging transgenic wheat and sugarcane plants expressing dsRNA to target genes with root lesion nematodes.

**O27.005 Current Research status and perspective of cereal cyst nematode, (*Heterodera aveane*，*H. filipjevi*) on wheat in China**

*D. Peng, W. Huang and H. Peng*
*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China*
*Email: pengdeliang@caas.cn*

The cereal cyst nematode (CCN), *Heterodera avenae* is one of the most important plant parasitic nematode and distributes throughout nearly all cereal growing areas in China. Since 2009, The occurrence and distribution of the the cereal cyst nematodes has been confirmed to occurrence in four new provinces including Ningxia, Tianjing, Xizang (Tibet) and Xijiang based on the morphological identification and molecular characterisation, so CCN has been confirmed to distribute 16 provinces in China. Yield losses were tested with inoculation and field, in Henan, Hebei, Beijing suburb, Qinghai, yield losses reach up to18%-35% in Henan, 15-20% in Hebei, 11-18% in Beijing Suburb and 10-28.24% in Qinghai. The population dynamics and life cycle of *H. avenae* were investigated in Beijing, Hebei, Jiangsu and Shandong from 2010 December to 2012 December. The results showed that there is one generation of *H. avenae* per year in those areas above mentioned. There are two cyst nematodes (*H. avenae* and *H. filipjevi*) occurrence in the wheat production area of China, the *H.avenae* is dominate species. The molecular diagnosis methods based on SCAR-PCR were developed to identify and early detect *H. avenae* and *H. filipjevi* from infested field. The pathotypes of twenties population of *H. avenae* from Beijing, Hebei, Jiangsu and Shandong were tested and identified by using the International Test Assortment (CIMMYT provide). The results showed that Qingyundian population (*H. avenae*) of Beijing was different from the 13 pathotypes which have been described and nominated. It`s slightly similar with pathotype Ha23. More than two huandrands cultivars were tested and evaluated resistance to *Heterodera avenae* and *H. filipjevi* in greenhouse and the field respectively. The results showed that no immune materials were found. Five cultivars including VP1620, BATAVIA, SUNR23, AUS4930 6.5/GS50a and Taikong 6 were high resistance to CCN Beijing population. A cDNA libarary from the second stage juvniles of H. avenae was constructed for exploring more candidate parasitism genes. 5800 ESTs were generated and 2568 unigenes were obtained. Three β-1,4-endoglucanase genes (*Ha-eng-1a, Ha-eng-2* and *Ha-eng-3*) expressed in the pharyngeal glands of the sedentary cyst nematode (*Heterodera avenae*,)were cloned. The cDNA of *Ha-eng-1a* encoded a deduced 463-amino acid sequence containing a catalytic domain and a cellulose binding module separated by a linker. The genomic DNA of Ha-eng-1a is 2,129 bp long, containing eight introns ranging from 56 bp to 157 bp and nine exons ranging from 70 bp to 299 bp. Southern blot analysis revealed that two copies of the *Ha-eng-1a* gene are present in *H. avenae*. *In situ* hybridization showed that the *Ha-eng-1a* transcripts specifically accumulated in the two subventral gland cells of the second-stage juveniles.

**O27.006 Cereal nematodes management strategies in wheat**

*A.A. Dababat, G. Erginbas-Orakci, H.J. Braun and A. Morgounov*
*CIMMYT Global Wheat Program, Ankara, Turkey*

*Email: a.dababat@cgiar.org*

Soil borne pathogens (SBPs) including the cereal cyst nematode (CCN) caused by *Heterodera* species and the Root Lesion Nematodes caused by *Pratylenchus* species are attack roots of cereal crops and resulting in a high yield loss and reduce grain quality. The damage caused by these nematodes is accelerated in areas where drought exists. A few control options are being used to reduce CCN damage through keeping the population level below damage threshold such as; chemical, biological, cultural, and genetic (resistance/tolerance) practices. Resistance is environmentally friendly and biologically effective once identified. However, up to now, resistance has only been identified against one of the CCN nematodes, *Heterodera filipjevi* in Turkey and foreign wheat germplasm though this resistance is not yet present in high yielding cultivars. Resistance to the other nematodes in the CCNs complex is still being sought. Therefore, alternative approaches limiting the damage caused by CN to wheat are needed. As a result of screening wheat germplasm against the CN hundreds of moderately resistant germplasm to *H. filipjevi* in winter wheat and to both *Pratylenchus* species in spring wheat germplasm are available. In 2012, germplasm with multi disease resistance including *H. avenae*, *Pratylenchus thornei* and *P. neglectus*, and *H. filipjevi* were distributed to international collaborators. The preliminary results of using seed treatments showed that seed treatment of wheat susceptible germplasm gave up to 47% reduction in number of *H. filipjevi* cyst per plant but did not reduce the number of cyst in the resistant germplasm since the cyst number was low and no room to decrease it further.

### O27.007 The research status of crop root-knot nematodes ( *Meloidogyne* spp. ) in China

*J.L. Liao , K. Zhuo and Y.H. Wen*
*College of Natural Resources and Environments, South China Agricultural University, No.483 Wushan Road, Tianhe District, Guangzhou, 510642, P. R. China*
*Email: jlliao@scau.edu.cn*

The root-knot nematode (*Meloidogyne* spp.) is one group of most important phytopathogenic nematodes in China, causing great damage to many different crops. The present research showed that *M. enterolobii* was one of main emerging important root-knot nematodes, infecting many kinds of crops in South China. The rapid and accurate systems based on DNA for detecting root-knot nematodes have been developed. Several effectors have been identified from the esophageal gland cells of root-knot nematodes, providing the valuable information for understanding the parasitism and pathogenicity of nematodes. Resources of microorganisms and plants for anti-nematodes were investigated in large scales. The potential bio-control agents were screened and their mechanism of action is explored. The nematode resistant cultivars and techniques based on grafting have been applied to the vegetable production in China. A special fund for agroscientific research in the public interest of China, study on the management of vegetable root-knot nematodes, led by Dr. Jian Heng has been launched.

### O27.008 Signaling in the compatible interaction between potato cyst nematode and potato

*S. Chen[1], D. Chronis[2], P. Lang[1] and X. Wang[1,2]*
*[1]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14853, USA; [2]USDA-ARS, Robert W. Holley Center for Agriculture and Health, Ithaca, NY 14853, USA*
*Email: Xiaohong.wang@ars.usda.gov*

Potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) are internationally recognized quarantine pests that have recently been found to be spreading in North America. These devastating potato pests are obligate endoparasites that invade roots of host plants and use their secreted effector proteins to transform root cells into a specialized feeding structure essential for the growth and development of the parasite. The majority of nematode effectors are originated from the nematode's esophageal gland cells and secreted through the nematode stylet during the parasitic interaction. It has been discovered that potato cyst nematodes secrete effector proteins that function in mimicking plant CLE signaling peptides and in suppressing plant immune responses to promote plant parasitism. Gene-silencing studies through host plant-derived RNA interference have confirmed the importance of these effectors in nematode parasitism. Several approaches including candidate host receptor searches, yeast two-hybrid screening, and proteomic analysis are being utilized to identify host targets of these effectors that modulate plant developmental and defense pathways. A better understanding of the function of these nematode-secreted effectors will not only advance our knowledge of the molecular basis of nematode parasitism but may also lead to the development of novel forms of engineered nematode resistance in potato.

### O27.009 Functional analysis of *Globodera pallida* SPRYSEC proteins

*Y.Y. Mei[1,2], S. Mantelin[2], A.Haegeman[1,2], G. Gheysen[1] and J.T. Jones[2]*
*[1]Department of Molecular Biotechnology, Ghent University, Coupure Links 653, 9000 Ghent, Belgium; [2]Cell and Molecular Sciences Group, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, United Kingdom*
*Email: John.Jones@hutton.ac.uk*

The potato cyst nematode, *Globodera pallida*, is the most economically important nematode to British agriculture and one of the most destructive pathogens in potato growing regions throughout the world. A lack of major resistance genes and removal of nematicides has resulted in an increasing need to better understand of the molecular basis of the interaction between host and nematode in order to develop sustainable control strategies. The interaction between the plant and cyst nematode is mediated by effectors that are synthesized in the nematode oesophageal glands and injected into the host cytoplasm through stylet. A large number of effectors have been identified from *G. pallida*. Of particular note is the SPRYSEC gene family that has more than 300 members with various localization patterns within plant cells. Many of these genes are expressed in the dorsal esophageal gland cells of J2s indicating their potential roles in plant parasitism. In this study, we investigated the role of SPRYSEC gene family members in suppression of plant defences. A panel of interesting potential host target proteins have been found after yeast two hybrid screening against an infected potato cDNA library. Several of the interacting proteins have been confirmed within the yeast cells or *in planta*.

**O27.010 Characterisation of resistance to the stem nematode (*Ditylenchus gigas*) in *Vicia faba***

*N. Stawniak and J.E. Thomas*
*National Institute of Agricultural Botany, Huntingdon Road, Cambridge, CB3 0LE, United Kingdom*
*Email: jane.thomas@niab.com*

Stem nematode can cause significant yield loss in faba beans. It is both seed-borne and soil-borne. Infested seed stocks may have to be discarded, leading to loss of high value material. Even low levels of infestation can lead to the introduction of the nematode into previously healthy soil, and restrict faba bean cropping in future years. Since nematicides are unavailable for use on the faba bean crop, resistance to the nematode provides the only feasible method of control. We have characterised resistance in a number of faba bean accessions in order to identify material suitable for inclusion in breeding programmes. Two lines (BPL 10 and INRA 29H) significantly reduced the incidence of stem swelling compared to commercial cultivars regarded as very susceptible in controlled inoculation tests. Harvested seed of INRA 29H contained no nematodes, whereas seed of BPL 10 had a mean of 45 nematodes per seed. However, a susceptible cultivar showed a mean of 1458 nematodes per seed at harvest. Nematode multiplication (Pf/Pi) in INRA 29H stem material was reduced to 5 compared to 48 for BPL 10 and 243 for cv. Wizard, which is currently a widely grown faba bean in the U.K. Both INRA 29H and BPL 10 are now being used in crossing programmes to introduce stem nematode resistance.

**O27.011 Distribution and genetic diversity of root-knot nematodes (*Meloidogyne* spp.) in potatoes from South Africa**

*E.M. Onkendi and L.N. Moleleki*
*Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa*
*Email: eddyonken@yahoo.com*

A molecular based assay was employed to analyse and accurately identify various root-knot nematodes (*Meloidogyne* spp.) parasitizing potatoes (*Solanum tuberosum*) in South Africa. Using the intergenic region (IGS) and the 28S D2-D3 expansion segments within the ribosomal DNA (rDNA) together with the region between the cytochrome oxidase subunit II (COII) and the 16S rRNA gene of the mtDNA, 78 composite potato tubers collected from seven major potato growing provinces were analysed and all *Meloidogyne* species present identified. During this study *M. incognita*, *M. arenaria*, *M. javanica*, *M. hapla*, *M. chitwoodi* and *M. enterolobii* were identified. The three tropical species; *M. javanica*, *M. incognita* and *M. arenaria* were identified as the most prevalent species, occurring almost in every region sampled. *M. hapla* and *M. enterolobii* occurred in Mpumalanga and KwaZulu–Natal respectively while *M. chitwoodi* was isolated from two growers located within the Free State. Results presented here form part of the first comprehensive surveillance study of root-knot nematodes to be carried out on potatoes in South Africa using a molecular based approach. The three genes were able to distinguish various *Meloidogyne* populations from one another, providing a reliable and robust method for future use in diagnostics within the potato industry for these phytoparasites.

**O27.012 Comparative analysis of complete mitochondrial genome sequences confirms independent origins of plant-parasitic nematodes**

*J.K. Park[1], T. Sultana[2], J. Kim[1], S.H. Lee[1] and T. Kim[1]*
*[1]Program in Cell Biology and Genetics and Department of Parasitology, College of Medicine, Chungbuk National University, Cheongju 361-763, Republic of Korea; [2]Department of Biological Sciences, Inha University, Incheon 402-751, Republic of Korea*
*Email: jkpyou@chungbuk.ac.kr*

The nematode infraorder Tylenchomorpha (Class Chromadorea) includes plant parasites that are of agricultural and economic importance, as well as insect-associates and fungal feeding species. Among tylenchomorph nematodes, the superfamilies Tylenchoidea and Aphelenchoidea represent the largest assemblages of plant-parasitic chromadorean members. Monophyletic grouping and/or phylogenetic positions of these two superfamilies within chromadorean nematodes have been the topic of debate over many decades. We investigated phylogenetic relationships of the Tylenchoidea

and Aphelenchoidea among other members of chromadorean nematodes based on comparative analysis of complete mitochondrial genome data, including three newly sequenced complete genomes from *Bursaphelenchus mucronatus, B. xylophilus* (Aphelenchoidea) and *Pratylenchus vulnus* (Tylenchoidea). Phylogenetic hypotheses for these mitochondrial genomes, based on different tree-building methods, did not support their monophyly: Aphelenchoidea was positioned basal to the Rhabditomrpha+Diplogasteromorpha+Ascaridomorpha +Panagrolaimomorpha clade, and Tylenchoidea was found to be the most basal taxon of the Chromadorean clade. Comparison of gene arrangement data corroborated the phyletic separation of these two groups: Similar gene arrangement patterns are found among the sampled species of the Rhabditomrpha/Diplogasteromorpha/Ascaridomorpha/Panagrolaimomorpha clade and aphelench species, with some minor exceptions. In contrast, only a single block (*rrnL-nad3*) is shared between aphelench (*Bursaphelenchus* spp.) and all three tylench (*P. vulnus, Radopholus similis* and *Heterodera glycines*) species. Additional mitochondrial genome sequences from as yet unsampled taxa will provide useful information for better characterizing the deep node phylogeny and mitochondrial genome evolution of nematodes.

### O27.013 Impact of source of resistance on the soybean cyst nematode virulence phenotype in a greenhouse study

*S.Y. Chen*
*University of Minnesota Southern Research and Outreach Center, 35838 120th St, Waseca, MN 56093, USA*
*Email: chenx099@umn.edu*

A greenhouse experiment was conducted to study the effect of source of resistance on the soybean cyst nematode (SCN, *Heterodera glycines*) virulence phenotype with the initial SCN populations HG-Type0 (avirulent), HG-Type2.5.7 (virulent to PI88788), and HG-Type1.3.6 (virulent to Peking). The soybean sequences were: 1) monoculture of soybean near-isogenic lines without (NIL-S) or with (NIL-R) *rhg1* from PI209332; 2) monoculture of either of the cultivars Latham EX547RRN, 91M90, and AR5084, carrying SCN-resistance from PI88788, Peking, and PI437654, respectively; 3) rotation of either two of the three cultivars; and 4) original populations in soil stored at -20 ℃. Soybean was planted in pots for eight periods each about 3-4 months before they were tested for SCN reproductive potential on PI88788, Peking, and PI437654. There was no difference in Female Index (FI) between the treatments of susceptible soybean and storage at -20 ℃, indicating that planting the SCN-susceptible soybean had no detectable selection on virulence phenotype. When the initial population was the avirulent HG-Type0, the PI88788-derived cultivar selected populations that overcame the resistance in PI88788, and the Peking-derived cultivar selected populations that overcame the resistance in Peking. In contrast, the PI437654-derived cultivar selected populations that increased FI on both PI88788 and Peking. NIL-R selected populations that overcame PI88788 and apparently also increased FI on Peking. When the initial population was HG-Type2.5.7, both Peking- and PI437654-derived cultivars increased FI on Peking. For the initial population HG-Type1.3.6, only PI88788-derived cultivar and NIL-R selected the populations that overcame PI88788. No selected populations could overcome the resistance in PI437654.

### O27.014 A real-time PCR assay for direct detection and quantification of *Pratylenchus neglectus* from soil

*G.P. Yan, R.W. Smiley, P.A. Okubara, A.M. Skantar and C.L. Reardon*
*Oregon State University, Pendleton, Oregon, 97801, U.S.A*
*Email: richard.smiley@oregonstate.edu*

*Pratylenchus neglectus* is one of the most widespread and economically important nematodes that attacks plant roots and restricts wheat productivity in the Pacific Northwest USA. It is challenging to quantify this nematode using microscopic methods in large numbers of field samples in which other nematode species are also present. A quantitative real-time polymerase chain reaction (qPCR) assay was developed to detect and quantify *P. neglectus* from DNA extracts of soil. The primers, designed from internal transcribed spacer region of rDNA, showed high specificity with a single melt curve peak to DNA from eight isolates of *P. neglectus*, but did not amplify DNA from 28 isolates of other nematode species. The assay was sensitive and capable of detecting genomic DNA of a single juvenile inoculated into one gram of soil. No significant difference ($P = 0.166$) was observed between the cycle threshold values of a single adult female, juvenile, and egg (containing faint outline of developing juvenile). Mixtures of nematodes in these life stages were therefore used to generate a standard curve ($R^2 = 0.96$, $P < 0.001$) by amplifying DNA extracted from soil to which nematodes were added. The standard curve was validated using sterilized soil inoculated with lower numbers of *P. neglectus*. A significant positive relationship ($R^2 = 0.66$, $P < 0.001$) was observed for nematode numbers quantified from natural field soils using qPCR and a traditional nematode extraction method. Real-time PCR potentially provides a useful platform for efficient detection and quantification of *P. neglectus* directly from field soils.

### O27.015 Identifying a novel source of resistance against *Heterodera filipjevi* in spring barley 'Steptoe' by analysis of a double haploid population Steptoe × Morex

*A. Westphal*[1] *and A. Graner*[2]
[1]*Institute for Plant Protection and Grassland, Julius Kühn-Institut, Messeweg 11/12, 38104 Braunschweig, Germany;* [2]*Leibnitz Institute of Plant Genetics and Crop Plant Research (IPK) Corrensstrasse 3, 06466 Gatersleben, Germany*
*Email: andreas.westphal@jki.bund.de*

Cereal cyst nematodes (CCN) damage small grain crops in many regions of the world. In Europe, these pests have become known for their infectivity on emerging spring cereals. A newly recognized species of the CNN complex is *Heterodera filipjevi*, which is similarly spread as *H. avenae* and causes damage in arid areas among others in the Pacific North West of the US, the Mediterranean and China. Resistance to *H. avenae* may not be effective against *H. filipjevi*. A resistant response to *H. filipjevi* was found in *Hordeum vulgare* 'Steptoe' while this cultivar was susceptible to *H. avenae*. The objective of the current study was to determine the chromosomal location of the resistance to *H. filipjevi* in Steptoe by use of a Steptoe × Morex double haploid population. In the greenhouse in small growth containers, a total of 93 double haploid lines, along with the resistant and susceptible parents, were tested if they permitted *H. filipjevi* to reproduce. Genetic mapping using a dense framework of single nucleotide polymorphism (SNP) markers allowed for precise positioning of the resistance locus on chromosome 3H. The development of markers appropriate for marker assisted selection will help breeders to effectively incorporate resistance into elite germplasm.

**O27.016 Investigating the role of thionins in the interaction between root knot nematodes and rice**

*H.L. Ji, T. Kyndt, C. Ullah and G. Gheysen*
*Department of molecular biotechnology, Ghent University, coupurelinks 653, 9000 Ghent, Belgium*
*Email: Ji.hongli@ugent.be*

In a transcriptome analysis on galls induced by *Meloidogyne graminicola* in rice, thionins were shown to be remarkably downregulated (Kyndt et al., 2012). Based on this information, thionin7 over-expression lines and its promotor-gus lines were generated. JA, SA and ABA were applied on the promotor-gus lines to test their inducing abilities on gus expression. Meanwhile, qRT-PCR was also conducted on several different rice thionin genes after these three hormones application, as well as in galls at different time points. The over-expression lines of thionin7 are being tested for susceptibility towards *M. graminicola* infection. To look into more detail into the transcriptome of the giant cells, inside the galls, we combined Laser Capture Microdissection with mRNA-Seq. The expression profiles indicated a significant induction of genes involved in the primary metabolism, on the other hand, many genes involved in secondary metabolism and plant defense were strongly suppressed in the giant cells, for instance, signaling genes, thionin-like peptides. These results will open a better understanding on the interaction of rice and its root-knot nematode *M. graminicola*.

**P27.001 Nematicidal activity of Eritrean weed plants against root-knot nematode *Meloidogyne incognita***

*K.K. Chaudhary*[1,2]*, A. Haile*[2]*, G. Kumar*[1] *and A. Varshney*[1]
[1]*Institute of Biomedical Education and Research, Mangalayatan University, Beswan, Aligarh, Uttar Pradesh-202145;* [2]*Department of Plant Protection, Hamelmalo Agricultural College, P O Box 397, Hamelmalo, Eritrea*
*Email: kamal22055@yahoo.co.in*

The nematicidal potential of ethanolic and aqueous extracts of ten wild plant species distributed on agricultural land of Eritrea against root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood was studied. Three concentrations of each plant extracts were tested. Rate of egg hatching and mortality of second stage juveniles of *M. incognita* were quantified at 24, 48 and 72h exposure. Significant mortality and egg hatching inhibition were observed with aqueous and ethanolic extracts of *Datura stramonium*. The inhibiting trend was followed by the two extracts of *Heliotropium indicum*. Juvenile mortality ranged from 8 to 100% in case of hot water extracts and 26 to 100% in case of ethanolic extracts. The nemato-toxicity of the tested plant extracts increased with increase of concentration and exposure time and vice versa. The hot water and ethanolic extracts of *D. stramonium* caused 75 to 100% mortality and 57 to 100% inhibition in egg hatching, respectively. The mortality was 74 to 100% in case of *H. indicum.* Among all the tested extracts, hot water and ethanolic extracts of *Lantana camara and Xanthium strumarium* were least effective.

**P27.002 Resistance and tolerance to *Heterodera avenae* in North American spring wheat**

*R.W. Smiley, J.M. Marshall and G.P. Yan*
*Oregon State University, Pendleton, Oregon, 97801, U.S.A.*
*Email: richard.smiley@oregonstate.edu*

The cereal cyst nematode, *Heterodera avenae*, reduces wheat production efficiency by>US$3.4 million annually in the Pacific Northwest states of Idaho, Oregon and Washington. Spring wheat trials were conducted in naturally-infested fields in Idaho and Washington during 2012. Twenty cultivars were planted as a split-plot design with each cultivar planted into six replicates of 1.8×9-m plots that were either treated with nematicides or were untreated. All plants exhibited the typical root knotting symptom in untreated soil. Mean number of white females was greater ($P$<0.01) in untreated (9/plant)

than in treated soil (<1/plant). In untreated soil, fewer white females were produced on Ouyen and WB-Rockland (1/plant) than on other cultivars (9-29/plant). Post-harvest density of *H. avenae* eggs (from cysts) was higher (>12,000/kg of soil) following growth of susceptible cultivars than following Ouyen and WB Rockland (<5,000/kg of soil). The latter density was similar to that in plots of all cultivars produced in treated soil, indicating a natural background density of *H. avenae* remaining from cysts that had been produced on cereal crops one or two years earlier. Ouyen contains *Cre1* resistance but was intolerant, with grain yield being lower in untreated than in treated plots. WB-Rockland was both highly resistant and tolerant. Two cultivars (Buck Pronto and UI Stone) were highly susceptible but very tolerant. These were the first field trials in North America that demonstrated the benefits that can be expected from developing cultivars with resistance plus tolerance to *H. avenae*.

**P27.003 The distribution and rDNA-ITS analysis of cereal cyst nematode (*Heterodera avenae*) in Shandong province, China**

*C.J. Liu[1,2], W.K. Huang[1], J.K. Cui[1], D.L. Peng[1] and H.M. Li[2]*
*[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; [2]College of Plant Protection, Nanjing University, Nanjing 210095, P. R. China*
*Email: pengdeliang@caas.cn*

The cereal cyst nematode (*Heterodera avenae*) is the most important nematode on wheat and cereal crops in China. The occurrance and distribution of Heterodera spp. were investigated by random sampling method from 19 counties of Shandong province. The specie was identified as *H. avenae* with the morphological and rDNA-ITS analysis. The cereal cyst nematode (*H. avenae*) was detected from 84.2 percent samples collected from Linyi, Laiwu, Zibo, Weifang, Dongying, Weihai, Yangtai. The highest cyst and egg number existed in Dongying city and Weifang city, the lowest cyst and egg number existed in Linyi city and Yantai city. This survey results will beneficial for making suitable management strategy and control measures.

**P27.004 Sensitive and direct detection of *Heterodera filipjevi* in soil and infected wheat by species-specific SCAR-PCR Assays**

*H. Peng[1], X. Qi[1], D. Peng[1], H. Long[2], X. He[1], W. He[1] and W. Huang[1]*
*[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; [2]Key Laboratory of Pests Comprehensive Governance for Tropical crops, Ministry of Agriculture, P. R. China, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Science, Danzhou 571737, P. R. China*
*Email: pengdeliang@caas.cn*

Cereal cyst nematodes (CCN), especially *Heterodera avenae* and *H. filipjevi*, are the most economically important plant-parasitic nematode on cereal crops in wheat production area of the world. Morphological identification these species is time-consuming and laborious because there are only slight differences. In this study species-specific SCAR-PCR assay for detection and identification of *H. filipjevi* from infected wheat roots and soil were developed. The species-specific primers were designed according to the randomly amplified polymorphic DNA (RAPD) markers amplified with random primer OPK16. A 646bp specific fragment of sequence was generated, which characterized amplified regions (SCAR) in *H. filipjevi*. The detection limitation of PCR assay was as low as 0.125 µl second-stage juvenile lysate, 3.9×10-3µl adult female lysate and 10-3 µl cyst lysate. The method was able to detect the various developmental stages of *H. filipjevi*, and a single of nematode in 0.5g soil. Two of six field samples (TYHN and XCHN) were detected as *H. filipjevi* by the method. In addition, we determined that the TYHN sample contained a mixed population of *H. filipjevi* and *H. avenae*. This present study is the first to provide a definitive diagnostic assay for *H. filipjevi* in wheat roots and soil using SCAR primer. The discovery of *H. filipjevi* in the Tangyin, Anyang city, Henan province represents a new record for the occurrence of this species in China.

**P27.005 Incidence of root knot nematode on okra in Layyah, Punjab, Pakistan**

*M. Hussain and A.A. Shahid*
*Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan*
*Email: manzoor.sahi@gmail.com*

Root knot nematodes (RKN), *Meloidogyne* spp., are serious pathogens of okra in Pakistan. A systemic survey was conducted to assess the reliable estimate of nematodes and their infestation level with okra plants at grower's field located at major vegetable production areas of each Tehsil of District Layyah. From each tehsil, four sampling sites were selected randomly from grower's fields. 20 samples of root and soil were taken from 12 different sites of each Tehsil. These sampling sites were located at Bhagal, Lalazar, Chowk Azam, Hira Minor, Fetehpur, Kazmi chowk, Qaziabad, Rajan Shah, Nawan Kot, Kapoori, Shergarh, and Rafiqabad. Nematode population in 20 g of roots and 100cm3 of soil samples was determined by the sieving-cum-modified Baermann funnel techniques. The incidence of infestation was assessed by [(Number of samples with RKN

total number of samples) x 100]. The perennial pattern of RKN samples revealed the presence of *M. incognita* singly or in combination of *M. javanica.* The incidence ranged from 70 to 95% with an average of 82.5%. The gall index ranged from 4 to 5 with a mean of 4.5 with maximum gall size. Both incidence and gall index varied from locality to locality. There was 86%, 85% and 87% root knot nematode incidence in the okra production areas of Layyah, Karor and Chobara, respectively. This survey yielded the first report of *Meloidogyne* spp. infestation in vegetables in District Layyah.

**P27.006 Influence of root knot nematode (*Meloidogyne Incognita*) infection on potato leaf NPK contents**

*A.S. Gondal, N. Javed and S.A. Khan*
*Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan*
*Email: amjadshahzad@live.com*

Potato crop requires proper crop nutrition and disease management strategies as it endures significant yield losses due to root knot nematode (*Meloidogyne incognita*) infection. The study aimed at determining the change in the leaf nutrient contents of different resistant and susceptible potato cultivars against RKN (*M. incognita*) infection. Five moderately resistant cultivars viz. FD-74-67, SH-332, SH-339, FD-1-3 and FD-49-62 and five susceptible cultivars viz. FD-19-2, FD-8-1, SH-692, SH-5 and FD-69-1 cultivars were accessed for change in leaf Nitrogen (N), Phosphorus (P) and Potassium (K) contents. Leaf nitrogen (N) and phosphorus (P) contents were determined by Kjeldahl Extraction Method and potassium (K) contents were determined by atomic absorption spectrophotometer. Susceptible cultivars were found to be deficient in these contents. Significant increase ($P<0.05$) in leaf NPK contents was observed in resistant cultivars under nematode stress compared to inoculated. Maximum leaf nitrogen (N) of 5.96% was noted in resistant cultivar FD-1-3 while minimum as 3.52% in susceptible potato cultivar FD-19-2. Lead phosphorus (P) content recorded maximum as 0.225 % in FD-1-3 and minimum as 1.48% in FD-19-2. Minimum leaf potassium (K) 0.33 and maximum 0.48 was recorded in FD-19-2 and FD-1-3, respectively.

**P27.007 Use of nutritional suppliments against root knot nematode (*Meloidogyne Incogtina*) infection in potato**

*A.S. Gondal, N. Javed and S.A. Khan*
*Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan*
*Email: amjadshahzad@live.com*

The effect of foliar application of micro-power®, humic acid and plant protector containing benzoic acid was examined for the management of root knot nematode (*Meloidogyne incognita*) in a susceptible potato cultivar FD-8-1. Application of plant protector significantly reduced the number of galls and egg masses and promotes overall plant growth followed by micro power and humic acid as compare to control. Foliar application of plant protector 4% endorsed the number of leaves, shoots development, tuber weight and decreased the root weight followed by micro-power 4% plant protector 2% and humic acid 4%. Minimum number of root galls and egg masses was recorded in case of plant protector 4% followed by micro-power 4%, micro-power 2% and plant protector 2%. Nematode fecundity rate was observed to be maximum in case of control with poor plant growth and maximum number of galls and egg masses. The significantly lower number of galls and egg masses and enhanced plant growth in case of plant protector containing benzoic acid at 4% concentration indicated to be best one.

**P27.008 The dynamics of *Heterodera avenae* in winter wheat in Hebei province**

*X.H. Li, J. Ma, B. Gao, R.Y. Wang and S.L. Chen*
*Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences/IPM Center of Hebei Province/Key Laboratory of Integrated Pest Management on Crops in Northern Region of North China, Ministry of Agriculture, P. R. China, Baoding 071000, Hebei Province, P. R. China*
*Email: chen_shulong@tom.com*

Cereal cyst nematode (*Heterodera avenae*) has become the main disease in wheat production in China and even worldwide. Understanding the dynamic of cereal cyst nematode in the field is important for the control of the disease. Three surveys were conducted in the main winter wheat production area in Hebei from October, 2009 to September, 2012. The results showed that few J2 were detected in soil in wheat sowing period (October 6th), and it reached the peak (12.3-18.6 J2/100 ml soil) in late November (23th to 27th, Nov.) before the soil freeze, then the number of J2 dropped to 0.1 J2/100 ml soil in 23rd of December and no J2 isolated in soil layers (0-14 cm) in January. Afterwards the number of J2 in soil increased again with the soil temperature increasing, and reached the peak in early April with 52-65 J2/100 ml soil, it decreased to 1.3 J2/100ml soil in May 12th. No J2 isolated from early June. Very low number of J2 with 0.4-3 J2/100 ml soil could be extracted in the soil from July to September. J2 could penetrate root from October 15 (0.7 J2/plant) to November 10th (0.3 J2/plant), no obvious peck found before soil freeze and the nematodes stop to penetrate during soil freeze. J2 started to penetrate root again from end of February and the higher number of J2 with 59-102 J2/plant were found from 6th-14th of April, then the number of J2 penetrated to the root decreased. Accordingly, third stage juveniles formed between October and November,

and peaked between late April and early May. The white females were detected from mid of May and higher number of cyst formed in late May. The eggs in the cyst from June to end of July were mainly in the embryonic development stage. In summary *H. avenae* occurs one generation per year in wheat in Hebei province and the main damage period is from late March to early April in spring.

**P27.009 Chemotaxis of *Ditylenchus destructor* to extracts from sweet potato**

*J. Ma, X.H. Li, R.Y. Wang, Y.L. Geng and S.L. Chen*
*Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Science/IPM centre of Hebei Province/Key Laboratory of IPM on Crops in Northern Region of North China, Ministry of Agriculture, 437 Dongguan street, Baoding, Hebei 071000, P. R. China*
*Email: chenshulong@gmail.com*

*Ditylenchus destructor* Thorne could cause severe damage to sweet potato, potato and other host plants in China. Host plant phytochemicals play important roles in the nematode behavior, thus identification of the compounds that attract the nematodes to the plant is important for the nematode control. In the present study, experiments were carried out to investigate chemotaxis of *D. destructor* to different host plant and determine the attraction intensity of different host plants. Sweet potato was consistently and significantly much more attractive than potato and carrot in all these assays. Compared with the stems, leaves and storage roots of sweet potato, stems showed the significantly strongest attraction ability to nematodes and our result showed that the attractant from sweet potato was stable to heat. Differences in the relative attraction to *D. destructor* among sweet potato cultivars were also studied, the results showed that the stems of susceptible cultivars Lizixiang, Jishu 98, Shangshu 19 were significantly more attractive than the resistant cultivars Xushu 25, Xushu18, Jishu 17-52; in contrast, there were no significant difference of the storage roots among different sweet potato cultivars. Furthermore, the compounds of sweet potato were extracted and studied in relation to the host-finding behavior of the nematode. The fractions extracted with butyl alcohol from stems were significantly more attractive than extractions by water, alcohol, petroleum ether and ethyl acetate. The results of this study suggest that extracts from sweet potato can potentially be used as baits in a trap for the control of *D. destructor* in the field.

**P27.010 Nematode detection using DIRT(s) extraction and 'anti-primer'**

*M.N.H. Tan[1, 3, 4], V.A. Vanstone[2], D.I. Berryman[1, 3] and M.G.K. Jones[1, 3]*
*[1]Plant Biosecurity Co-operative Research Centre, PO Box 5012, Bruce ACT 2617; [2]Department of Agriculture & Food Western Australia, Baron-Hay Court, South Perth WA 6151; [3]Plant Biotechnology Research Group, School of Biological Sciences and Biotechnology, WA State Agricultural Biotechnology Centre, Murdoch University, Perth, WA 6150, Western Australia; [4]Animal & Plant Health Centre, Agri-Food & Veterinary Authority of Singapore, 6 Perahu Road, Singapore 718827*
*Email:matthew_tan@ava.gov.sg*

PCR are routinely used in many diagnostic environments and can be affected by inhibitors from DNA extracted from soil or plant materials. Special treatments for inhibitors (humic and fulvic acids) are typically required. These inhibitors can affect the quality of extracted DNA from soil. 100g of soil is usually the limit because of inhibitors and multiple steps were required to purify the DNA and that can be expensive. In this study, DNA Isolation Rapid Technique from soil, which refers to as, DIRT(s) is described and can be done in 30 min. The whole procedure - from retrieving nematodes from infected soil to DNA extraction using the blender and finally retrieving results can be completed in 4 hours. Using the mist chamber to extract nematodes from infected soil, followed by DNA extraction, typically require more than a day. The aim of this study demonstrate the efficiency of the DIRT(s) extraction compared with mist chamber particularly in achieving faster and possibly better results.

**P27.011 Rotation crop evaluation for management of the soybean cyst nematode**

*B.S. Yu, J.J. Wang and J.R. Li*
*Soybean Research Institute, Heilongjiang Academy of Agricultural Sciences, Harbin, 150086, P. R. China*
*Email:bsyu100@163.com*

Crop rotation is an effective tactic for soybean cyst nematode (SCN) management. In Heilongjiang province of China, corn is almost exclusively used as a nonhost rotation crop with soybean. This study was conducted to determine the effectiveness of crops common to or having potential use in Heilongjiang province as rotation crops for managing SCN. Five potential rotation crops of marigold (*Tagetes erecta*), red clover (*Trifolium pratense*), barley (*Fructus hordei* Vulgaris), maize (*Zea mays* L) and flax (*Linum usitatissimum* L) and susceptible soybean were grown in the infected field at Harbin in 2010; corn, wheat and susceptible soybean was grown on different plots in 2011; and susceptible soybean were grown in all plots in 2012. Nematode populations were measured both years before planting and after harvest; soybean yield was measured in 2012. There was large variability in SCN population and soybean yield at the site. Nevertheless, significant treatment effects were detected at all treatments. While all of the rotation crops lowered SCN populations compared with SCN-suscep-

tible soybean. Leguminous non-hosts or poor hosts such as Marigold and Red clover were best in reducing SCN population density. Corn, the most common rotation crop was among the least effective in reducing nematode populations. There was yield benefit from SCN management. The data suggest that three year of rotation of soybean with some crops such as marigold before planting a susceptible soybean may be sufficient in managing SCN.

**P27.012 Cloning of dormancy related gene *daf-16* and dynamic analysis of expression in adverse situation in pine wood nematode, *Bursaphelenchus xylophilus***

*D.D. Wang[1,2], G.H. Chen[2], Z.C. Mao[2], Y.H. Yang[2], J. Ling[2] and B.Y. Xie[2]*

*[1]Yangtze Normal University, College of Life Science and Technology, P. R. China; [2]Institute of Vegetables and Flowers, Chinese Academy of Agricultural Academy, P. R. China*

*Email: wddwill@yahoo.com.cn*

Dormancy is the important phenotypic plasticity in *Bursaphelenchus xylophilus* under adverse situation, and play the vital significance in its lifecycle. FOXO transcription factors Encoded by *daf-16* gene is a Important regulators for dauer formation in *Caenorhadits.elegans.* We got *daf-16* gene sequence and analysed the mRNA expression of *daf-16*-1, *daf-16-2* gene and protein expression of *daf-16-2* gene level under adverse situation. The result show, there are two *daf-16* gene in *B. xylophilus* , *daf-16-1* （ JN628938 ） and *daf-16-2* （GU584878）. *daf-16-1* gene has two splicing way, form1 cDNA full length is 2043 bp and contains a 1614 bp ORF, 5 'UTR of 224 bp, 3' UTR of 205 bp, form2 cDNA full length is 1847 bp and contains a 1440 bp ORF, 5 'UTR of 479 bp, 3' UTR of 349 bp; *daf-16-2* gene cDNA full length is 2730 bp and contains a 1911 bp ORF, 5 'UTR of 470 bp, 3' UTR of 349 bp. The two gene were both a single copy in *B. xylophilus.* The mRNA expression level of *daf-16-1* were up-regulated under low and high temperature, down-regulated in lack of food; The mRNA expression level of *daf-16-2* were up-regulated under high temperature and lack of food, down-regulated in low temperature; The protein expression level of *daf-16-2* were down-regulated under low temperature and lack of food, no effect on high temperature. It shows that when outside environment changes daf-16 gene may play an important regulatory role in *B. xylophilus*.

**P27.013 SCAR molecular markers correlated with populations of *Meloidogyne incognita* virulent to resistance gene *Me3***

*G. Wang, E.F. Li, Z.C. Mao, B.Y. Xie and D.X. Feng*

*Institute of Vegetables and Flowers, CAAS, No.12 Zhongguancun South St., Haidian District, Beijing, 100081, P. R. China*

*Email: xiebingyan@caas.cn*

Molecular makers of virulent populations against *Me3* in *Meloidogyne incognita* was studied in order to detect the virulence mutation rapidly and effectively. Root-knot nematode populations including avirulent population, populations overcoming resistant genes *Me3* and the mixed group of the two population as the experiment materials, polymerase chain reaction was done with 100 primer pairs designed according to *M. incognita* genome and 19 pairs reported in literature to screen specific band among three populations. And subsequently SCAR primers were designed and a multiplex PCR reaction system was built. Seven primer pairs amplifying stability bands were screened. Through BLAST in NCBI, two of which were converted into SCAR markers differentiating the three populations. Multiplex PCR from avirulent population and Me3-virulent isolates generated a fragment of 999 and 629 bp, respectively, while from the mixed group generated both of the above fragments. Virulent mutation markers were successfully developed in *M. incognita*, and one-step multiplex PCR can be used for identification of Me-virulence.

**P27.014 The effect of transgenic cucumber with dsRNA of mapk to root-knot nematode**

*G.H. Chen, Y. Wang and B.Y. Xie*

*Institute of Vegetables and Flowers, CAAS, NO.12 South Zhongguancun Street, Haidian District, Beijing, 10081, P. R. China*

*Email: chenguohua01@caas.cn*

Root-knot nematodes (*Meloidogyne* spp. RKN) causes devastating disease on cucumber, resistant breeding is the best strategy to control the nematodes. In this study, we selected RNAi lethal genes from south root-knot nematode through comparative genomics, and verified the lethal effects of the candidate genes on RKNs through TRV-VIGS technique in tomato. The results showed that Mapk exhibited strong lethal effects to RKNs，and the number of egg masses reduced 60.2%. Mapk gene was further transferred to cucumber (*Cucumis sativus*) cotyledon by *Agrobacterium tumefaciens* to obtain the transgenic cucumber lines to resistant to the root-knot nematode. The transgenic cucumber with Mapk dsRNA could inhibit expression of Mapk gene of root knot nematode, and showed good control efficiency to the nematode. The result showed the number of egg masses reduced 49.4% in pot experiments and few egg masses in the fields testing. From the index of diversity of bacterial, fungus, archaea and nematode, there was no side-effect in two types of soil by transgenic cucumber and non-transgenic cucumber.

**P27.015 Effect analysis of steam fumigation for controlling *Ditylenchus destructor***

*Y.L. Li, X.Q. Wang, H. Sun, B. Hu and J.Q. Zheng*
*Institution of Beijing Plant Protection, No.9 North Third Ring Middle Road, Xicheng District, Beijing, 100029, P. R. China*
*Email: lylong77@163.com*

Sweet potato stem nematode disease, *Ditylenchus destructor*, is the most serious pests of sweet potato, which is an important grain crop in the world. Most of crop pest can be controlled effectively in the high temperature condition. The effect and applicability of steam fumigation were studied on this paper by measuring the soil temperature, the mortalities of *Ditylenchus destructor* from sweet-potato tuber buried in corresponding depth. The results showed that, on the condition of 40 minutes` steam fumigation, the maximum soil temperature in 10 cm and 20 cm depth were respectively 60.6℃ and 37.9℃, the mortalities of nematode in the corresponding sweet-potato tuber were respectively 100% and 88.1% while the ambient temperature was 23℃. The maximum soil temperature in corresponding depth were respectively 21.7℃ and 16.0℃, the mortalities of nematode in the tuber were respectively 26.4% and 24.8% while the ambient temperature was 7℃. The method of steam fumigation showed preferably effect for controlling *Ditylenchus destructor* in surface soil, meanwhile, the higher the ambient temperature, the better the control effect of steam fumigation. Except for *Ditylenchus destructor*，this method can be used well for green management of other crop pest.

**P27.016 Investigation of cereal cyst nematode in a naturally infested winter wheat field for nematode management**

*H.Y. Wu[1,2], W.K. Huang[2] and D.L. Peng[2]*
*[1]Agricultural College of Guangxi University, Nanning, Guangxi 530004, P. R. China; [2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Chinese Academy of Agricultural Sciences, Beijing* 100193*, P. R. China*
*Email: whyzxb@yahoo.com.cn*

The cereal cyst nematode (CCN; *Heterodera avenae*) is found in at least 16 provinces in China. CCN often damages wheat and barley, causing severe economic losses. The Shandong winter wheat region is the second largest wheat production base in China. Thus, feasible management approaches in this location should be explored. This study investigated the horizontal and vertical distributions of the CCN population in a naturally infested field under local climatic and planting pattern conditions. The spatial distribution between planting rows and spaces was also determined. The results showed that the distribution of cysts was uneven at the horizontal scale. When the row spacing was 20 cm, higher cyst population densities were observed in the planting row and in the middle of two planting rows. Moreover, about 78.9% cysts were distributed in the 0–20 cm soil layer. These results suggested that the specific site for the effective application of chemicals or biological control agents was in the middle of two planting rows at a depth of at least 20 cm. This scientific information can serve as a reference for the development of an effective site-specific management strategy for the local control of CCN in large wheat field areas using a mechanical tool.

**P27.017 Toward management of cereal cyst nematode with host resistance: identification of effective resistant sources in China**

*H.J. Li[1], L. Cui [1], H.L. Li [2], X.M. Wang [1] and W.H. Tang[3]*
*[1]Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China; [2]College of Plant Protection, Henan Agricultural University, Zhenzhou 450002, P. R. China; [3]Department of Plant Pathology, China Agricultural University, Beijing 100193, P. R. China*
*Email: lihongjie@caas.cn*

Recent prevalence of cereal cyst nematode (CCN, *Heterodera avenae* and *H. filipjevi*) throughout the important wheat growing regions of China has attracted serious attentions. Unfortunately, the shortage of resistant resources has limited the use of host resistance to control CCN. Since 2008, a project has been initiated to identify effective sources of resistance to *Heterodera* spp. indigenous to China. Based on a three-year field test, resistance of the wheat cultivar Madsen from Washington State University, USA was effective against both species of *Heterodera*. Results of histological analysis indicated that the number of juveniles invaded the roots of Madsen was fewer than the susceptible control Wenmai 19. Inoculation tests with 11 pathotypes of *H. filipjevi* and *H. avenae* from Henan, Anhui, and Shandong provinces demonstrated that Madsen was effective against all the populations of both species of *Heterodera*. Genetic analysis indicated that a single dominant gene was associated with the resistance of Madsen to *H. filipjevi*, which permits the transfer of its resistance to CCN in to more adapted local cultivars. Some advanced lines have been produced, which have shown to be resistant to *H. filipjevi* and *H. avenae* as effective as the donor parent Madsen. In addition to resistance to *H. filipjevi* in Madsen, a number of *Triticum durum* and wheat-*Thinopyrum* derivatives, *Triticale*, *T. dicoccum*, and *T. dicoccoides* accessions exhibited excellent resistance against CCN in the field tests. Since most commercial wheat cultivars currently grown were susceptible, these resistant germplasms provide valuable sources for improvement of CCN resistance in China.

**P27.018 The comparative genome analysis of *Fusarium oxysporum***

*J. Ling and B.Y. Xie*
*Institute of Vegetables and Flowers, CAAS, No.12 South Zhongguancun Street, Haidian District, Beijing, 10081, P. R. China*
*Email: lingjian@caas.cn*

The fungus of *Fusarium oxysporum* is economically important soil borne plant pathogens, causing vascular wilt disease in a wide variety of crops. We have sequenced 10 fungus of *F. oxysporum* genomes that their host including cotton, cucumber, water-melon, melon and cabbage. In the 10 genomes, the average genome size is about 51.2 Mb and the average annotated genes are about 14821. By using the genome of *F. oxysporum* f. sp. *lycopersici* as the reference genome, we investigated the co-syntenic regions of *F. oxysporum*. The syntenic regions shared by above 10 genomes cover 90.3% of the genome of *F. oxysporum* f. sp. *lycopersici*, and the most non-syntenic regions of *F. oxysporum* located on the 3, 6 and 15 chromosome, which may be related with the host specificity. Furthermore, we found the expansion and contraction of some special gene family are associated with the host-special *F. oxysporum*. By using comparative genomic analysis, we identified several dozen genes that are associated with the host specificity, pathogenicity and vascular colonization. Now we are using genetics experiments to study the functions of these genes

**P27.019 Soybean germplasm resources reaction to races 14 of soybean cyst nematode**

*J.S. Chen[1,2], F. Zhu[1], X.F. Zhu[1], Y.Y. Wang[1], Y.X. Duan[1] and L. Chen[1]*
*[1]Nematology Institute of Northern China, College of Plant Protection, Shenyang Agricultural University, Shenyang 110866, P. R. China; [2]Daqing Branch， Heilongjiang academy of agricultural science, Daqing 163316, P. R. China*
*Email: chenlijie0210@163.com*

Soybean cyst nematode is one of the most devastating pests of soybean， which causes extensive economic losses in china and worldwide. Resistant soybean varieties are the most effective tool available for management of SCN. The objectives of this study were to screen sources of resistance to SCN and determine the characteristics of the resistance. 167 accessions of soybean varieties and germplasms were evaluated for the resistance to the race 14 of soybean cyst nematode. The results showed that, 3 soybean varieties and germplasms were resistant, accounting 1.8% of the total evaluated cultivars; 20 varieties and germplasms were moderate resistant, 11.98% of the total. The results showed that most of the major soybean varieties and germplasms were susceptible or highly susceptible to race 14 of SCN. The resistant varieties and germplasm resources, which were identified in this study provided material platform for breeding against soybean cyst nematode in soybean production of China.

**P27.020 Clone and function analysis of *MiAsg* gene of *Meloidogyne incognita***

*M. Mei, Y.H. Huang, Z.C. Mao and B.Y. Xie*
*Institute of Vegetables and Flowers, CAAS, No.12 Nandajie Zhongguancun, Haidian District, Beijing, 100081, P. R. China*
*Email: qdjzm@yahoo.com.cn*

Root-knot nematode（*Meloidogyne spp.*）is a worldwide disease, causing serious damage to agricultural production. In preliminary studies, we predict the number of RNA interference (RNAi) phenotype genes of nematode in its genome using bioinformatics methods. In this study, we designed special primers based on the predicted Mitochondrial ATP synthase g subunit gene (*Asg*) sequence and cloned the *Asg* gene in *M. incognita*, which was transferred into tomato plantlets using virus-induced gene silence (VIGS) technique after analyzing the sequence characters to investigate the influence of the *MiAsg* gene silence on the amounts of root knot on the tomato plantlet roots. The results showed that the similarity between the cloned *MiAsg* gene and the predicted Asg was as high as 100%. Sixty days after inoculation with *M. incognita*, the amounts of root knot on tomato plantlets of which the *MiAsg* gene was silenced decreased by 59.6% compared to that of the empty vector control, by 59.5% compared to that of the water control. The study revealed the *MiAsg* gene silence had a good effect on prevention and control of root-knot nematode disease, also showed that these *MiAsg* genes may be involved in the pathogenesis of nematode, which provided new ideas and ways to the research of nematode pathology and nematode disease control.

**P27.021 Characterization of introns and alternative splicing in *Bursaphelenchus xylophilus* by comparative analysis of transcriptomic sequences and genomic sequences**

*L. Huang, X.L. Ding, L.H. Zhu, X.Q. Wu and J.R. Ye*
*Institute of Forest Protection, College of Forest Resources and Environment, Nanjing Forestry University, Nanjing, 210037, P. R. China; Jiangsu Key Laboratory for Prevention and Management of Invasive Species, Nanjing, 210037, P. R. China*
*Email: jrye@njfu.com.cn*

The pine wood nematode *Bursaphelenchus xylophilus*, is a disastrous pathogen of the pine forests in East Asia and European. Although the genomic sequences of *B. xylophilus* have been reported, little information regarding the introns therein is available. In the study, we

investigated the introns of *B. xylophilus* by comparative analysis of genomic sequences and transcriptomic sequences. A total of 31295 introns were identified, of which 89% contained canonical splice site pair GT-AG. The mean introns length was 97bp and the density of the introns in the genomic sequences was 1.51. 5' splice sites contained the conserved sequence of GTAAGT with the splice strength of 2.9. Conserved nucleotides sequence of TTCTAAT was contained in the branch sites. The conserved splice model of 3' splice sites was TTAG|GT. And the polypyrimidine tract was analyzed in the study. Resulted showed that these splicing characters shared a highly similarity within Nematoda. Furthermore, the alternative splicing was identified in a few of genes. This indicated that the alternative splicing regulation may be one of important ways of gene regulation in *B. Xylophilus*.

**P27.022 Morphological identification and description of rice root nematodes (*Hirschmanniella* ssp.) occurring in Jiangxi province of China**

*R.Q. Cui, X.T. Sun and C.Z. Hu*
*College of Agronomy, Jiangxi Agricultural University, Nanchang, Jiangxi Province, 330045, P. R. China*
*Email: cuiruqiang@sohu.com*

A total of 96 rice root samples were collected from 24 cities and counties in Jiangxi province where rice is grown. According to the morphological characters and morphometrics, 7 species in genus *Hirschmanniella* Luc and Goodey 1963 were identified. They were *H. gracilis*, *H. kejiaensis*, *H. orzyae*, *H. divewrsa*, *H. belli*, *H. caudacrena* and *H. microtyla*. With the exception of *H. orzyae*, the six species are considered as the first that recorded in Jiangxi province. The results of geographical distribution survey indicated that on rice widely distributed in Jiangxi province. There are two populations of different species at least in one paddy, at most up to four. Moreover it occurred in highest population densities. Population densities varied from place to place, the highest density was 389 *Hirschmanniella* spp. per 10 gram rice root and the lowest density was 26 *Hirschmanniella* spp. per 10 gram rice root.

**P27.023 Nematicidal activity of oxalate and citric acid isolated from a nematicidal fugnus, *Aspergillus* sp. Y-61, on *Meloidogyne incognita***

*T. Liu, D. Dong, C.G. Lu, T.T. Zhang, J.Y. Qiu and C.W. Liu*
*Institute of Plant and Environment Protection, Beijing Academy of Agricultural and Forestry Science, 100097, P. R. China*
*Email:lting11@163.com*

An isolate of the Fungus *Aspergillus* sp. Y-61 produced secondary metabolites that inhibited egg hatch and increased juvenile mortality of the root-knot nematode *Meloidogyne incognita* in vitro. 16S rDNA gene sequencing showed that the isolate sequence was 100% identical to *Aspergillus niger*. The culture filtrates form different culture media were tested for nematocidal activity. The maximal activity against *M. incognita* was obtained by using modified basal (MB) medium. The nematicidal assay-directed fractionation of the culture broth delivered oxalate (1) and citric acid (2). Oxalate, a low molecular weight compound, shows a broad range of biological activities. The nematicidal activity of fervenulin (1) was assessed using the broth microdilution technique. The lowest minimum inhibitory concentrations (MICs) of the compound against egg hatch of *M. incognita* was 15.8 μg/ml and juvenile mortality of *M. incognita* increasing was observed at 74.22 μg/ml. Moreover, at the concentration of 120 μg/ml oxalate (1) showed killing effect on second-stage nematode juveniles of *M. incognita* up to 100% after incubation for 24 h. Citric acid (2), another bioactive compound produced by *Aspergillus* sp. Y-61, showed weak nematicidal activity with *M. incognita*.

**P27.024 Efficacy of some organic amendments for the control of root-knot nematode of tomato**

*J. Ma, D. Dong, C.G. Lu, J.Y. Qiu, T. Liu and C.W. Liu*
*Institute of Plant and Environment Protection, Beijing Academy of Agricultural and Forestry Science, 100097, P. R. China*
*Email:lting11@163.com*

Application of organic soil amendments is a traditional control method for plant–parasitic nematodes and it is considered a part of nematode-management programs. Six organic amendments, namely, Seasame cake, wheat bran, bean pulp, chicken manure, Mushroom residue and EM Enrichment Broth were screened in controlling root-knot nematode of potato in the pots. The amendments were applied at the rate of 5% (W/W). All the amendments exhibited varying degrees of reduction compared to the control. The control effects of Seasame cake, wheat bran, bean pulp, chicken manure, Mushroom residue and EM Enrichment Broth were 89%, 100%, 74%, 67%, 37% and 30% after 60 days, respectively. Seasame was found to be more effective and increased height to 23 cm as compared with the check was 18 cm. With increasing emphasis on cost reduction of industrial processes and value addition to agro-industrial residues, seasame cake could be an ideal source of proteinaceous nutrients and as support matrix for various biotechnological processes. The organic amendments could be used as the alternatives of nematicides and prove to be one component in integrated root-knot nematode management for tomato in conventional and organic production systems.

**P27.025 Isolation and clone of some RXLR candidate effecter genes of *Meloidogyne incognita* Me3 virulent population**

*W. Y. Zhang, Z.C. Mao, Y.S. Wang and B.Y. Xie*
*Institute of Vegetables and Flowers, CAAS, No.12 Nandajie Zhongguancun, Haidian District, Beijing, 100081, P. R. China*
*Email:349091034@qq.com*

*Meloidogyne* spp. has a wide range of hosts and does huge harm, causing great financial losses every year. Base on the genomes re-sequencing of wild type and Me3 virulence mutant type Southern root-knot nematode (*M. incongnita*). 24 RXLR effectors were predicted, and 9 genes were isolated from virulence mutant type respectively. Two of them, 14v, and 19v, which signal peptide in sequences were removed and were clone into PVX by *Cla*I and *Sal*I, respectively. The obtained vectors were transformed to *Agrobacterium tumefaciens* strain GV3101 by electroporation, the cell suspension (OD600=0.4) was infiltrated to the leaves of N. *benthamiana*. After infiltration BAX 1 hour, the 14v, and 19v was overlap infiltrated to the BAX area, and the GFP as a negative control. After 7 days, the overlap infiltration results shows that 14v, 19v can suppress PCD caused by BAX, but single infiltration of 14v, 19v didn't show PCD symptom. These results revealed that some secretary proteins from virulence nematode could suppress the PCD. This could support an important basis to reveal the Immunosuppressive of virulent root-knot nematodes.

**P27.026 The control effect of five biological agents to the cucumber root-knot nematodes in greenhouse**

*X. Xi[1], Q. Bai[1], Q. Zhang[1]*
*Institute of Plant Protection, Inner Mongolia Academy of Agriculture and Husbandry Animal Huhhot 010031, P. R. China*
*Email:xixianmei@sina.com*

Vegetable root-knot nematode has already become the major threat for greenhouse vegetable production in Chifeng city of Inner Mongolia, and caused serious yield losses on cucumber. The field experiment was conducted to exam the effect of five biological agents against the cucumber root-knot nematode in greenhouse during 2011-2012. Xianchongbike (living bacteria count ≥10 billions/g 3kg/hectare) and genwuxian (living bacteria count ≥0.02billions/g, 75 kg/hectare) were manually applied, and Genxiantong (Bacillus 3000×), Xianmie (Bacillus 90l/hectare) and 1.8%Avermectins EC (500×) were applied by drenching at the stage that the cucumber seedlings were transplanted into greenhouse. All the bioagents were re-applied once 10 days after the transplantation. Each treatment had 4 replicates, and the cucumber seedlings treated with water were used as the control. The results showed that all these bioagents could increase the cucumber production, raise the commercial rate and effectively controlled the cucumber root-knot nematode under the green-house conditions. Among them, Xianmie was the best one with a disease control efficiency up to 56.25%.

**P27.027 Systematic analysis of *Bursaphelenchus xylophilus* microRNAs from different infection stages of pine wilt disease**

*X.L. Ding, J.R. Ye, X.Q. Wu, L. Huang, L.H. Zhu and S.X. Lin*
*Institute of Forest Protection, College of Forest Resources and Environment, Nanjing Forestry University, Nanjing, 210037, P. R. China; Jiangsu Key Laboratory for Prevention and Management of Invasive Species, Nanjing, 210037, P. R. China*
*Email: njfu_jrye@163.com*

Pine wilt disease caused by *Bursaphelenchus xylophilus* is the most destructive pine disease in East Asia and Europe. We classified this disease into three infection stages: First stage (F): 15 days after infection. Middle stage (M): approximately 10 days later. Last stage (L): Another 10 days later. We also used *B. xylophilus* cultured on *Botrytis cinerea* served as a Control stage. Here, deep sequencing technique was employed to acquire comprehensive understanding of roles that miRNAs played during the pathogenic process of pine wilt disease. Four *B. xylophilus* miRNAs libraries of previously annotated stages were constructed and sequenced. After systematic bioinformatics analysis, we obtained 110 evolutionary conserved miRNAs, 10 miRNA metazoan homologs with typical hairpin structures and 325 novel miRNA candidates. 107 miRNA* sequences were also identified from the opposing arms of their corresponding mature miRNAs hairpin structure. Moreover, we also identified 28 miRNAs which might closely associate with pine wilt disease. Interestingly, most of the conserved miRNAs expressions reached their climax in the Middle stage of pine wilt disease. Target prediction and GO enrichment analysis revealed that most of the miRNAs involved in the regulation of hydrolysis activity which should be responsible for the degradation of pine cell walls and facilitate the feeding and migration of *B. xylophilus*. In addition, one putative conventional mirtron gene was able to identify from self-generated intron data together with five mirtron candidate. Our research provided the first profiling of *B. xylophilus* miRNAs during the different infection stages of Pine Wilt Disease and suggested that miRNAs might be key regulatory factors in this disease. These findings would lead to further exploration on the pathological and biological regulation of miRNAs *in B. Xylophilus*.

**P27.028 Effects of endophytic bacteria on the individual development and virulence of Pine Wood**

**Nematode *Bursaphelenchus xylophilus***

*X.Q. Wu, X.J. Tian, X. Fang, Y. Xiang, J.R. Ye, W.M. Yuan and X.F. Chu*
*Institute of Forest Protection, College of Forest Resources and Environment, Nanjing Forestry University, Nanjing, 210037, P. R. China; Jiangsu Key Laboratory for Prevention and Management of Invasive Species, Nanjing, 210037, P. R. China*
*Email: xqwu@njfu.edu.cn*

Pine wilt disease is a destructive disease caused by *Bursaphelenchus xylophilus*. Recently, the roles played by bacteria in pathogenesis have attracted much attention. But little is known about the interrelationship between *B. xylophilus* and their endophytic bacteria. In this research, several endophytic bacteria isolated from *B. xylophilus* strains, were chosen to study its effects on individual development and virulence of *B. xylophilus*. The results showed that the endophytic bacteria isolated from the highly virulent *B. xylophilus* could partly promote the development of the nematodes, while those from the low virulent *B. xylophilus* inhibit the development of nematodes; the period of embryo development is one of the key factors for the virulence of *B. xylophilus*; both bacteria-free and wild type *B. xylophilus* were pathogenetic to six-month-old microcuttings of *Pinus desiflora*. A mixture of bacteria, *Stenotrophomonas maltophilia* NSBx.14 and *Achromobacter xylosoxidans* ss. *xylosoxidans* NSBx.6, isolated from highly virulent *B. xylophilus* strain, were used for treatment of aseptic *B. xylophilus*, the virulence of *B. xylophilus* was promoted to be the level just lower than that of *B. xylophilus* wild type. Endophytic bacteria of highly virulent *B. xylophilus*, especially *S. maltophilia*, seemed to possess the ability to increase virulence of nematodes. The disease severity index of *P. desiflora* microcuttings was positively correlated to reproduction amount of *B. xylophilus* in host pine. This research suggests that the pathogenicity of *B. xylophilus* is not affected by loss of bacteria and that endophytic or epiphytic microbe might affect the development and virulence level of the *B. xylophilus*.

**P27.029 Identification of the root-knot nematode species on *Zanthoxylum bungeanum* and management with combinations of different biocontrol agents**

*X.Z. Wang , L.F. Wang, C.G. Piao, J.W. Ma, D.P. Gou and Y. Wang*
*Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry/Key Laboratory of Forest Protection of State Forestry Administration, Beijing, 100091, P. R. China*
*Email: nema@caf.ac.cn*

The seed of *Zanthoxylum bungeanum* Maxim. (Sichuan pepper), is well used as a kind of spice in China and the traditional Chinese medicine for its therapeutic properties. In recent years, a new disease threats the growth of *Z. bungeanum* in Aba Tibetan and Qiang Autonomous Prefecture (China's largest *Z. bungeanum* producing area). It may even kills *Z. bungeanum* when the disease is severe, causing severe decline in yield, and leading to great economic losses. A survey for plant-parasitic nematodes was conducted in this area from May to September 2011. Based on our results, the nematode species on *Z. bungeanum* was identified as *Meloidogyne hapla* Chitwood by the morphological, biochemical and molecular methods. To our knowledge, this is the first report of *M. hapla* on *Z. bungeanum* in China. In the meantime, the nematicidal effect of *Paecilomyces lilacinus* (Thom) Samson and *Pochonia chlamydosporia* Goddard singly or in combination was tested against *M. hapla* between 2011 and 2012 in this area. Under field conditions, all treatments reduced the disease severity and enhanced plant growth compared to untreated control while the spore concentration of fungi was $10^7$-$10^9$ spores per plant. All treatments not only caused the root galling index reduced by 68.7%-89.7%, but caused mortality of *M. hapla* as 72.0%-84.0% of juveniles in per cubic meter of soil after 120 days of exposures, compared to the control. Generally, the results indicate that *Paecilomyces lilacinus* and *Pochonia chlamydosporia* have a lethal effect on *M. hapla*, and could prove to be components in integrated root-knot nematode management in *Sichuan pepper* fields.

**P27.030 Gene cloning and analysis of expression pattern of auxin response factors (ARF) during the process of peed sites in cucumber infected by root-knot nematode**

*Y. Li[1,2], Z.C.Mao[2], J. Ling[2] and B.Y. Xie[2]*
*[1]College of Forestry, Beihua University, Jilin 132013, P. R. China; [2]Institute of Vegetables and Flowers, Chinese Academy of Agricultural Academy, Beijing 100081, P. R. China*
*Email: liying1019@126.com*

In order to reveal the regulatory mechanisms of auxin response factors (ARF) during the process of feed sites forming in cucumber infected by root knot nematode, the quantities, phylogenetic and expression pattern of ARF genes in root, stem, leaf, female flower, male flower, fruit, stem tip and tendril were analyzed by bioinformatics methods of HMMER, BLAST, PHYLIP and RT-PCR methods, based on the whose genome sequences of cucumber materials 9930. The result showed that 15 ARF genes in whole sequences of cucumber were classed into A, B and C. Three genes CuARF7/10/12 were expressed at some extent in eight organs of cucumber. Expression of others genes were rather changeable. This reflected that expression these genes possessed tissue specificity. The results obtained by QRT-PCR method demonstrated that genes of

CuARF7/10/12 were not expressed, expression of genes CuARF1/3/6/9/11/14/15 were not markedly change, the genes of CuARF2/4/8 were up-regulated expressed, the genes of CuARF5/13 were down regulated expressed at 7d, 14d and 21d of cucumber infected by root knot nematode. The genes of CuARF2/4/5/8/13 were all responds at some extent to infection by root knot nematode, but the peaks of genes were occurred in different times. The discrimination of time of gene expression was probably caused by the order of respond of genes to infection by root knot nematode.

**P27.031 Clone and VIGS effect analysis of lactate dehydrogenase gene in *Meloidogyne incognita***

*Y.H. Huang, M. Mei, Z.C. Mao and B.Y. Xie*
*Institute of Vegetables and Flowers, CAAS, No.12 Nandajie Zhongguancun, Haidian District, Beijing, 100081, P. R. China*
*Email: albertluke@126.com*

The aim of the present study was to clone and analyze the lactate dehydrogenase gene in *Meloidogyne incognita*, and identify the control effect of *MiLdh* gene silence on the diseases caused by *Meloidogyne* spp. In preliminary studies, we predicted the number of RNA interference (RNAi) phenotype genes of nematode in its genome using bioinformatics methods.In this study, we designed special primers based on the predicted *MiLdh* gene sequence and cloned MiLdh gene which was transferred into tomato plantletsusing virus-induced gene silence (VIGS) technique after analyzing the sequence characters to investigate the influence of the *MiLdh* gene silence on the amounts of root knot on the tomato plant inoculated with *M. incognita.*The results showed that the identity between the cloned *MiLdh* gene and the predicted *MiLdh* gene was as high as 94.9% in nucleotide sequence and 95.5% in the deduced amino acid sequence. Sixty days after inoculation with *M. incognita*, the amounts of root knot on tomato plantlets of which the *MiLdh* gene was silenced decreased by 48.6% compared to that of the empty vector control, by 48.4% compared to that of the water control. The study revealed the *MiLdh* gene silence had a good effect on prevention and control of root-knot nematode disease, also showed that these *MiLdh* genes may be involved in the pathogenesis of nematode, which provided new ideas and ways to the research of nematode pathology and nematode disease control.

**P27.032 Protoplast-mediated transformation of the endophytic fungus *Acremonium implicatum***

*Y.R. Yao, X.L Tian and B.Y. Xie*
*Institute of Vegetables and Flowers, CAAS, No.12 Nandajie Zhongguancun, Haidian District, Beijing, 100081, P. R. China*
*Email: yyr1012@126.com*

*Acremonium implicatum*, a strain of endophytic fungus separated from tomato, showed strong nematicidal activity against *Meloidogyne incognita* in vitro and pot experiments. To make full use of the nematicidal acvivity of the endophytic fungus *A. implicatum*, we developed an efficient protoplast-mediated transformation system for it, using green fluorescent protein (GFP) expression. We found that the protoplast-mediated transformation system significantly improved the rate of transformation. The transformants was grown in agar media for ten generations. In all cases, resistance to the selection pression (hygromycin B) was maintained. The pot experiments with the transformation revealed that the nematicidal activity against *Meloidogyne incognita* did not change. This technology will help conduct further study of the *A. implicatum.*

**P27.033 The secretory protein gene *NSP-1* of virulent *Meloidogyne incongnita* contribute to immunity suppression**

*Z.C. Mao, W.Y. Zhang, Y.S. Wang and B.Y. Xie*
*Institute of Vegetables and Flowers, CAAS, No.12 Nandajie Zhongguancun, Haidian District, Beijing, 100081, P. R. China*
*Email: maozhenchuan@caas.cn*

Virulent root-knot nematodes (*Meloidogyne* spp.) are able to complete their life cycle in resistance plant, and might lead to resistance break-up. The secretary proteins were important for the pathogenicity of nematodes. The comparative transcriptomes analysis of *Me3* virulent nematode near-isogenic lines (*Meloidogyne incongnita* ) and a real-time quantitative polymerase chain reaction revealed that the *NSP-1* was isolated and expressed in Me3 virulent nematode specially. *NSP-1* gene encodes a secreted protein, which includes a signal peptide and RXLR-like domain. The in situ hybridization showed that NSP-1was located in esophageal gland. With the overlap infiltrate of PVX expression model system on *Nicotiana benthamiana*, we found that after infiltration 7 d, the *BAX* induced hypersensitive (Necrosis), but on the overlap infiltration area of *NSP-1* and *BAX*, the leave was normal. It was showed that the *NSP-1* had a function to suppress PCD triggered by *BAX*, and could be a key candidate gene to root-knot nematode virulence adaptive evolution and effecter triggered immunity suppression.

**P27.034 Hsp90 gene expression of soybean under *Heterodera glycines* stress**

*F. Zhu[1], J.S. Chen[1, 2], X.F. Zhu[1], Y.Y. Wang[1], F. Wang[1], Q. Zuo[1], D. Wang[1], Y.X. Duan[1] and L. Chen[1]*
*[1]Nematology Institute of Northern China, College of Plant Protection, Shenyang Agricultural University, Shenyang 110866, P. R. China; [2]Daqing Branch，Heilongjiang Academy of Agricultural Science, Daqing*

*163316, P. R. China*
*Email: chenlijie0210@163.com*

The heat shock protein 90, Hsp90, has been identified and found to be highly conserved among different species. To study the gene expression induced by *Heterodera glycines* stress in Soybean, qRT-PCR experiment using gene-specific primers were carried out (forward, 5′-GTTGTGGATTCTCCTTGCTGTCT-3′, and reverse, 5′-AATCTCCATCGTCTTCTTGCTTG-3′). The *Gm-hsp90* gene of soybean was amplified from two varieties (susceptible cultivar Liaodou15 and resistant cultivar Huipizhi black coat soybean (ZDD2315) per 5 days until 35 days after inoculation by *H. glycines*. In these assays *Gm-hsp90* mRNA accumulation was detected in all soybean roots un-infected and infected by *H. glycines*. Furthermore, we observed that the altered mRNA levels of *Gm-hsp90* enhanced after nematode infection, and the mRNA abundance increased in both susceptible and resistant soybean roots. However, the *Gm-hsp90* expression levels were different in inoculation time by quantitatively measured, and the *Gm-hsp90* expression levels in resistant Huipizhi black coat soybean were higher than that in susceptible Liaodou 15 except 35 days post inoculation (dpi). The first peak of Huipizhi Heidou *hsp90* expression appeared in 10dpi, nearly 1.5 times compared to that of Liaodou 15. The second peak of that appeared in 30dpi, more 1.9 times than that of Liaodou 15. Meanwhile, the plant expression vector *pCAMBIA1302* containing *Gm-hsp90* gene was constructed. A subcellular localization study using GFP fusion protein indicated that *Gm-hsp90* is localized in the cytomembrane. The research revealed the resistant cultivars *Gm-hsp90* transcript more than susceptive soybean under nematode stress. And *Hsp90* participate in resistance response of soybean against to *H. glycines* at the molecular level.

### P27.035 Comparison of transcriptome pre- and post-parasitic stages of the nematode *Heterodera avenae*

*D. Yang, C.L. Chen, S.S. Liu, Q. Yang and H. Jian*
*Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: hengjian@cau.edu.cn*

As a worldwide plant pathogen, *Heterodera avenae*, is an obligate parasitein cereal crops.In China, the occurrence of *H. avenae* had distributed in 13 provincesand about 4 million hectares wheat fieldswere infested, and reducedthegrain yield in significant proportions. However, a lack of genomic information and less genes information in the public databaseshas hindered the comprehensive elucidation of the molecular mechanisms coordinating its parasitism and pathogenicity. Using 454 Flx+ pyrosequencing, we analyzed the transcriptome of *H. avenae* and collected totally 1,066,719 reads including 551,935 from the pre-parasitic stages and 514,784 from the post-parasitic stages. Thesewere assembled into 10,841 contigs with a mean length of 1,440 bp, among which 2892 contigs were differentially expressed between pre- and post-parasitic stages, and remained 71,401 singletons with an average length of 430 bp. Homology searches revealed that 59% of all contigs had significant matches with annotations to NCBI Nr database.In addition, 2855 (26%) and 1856 (17%) of those contigs were functionally classified using GO hierarchy and KEGG pathway respectively. The post-parasitic up-regulated genes mainly enrichedin thesome metabolism pathways such as Amino Acid Metabolism, Carbohydrate Metabolism, Lipid Metabolism and Biosynthesis of Other Secondary Metabolites, because life cycle of parasitic nematode turned into a more active phase after infection. We also identifiedsomeeffectors in pre-parasitic stages expressed highly such as mimicking plant annexin 4F01,plant cell wall degradation enzymes-GHF5 beta-1,4-endoglucanase, pectate lyaseand so on. Furthermore, a lists of new putative effectors were found and under investigation.

### P27.036 Temperature-manipulated development of novel cellular and mycelium stages of *Pasteuria penetrans*

*X.X. Bu, H. Jian, Z.X. Chen, X.Z. Liu and Q. Liu*
*Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: hengjian@cau.edu.cn*

The development of *Pasteuria. penetrans* in root-knot nematodes had been further studied using light and scanning electron microscopy. Some rod-like bacilli were observed when root-knot nematodes infected with *P. penetrans* were cultured for 12 days at 25℃/35℃ 10h dark/14 h light. Rod-like bacilli were 0.6-0.9 μm in length and about 0.6-0.9 μm in diameter and some of them accumulated to form cellular masses. Numerous of thalli had produced two days later and gathered around the metacopus or the intestine of nematode. At this time, mycelia like zingiber hitherto not documented were observed under scanning electron microscopy. The mycelia like zingiber should be long to vegetative thalli and they were still detected after 700 accumulated degree days. It suggested that vegetative growth and differentiation may simultaneously occur in most thalli. The development of thalli would be stopped if they were cultured at 16 ±1℃. This status can remain no less than 30 days. *P. penetrans* can recover to develop and produce mature endospores if they were transferred to normal condition. The development rate after transferred to the natural condition was still the same as the control culture at the same condition all along. Comparing the yield of spores developing from low temperature treated mycelia with the control, and the result indicated there was no significant different. But the sporulation of sporangia would

not be stopped, even if they were cultured at 16 ±1℃.

**P27.037 Influence to race of soybean cyst nematode by resistant cultivars**

*B.S. Yu[1], J.J. Wang[1], J.R. Li[1] and Y.M. An[2]*
*[1]Soybean Research Institute, Heilongjiang Academy of Agricultural Sciences; [2]Heilongjiang Academy of Agricultural Sciences, Harbin, 150086, P. R. China*
*Email:bsyu100@163.com*

The soybean cyst nematode (SCN, Heterodera glycines Ichinohe ) occurs in many soybean producing countries, and the soybean cyst nematode race 3 is most prevalent in Heilongjiang Province. An SCN race 3 population originally collected from a field in Heilongjiang Province was cultured in pots separately on ten SCN - resistant varieties ( Harbinxiaoheidou, Huipizhiheidou, Peking, Kangxian 1, Kangxian 2, Kangxian 3, Kangxian 4, Kangxian 5, Kangxian6,Fengdou3) for 10 generations in 2010 - 2012. The resulting populations were analyzed for their parasitic ability on the four race differential lines Pickett, Peking, PI88788, and PI 90763. The original SCN race 3 population changed to race 6 after 10 generations of continuous culture on Kangxian 1, Kangxian 2, Kangxian 3, Kangxian 4, and Kangxian 5 Kangxian6, Fengdou3; to race 10 on Huipizhiheidou; to race 14 on Peking; and to race 15 on Harbinxiaoheidou. The above- mentioned identification results indicated that new SCN races were generated from the original species after forcibly propagation on resistant varieties, which can even become susceptible, thus we conclude that rotation is an effective way to maintain the resistance stability of the resistant varieties in soybean production.

**N27.001 Application of microwave to control root knot nematode of watermelon in greenhouse**

*W.K. Huang, G.F. wang, J.K. Cui, L.A. Kong and D.L. Peng*
*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, P. R. China*
*Email: pengdeliang@caas.cn*

With the development of facility farming, the root-knot nematode (*Meloidogyne* spp., RKN) has been the most damaging agricultural pests attacking a wide range of crops in nursery systems. It can cause dramatic yield losses mainly in tropical and sub-tropical agriculture. In China, about 50% of vegetables in greenhouse were found to be infected by RKN, which caused over 400 $ million losses each year. Nematode management methods for these parasites include plant resistance, nematicides, biofumigation and crop rotation. However, highly effective nematicides such as fenamiphos, dibromide and methyl bromide are currently in the process of being canceled due to environment contamination and human toxicity considerations. Therefore, considerable research has been conducted to find replacements for these nematicides used in high value vegetable greenhouse. The use of microwave energy to control microbes in electronically heated foods is well documented. Use of radio-frequencies to control microorganisms in plant materials and soils has received less attention. Rahi1 and Rich (2008) showed that an exposure time of 45 seconds was the most efficient in yielding soil temperatures high enough to kill plant-parasitic nematodes. Irradiation of soil infested with *Rotylenchulus reniform* nematodes for 45 seconds resulted in a 99% extermination of the organisms in all treatments. Barker et al.(1972) found that a 5 minutes exposure was needed to kill eggs of *Heterodera glycines*, and mortality was greatly affected by both soil moisture content and soil. O'Bannon and Good (1971) showed that microwaves could control root-knot nematodes (*Meloidogyne* spp.) in small samples of soil when a temperature of 72℃ was achieved. Heald et al. (1974) reported that nematodes were controlled in a fine sandy loam soil infested with the reniform nematodes at depths of 5 cm. Results of these studies can serve as a foundation for later field trials and be valueable to thousands of greenhouse growers and operators who could employ microwaves for sterilizing soil. To get a safety, economic and effective control measure in nursery system in Beijing, an application of microwave energy was finished before planting in Daxing in 2012. Nematode number in soil, root length of watermelon seedlings and root gall index of plants were counted after treatment with 2500 MHz frequency power. Result showed that nematode number in soil was decreased by 85.4% and 50.1%, respectively, at 10 days and 30 days after treatment. The root length of seedlings was 30 cm longer than the untreated control at one month after planting. A significantly root gall index decrease was also observed in microwave treatment compared with the untreated control. Therefore, treatment with microwave energy before planting was effective in controlling root knot nematode in nursery systems. Better technology and economic benefits should be evaluated in further research.

**N27.002 Effect of abamectin on cereal cyst nematode of wheat**

*T. Wang, W. Zhao, W.K. Huang, D.L. Peng and R.D. Qi*
*Institute of Plant Protection and Agro-products Safety, Anhui Academy of Agricultural Sciences, No.40 Nongkenan Road, Hefei, 230031 Anhui, P. R. China*
*Email: rende7@126.com*

Cereal cyst nematode (CCN) is globally and economically important in wheat production systems, and distributed widely in main wheat growing areas in China. In order to investigate the effect of Abamectin against CCN and its application method, the plot trials were

conducted by soil treatment, seed processing and root irrigation with Abamectin emulsifiable concentrate (EC), controlled releasing Abamectin capsule suspension (CS) and Abamectin granules (GR), respectively. Three groups of results were obtained as follows: (i) the control efficacies of Abamectin against CCN by root irrigation were 22.3% and 31.4% with Abamectin EC (225 g a.i./ha) and Abamectin CS (225 g a.i./ha) before overwintering, respectively, and 73.6% and 70.4% during regreening stage of wheat, respectively; (ii) the control efficacies against CCN were 21.8%, 16.6% and 27.8% by soil treatment with Abamectin G, Abamectin EC and Abamectin CS before sowing, respectively; (iii) the efficacies against CCN were 23.4% and 52.4% by seed dressing with Abamectin EC and Abamectin CS at the ratio of 2% of seed weight, respectively. The results suggested that Abamectin was an available nematicide to control CCN, and taken together, seed processing with the controlled releasing Abamectin CS must be the most effective method considering the ease of operation and efficacy based on our study.

### N27.003 Identification of cyst-forming nematodes (Nematoda: Heteroderidae) from China based on morphology and rDNA

*D. Wang, L.J. Chen, X.F. Zhu, Y.Y. Wang, F. Zhu and Y.X. Duan*
*Nematology Institute of Northern China, College Plant Protection, Shenyang Agricultural University, Shenyang, 110866, P. R. China*
*Email: duanyx6407@163.com*

The cyst-forming nematodes are a group of important plant pathogens worldwide. We collected and identified eight species of the two genus *Heterodra* and *Cactodera* of cyst-forming nematodes from 2010 to 2012 in China. The species included *H.avenae, H. filipjevi, H. glycines, H. elachista, H. ripae, Cactodera cacti, C. eremica* and *C. estonica*, which were detected and identified by comparative morphological and morphometric and molecular approaches in our research. Molecular approaches based on D2D3 of the 28S rDNA and rDNA-ITS were applied to study molecular characterization and phylogenetic relationships. The restriction fragment length polymorphism profiles of eight species were obtained with 10 restriction enzymes (*Alu*I, *Ava*I, *Bsh1236*I, *Cfo*I, *EcoR*I, *Hinf*I, *Msp*I, *Pst*I, *Rsa*I, *Taq*I) after digestion of PCR products of ITS- rDNA regions. Among these species, *H. ripae* and *C. eremica* were new records in China.

### N27.004 The test on the dormancy stages in newly formed brown cysts of *Heterodera avenae*

*H.H. Zhao[1], H.Y. Ding[1], C. Liang[1] and D.L. Peng[2]*
*[1]College of Agronomy and Plant Protection, Qingdao Agricultural University, No.700 Changcheng Road, Chengyang District, Qingdao 266109, P. R. China;*
*[2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, CAAS, No. 2 West Yuanming Yuan Road, Haidian District, Beijing, 100193, P. R. China*
*Email: hhzhao@qau.edu.cn*

*Heterodera avenae* (CCN) is the most important nematodes damaging wheat in China. It was numerously reported that juvenile emergence from eggs stopped in newly formed brown cysts and the break of dormancy required low temperature treatment for a relative long time. Free juveniles were also found out of the egg shell in newly formed brown cysts with different juvenile-egg ratio during our CCN investigations. The test was implemented to determine the activity of free juveniles and eggs in newly formed brown cysts. Free juveniles and eggs were isolated under room temperature (22-30℃), afterwards some of them were inoculated onto wheat seedlings immediately and some were treated at low temperature (5℃) for different length of time before the inoculation. Wheat seedlings were earlier planted in paper cups, and incubated under 15-16℃ in light incubator after each inoculation. Wheat roots were taken out and stained to detect infection situations after 3-4 weeks of incubation. It was found that no infection occurred for both juveniles and eggs without low temperature treatment; infection occurred only for the juveniles which had been treated with low temperature for 20, 30 and 45 days, and for the eggs treated with low temperature for 10, 20, 30 and 45 days. The results indicated that dormancy happened on the juveniles as well as eggs in newly formed brown cysts and the encysted juveniles required low temperature treatment to activate the ability of infection or even emergence from cysts.

### N27.005 Morphological and rDNA diversity of *Ditylenchus destructor* in China

*C.L. Xu, H. Xie, K.M. Li and J. Zuo*
*Laboratory of Plant Nematology, College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, P. R. China*
*Email: xiehui@scau.edu.cn*

*Ditylenchus destructor* seriously affects the yield of potato (*Solanum truberosum*), peanut (*Arachis hypogaea*) and other economic plants at abroad. In recent years, this nematode has been a destructive pathogen on sweet potato (*Ipomoea batutas*) and some medical plants in China. It was reported that the parasitism, pathogenicity and rDNA-ITS sequences of *D. destructor* in China showed diversity with what reported at abroad. In order to find out more information about *D. destructor* in China, 28 populations of *D. destructor* from 4 different hosts, including 25 populations from different varieties of sweet potato, one from *Astragalas mongholicu*, one from *Dioscorea opposite* and one from *Anthurium rzerianum*, collected from 12 provinces (city or auto-

nomic region) in China, were studied based on morphological identification and rDNA-ITS and 18S gene sequences analysis. The results showed that there were no big differences within these populations, except some populations showed more diversity in the types of oesophagus overlapping intestine, and one populations showed only one tail type. All the populations were separated into 3 types according to the divergence of ITS sequences. Type Ⅰ had 100% similarity with submitted sequence of *D. destructor* (GenBank Accession No. AY987007), type Ⅱ had 97.2% similarity with AY987007, and type Ⅲ was characterized by losing 188bp in the ITS1 region compared to AY987007 and similar to those submitted sequences of *D. destructor* from China. The alignment results of 18S gene sequences demonstrated similar results to the divergence of ITS sequences.

## Concurrent Session 28-New Careers and Roles for Plant Pathologists

### O28.001 Plant pathologists in the international development organizations in the XXI century

*W. El Khoury*
*Policy and Technical Advisory Division, International Fund for Agricultural Development (IFAD), Rome, Italy*
*Email: w.elkhoury@ifad.org*

With the expected increase of the world population to over 9 billion by 2050, agricultural production has to increase up to 70 % to feed the world. Despite the progress in increasing production and food availability, around one billion people still suffer from chronic hunger. Reduction in losses caused by pests and diseases at pre- and postharvest stages is crucial to achieve the needed increase in food. Crop losses due to plant diseases vary greatly between countries, crops and years, and there are no systematic assessments of these losses in most developing countries where losses are highest and where they incur large and direct impact on food security and farmers' income. Several international development organizations, including UN agencies, international agricultural research centers, and international NGOs concerned with agriculture are working more through a holistic approach in their programmes addressing food security, nutrition, environmental sustainability and enhanced the resilience of the farming systems to the increasing climatic, environmental, economic, and social shocks. The focus of these programmes is on the most vulnerable in terms of poverty, gender and youth, fragile ecosystems and regions where effects of climate change are expected to be highest. Plant pathologists play a critical role in this global agricultural development agenda, especially in priority areas of sustainable crop intensification, including integrated pest and disease management, soil health, and ecosystem services, postharvest losses, safety including mycotoxins and pesticide residues in food and water, resistant breeding and transboundary diseases and epidemics as affected by international trade, human movement and climate change.

### O28.002 Microbial forensics: new applications and opportunities for plant pathologists

*J. Fletcher*
*National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK 74078, U.S.A.*
*Email: jacqueline.fletcher@okstate.edu*

Crops, forests and rangelands are vulnerable to direct targeting by individuals, groups or states intending to cause harm. Preparedness requires comprehensive national security plans that include robust capacity for microbial forensic investigation and criminal attribution. Elements such as characterization of a specific microbe and how it was produced and introduced support the goal of linking the crime to its perpetrator(s). Over the past decade in the U.S., targeted enhancement of capabilities in microbial forensics have included the emerging discipline of plant pathogen forensics. The latter effort is hampered by the lack of key personnel trained in applied plant pathology, diagnostics and forensic science. More knowledgeable field and laboratory personnel are needed to achieve effective plant biosecurity systems and, in the case of a criminal event, to respond quickly as consistent with the needs of a forensic investigation. An Oklahoma State University targeted graduate education program in forensic plant pathology blends coursework and research in both plant pathology and forensic sciences, and includes summer internships in key forensic or homeland security laboratories and companies to provide real-world experience and opportunities for networking. Program graduates are employed in a variety of security-related positions in government, higher education and industry. Resources targeted to applied plant pathology graduate programs and training programs for other key responders are critically needed.

### O28.003 Plant pathologists need interdisciplinary expertizes for life-long employability

*P.J.G.M. de Wit*
*Laboratory of Phytopathology, Wageningen University, P.O. Box 6708 PB, Wageningen, The Netherlands*
*Email: pierre.dewit@wur.nl*

There is an increasing demand for arable and vegetable crops to feed the increasing world population. High yielding crops and vegetables are prone to new disease outbreaks as breeding for both high yield and disease resistance is often not compatible because there is a fitness cost to disease resistance. There might be physical, physiological, biochemical or metabolic trade-offs of successful defense against pathogens. Especially protection against post-harvest and soil-borne diseases is particularly difficult to achieve not only by chemical treatment but also by disease resistance breeding and biocontrol agents.

New disease outbreaks require joint actions by multidisciplinary trained plant pathologists to come up with sustainable solutions. MSc and PhD students need to be trained in all facets of biology with not only expertise in classical plant pathology, but also in diagnostics, genetics, plant breeding, molecular biology, genomics and bioinformatics. Multidisciplinary trained MSc and PhD students are required in academia governmental jobs and private industry to prevent or cope with new disease outbreaks. In Msc and PhD education we need to teach all these expertizes. I will give an overview of the jobs that were taken by MSc and PhD students who were trained in different aspects of plant pathology during the

last two decades at Wageningen University and affiliated academic institutions.

**O28.004 Research for action: from research to business**

*M.L. Gullino[1, 2, 3], M. Pugliese[1, 2, 3] and A. Garibaldi[1, 3]*
*[1]Agroinnova – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy; [2]Disafa – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy; [3]AgriNewTech srl, Torino, Italy*
*Email: marialodovica.gullino@unito.it*

University spin-offs are companies founded by research staff members to exploit technological inventions developed at Academic level. A spin-off company, AgriNewTech, started in Italy with the aim to transfer experiences to growers and composting plants related to the capacity of compost to suppress some important diseases of vegetables and flowers. The company provides innovative services for increasing the quality of compost, in particular for its use for controlling soil-borne plant pathogens in the agricultural sector, based on the enrichment with selected microorganisms. Business is oriented to new fertilizers and substrates, based on high-quality compost and microorganism isolated from composts, able to prevent plant disease and reduce the use of chemicals. The technology is strategically important as an alternative to Methyl Bromide (Montreal Protocol) for controlling soil-borne pathogens. Main markets are composting industry and the agricultural sector. Main customers are SMEs producing organic wastes, composting plants and farms. Spin-off companies must be considered an interesting option for new careers for plant pathologists.

## Concurrent Session 29-Plant Diseases and Control in Protected Cultivation

### O29.001 Alternative strategies for the control of soil-borne pathogens of vegetable crops in green house

*M. Pugliese[1,2], G. Gilardi[1], P. Colla[1], M.L. Gullino[1,2] and A. Garibaldi[1]*
*[1]Agroinnova – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy; [2]Disafa – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*
*Email: massimo.pugliese@unito.it*

The European directive 2009/128/EC on sustainable use of pesticides and the Regulation 1107/2009 on Plant Protection products (replacing the Dir. 91/414/EEC) provide a strong limitation to the use of chemicals, in particular fumigants, in Europe. The agricultural sector is encouraged to innovate and change strategies for crop protection, with the need to revisit the methods of management to satisfy the requirement of environmental sustainability, by the adoption of integrated systems that combine, in a rational way, chemical, physical, genetic, cultural and biological control strategies. Some examples of the experiences carried out in Italy are reported. Trials have been carried out to evaluate the effect of chemical and not chemical disease management strategies to control soil-borne pathogens of solanaceous (pepper and tomato), cucurbit (melon, zucchini and cucumber) and leafy vegetables (lettuce, rocket) crops in Piedmont (Northern Italy). Alternative strategies included the use of suppressive substrates and fertilizers (compost, biochar…), soil solarization, biofumigation, grafting, biological control agents, as well as silicates and management of electrical conductivity in soil-less crops. Different strategies are critically discussed, and new trends for managing soil-borne diseases are suggested.

### O29.002 Integrated management of replanting diseases of vegetables in greenhouse in North China

*S.D. Li, Z.Q. Miao, R.J. Guo and M.H. Sun*
*Key Laboratory of Pest Management in Crops, Ministry of Agriculture, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China*
*Email: lisd@ieda.org.cn*

Replanting of cucumber resulted in deterioration of soil microflora, decline of soil fertility, and serious occurrence of soilborne diseases caused by *Fusarium* spp., *Rhizoctonia solani*, and *Meloidogyne incognita*. We developed an integrated management of the problems to re-establish beneficial microflora of the continuous cropping soil. Three delivery systems of BCAs of *Bacillus subtilis*, *Gliocladium roseum* and *Paecilomyces lilacinus* are developed. The first way is to sprinkle BCAs on corn stalk and buried under soil. The treatment could promote plant growth, increase the soil temperature at 20 cm depth by 5-8℃ in winter, reduce disease incidence and enhance the yield by 30%, in comparison with "no treatment". The 2nd way to introduce BCAs into vegetable rhizosphere is to mix them into nursery substrate. Concentrations at $10^7$ and $10^5$ cfu/g matrix for bacterial and fungal BCAs, respectively, could promote seedling growth and suppress root diseases significantly, and reduce *F. oxysporum* population for 10 times in comparison with the "no treatment", when it was detected by real-time PCR. Wilt disease delayed for 2 weeks, and the control efficacy was 58.6% after transplanting for 5 weeks. The 3rd way is to mix the BCAs into organic manures. Treatment with fugal and bacterial BCA at $2.5\times10^5$ cfu/g and $1\times10^7$ cfu/g, respectively, could increase height, stem width and yield of cucumber by 10%, 13% and 19%, respectively, in comparison with the "no treatment". The research suggests a great potential for integrated management of vegetable replanting problems in regions of North China with cold weather.

### O29.003 Molecular surveillance and systematic management of bacterial canker in greenhouse tomatoes

*S.A. Miller, M.L. Lewis Ivey, X. Xu, M. Bledsoe and G. Hoyos*
*Department of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH USA 44691; Village Farms International, Inc., Heathrow, FL USA; EnviroLogix Inc. Portland, ME, USA*
*Email: miller.769@osu.edu*

Bacterial canker is one of the most economically damaging diseases of greenhouse tomatoes and outbreaks can be devastating. *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) is seedborne and easily spread mechanically. Molecular fingerprinting tools that exploit Cmm genetic diversity offer the ability to trace strains within production systems. We designed a multivariate matrix using geographical information, propagation and production flow diagrams and varietal and seed source data superimposed with rep-PCR fingerprints and dnaA sequence analysis of Cmm strains. The multivariate matrix allows Cmm phenotypic and genotypic information to be recorded and transmitted at any point in a production system and the point of origin of each strain can be identified. We used the DELPHI process with a panel of experts to identify the most critical points of entry and dissemination of Cmm in tomato propagation and production greenhouses. These include irrigation water, Cmm-infected seedlings (propagation), tools, gloves, footwear, equipment, weeds and volunteer plants, and crop debris. Sampling strategies were designed and implemented to determine the relevance of each of these

sources within the production system. Cmm was detected using culturing on semi-selective media, endpoint and quantitative PCR, and a commercially available isothermal DNA amplification kit (DNAble® LFD), alone or combined with an immunomagnetic separation assay. Identification of major risk factors for outbreaks of bacterial canker in greenhouse tomatoes will allow the development and implementation of effective system-wide management strategies.

### O29.004 Root zone habitat management in closed hydroponic systems for control of *Oomycete* pathogens

*B.W. Alsanius*[1], *A.K. Rosberg*[1], *S. Khalil*[1], *M. Hultberg*[1] *and W. Wohanka*[2]
[1]*Swedish University of Agricultural Sciences, Dept of Biosystems and Technology, PO Box 103, SE-230 53 Alnarp, Sweden;* [2]*Geisenheim University, Dept of Phytomedicine, von Lade Str 1, DE-65366 Geisenheim, Germany*
*Email: beatrix.alsanius@slu.se*

Protection of surface and ground water as well as coastal areas is well established in Swedish and European legislation. The degree of production intensity in both out- and indoor horticultural production systems contributes to nutrient leakage and/or release, and thereby to eutrophication. However, closed systems encourage the propagation and dispersal of oomycetes. As few fungicides are registered under Swedish conditions for controlling root diseases in crops with continuous harvesting, management of abiotic factors to reinforce antagonistic mechanisms associated the ambient microbiota associated to the root and growing medium (root zone habitat management) and/or by introduced biocontrol agents might be an attractive strategy. Antagonistic interactions towards *Pythium aphanidermatum* using peat as a growing medium indicated a strong interaction between disease suppressiveness and utilization of specific organic carbon, nitrogen, phosphorous and sulfur sources. The nitrogen source and concentration also affected microbial metabolite formation, such as 2,4 diacetyl phloroglucinol and biosurfactants. A reduced water content in the growing medium decreased root attack by *P. ltimum* and promoted the impact of *Trichoderma* based biocontrol agents. Increased electrical conductivity as well as micronutrient supply favored formation of lytic enzyme activity in the presence of *Trichoderma* based biocontrol agents.

### O29.005 *Pepino mosaic virus* genotype shift in North America and rapid genotype identification using loop-mediated isothermal amplification

*K.S. Ling*[1], *R. Li*[1] *and M. Bledsoe*[2]
[1]*U.S. Department of Agriculture-Agricultural Research Service, U.S. Vegetable Laboratory, Charleston, SC, USA;* [2]*Village Farms, Heathrow, FL, USA*
*Email: kai.ling@ars.usda.gov*

Pepino mosaic, once an emerging disease a decade ago, has become endemic on greenhouse tomatoes worldwide in recent years. Three distinct genotypes of *Pepino mosaic virus* (PepMV), including EU, US1 and CH2 have been recognized. Our earlier study in 2006-2007 demonstrated a predominant EU genotype in Canada and United States. The objective of the present study was to monitor the dynamic of PepMV genetic composition and its current status in North America. Through yearly monitoring during 2009-2012, we detected a dramatic shift in the prevalent genotype of PepMV from EU to CH2 in North America since early 2010 and an additional shift from CH2 to US1 in Mexico only two years later. We demonstrated that for detection of PepMV-infested commercial tomato seeds a previously developed real-time RT-PCR was more sensitive than traditional ELISA. According to coat protein gene, PepMV variants with identical or similar genomic sequence were identified using RT-PCR from commercial tomato seed sources used for scion or rootstock. A reverse transcription loop-mediated isothermal amplification (RT-LAMP) system was developed and shown to achieve rapid genotype identification for the respective genotypes (EU, US1 and CH2). Through systemic monitoring and genetic diversity analysis, we demonstrated that PepMV genotype shift in North America likely resulted from the introduction of contaminated tomato seed lots. Therefore, it is recommended that seed health test should be conducted using more sensitive RT-PCR or real-time RT-PCR to complement ELISA, and if necessary, using RT-LAMP to determine the specific genotype.

### O29.006 The screen of nematocide from biomass and pesticide for the synergistic effect

*J.Y. Qiu, T. Liu, W.C. Liu and J. Ma*
*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, P. R. China*
*Email: qiujiyan@126.com*

The feasibility of using mini-dose nematocide to control the tomato root knot nematode (*Meloidogyne incognita*) in protected cultivation by nematocide treated with biocontrol agents were screened and the best effective control combination, which including both nematocide of abamectin granules, lythidathion granules and plant-dregs such as sesame dregs, soybean dregs, EM broth, chicken manure, wheat bran, making different treat-combination to screen out the best combination and the efficiency of root knot nematode (*M. incognita*), by pot treated with mixing egg masses soil and different treat-combination at the time of tomato seedling planting. The result showed the treatment of 10% lythidathion granules

plus sesame dregs have higher efficiency for control of root knot nematode (96%), plus soybean dregs the efficiency were 89%, plus wheat bran the efficiency were over 90% (inhibited growth), plus EM broth the efficiency were around 60% and mini-dose lythidathion were up to 30%. The 0.5% abamectin granules plus sesame dregs have higher efficiency for control of root knot nematode (83%), plus chicken manure the control efficiency were 67%, plus soybean dregs the efficiency were 77%, plus wheat bran the efficiency were inhibited growth problem, plus EM broth the efficiency were 69% and mini-dose abamectin were up to 10%.The control efficiency in all of co-treats were higher than the single treatment and showed the synergistic effect from using mini-dose nematocide with plant-dregs.

**O29.007 Biochar increases beneficial and non-beneficial soil microbes including *Rhizoctonia solani,* the causal agent of seedling damping-off**

*T. Copley and S. Jabaji*
*McGill University Macdonald Campus, Plant Science Department, Raymond Building, 21 111 Lakeshore, Ste-Anne-de-Bellevue, Quebec, Canada H9X 3V9*
*Email: tanya.copley@mail.mcgill.ca*

Biochar is known to increase beneficial soil properties such as nutrient and water retention, as well as plant growth promoting microorganisms. Several studies have shown that soil-applied biochar induces systemic resistance to foliar pathogens. To date, however, no studies have looked at the effect of biochar on soilborne pathogens especially those that cause seedling damping-off. The following study is aimed to understand the effect of maple bark biochar amendments applied at 0%, 1%, 3% and 5% (w/w) in soilless potting mix on: 1- bulk microbial populations, and 2- *Rhizoctonia solani* AG-4 A76 growth and damping off disease incidence in eleven seedlings, including pepper, tomato, cucumber, soybean alfalfa, pea, radish, leek, lettuce, carrot and sugarbeet. Increasing levels of biochar in potting mix were found to significantly increase the colony-forming units (CFUs) of taxonomically different microbial populations per gram of potting mix and also the DNA copy numbers of *R. solani* using qPCR. Interestingly, *in vitro* biochar amendment in agar increased *R. solani* growth rates and caused alterations in *R. solani* metabolite compositions as determined by GC-MS. Seedlings grown in the presence 3% and 5% of biochar were more susceptible to post-emergence *Rhizoctonia* rot. To the best of our knowledge, the results of this study are the first to show that disease incident of soilborne pathogens, particularly *R. solani*, increased as a result of biochar application. Caution should be exercised when applying biochar to pathogen prone potting mix.

**O29.008 Efficacy of phosphate-mobilizing bacteria to control plant pathogens in tomato when applied with an animal bone charcoal formulation**

*J. Postma[1], E.H. Nijhuis[1] and E. Someus[2]*
*[1]Plant Research International, P.O.Box 69, 6700 AB Wageningen, The Netherlands; [2]Terra Humana Ltd., Széchenyi u. 59, H-1222 Budapest, Hungary*
*Email: joeke.postma@wur.nl*

The aim of this research is recycling of high phosphorus containing organic waste from food industry (bone meal) into a safe biotechnological crop protection and plant fertilizer product. Carbonization of animal bone meal generates a porous product, which is suitable for microbial colonization. This animal bone charcoal (ABC) can be used as microbiological carrier for biological control agents or other beneficial micro-organisms, meanwhile delivering P for plant growth. Several naturally occurring soil bacteria with antagonistic properties were able to solubilise phosphate: e.g. strains of *Burkholderia, Pseudomonas, Serratia, Bacillus, Paenibacillus, Arthrobacter,* and *Streptomyces*. These bacteria were further tested for their capability to colonize the bone char with an additional carbon source, and to survive in the dried product. Few promising bacterial isolates were further tested for biocontrol efficacy in plant assays in the greenhouse. Tests were performed with young tomato plants in potting soil and rockwool, which were infested with *Pythium aphanidermatum* and *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) causing respectively damping off and crown and root rot. Scanning electron microscopy (SEM) pictures showed the intensive colonization of the bacteria in the interior of ABC. Of the tested strains, *Pseudomonas chlororaphis* 4.4.1 was most effective in controlling the diseases; it controlled *P. aphanidermatum* and FORL in tomato in each of the tests. Meanwhile, the strain appeared to be a very good root colonizer; 1-8% of the cultural bacteria on the roots or in rhizosphere soil consisted of the introduced strain.

**P29.001 The use of silicates recycled from photovoltaic industries for controlling powdery mildew of cucurbit crops**

*M. Pugliese[1,2], E. Cogliati[1], M.L. Gullino[1,2] and A. Garibaldi[1]*
*[1]Agroinnova–University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy; [2]Disafa–University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*
*Email: massimo.pugliese@unito.it*

Silicon is the second most abundant element on earth's surface and its use can stimulate natural defense mechanisms in plants. The effect of silicates recycled from photovoltaic industries applied against powdery mildew on zucchini (*Cucurbita pepo)* and on cucumber (*Cucumis sativus*) was evaluated under greenhouse conditions by foliar and root application. Potted plants were

inoculated with a spore suspension containing $1\times10^5$ cfu/ml. In the first case, the following treatments were carried out, 3 and 10 days after pathogen inoculation: propiconazole (15 ml/hl); *Bacillus subtilis* (250 g/hl); 1% and 0.1% recycled sodium silicate; tap water as control. In the second case, recycled sodium silicate was added at the concentration of 100, 200 and 400 mg/l to nutrient solutions of cucumber plants grown soilless. Disease incidence and severity were assessed 7, 14 and 21 days after pathogen inoculation. The application of 1% sodium silicate significantly reduced powdery mildew on zucchini and increased above-ground biomass of plants at a level similar to chemical control. The other treatments, including *B. subtilis*, reduced disease severity compared to water control, but were less efficient. In soilless crops, the application of at least 200 mg/l of silicates to the nutrient solutions significantly reduced the disease. The use of recycled silicates from photovoltaic industries is a valid alternative for the control of powdery mildew. However, silicates might not be sufficient at high disease incidence levels, and their use is more suitable within an integrated disease control strategy.

**P29.002 Characterization of major resistance genes to *Leptosphaeria maculans* in Canadian *Brassica napus* accessions**

*X. Zhang, W.G. Dilantha Fernando, G. Peng and H.R. Kutcher*
*Department of Plant Science, University of Manitoba, 222 Agriculture Building, 66 Dafoe Road, Winnipeg, MB R3T 2N2, Canada*
*Email: zhangx39@cc.umanitoba.ca*

Blackleg, caused by the fungal pathogen *Leptosphaeria maculans* is one of the major diseases of canola (*Brassica napus*, oilseed rape) worldwide. The disease was mainly controlled by cultivation of resistant cultivars and adequate crop rotation. Breakdown of resistance genes by virulent isolates has been observed throughout the Canadian Prairies in recent years. Until now there has been no systematic study done to investigate the R-genes in Canadian canola cultivars. In this study, a set of 22 *L. maculans* isolates with well-characterized avirulence alleles were employed to characterize R-genes of 100 Canadian *Brassica napus* accessions (including commercial cultivars and advanced breeding lines). Either known R-genes or unknown R-genes were identified in 84 cultivars/lines. Six known R-genes (*Rlm1, Rlm2, Rlm3, Rlm5, Rlm9, Rlm10*) were identified in 68 cultivars/lines, and 16 cultivars carried unknown R-genes. However, 16 cultivars/lines carry none of the R-genes that were assessed in this study. Within six detected R-genes, *Rlm3* was identified in 65 accessions, followed by *Rlm2* in 14 accessions, and *Rlm1* in 8 accessions, whereas other R-genes were rarely detected. Accessions carry R-genes can be divided into five groups based on their R-gene components: *Rlm1* resistance, *Rlm2* resistance, *Rlm3* resistance, other known R-gene resistance and unknown R-gene resistance. The results highlighted that rotations of cultivars with different R-genes are possible in the Canadian Prairies.

**P29.003 Effects of soil inoculum on the occurrence of rice bakanae disease in paddy fields**

*C.C. Hsu, M.H. Lai, J.W. Huang and C.Y. Chen*
*Department of Plant Pathology, National Chung-Hsing University, Taichung, 40227, Taiwan, China*
*Email: helianthus02@gmail.com*

Rice bakanae disease, caused by *Fusarium fujikuroi*, is prevalent on rice-growing regions. Symptoms occur on all the growing stages of rice, including excessive growth of stem, thin and chlorotic leaves, and progressive drying from below, which eventually leads to death of plants. This disease is monocyclic and infection is through roots. It has long been considered to be seed-borne. However provided that the infection is initiated from seeds or seedlings, most affected rice seedlings shall die before or soon after transplanting from seedbeds into paddy fields. With this respect, the disease occurring on adult rice plants in paddy fields is so prevalent that the phenomenon cannot simply be explained by the pathogen being passed from seedlings surviving from infection to adult plants. Consequently it is believed that soil inoculum in paddy fields may play more important role on the occurrence of bakanae disease than what we have thought before. Diseased rice plants appearing in paddy fields after transplanting may largely arise from inoculum in soil. The aim of this study is to determine the role of soil inoculum in this disease, and whether the inoculum concentrations affect the patterns of symptoms and timing of appearance of symptoms. Seedlings of 21 day-old were transplanted into artificially infected soil compounded with different inoculum concentrations. The results revealed that soil inoculum can be the source of infection in the paddy fields with the higher inoculum concentration causing the higher disease incidence and earlier appearance of symptoms.

**P29.004 Pathogenicity and environmental spore load f *Fusarium* species causing internal fruit rot in bell pepper**

*K. Van Poucke, L. Van Herck, C. Sauviller, M. Frans, R. Aerts and K. Heungens*
*Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit, Burg. Van Gansberghelaan 96/2, 9820 Merelbeke, Belgium*
*Email: kris.vanpoucke@ilvo.vlaanderen.be*

Internal fruit rot of bell pepper is mainly caused by members of the *Fusarium lactis*species complex (FLASC), which contains multiple sequence types (STs). Less important causal agents are *F. proliferatum* and *F.*

*oxysporum.* The relative pathogenicity of the FLASC STs and the other *Fusarium* species on a series of cultivars was tested via inoculation of pin-wounded fruit or via inoculation of the flowers, the natural infection pathway. *F. oxysporum* produced the largest lesions on inoculated fruit; in general, yellow pepper cultivars were most susceptible. The presence of *F.* inside fruit of flower-inoculated plants was evaluated after two weeks and at harvest. All *Fusarium* species were equally able to infect via flowers, but infection incidence was dependent on the cultivar. To determine the spore load of FLASC STs, *F. oxysporum* and *F. proliferatum*, we developed specific real-time PCR assays and applied them to samples taken with a Burkard volumetric spore sampler during the 2012 growing season. In general, spores were present in high numbers during spring and became less abundant later in the year. FLASC spores were the most numerous, explaining the higher incidence of this species in infected fruit. The real-time PCR detection technique was also used on surface samples. Horizontal surfaces usually contained large numbers of spores. Also, samples of organic residue that had dropped onto the floor were tested. In many cases, dried-up aborted fruits harboured very large numbers of FLASC spores, indicating that they might be an important source of inoculum.

**P29.005 Development of management methods of major disease on black raspberry (*Rubus coreanus*)**

*J.H. Kim[1], J. Kim[1], S.S. Cheong[1], Y.R. Kwon[1] and W. Hyu[2]*
*[1]Jeollabuk-do Agricultural Research and Extension Services, Iksan 570-704, Republic of Korea; [2]Faculty Biological Resources Science, Chonbuk National University, Jeonju 561-756, Republic of Korea*
*Email: kimjuhee@korea.kr*

This experiment was carried out to analyze the cause of withering death and disease occurrence. Increased with the age of the plants was an increased incidence of late blight. leaf spot and anthracnose that occurs in the leaves increased rapidly in the more than three years old trees. Anthracnose by *Colletotrichum* spp., and fusarium wilt by *Fusarium* spp. were isolated from dead aboveground plant and *Phytophthora* spp. and fusarium wilt by *Fusarium* spp. in the underground parts of the plants were isolated. By mulching cultivation resulting in field, anthracnose and leaf spot occurrence was decreased 54.6-75.6% by muching cultivation in field, anthracnose and Phytophthora blight occurrence was decreased 54.6%. Weed stop, black vinyl, rice straw and free mulching cultivation was sequentially effective for disease control. As the cumulative rainfall increases, disease occurrence of black raspberry in field by sprayed fertilizer around the roots and boundary soil surface was increased. Wilt and death by occurrence of Phytophthora and anthracnose on black raspberry can be overcome by improving the cultivation environment due to the fertilizer of standard amount and improvement of drain.

**P29.006 First report of bacterial blight, heterogenecity of disease incidence, and biocontrol approach on greensoybean in Thailand**

*S. Prathuangwong[1,2], D. Athinuwat[3], W. Chuaboon[1], M. Choorin[1], L. Kladsuwan[1] and S. Marach[1,2]*
*[1]Department of Plant Pathology, Kasetsart University, Bangkok, 10900, Thailand; [2]Center for Advanced Studies for Tropical Natural Resources, Kasetsart University, Bangkok, 10900, Thailand; [3]Major of Organic Farming Management, Faculty of Science and Technology, Thammasart University, Bangkok, 12120, Thailand*
*Email: g5517100155@ku.ac.th*

In 2012 growing season, the incidence and etiology observed in production area were exhibited a new disease, bacterial blight caused by *Pseudomonas syringae* pv. *glycinea* (Psg) with 12% of total soybean fields investigated (45 fields). PCR and sequencing assays revealed the causal pathogen was 99% identical to Psg (No.AEGG00000000.1). This disease is the first report on greensoybean in Thailand. Samples of other diseases included bacterial pustule (caused by *Xanthomonas axonopodis* pv. *glycines*; Xag), rust (*Phakopsora pachyrhizi*), anthracnose (*Collectotrichum truncatum*), sudden death syndrome (*Fusarium solani*), and viruses (SMV and SCLV) with 22.6, 20.8, 11.7, 8.8, and 8.1% incidence respectively. The left 16% incidence were Cercospora leaf spot, brown stem rot, downy mildew, root and stem rot, stem canker, charcoal rot, Septoria brown spot, Phyllosticta leaf spot, frogeye leaf spot, and Myrothecium leaf spot infection. The 3 diseases in this study: bacterial pustule, rust, and anthracnose had incidentally increased approximately 13.8, 9.6, and 0.4% respectively, where decrease of *F. solani* was observed compared in 1994 study. The results suggest that bacterial pustule is a predominant disease of greensoybean production and the change in all disease incidences may be due to differences in environmental conditions and appropriate rotation strategy. The 4-known biocontrol agents were tested for pathogen inhibition using disc diffusion and dual culture assays, that *Pseudomonas fluorescens* SP007s and *Bacillus amyloliquefaciens* KPS46 provided the most effective results against Xag and Psg, where *Paenibacillus pabuli* SW01/4 and *Trichoderma harzianum* CB-pin01 had slightly effect, but the strain CB-pin01 significantly inhibited *F. solani.*

**P29.007 Effect of false smut on yield of rice and resistance identification of rice cultivars against *Ustilaginoidea virens***

*X.J. Yang[1], T.B. Lin[2], H.C. Ruan[1], L. Gan[1], Y.X. Du[1], N.N. Shi[1]* and *F.R. Chen[1]*
*[1]Institute of Plant Protection, Fujian Academy of Agricultural Sciences, Fuzhou, Fujian, 350013, P. R. China;*

*[2]College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, P. R. China*
*Email: chenfuruzb@163.com*

False smut is a heavy disease in Fujian province in China, caused by *Ustilaginoidea virens.* It was reported that the disease reduced crops quality but less effected on yield loss previously, and planting resistant rice cultivar was main measure to control false smut disease. In this study, we investigated the effect of false smut ball quantity on blighted grain rate and yield loss of rice in fields, and performed the evaluation of resistance of 66 rice varieties against *U. virens* using natural inducing methods. The results showed the quantity of false smut ball was correlated strongly to increasing blighted grain and yield loss rate. The loss rate of yield is greater than 35%, when the number of false smut ball is over five per panicle. The resistance of variety was evaluated according to the rate of diseased ears combined infected grains rate. The resistant cultivar against false smut has not been found in 66 experimental varieties, and seven out of 66 varieties were identified as moderate resistance, including variety of Guangyou 2186, Fuliangyou 686, Fufengyou 366, Zhenyou 998, Yueliangyou 673, Guangxiang 8 you 168 and Guangyou 7017; 22 varieties were identified as moderate bingsui and 37 varieties were susceptibility.

**P29.008 Fungi and bacteria dominant populations in healthy and symptomatic barks of *Populus* × *euroamericana* canker disease**

*Y. Li, C.G. Piao, W. He* and *M.W. Guo*
*Research Institute of Forest Ecology Environment and Protection, Chinese Academy of Forestry, No.1 Dongxiaofu, Haidian District, Beijing 100091, P.R. China*
*Email:lylx78@gmail.com*

In 2006, a new canker was observed on trees of *Populus* × *euramericana* cv. '74/76' and *P.* × *euramerican* 'zhonglin 46' in the Henan and Shandong provinces of China. The disease occurred mainly in the summer. Diseased plants showed stem barks or cracked twigs and exuded frothy, and when the disease progressed, many long-shaped cankers (50-150 cm×3-8 cm, width×length) with abundant white, odorous exudates rapidly appeared. In order to detect the causal agent of the poplar canker disease observed in China, changes of dominant population of fungi and bacteria of healthy and diseased barks of *Populus×euroamericana* were analyzed. The healthy and diseased poplar tissues of *P.* × *euramerican* 'zhonglin 46' were collected and used to the isolation of cultivable fungi and bacteria in 2009. The results showed that dominant species of fungi from healthy and diseased poplar bark were *Alternaria alternata* and *Fusarium solani* respectively, and the strains of *F. solani* from diseased poplar bark are more than 85% of all the fungal isolates. And bacterial dominant species of diseased poplar plants bark was *Lonsdalea quercina*, and the isolates number of which were more than 67% of the all bacterial isolates, while preponderant species of three healthy poplar bark were different, i.e. *Microbacterium* sp., *Cellulomonas* sp. and *Janibacter melonis*. The results suggested that *F. solani* and *L. quercina,* the preponderant species of symptomatic bark of *Populus×euroamericana*, may be two important causal agents of the canker disease of *Populus×euroamericana.*

**P29.009 *AaLAEA*, a methyltransferase gene homolog, controls biosynthesis of secondary metabolites and pathogenicity in the multi pathotypes of *Alternaria alternata***

*K. Takao, Y. Akagi, T. Tsuge, E. Nanba and M. Kodama*
*Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan*
*Email: totoro518jp@yahoo.co.jp*

Filamentous fungi are considers promising resources for the discovery of novel bioactive compounds because of their ability to produce various kinds of secondary metabolites. Recently, it was reported that a nucleoprotein, LaeA, is required for the expression of secondary metabolite genes in the model fungus *Aspergillus nidulans*. Deletion of *LaeA* (Δ*LaeA*) blocked the expression of metabolic gene cluster, including the penicillin, aflatoxin and sterigmatocystin gene clusters in *Aspergillus* spp. Furthermore, mutants of *LaeA* display reduced pathogenicity in a human pathogen *A. fumigatus* and plant pathogens *Cochliobolus heterostrophus* and *Fusarium verticillioides. Alternaria alternata* is one of the most cosmopolitan fungal species, generally is saprophytic. *A. alternata* pathogens include at least 7 pathogenic variants (pathoytpes), which produce host-specific toxins (HSTs) and cause severe diseases on each host plant. In this study, we identified *LaeA* homologues (*AtLAE, AsLAEA* and *AaLAEA*) that encode a methyltransferase in each pathotype (tomato, strawberry and apple) of *A. alternata*. AAL-toxin production and pathogenicity of *AtLAEA* mutant were significantly reduced. Gene expression of the AAL-toxin biosynthetic gene cluster was reduced and spore production and hyphal growth were also affected. Production of host-specific AF- and AM-toxins was reduced in the *AsLAEA* and *AaLAEA* mutants, respectively, with a decrease of virulence on each host plant. The mutants also showed decreased aerial hyphal growth and sporulation. Thus, *LAEA* genes positively regulate host-specific toxins biosynthesis, pathogenicity and hyphal growth of the *A. alternata* pathotypes.

**P29.010 A canker disease of *Populus* × *euramericana* in China, caused by "*Lonsdalea quercina* subsp. *populi***

*Y. Li, W. He, C.G. Piao and M.W. Guo*

*Research Institute of Forest Ecology Environment and Protection, Chinese Academy of Forestry, No.1 Dongxiaofu, Haidian District, Beijing 100091, P.R. China; Beijing Forestry University, Beijing 100083, P. R. China*
*Email:lylx78@gmail.com*

In 2006, a new canker was observed on trees of *Populus* × *euramericana* cv. '74/76' and *P.* × *euramerican* 'zhonglin 46' in the Henan and Shandong Provinces of China. The disease, which is characterized by canker with white exudates dripping from the bark, occurred mainly in the summer. A particular gram-negative rod-shaped bacterium was repeatedly isolated from the infected samples and proven to infect trees of *P.* × *euramericana* by Koch's postulates. Through a polyphasic taxonomic approach (using sequence-, DDH-, chemotaxonomic- and phenotypic data), the poplar isolates were identified as "*Lonsdalea quercina* subsp. *populi*", a subspecies very recently described based on isolates from oozing bark canker of poplar (*Populus* × *euramericana*) trees in Hungary.

**P29.011 Resistance spectrum assay and fine mapping of the rice blast resistance gene *Pita²***

*S. Chen[1,2], J. Su[1,2], W. Wang[1,2], C. Wang[1,2], J. Yang[1,2], A. Feng[1,2], L. Zeng[1,2] and X. Zhu[1,2]*
*[1]The Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, P. R. China; [2]Guangdong Provincial Key Laboratory of High Technology for Plant Protection, Guangzhou, 510640, P. R. China*
*Email: zhuxy@gdppri.com*

Rice blast, caused by *Magnaporthe oryzae*, is one of the most important plant fungus diseases worldwide. Deployment of the broad-spectrum resistance genes is the most effective and economical approach to control the disease. To clarify the resistance genes *Pita²* and *Pita*, we performed the resistance assessment by rice blast inoculation on IRBLta2-Re and IBLta-CP1, the monogenic lines (MLs) of rice blast resistance genes *Pita²* and *Pita*. The analysis by using 196 rice blast isolates derived from China indicated that the resistance spectrum of IRBLta2-Re was broader than that of IRBLta-CP1. To further discriminate the difference between these two genes, we conducted the fine mapping of *Pita²* and the genome sequences analyzing of *Pita*/*pita* alleles in the MLs. 1250 $F_2$ individuals from the cross between CO39 and IRBLta2-Re were used as the mapping population. The $F_2$ population was inoculated with the blast isolate GD08-T4 which was incompatible to IRBLta2-Re, but compatible to CO39 and IRBLta-CP1. In the phenotypic data analysis, the $F_2$ population segregated in a 3:1 (R: S) ratio for resistant and susceptible plants, respectively. Among the total of 50 microsatelite and 12 position specific microsatelite (PSM) markers distributed by two sides of the *Pita* gene within 2000 kb region were selected in the parents polymorphism screening, 10 of polymorphic markers were selected to test all of the 315 S individuals. We identified 8 markers, such as RM27877, RM27891, PT2, PT9, PT12, RM27956, RM27991 and RM28009, were tightly linked to the resistance gene, among which PT3 and PT5 are co-segregated with the target gene. Indeed, a contig map corresponding to the *Pita²* and *Pita* genes was constructed based on the fine mapping and bioinformatics assay. The *Pita²* gene was assumed to be in an interval of approximately 178 kb which containing a total of 5 NBS-LRR genes, and was 500 kb away from the *Pita* allele. Single amino acid difference (SAAD) of *Pita/pita* locus was analyzed by the sequence comparison between corresponding locus in IRBLta2-Re and IRBLta-CP1, the results showed that both IRBLta2-Re and IRBLta-CP1 had the *Pita* gene.

## Concurrent Session 30-Plant Food Security A Network of Excellence on Biosecurity

### O30.001 Plant and food biosecurity: need for International cooperation and communication

*M.L. Gullino[1,2], P. Colla[1], M. Bonifert[3], A. Kocic[3] and A. Bertin[4]*
*[1]Centre for the Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Torino, 10095 Grugliasco, TO, Italy; [2]DISAFA, University of Torino, 10095 Grugliasco, TO, Italy; [3]Regional Environmental Center for Central and Eastern Europe, Hungary; [4]SPIN-TO, Italy*
*Email: marialodovica.gullino@unito.it*

Biosecurity refers to protection from harm caused by biological agents and it encompasses food safety, animal life and health, and plant life and health, including associated environmental risks. From 2005 a consortium of European researchers explored this topic focusing on the risks posed to European agriculture and forestry by the deliberate introduction of plant pathogens. In 2011 the European Commission funded the Project Plant and Food Biosecurity with the goal of creating a virtual Centre of Competence to prevent, respond to and recover from to both intentional and unintentional biosecurity threats to European agriculture, farming and the agro-food industry. PLANTFOODSEC focuses on biological threats having the capacity to affect and damage agriculture, infect plants and ultimately affect food and feed at any stage in the supply chain. The Network combines additional expertise and it includes 13 partners coming from 8 Countries (Italy, UK, Germany, France, Hungary, Turkey, Israel and USA) within an International cooperation perspective to benefit from the experience gained by non-EU partners in this field. To date, PLANTFOODSEC has identified regulatory threats, prioritized target crops and target pathogens (including human pathogens on plants and mycotoxins), designed a virtual diagnostic network and set-up risk assessment and decision-making tools for use by law enforcement offices. The project is identifying priorities for research and regulatory policy and a particular effort is now devoted in reaching the stakeholders and in raising the awareness of policy makers to enhance European preparedness and response capabilities.

### O30.002 Plant and food biosecurity and communication - fostering collaboration from virtual to 'real' networks

*A. Kocic, M. Bonifert and Z. Bauer*
*Regional Environmental Center for Central and Eastern Europe (REC), 2000 Szentendre, Hungary*
*Email: AKocic@rec.org*

Aiming at the enhancement of knowledge in the field of plant and food biosecurity, the ambition is to address and engage targeted stakeholders at EU or global level, to identify priorities for research and regulatory policy and to provide relevant baseline assessments, all within the framework of present and future network capabilities. One possible method to achieve the goal of encouraged collaboration is the conversion from 'virtual' to 'real' networks. Within PLANTFOODSEC, the awareness raising initiatives range from presenting current risks on plant and food systems that are facing Europe to the reflection that the current work will require future specific continuations building on increasingly focused research efforts. Taking all above mentioned aspects into consideration, communication is considered to be the key tool. Several efforts and streamlined communication actions seem to be adaptable regarding plant and food biosecurity. Whereas some lead towards the implementation of stakeholder forums, others focus on the harmonisation and integration of biosecurity systems, in which the "one health" paradigm strengthens the existing links and synergies across different sectors in Environment and Health. In sustainable diagnostic networks, communication ensures the widening of user bases with involved agronomists, researchers and advisors throughout the EU, in order to increase the ability to log disease outbreaks and to enable early warning systems. Eventually, the development of a specific communication strategy represents a key product in which all involved factors and stakeholders are to be consolidated under one common scientific umbrella – both for now and the future.

### O30.003 Virtual diagnostic networks: Creating a platform for collaborative diagnostics

*J. Stack, J.E. Thomas and P. Verrier*
*Kansas State University, Manhattan, Kansas State 66506, United States*
*Email: jstack@ksu.edu*

Plant pathogen diagnostic laboratories use multiple information sources and a wide variety of techniques to deliver diagnostic, advisory, and sometimes regulatory services. In the United States, the National Plant Diagnostic Network was established within the USDA to link, strengthen and coordinate existing, independent state-associated diagnostic laboratories. The system aims to enhance plant pathogen detection and identification and to mitigate plant diseases by training and informing first detectors and diagnosticians, providing a mechanism for the reporting of disease outbreaks, and standardizing technologies and protocols. In Europe, a similar structure is under development as part of the European Union funded project, Plant and Food Biosecurity. The network will bring together information on member states' diagnostic laboratories, their facilities and expertise, and will provide a community resource covering training, workshops, protocol updates and emerging disease issues.

Importantly, the network will focus on advisory and commercial laboratories, and will create a route for agronomists, advisors and diagnostic service providers to add and obtain information on developing situations through the growing season. A mechanism for inter-laboratory "expert to expert" consultations will also be developed. The system is not designed to provide formal alerts or notification of pathogen outbreaks to regulatory authorities, though in the future national moderators could scan data to identify unusual occurrences of disease which might signal serious developing threats.

### O30.004 Exclusion of high-consequence pathogens from crop production systems

*A. Gamliel*
*Institute of Agricultural Engineering, Agricultural Research Organization (ARO) Volcani Center, Bet Dagan, 50250 Israel*
*Email: agamliel@agri.gov.il*

Introduction of harmful crop pathogen may pose a threat to a country's agricultural, economy and trade. Therefore, the main goal when a harmful pathogen is detected is its ultimate elimination from the production system and its products. Successful elimination of pathogen involves a concerted complex of detection, risk assessment, adoption of the appropriate strategy, and careful execution of all the control procedures. Successful management relies on preparedness, rapid response and the use of the appropriate strategy. Exclusion of high-consequence pathogens incorporates and assembles all the agricultural practices which are relevant to the crop production and which are also of impact with regard to pest onset or suppression. Exclusion of a pest includes and considers a rapid and effective containment of the pest at all potential reservoirs (volunteer plants, vector etc.). Followed by containment, eradication measures are exerted to eliminate the pest from the intruded environment. The third stage of recovery, includes additional pest management measures in order to maintain eradication efficacy and minimize options for pest, reeruption. Adoption of the strategies to eliminate an invasive pathogen depends on realistic assessment of the effectiveness of the various approaches, and the feasibility for their success. A quantitative assessment of all the factors that influence the eradication process can lead to the adoption of an appropriate eradication approach and strategy. However, other social, political and demographic constraints may influence the selection of the most appropriate strategies and measures.

### O30.005 Quantification and interpretation of risk for security, trade, food and environment

*J.D. Mumford, F. Suffert, A.W. Leach, J. Holt, I. Sache, C. LeFay-Souloy, M. Barbier and R.A. Hamilton*
*Centre for Environmental Policy, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, United Kingdom*
*Email: j.mumford@imperial.ac.uk*

Risk analysis processes for unintentional introductions of pathogens are well established for international trade, food safety and environmental conservation. Additional risk analyses related to intentional introductions due to terrorism or other criminal acts should build on currently recognised systems to ensure that agroterrorism risks can be reasonably compared with other risks to crops and food supply chains. The motivation and capabilities of perpetrators and the vulnerability of ecological and social systems to terrorism and criminal acts are particular points that distinguish agroterrorism risk analysis from more conventional risk analyses for plant pathogens or other pests. It is appropriate to develop securitization-oriented risk analyses based on scenarios related to perpetrator motivation, feasibility and receptor systems, rather than focusing solely on biological agents or pathways. An assessment system has been developed based on an evolution of the EPPO PRA Scheme. This has been demonstrated by risk assessors familiar with agricultural trade risk analysis for a broad range of potential agroterrorism and bio-crime scenarios. The process is compatible with agricultural PRAs and uses robust scoring, confidence and justification steps to elicit knowledge that generates systematic risk (likelihood*impact) measures for agroterrorism scenarios.

### O30.006 The potential for mis-use of scientific research: ethical conundrums & global solutions

*J. Fletcher, J. Leach and P. Lewis*
*National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK 74078, U.S.A.*
*Email: jacqueline.fletcher@okstate.edu*

Life sciences research is designed to increase understanding of the living world and to use research findings for beneficial purposes. Agricultural (including plant pathology) research is intended to enhance agricultural systems and production, thereby strengthening global food security. Life science research is essential also to strong national security and economic health. However, "good" science can be applied to "bad" uses. In 2004 the National Research Council of the U.S. National Academy of Science stated "*…the same technologies can be used legitimately for human betterment and misused for bioterrorism*." Dual use research (DUR) is research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health, agriculture, plants, animals, the environment, or materiel. The U.S. National Science Advisory Board for Biosecurity (NSABB), within the National Institutes of Health, is charged to provide advice

regarding biosecurity oversight of dual use research to the Federal government, taking into consideration national security concerns and the needs of the research community. The NSABB has offered critical recommendations on: (1) a proposed framework for the oversight of dual use life science research (2) biosecurity concerns related to synthetic biology and select agents; (3) outreach and education on dual use research issues, including international engagement and attention to amateur biologists and scientists in non-life science disciplines; (4) enhancing personnel reliability and strengthening a culture of responsibility; (5) development and dissemination of codes of conduct for dual use research.

**P30.001 Plant and food biosecurity: a European union network of excellence**

*M.L. Gullino[1,2], J.E. Thomas[3], C. Henry[4], H.W. Dehne[5], F. Suffert[6], M. Bonifert[7], J.D. Mumford[8], H. Alpas[9], A. Bertin[10], F. Marelli[11], A. Gamliel[12], J. Fletcher[13] and J. Stack[14]*

*[1]Centre for the Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Torino, 10095 Grugliasco, TO, Italy; [2]DISAFA, University of Torino, 10095 Grugliasco, TO, Italy; [3]National Institute of Agricultural Botany, UK; [4]Food and Environment Research Agency, UK; [5]Rheinische Friedrich-Wilhelms-Universitaet Bonn, Germany; [6]Institut National de la Recherche Agronomique, France; [7]Regional Environmental Center for Central and Eastern Europe, Hungary; [8]Imperial College of Science, Technology and Medicine, UK; [9]Middle East Technical University, Turkey; [10]SPIN-TO, Italy; [11]United Nations Interregional Crime and Research Institute, Italy; [12]The Agricultural Research Organization of Israel, Volcani Centre, Israel; [13]Okhlaoma State University, USA; [14]Kansas State University, USA*

*Email: marialodovica.gullino@unito.it*

Biosecurity refers to protection from harm caused by biological agents and it encompasses food safety, animal life and health, and plant life and health, including associated environmental risks. From 2005 a consortium of European researchers explored this topic focusing on the risks posed to European agriculture and forestry by the deliberate introduction of plant pathogens. In 2011 the European Commission funded the Project Plant and Food Biosecurity with the goal of creating a virtual Centre of Competence to prevent, respond to and recover from to both intentional and unintentional biosecurity threats to European agriculture, farming and the agro-food industry. PLANTFOODSEC focuses on biological threats having the capacity to affect and damage agriculture, infect plants and ultimately affect food and feed at any stage in the supply chain. The Network combines additional expertise and it includes 13 partners coming from 8 Countries (Italy, UK, Germany, France, Hungary, Turkey, Israel and USA) within an International cooperation perspective to benefit from the experience gained by non-EU partners in this field. To date, PLANTFOODSEC has identified regulatory threats, prioritized target crops and target pathogens (including human pathogens on plants and mycotoxins), designed a virtual diagnostic network and set-up risk assessment and decision-making tools for use by law enforcement offices. The project is identifying priorities for research and regulatory policy and a particular effort is now devoted in reaching the stakeholders and in raising the awareness of policy makers to enhance European preparedness and response capabilities.

**P30.002 The use of cold atmospheric plasma to decontaminate the surface of soft fruits**

*P. Varghese, O. Nwaiwu, J. Hort, M. Dickinson, J. Hobman and C.E.D. Rees*

*Division of Food Sciences, School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, Leics, LE12 5RD, UK*

*Email: stxpv4@exmail.nottingham.ac.uk*

UK households dispose of 75,000 tonnes of soft fruit annually, often because of product deterioration due to mould spoilage. Cold atmospheric plasma (CAP) was evaluated as a method to decontaminate the surface of soft fruit to extend shelf life. The CAP was generated under atmospheric conditions, using a mixture of Helium and Oxygen gases and high voltage pulses, and excitation/ionisation of gases leads to the formation of reactive species. To investigate the effect on soft fruit, samples were exposed to CAP for different lengths of time and in different containers. The effect of CAP treatment on the organoleptic properties of strawberries treated for 1 min was determined by sensory evaluation. No difference was detected between treated and untreated fruit. Samples were incubated at 25 ℃ and were visually scored for spoilage and CAP treatment was found to inhibit fungal growth and decrease spoilage. A second aim was to isolate filamentous fungi that naturally contaminate soft fruits and determine their sensitivity to CAP treatment. Fungi growing on the samples were recovered and identified *as Botrytis cinerea, Rhizopus* (strawberry) and *Alternaria* (Blueberry). Of these, *Botrytis* was the most sensitive to CAP treatment. This work shows that there is potential to use CAP to extend the shelf life of soft fruit, but further work is needed to optimise treatments, the configuration of the plasma plume and understand the effect of environmental conditions on CAP treatment. A complete understanding of the mechanism of microbial inactivation is required to facilitate the commercial application of CAP.

**P30.003 Risk and vulnerability analysis of plant disease epidemics on a societal level**

*A. Djurle*

*Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, SE-750 07 Uppsala, Sweden*
*Email: Annika.Djurle@slu.se*

Yield or crop losses are usually quantified at the farmer's level. It is well known that losses also occur at other levels, but their quantification is more difficult and rarely made. As long as the losses of an individual farmer are within a 'normal' range the possible production deficit can be compensated for via import or transfer between regions within a country. In the perspective of food security in combination with severe yield losses due to some disease, or an unexpected pathogen incursion, societies may be taken by surprise unless there is some general preparedness and routines for handling unwanted events. The level of preparedness can be evaluated via a risk and vulnerability analysis. Within this framework risks, vulnerabilities, and critical dependencies are identified, followed by analyses of how to handle the event, possible action plans, description of the results, feed-back and reporting. A model for analysis of risks and vulnerabilities in the food chain is presented and a severe epidemic of a wheat disease is used as an example.

**P30.004 Effect of *Chlorella vulgaris* on enhancement of storage and freshness in organic strawberry fruit and fresh-cut leafy vegetables**

*H.J. Jee, M.J. Kim, C.K. Shim, Y.K. Kim, J.H. Park, S.J. Hong and E.J. Han*
*Organic agriculture Division, National Academic of Agricultural Science, Rural Development of Administration, Republic of Korea*
*Email: hjjee@korea.kr*

This study was aimed to enhance storage and freshness of strawberry fruits and foliage vegetables treated with *Chlorella vulgaris* as a bio-fertilizer. The soluble liquid content of Seolhyang and Yukbo Strawberry fruits was enhanced by 22.2% and 11.5%, respectively, compared to control treatment. The decay rates of Seolhyang and Yukbo Strawberry fruits treated with foliar spray were lower than soil drench treatments during cold storage. Surface color change and chlorosis of leave of fresh-cut vegetables were observed in the sample treated with water spray at 10 days after cold storage. However, the decay rate of fresh-cut leafy vegetables, lettuce, kale, red kale, white kale and beet treated with foliar spray of 15 % of *C. vulgaris* were significantly lower than water spray treatment during storage at 4℃.

**P30.005 Effecting change in policy and public perception to achieve global food security**

*L. Korsten*
*Department of Microbiology and Plant Pathology, University of Pretoria, Private Bag X20, Hatfield, Pretoria, South Africa, 0028*
*Email: lise.korsten@up.ac.za*

Achieving food security in its totality continues to be a challenge not only for developing countries, but also for the developed world. The difference in achieving equilibrium between food security and -insecurity lies in the magnitude of the problem. This can be expressed in terms of natural resources, infrastructure, food production capacity, distribution systems and networks, political stability, access to food, proportion of the population affected and trained human capital in critical knowledge fields. Most developing countries lack adequate human capital and expertise in agriculture and food production systems for sustained economic growth and well-being. Compliance with the Agreement on Sanitary and Phytosanitary (SPS) measures further complicate matters as substantial knowledge and expertise are required in specialised fields such as pest risk assessment, postharvest technology, food safety and biosecurity. Currently, South Africa and the rest of Africa are not training adequate numbers of plant pathologists and are not able to provide government or industry with adequate human capital to support free trade and food security. Most developing countries are therefore not able to address new challenges associated with SPS measures and food security. One of the objectives of the International Society for Plant Pathology is to effect changes in public policy and opinions on global food security. This paper presents an intervention strategy to improve public awareness of plant pathology, postharvest technology and food security.

**P30.006 *Fusarium proliferatum* - *Allium cepa* pathosystem: a model encompassing crop protection, agricultural biosecurity and animal/human health**

*A. Gamliel, J.P. Stack, J. Fletcher, Y. Issac, I. Moncrief, H. Dehne and M.L. Gullino*
*Institute of Agricultural Engineering, Agricultural Research Organization (ARO) Volcani Center, Bet Dagan, 50250 Israel*
*Email: agamliel@agri.gov.il*

The *Fusarium proliferatum (FP)* - *Allium cepa* pathosystem offers an excellent case-study of an epidemic chain involving plant, animal and human health. The fungus is an opportunistic pathogen of humans, producing an array of mycotoxins (e.g., fumonisin, fusaproliferin and moniliformin) with toxicities to humans and animals, and consequently is a food and feed safety concern. *F. proliferatum* is also a plant pathogen and endophyte with a very wide host range across many plant families; symptoms can be evident or not. Distributed globally, *F. proliferatum* was first reported in Israel in 2008 in infected onion bulbs and has since been isolated from corn, garlic, cucumber, tomato, watermelon, and pumpkin

throughout Israel. *F. proliferatum* is systemic in onion (though not in all its plant hosts), invading sets and inflorescences. *F. proliferatum* may be disseminated in seeds of onion and corn at rates of 0.1-10%. The pathogen has also been isolated from many volunteer plants in and adjacent to cultivated fields, including *Phragmites australis* (wild cane), *Sonchus oleraceus*, and species of *Portulaca*, *Amarantus*, *Chenopodium*, and *Tamarix*. Effective management of *F. proliferatum* requires an integrated approach, including the production of pathogen-free seeds and sets, supplemental treatments during crop production, and a validated system of traceback. Production of mycotoxin-free products should be an essential goal of food production. However disease and toxin management will not be fully accomplished and critical control points identified, without a more complete understanding of the entire pathosystem, including pathogen establishment and disease spread.

**P30.008 Pathogenicity, virulence and mycotoxin production of *Fusarium proliferatum* isolates**

*Z. Guo*[1], *B. Altincicek*[1], *P. Karlovsky*[2] *and H.W. Dehne*[1]
[1]*Institute of Crop Science and Resource Conservation (INRES–Phytomedicine), University of Bonn, Nussallee 9, D-53115 Bonn, Germany;* [2]*Department of Crop Sciences, Molecular Phytopathology and Mycotoxin Research, University of Göttingen, Grisebachstrasse 6, D-37077 Göttingen, Germany*
*Email: s7ziguoo@uni-bonn.de*

*Fusarium proliferatum* (Matsushima) Nirenberg is an important pathogen infecting numerous crop plants thereby reducing yield and quality. In various climatic zones, *F. proliferatum* colonizes an extraordinarily broad range of host plants including maize, wheat, barley, rice, asparagus, banana, date palm, garlic, onion, miscanthus and more. *F. proliferatum* is capable of synthesizing a variety of mycotoxins, including most notable fumonisins, beauvericin, enniation, fusaric acid, fusarin, fusaproliferin and moniliform. According to the combination of morphological, chromatographic and genetics approaches, the varieties of 32 different isolates of *F. proliferatum* pathogenicity on detached maize leaves, virulence on *Tenebrio molitor* larvae, production of mycotoxins and intraspecific polymorphism will be monitored. Using HPLC methods, the chemotype of various *F. proliferatum* isolates will be demonstrated. Our results indicated that significant variations in growth rates existed between *F. proliferatum* isolates on PDA under tested conditions. The detached leaf assay revealed varying pathogenicity of *F. proliferatum* isolates on maize. Varying virulence on *T. molitor* larvae was correlated with the production of specific mycotoxins. Phylogenetic analysis using the gene marker FUM1, elongation factor 1-α (EF-1α), β-tubulin, calmodulin (CAM) and RNA polymerase second largest submit (RPB2) will be performed to determine genotypes of tested *F. proliferatum* isolates.

**P30.009 Development of generic disease recovery plans using an iterative, deliberative process**

*N. McRoberts*[1], *J. Brown*[2], *F. Nutter*[3], *J. Stack*[4], *C. Thomas*[1] *and R. Martin*[5]
[1]*University of California, Davis, CA 95616, USA;* [2]*University of Arizona, Tuscon, AZ 85721, USA;* [3]*Iowa State University, Ames, IA, 50011, USA;* [4]*Kansas State University, Manhattan, KS, 66506, USA;* [5]*Purdue University, West Lafayette, IN, 47907, USA*
*Email: nmcroberts@ucdavis.edu*

Invasion by, or *in situ* emergence of, novel plant pathogens poses an on-going challenge to food and non-food crop production world-wide. One of the key elements in the effort to mitigate the effects of the occurrence of novel diseases is the development of disease recovery plans. In their simplest form disease recovery plans provide a categorization of diseases against a set of ecological and economic criteria, which together provide guidance for response agencies as to appropriate actions to be taken at different organizational scales. Since, by definition, novel diseases are new to the regions they invade there is often little specific information available on which to base recovery plans. If specific plans are developed *de novo* in each case the time lag between the first appearance of a disease and the completion of the recovery plan can be considerable, and may adversely impact the efficacy of the response. To reduce this effect it would be helpful to be able to develop recovery plans based on generic properties of broad classes of disease, allowing for specific variations to be introduced on the fly as data accumulate. The potential for such an approach depends on the robustness of the concept of generic disease types, both as actual ecological and economic entities and as abstract concepts which different people will consistently recognize as coherent groupings. We describe a research process, based on a combination of expert evaluation and objective multivariate statistical analysis, in which we established the existence of generic disease types from a set of disease recovery plans in the USA. The implications for the future development of specific disease recovery plans are discussed.

**P30.010 Biosecurity implications of new technology and discovery in plant virus research**

*R.M. MacDiarmid, B. Rodoni, U. Melcher, F. Ochoa-Corona and M. Roossinck*
*The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169, Auckland, 1142, New Zealand*
*Email: Robin.macdiarmid@plantandfood.co.nz*

Human activity is causing new encounters between viruses and plants. Anthropogenic interventions include

changing land use, decreasing biodiversity, trade, the introduction of new plant and vector species to native landscapes, and changing atmospheric and climatic conditions. The discovery of thousands of new viruses, especially those associated with healthy appearing native plants, is shifting the paradigm for their role within the ecosystem from foe to friend. The cost of new plant virus incursions can be high and result in the loss of trade and/or production for short or extended periods. We present and justify three recommendations for plant biosecurity to improve communication about plant viruses, assist with the identification of viruses and their impacts, and protect the high economic, social, environmental and cultural value of our respective nations' unique flora: 1) As part of the burden of proof, countries and jurisdictions should identify what pests already exist in, and which pests pose a risk to, their native flora, 2) Plant virus sequences not associated with a recognized virus infection are designated as "uncultured virus" and tentatively named using the host plant species of greatest known prevalence, the word 'virus', a general location identifier, and a serial number, 3) Invest in basic research to determine the ecology of known and new viruses with existing and potential new plant hosts and vectors and develop host-virus pathogenicity prediction tools. These recommendations have implications for researchers, risk analysts, biosecurity authorities, and policy makers at both a national and an international level.

## Concurrent Session 31-Plant Pathogenic Bacteria

### O31.001 Functional structure of *Pseudomonas syringae* type III effector repertoires in the context of plant immunity

*S. Chakravarthy, H.L. Wei, J.N. Worley, T. Helmann and A. Collmer*
*Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14853 USA*
*Email: arc2@cornell.edu*

The virulence of *Pseudomonas syringae* pv. *tomato* DC3000 is promoted by the phytotoxin coronatine and multiple effector proteins injected into plant cells by the type III secretion system, which collectively suppress immunity elicited by flagellin and other bacterial PAMPs. DC3000 can cause bacterial speck disease in *Nicotiana benthamiana* (if the *hopQ1-1* effector gene is deleted), but growth of a DC3000 polymutant, DC3000D28E (deficient in 28 effector genes), in this model plant was 4 logs lower and symptomless in comparison with DC3000 Δ*hopQ1-1*. Various combinations of effector genes were restored to DC3000D28E by a programmable or random *in vivo* assembly shuttle (PRIVAS) system, and the minimal requirements for virulence in *N. benthamiana* were explored with subsets of PRIVAS-restored effectors and mutations altering flagellin and coronatine production. Success of syringe-infiltrated pathogen required a combination of effectors that target vesicle trafficking and PAMP perception and was enhanced by deletion of *fliC* (flagellin). Success of dip-inoculated pathogen was strongly enhanced by restoring PSPTO4723 (*cmaL*) to DC3000D28E, which had been deleted along with a cluster of effector genes during mutant construction and was discovered to direct the production of L-*allo*-isoleucine, the starting unit for coronamic acid production. Because coronamic acid and coronafacic acid are ligated to produce coronatine, analysis of DC3000D28E derivatives differentially expressing *cmaL* enabled documentation of contributions of the coronatine holotoxin to virulence that are discernible only when redundancies with effectors in the complete repertoire are eliminated, thus revealing overlap and specializations in action of the toxin and effectors in pathogenesis.

### O31.002 Regulation of the T3SS formation and T3SS effector secretion in *Xanthomonas oryzae*

*Z.Y. Liu, Y.R. Li, X.B. Xue, L.L. Cai, L.F. Zou, H.S. Zou and G.Y. Chen*
*School of Agriculture and Biology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, P. R. China*
*Email: gyouchen@sjtu.edu.cn*

Two pathovars, pv. *oryzae* and pv. *oryzicola* in *Xanthomonas oryzae*, the causal agents of bacterial leaf blight and bacterial leaf streak in the model plant rice, possesse similar hypersensitive response (HR) and pathogenicity (*hrp*), *hrp*-conserved (*hrc*), *hrp*-associated (*hpa*) clusters (*hrp-hrc-hpa*) that encodes a type III secretion system (T3SS) through which T3SS effectors are injected into host cells to trigger plant diseases or defences. Mutations in this cluster usually abolish the bacterial ability to cause HR in nonhost tobacco and pathogenicity in host rice. In *Xanthomonas* spp., these genes are generally assumed to be regulated by the key master regulators HrpG and HrpX. However, recent results in my lab have revealed novel findings: (1) HrpD6, controlled by HrpX, is a regulator for *hpa1*, *hpa2*, *hrcC*, *hrcT* and *hpaB*; (2) the expression of *hrcC*, *hrpD5*, *hrpE* and *hpa3* is occasionally HrpG- and HrpX-independent; (3) the expression of *hrcT* is HrpG-independent and HrpX-dependent; (4) HrcC, HrpE and Hpa3 are not only involved in the T3SS secretion, but also in carbon metabolism; (5) fructose-bisphophate aldolase exhibits functional roles between carbon metabolisms and the *hrp* system of the pathogen. Thus, the unknown regulators for HrcC, HrpD5, HrpE and Hpa3 and their roles in consistence with the T3SS are undertaking.

### O31.003 Characterisation of virulence factors in the horse chestnut pathogen *Pseudomonas syringae* pv. *aesculi*

*F. Dorati, E. Colombi, M. Cerri, M. Sanchez-Contreras, D. Paliwal, G.A. Barrett, G.J. Holloway, D.J. Studholme, S. Tegli, N.R. Waterfield, D.L. Arnold and R.W. Jackson*
*School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AJ, UK*
*Email: R.W.Jackson@reading.ac.uk*

Bleeding canker of European Horse Chestnut (*Aesculus hippocastanum*) has swept through continental north western Europe and into the British Isles in the last decade. Symptoms of the disease include necrotic-like lesions on the trunk and branches of trees, which ooze a brown-black exudates giving the appearance of bleeding. The causal agent is *Pseudomonas syringae* pv. *aesculi* (*Pae*), which can infect xylem and phloem of the tree, and if the pathogen girdles the entire tree, it can kill it. We sought to improve our understanding of the ecology and pathogenicity of the pathogen. With the knowledge that *Pseudomonas syringae sensu lato* can be found widespread within the environment, we firstly investigated the dynamics of the pathogen when challenged with common bacterial predators, including amoeba, nematodes and insects. Since we observed enhanced predation resistance compared to control strains, we employed a large scale genetic screen to identify a number of genetic factors that appear to play an important role in predation survival; extracellular polysaccharide appears to be a key factor. Aided by the genome sequence of *Pae*, we were also able to identify putative

virulence genes and through a series of pathogenicity tests, we have identified a number of genes that are important for virulence to plants. Of note was the discovery of a single point mutation in *hopAB1* that appears to be responsible for diversification of pathogens to different tree hosts.

### O31.004 Banana *Xanthomonas* wilt (BXW) in East and Central Africa

*L. Tripathi[1], J.N. Tripathi[1], A. Kiggundu[2] and W. K. Tushemereirwe[2]*
*[1]International Institute of Tropical Agriculture (IITA), C/o ILRI, PO Box 30709, Nairobi, Kenya; [2]National Agricultural Research Laboratories (NARL), PO Box 7065, Kampala, Uganda*
*Email: L.Tripathi@cgiar.org*

The banana Xanthomonas wilt (BXW) disease caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (Xcm) is threatening banana production across the great Lakes region of East and Central Africa. The pathogen is highly contagious and its spread has endangered the livelihood of millions of farmers who rely on banana for food and income. The economic impact of the disease is potentially disastrous because it destroys whole plants leading to complete yield loss of banana. BXW can be managed by following cultural practices; however, the adoption of these practices has been inconsistent as these techniques are labor intensive. The development of disease resistant bananas remains a high priority since farmers are reluctant to employ labor-intensive disease control measures and there is no host plant resistance among banana cultivars. Researchers at the International Institute of Tropical Agriculture (IITA) and the Uganda-based National Agricultural Research Organization (NARO), in partnership with the African Agricultural Technology Foundation (AATF) have successfully developed transgenic bananas constitutively expressing the Hypersensitive Response Assisting Protein (*Hrap*) or Plant Ferredoxin Like Protein (*Pflp*) gene provided by Academia Sinica Taiwan. These transgenic lines have exhibited strong resistance to BXW in the laboratory and screen house tests. The best 65 resistant lines were also evaluated in a confined field trial in October 2010 at NARL, Uganda after approval from National Biosafety Committee. This is a significant step toward development of transgenic banana varieties resistant to BXW, which will boost the available arsenal to fight this epidemic disease and save livelihoods in Africa.

### O31.005 Glancing at host adaptation in *Ralstonia solanacearum* through comparative genomics of highly host-adapted lineages

*F. Ailloud, G. Cellier, D. Roche, C. Allen and P. Prior*
*Cirad-Inra UMR PVBMT, 7 chemin de l'IRAT, Saint-Pierre, La Réunion, 97410, France*
*Email: philippe.prior@cirad.fr*

*Ralstonia solanacearum* is a vascular soil-born plant pathogen with an unusually broad host range. This globally distributed, economically destructive organism has thousands of distinct lineages within a heterogeneous and taxonomically disputed species complex. Some of those lineages can be assigned to ecotypes that include highly host-adapted strains such as the banana Moko disease-causing strains (IIA-6, IIA-24, IIB-3, IIB4), the cold-tolerant potato brown rot strains (IIB-1) and the recently emerged IIB-4NPB strains (Not Pathogenic to Banana). The polyphyletic nature of the Moko ecotype and the unexpected closeness of some its lineages to the paraphyletic brown-rot and NPB ecotypes make those highly adapted strains a robust model for study of host adaptation and speciation in general. High-quality genomes of 10 new strains belonging to our model ecotypes were produced to complement the 17 publicly available ones. Using a panel of bioinformatics methods, we looked for genetic or evolutionary features that discriminate between ecotypes. There were relatively few divergent features. Those related to known virulence factors were further analysed for functional clues about host adaptation and ecotype emergence mechanisms. These analyses yield no clear signal, suggesting that the large biological differences between these closely related strains result from differences in gene expression rather than from differences in gene content. Transcriptomic analyses of these strains during host infection are underway to test this hypothesis.

### O31.006 *Ralstonia solanacearum* nitric oxide reductase is required for expression of the Type 3 secretion system

*C. Allen, B. Dalsing, J.M. Jacobs and A. S. Milling*
*Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706, USA*
*Email: cza@plantpath.wisc.edu*

The transcriptome of the bacterial wilt pathogen *Ralstonia solanacearum* indicated active inorganic nitrogen metabolism during tomato pathogenesis. Mutating *norB*, encoding *R. solanacearum*'s nitric oxide (NO) reductase, eliminated the bacterium's ability to respire on NO. NO is both a product of bacterial N metabolism and a potent plant defense signal. AniA, the enzyme that produces NO, is expressed 125-fold less in *norB*- cells, so *norB*- likely has lower NO levels than wildtype (WT). *norB*- was severely compromised in virulence, colonization and competitive fitness on tomato. *R. solanacearum* triggers a hypersensitive response (HR) on tobacco following recognition of a Type 3-secreted (T3S) effector. Unexpectedly, *norB*- induced no HR on tobacco, although it multiplied well. Moreover, *norB*- bacteria triggered lower expression of plant defense genes, indicating that

the plant did not recognize the *norB*- mutant and suggesting that NO levels were lower in *norB*- infections. Microscopic analyses with the NO dye DAF-FM DA found less NO in *norB*- infused leaves. Further, *hrpB* (transcriptional activator of T3S) and *popA* (a HrpB-regulated effector) were essentially not expressed in *norB*-. HrpB contains a predicted S-nitrosylation site at Cys112. We therefore hypothesized that failure to S-nitrosylate HrpB explains loss of T3S in the *norB*- mutant. Consistent with this hypothesis, infusing tobacco with *norB*- cells treated with the S-nitrosylation inducer Cys-NO restored WT levels of NO and triggered WT tobacco defenses. These data indicate that NorB mediates *R. solanacearum* NO production, Type 3 secretion, and plant recognition. Endogenous NO production appears to activate T3S via S-nitrosylation of HrpB.

**O31.007 New insights into the citrus huanglongbing complex and potential solutions to this devastating disease**

*Y.P. Duan, L.J. Zhou, M.Q. Zhang, C. Vahling-Armstrong, Q.X. Hao and H.S. Zhou*
*USDA-ARS-USHRL, Fort Pierce, Florida 34945, USA*
*Email: Yongping.duan@ars.usda.gov*

Citrus huanglongbing (HLB) is both a century-old and emerging disease that impedes citrus production worldwide. '*Candidatus* Liberibacter asiaticus' (Las) is the globally prevalent species of HLB bacteria. Here we describe our molecular characterizations of Las, and our newly-developed control methods for citrus HLB. From a genomics standpoint, Las has a significantly reduced genome (1.26Mb) and unique features adapted to an intracellular life style. Although the genome is small, Las contains at least two prophages that make up ~1/16 of the entire genome. Frequent recombination and re-assortment of the prophages may contribute to evolving diversity and plasticity of Las. Nine different types of Las populations may co-exist in a single infection or in different hosts and different geographical locations. Furthermore, different Las populations may account for titer variations, such as the extreme low titer of Las bacteria (detected by our qPCR method) from seed-transmitted citrus and infected *Murraya paniculata*. Las encodes a functional ATP translocase and acts as an energy parasite. Tomodulate host energy biosyntheses and/or defense responses, Las encodes two novel autotransporter proteins that target the host mitochondria. To compete for the limited zinc availability, Las encodes a ZuABC high affinity zinc uptake system. Las encodes a functional flagellin that slowly triggers the citrus basal defense response. Although HLB is extremely difficult to manage, our newly-developed thermotherapy and chemotherapy methods provide potential components of an integrated control strategy for this devastating disease.

**O31.008 Tracking down the movement of virulence factor: Direct visualization of *Agrobacterium*-delivered VirE2 protein inside host cells**

*X. Li, Q. Yang, H. Tu and S.Q. Pan*
*Department of Biological Sciences, National University of Singapore, Singapore 117543*
*Email: dbspansq@nus.edu.sg*

*Agrobacterium tumefaciens* causes crown gall tumors on natural host plants by transferring T-DNA into plant cells. It can also transfer T-DNA into various non-natural-host recipients, including yeast, algae and fungal cells. The DNA is delivered as a nucleoprotein complex composed of a single-stranded DNA molecule (T-DNA) as well as several bacterial virulence proteins, including VirD2 and VirE2. However, either the DNA or protein molecules have never been directly visualized. In this report, we adopted a split-GFP approach; the small GFP fragment (GFP11) was inserted into VirE2 at a permissive site to create the VirE2-GFP11 fusion, which was expressed in *A. tumefaciens*; and the large fragment (GFP1-10) was expressed in the recipient cells. Upon delivery of VirE2-GFP11 into the recipient cells, GFP fluorescence spots were visualized. VirE2-GFP11 was functional like VirE2; the GFP fusion movement could indicate the trafficking of *Agrobacterium*-delivered VirE2. As the natural host, 74% plant cells received the VirE2 protein in a leaf-infiltration assay; it took 30 min for VirE2 to move from the membrane into the nucleus. As a non-natural-host recipient, 51% of yeast cells received VirE2, which did not move towards the nucleus. Only 0.2% yeast cells transiently expressed the transgene delivered by *Agrobacterium*. This indicates that *Agrobacterium* is a more efficient vector for protein delivery than genetic transformation for a non-natural-host recipient. We hypothesize that *Agrobacterium* might have acquired the capacity to deliver proteins before successful delivery of DNA into the nucleus; the nucleoprotein trafficking inside the recipient cells could be the limiting factor.

**O31.009 *Xanthomonas vasicola* pv. *vasculorum* jumps hosts from sugarcane to *Eucalyptus* in South Africa**

*T.A. Coutinho[1], L. van der Westhuizen[1], S. MacFarlane[2], J. Roux[1], G.D. Carstensen[1] and S.N. Venter[1]*
*[1]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Pretoria 0028, South Africa; [2]South African Sugarcane Research Institute (SASRI), Private Bag X02, Mount Edgecombe 4300, South Africa*
*Email: gabrielle.carstensen@fabi.up.ac.za*

Three bacterial pathogens have previously been shown to cause blight and die-back of *Eucalyptus* species, namely *Pantoea ananatis*, *Xanthomonas axonopodis*

and *X. dyei* pv. *eucalypti*. *P. ananatis* has only been reported infecting this host in South Africa while *X. axonopodis* occurs in South America, and *X.d.* pv. *eucalypti* only occurs in Australia. In 2003 a newly established compartment of an *E. grandis* clone in the Mtunzini area of South Africa showed extensive leaf blight and die-back. The plantation was located in an area where sugarcane is extensively cultivated. Bacteria were commonly found exuding from leaves and petioles. Isolations from disease material consistently yielded *P. ananatis* and a *Xanthomonas* sp. The objective of this study was to identify the *Xanthomonas* sp. isolated. 16S rRNA and *gyrB* sequencing was performed on all strains. Strains of *Xanthomonas* from the Mtunzini outbreak grouped with strains of *X. vasicola* pv. *vasculorum*, known to infect sugarcane and maize. This pathovar has previously been reported from South Africa infecting these two hosts. Pathogenicity tests were undertaken using susceptible *Eucalyptus* seedlings and *X. vasicola* pv. *vasculorum* strains were found to be pathogenic. A strain of *X. vasicola* pv. *vasculorum* previously isolated from sugarcane was included in this inoculation trial and produced typical symptoms of blight. Three sugarcane cultivars were inoculated with the strains from eucalypts and produced typical symptoms of gummosis usually associated with this bacterium on sugarcane. It thus appears that *X. vasicola* pv. *vasculorum* has made a significant host jump from sugarcane to eucalypts in South Africa.

### O31.010 The diffusible factor (DF) synthase XanB2 is a bifunctional chorismatase that links the shikimate pathway to ubiquinone and xanthomonadins biosynthetic pathways in the phytopathogen *Xanthomonas*

*L. Zhou, J.Y. Wang and Y.W. He*
*State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, P. R. China*
*Email: yawenhe@sjtu.edu.cn*

The diffusible factor (DF) synthase XanB2, originally identified in *Xanthomonas campestris* pv. *campestris (Xcc*), is highly conserved across a wide range of bacterial species, but its substrate and catalytic mechanism have not yet been investigated. Here, we show that XanB2 is a unique bifunctional chorismatase that hydrolyzes chorismate, the end product of the shikimate pathway, to produce 3-hydrobenzoic acid (3-HBA) and 4-HBA. 3-HBA and 4-HBA are respectively associated with the yellow pigment xanthomonadin biosynthesis and antioxidant activity in *Xcc*. We further demonstrate that XanB2 is a structurally novel enzyme with three putative domains. It catalyzes 3-HBA and 4-HBA biosynthesis via a unique mechanism with the C-terminal YjgF-like domain conferring activity for 3-HBA biosynthesis and the N-terminal FGFG motif-containing domain responsible for 4-HBA biosynthesis. Furthermore, we show that *Xcc* produces coenzyme Q8 (CoQ8) via a new biosynthetic pathway independent of the key chorismate-pyruvate lyase UbiC. XanB2 is the alternative source of 4-HBA for CoQ8 biosynthesis. The similar CoQ8 biosynthetic pathway, xanthomonadin biosynthetic gene cluster, and XanB2 homologs are well conserved in the bacterial species within *Xanthomonas*, *Xylella*, *Xylophilus*, *Pseudoxanthomonas*, *Rhodanobacter*, *Frateuria*, *Herminiimonas*, and *Variovorax*, suggesting that XanB2 may be a conserved metabolic link between the shikimate pathway, ubiquinone and xanthomonadin biosynthetic pathways in diverse bacteria.

### O31.011 Variability at the molecular and pathogenicity levels within strains of *Pseudomonas syringae* pv. *actinidiae*

*J.L.Vanneste[1], J.R. Chapman[2], J. Yu[1], R.K. Taylor[2], D.A. Cornish[1] and B.S. Weir[3]*
*[1]The New Zealand Institute for Plant & Food Research Ltd, Private Bag 3123, Hamilton 3240, New Zealand; [2]Plant Health and Environment Laboratory, Ministry for Primary Industries, PO Box 2095, Auckland 1140, New Zealand; [3]Landcare Research, Private Bag 92170, Auckland 1142, New Zealand*
*Email: Joel.Vanneste@plantandfood.co.nz*

All strains of *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of bacterial canker of kiwifruit, share the same host range (plants of the genus *Actinidia*), on which they are all able to cause necrotic leaf spots. However, some strains cause additional symptoms such as dieback, cankers and production of exudate. There are other differences at the biochemical, molecular, pathological and colony morphology levels between strains of Psa. Differences between those Psa strains were confirmed by multi-locus sequence typing analysis using seven housekeeping genes. This analysis revealed four distinct groups called biovars. Strains of biovar 1 and biovar 2 share a similar BOX PCR pattern but they have a different *cts* haplotype. Furthermore, strains of biovar 1 produce phaseolotoxin and those of biovar 2 produce coronatine. Strains of biovars 1 and 2 have mostly been isolated from Japan and Korea respectively. Strains of biovar 3 or 4, the two biovars found in New Zealand, do not produce either toxin; they each have a unique *cts* haplotype and BOX PCR pattern. Strains of biovar 3 have been isolated in Europe since 2008. Strains of biovar 4 have not been associated to symptoms other than leaf spots. The same grouping into four biovars was found when analysing the DNA sequence of the seven effector genes. The four biovars of Psa are more related to one another than to any other pathovar of *P. syringae* except *P. syringae* pv. *theae* (Pst). Strains of Psa biovar 4 are more closely related to Pst than to strains of the other biovars.

**O31.012 Elucidation of role in virulence of type tree effectors from *Xanthomonas axonopodis* pv. *manihotis***

C.A. Medina[1], J.S. Ramirez[1], R. Bart[2], B.J. Staskawicz[2] and A.J. Bernal[1]
[1]Laboratorio de Micología y Fitopatología. Universidad de los Andes. Bogotá, Colombia; [2]Plant and Microbial Biology, UC Berkeley, California, USA
Email: ca.medina968@uniandes.edu.co

*Xanthomonas axonopodis* pv. *manihotis* (*Xam*) is the causal agent of cassava bacterial blight (CBB), one of the most devastating diseases in this crop. This disease can cause losses of up to 100% under the appropriate conditions. Previous studies have shown the presence of 19 type three effectors (T3E) in the genome of the type strain Xam CIO151. Together, these proteins have a key role in the virulence of this strain. Here we performed a systematic mutational analysis to understand the role of T3E on the virulence of this pathovar. Using a double crossing-over strategy, we generated single mutants (*Δ*) for the T3E *avrBs2, xopN, xopQ, xopX, xopZ,* and *hpaF*. We subsequently combined these mutations, generating double and triple mutants. The mutants were tested in their ability to cause disease on the susceptible cassava cultivar MCOL2215. A reduction in disease symptoms was observed with single mutants for *avrBs2, xopX* and *xopZ* when they were compared against the wild type strains. Interestingly, the double and triple mutants had an additive effect in virulence showing a reduction in levels of virulence similar to *ΔhrpX*, a mutant of the central transcriptional regulator of the T3SS. This study has demonstrated the role of some T3Es of *Xam* in virulence. We have also demonstrated that some pairs of effectors have an additive effect in virulence while others show redundancy. Our findings are critical in the elucidation of the role of type three effectors of *Xam* in virulence, and could be useful for the development of durable and sustainable resistance to CBB in cassava.

**O31.013 HopAD1 of *Pseudomonas syringae* pv. *tomato* DC3000 is a suppressor of flagellin-induced immunity and an elicitor of cell death in *Nicotiana benthamiana***

H.L. Wei, S. Chakravarthy, T. Helmann and A. Collmer
Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY USA 14853, USA
Email: hw359@cornell.edu

*Pseudomonas syringae* pv. *tomato* DC3000 injects multiple effector proteins into plant cells via the type III secretion system (T3SS), enabling it to cause bacterial speck disease in *Arabidopsis*, tomato, and *Nicotiana benthamiana* (if effector gene *hopQ1-1* is deleted). Polymutant DC3000D28E lacks the 28 effector genes thought to produce the repertoire of active effectors, grows no better than T3SS-deficient DC3000 mutants, and can be restored to substantial virulence in *N. benthamiana* with just 8 of the missing effectors. Although these observations suggest that DC3000D28E is functionally effectorless, the mutant has two properties that indicate it may produce one or more other active effectors: it elicits cell death at high levels of inoculum in *N. benthamiana*, and flagellin-elicited production of reactive oxygen species in *N. benthamiana* is not observed unless T3SS genes are mutated. The known effector genes remaining in DC3000D28E were deleted, revealing that one of them, *hopAD1* (PSPTO4691), is responsible for both of these plant interaction phenotypes. Thus, HopAD1 appears to be a suppressor of flagellin-induced immunity but also a potential elicitor of effector-triggered immunity in *N. benthamiana* (although a previous observation that HopAD1 is toxic when expressed in yeast raises the possibility that the cell death in *N. benthamiana* is not defense related). Deletion of *hopAD1* from DC3000D28E or a Δ*fliC* (flagellin) derivative did not substantially improve bacterial growth in *N. benthamiana*, and ongoing experiments are exploring the impact of deleting *hopAD1* from DC3000D28E expressing the minimal functional repertoire of 8 effectors and from wild-type DC3000 and DC3000 Δ*hopQ1-1*.

**O31.014 The emergence of *Xanthomonas citri* pv. *citri* in Mali results from at least two independent introductions**

A. Leduc[1], C. Vernière[1], V. Ravigné[1], M. Magne[1], C. Boyer[1], K. Vital[1], P. Grygiel[1], C.C. Juhasz[1], Y. Traoré[2], I. onni[3] and O. Pruvost [1]
[1]CIRAD-Université de la Réunion, UMR PVBMT, Saint Pierre, La Réunion, F-97410 France ; [2]Institut Polytechnique Rural, Katibougou, Mali; [3]Institut du Développement Rural, Université Polytechnique de Bobo-Dioulasso, BP1091, Bobo-Dioulasso 01, Burkina Faso
Email: alice.leduc@cirad.fr

Citrus is threatened by various diseases included Asiatic Citrus Canker, caused by the bacterium *Xanthomonas citri* pv. *citri* (Xcc). This pathogen is listed as a quarantine organism in Europe and is re-emerging in many countries in Africa, including Mali in 2008. Pathogenicity tests showed that Malian strains were related to pathotype A which is pathogenic on a wide range of *Citrus* species. A Multilocus Sequence Analysis (MLSA) based on six housekeeping genes indicated the presence in Mali of two Sequence Types (ST): ST2 in 4 provinces and ST3, only present in Bamako. A strain collection (n=714) was genotyped with two molecular typing systems using Variable Number of Tandem Repeats (VNTRs). The first system, called MLVA-31, has 31 minisatellite markers and aims to analysis the genetic diversity of a worldwide collection of Xcc. The MLVA-14 called second system has 14 microsatellite markers and is useful for outbreak investigation and epidemics understanding. Both systems data suggested a

hypothetical epidemiological link between Malian population and a collection of 46 strains recently sampled in Burkina Faso. Furthermore, analysis of the Malian collection using MLVA-1 revealed a relatively low global genetic diversity (Ht = 0.37). In addition, presence of major clonal complexes supports the hypothesis of a recent emergence. Characterization and structuration of genotyping data defined two independent introductions of the pathogen in Mali. Our results provide additional information on the epidemiology of Xcc and its re-emergence in Africa and point out the importance of MLVA-based genotyping tools in molecular epidemiology analyses and global surveillance.

**O31.015 Small RNA plays big: Role of the small RNA ArcZ (RyhA) in regulating *Erwinia amylovora* pathogenesis**

*Q. Zeng and G.W. Sundin*
*Department of Plant, Soil, and Microbial Sciences, Michigan state university, East Lansing, MI, 48824, USA*
*Email: zengquan@msu.edu*

*Erwinia amylovora* is a bacterial plant pathogen and the causal agent of fire blight, an economically-significant disease that occurs on apples and pears throughout the United States. Pathogenesis of *Erwinia amylovora* requires motility during initial invasion, the type III secretion system (T3SS), and production of the exopolysaccharide (EPS) amylovoran. Production of the T3SS, EPS synthesis and biofilm formation, and motility functions all require multi-genic systems that are tightly regulated. Pathogen cells must rapidly sense their spatial location in the host environment, and respond to these cues by switching virulence genes on and off. When environmental situations dictate rapid on/off switching, post-transcriptional regulation offers an additional mechanism to more precisely fine-tune protein synthesis. In this study, we determined that an intergenic, non-translated small RNA molecule (sRNA) ArcZ, in concert with the sRNA chaperone Hfq, functions in *E. amylovora* pathogenesis to regulate the T3SS, cellular motility, amylovoran production, and biofilm formation. By up-regulating the master flagellar regulator *flhDC*, ArcZ and Hfq positively control bacterial motility. RNA secondary structure analysis revealed that *flhDC* forms a potential stem loop structure in its RBS region, and ArcZ de-represses this translational inhibition of *flhDC* by base-pairing with the 5'-untranslated regions (5' UTR) of *flhDC*. Mutational analysis identified that nucleotides 150 to 161 are the critical nucleotides of ArcZ for its interaction with *flhDC* and activation of bacterial motility. This novel sRNA-mRNA interaction model enables us to understand how *E. amylovora* precisely controls the expression of various virulence factors during plant infection.

**O31.016 Early steps in the infection process in two *Xanthomonas* spp. models: chemotaxis and biofilm formation**

*J. Garita[1,2], M. Sena[1], P. Sabuquillo[1], M.I. Bianco[3], E. Ferragud[1], C. Redondo[1] and J. Cubero[1]*
*[1]Instituto Nacional de Investigación y tecnología Agraria y Alimentaria (INIA), Crta de La Coruña km 7, 5, Madrid 28002, Spain; [2]Instituto Valenciano de Investigaciones Agrarias (IVIA). Crta Moncada Náquera Km 4,5, Valencia 46113, Spain; [3]Fundación Instituto Leloir, Laboratorio Genética Bacteriana, Buenos Aires, Argentina*
*Email: cubero@inia.es*

Chemotaxis and motility, as well as biofilm formation, are crucial steps in the interaction between some bacteria and the plants. *Xanthomonas* is a genus of bacteria characterized for a high strain-host specialization due to the different virulence mechanisms among the species or even among pathovars within the same species. In the present work we have studied differential patterns in chemotaxis and in the ability to form biofilms, as early virulence events, in different bacterial models that include *Xanthomonas arboricola* pv. *pruni* (*Xap*), causal agent of bacterial spot of stone fruits, and *Xanthomonas citri* subsp. *citri* (*Xcc*), which cause citrus bacterial canker. Differences in surface (swarming type) or in liquid medium movements (swimming type) among strains of *Xap* were found and correlated to their different growth and ability for biofilm formation as well as taxonomic classification performed by multilocus sequencing and carbon source utilization. Moreover, for *Xanthomonas citri* subsp. *citri*, chemotaxis motility and biofilm formation was also correlated with the host range. Narrow *Xcc* host range strains showed more swimming ability and less capacity for biofilm formation than wide host range strains. In addition, narrow and wide host range strains of *Xcc* differed in their different responses to the plant stimulus in chemotaxis or biofilm formation processes. Our results confirmed the role of such processes in the different virulence among the *Xanthomonas* studied.

**P31.001 Pathogenic diversity of *Xanthomonas oryzae* pv. *oryzae* strains from Punjab province of Pakistan**

*H.M.I. Arshad, J.A. Khan, K. Saleem, S.S. Alam and S.T. Sahi*
*Senior Scientist, Plant Protection Division, Nuclear Institute for Agriculture and Biology, P.O. BOX-128, Jhang Road, Faisalabad, 38000, Punjab, Pakistan*
*Email: Iagill_1@yahoo.com*

Bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is damaging the rice crop in Punjab province of Pakistan due to the lack of information on existence of local pathotypes and varietal rotation in a particular agro-ecological zone. For obtaining the information on exact situation of *Xoo* pathotypes in Punjab, a total of 300 *Xoo* strains, collected from 17 districts were

evaluated for their pathotypes grouping based on their virulence on six rice differentials carrying different Xa genes. Rice differentials and their genes used for virulence analysis were IRBB-4 (Xa 4), IRBB-5 (xa 5), IRBB-10 (Xa 10), IRBB-11 (Xa 11), IRBB-62 (Xa 4, Xa7, Xa21) and IR-24 (as a susceptible check). Among 300 strains of *Xoo*, 30 Pathotypes were identified. Pathotype 1, virulent to all resistant genes tested, comprised 13% of strains distributed in 12 districts with the percentage of 3, 5, 10, 33, 10, 8, 5, 10, 3, 3, 8 and 3 in Narowal, Sialkot, Sheikhupura, Hafizabad, Gujrat, Mandi Bahuddin, Nankana, Faisalabad, Chiniot, Okara, Sahiwal, Pak Patan respectively. The majority of strains (29%) in Pathotypes 2, 3, 9, 11, 12, 14, 15, 17 & 18 were virulent on three to four resistant genes including IRBB 62. The strains (6%) of pathotypes 10, 16 & 22 were virulent on three to four single resistant genes but avirulent to IRBB62 (a pyramid of Xa4, Xa7 and Xa21). Pathotype 30 comprised 6.% of strains, avirulent to all genes tested, distributed in Gujrawala, Sheikhupura, Hafizabad, Gujrat, Mandi Bahuddin, Nankana and Faisalabad.

**P31.002 Bacterial elicitors of the plant defence compounds, glucosinolates**

*S. Sahin, V.C Trenerry, J. Luck, I. Potter, R. Jones and S. Rochfort*
*School of Molecular Science, La Trobe University, Melbourne, Victoria, 3086, Australia*
*Email: sabiha_sahin@live.com.au*

A plant's defence system is activated though the physical and chemical process of infection by plant pathogens. Activation can lead to the up-regulation of pathogenesis-related compounds, including phytochemicals such as the glucosinolates which are important in plant-pathogen interactions. This study focuses on the potential to accumulate these compounds within plant systems as a strategy to offer improved disease resistance in Brassicas using two *Xanthomonas* species. The infection of seedlings with *Xanthomonas campestris* pv. *campestris* (*Xcc*) down-regulated all measured aliphatic glucosinolates within these plants. However, the up-regulation of aliphatic glucosinolates was observed in cabbage sprouts inoculated with non-host pathogen *X. oryzae* pv. *oryzae* (*Xoo*). This suggests that the presence of these bacteria can modulate the levels of defence-related chemicals resulting in either plant disease or plant immunity. Furthermore, we demonstrated that the elicitation of these compounds differed between host and non-host interactions. This study demonstrated that putative bacterial elicitors up-regulated 4-methoxy glucobrassicin within cabbage sprouts post-inoculation. The results indicated that the accumulation of this compound is not sufficient to protect the plant from disease and that the bacteria may be capable of inhibiting the production of 4-methoxy glucobrassicin hydrolysis products than are critical in defence. Thus, the levels of hydrolysis products in plant-bacterial interactions could provide further insight into the host specificity of plant bacteria and the mechanisms of plant defence and should be the focus of further investigation.

**P31.004 Soft rot of root chicory in Hokkaido and its causal bacteria**

*W.W. Lan, Y. Nishiwaki, S. Akino and N. Kondo*
*Laboratory of Plant Pathology, Division of Agrobiology, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido, 060-8589, Japan*
*Email: lanweiwei1016@gmail.com*

In August 2010, bacterial soft rot was found on root chicory (*Cichorium intybus* L. var. *sativum* Bisch.) in Hokkaido, Japan. Severely infected plants in fields show discoloration, wilting of foliage, and black necrosis of petioles near the crown. Wilted leaves subsequently collapse and die, forming a dry, brown or black rosette. The root and crown become partially or wholly soft-rotted. On the infected root area, the slimy mass turns dark brown or black. The causal bacteria are gram-negative rods, peritrichously flagellated and facultatively anaerobic. These bacteria were exclusively isolated from the rotted roots, and typical symptoms were reproduced after inoculation with the strains. Consequently, the bacteria were identified as *Dickeya dianthicola*, *Pectobacterium carotovorum* subsp. *carotovorum*, and *P. carotovorum* subsp. *odoriferum* based on further bacteriological characterization and the sequence analysis of malate dehydrogenase gene and the 16S rRNA gene. These bacteria should be added along with the previously reported *Dickeya* (=*Erwinia*) *chrysanthemi* in Saitama Prefecture, Japan, as causal pathogens of bacterial wilt in chicory.

**P31.005 Exploring the elimination of viruses and *Liberibacter* from potato, using cryotherapy and tissue culture**

*P.J. Fletcher, J.D. Fletcher, F.A. Shah and S.A. Harrow*
*The New Zealand Institute for Plant & Food Research Limited, Private Bag 4704, Christchurch 8014, New Zealand*
*Email: pam.fletcher@plantandfood.co.nz*

Traditionally, in New Zealand, viruses have been eliminated from potato (*Solanum tuberosum* L.) by using a combination of chemical and heat therapy of *in vitro* growing shoot tips or *in vivo* growing meristems. Cryotherapy, a new and possibly more effective method for virus elimination, is being explored. A previous investigation, using a protocol based on cryogenic encapsulation-vitrification, successfully eliminated viruses PVS and PVA from potato. More than 500 shoot tips from thirteen potato cultivars were used in these experiments. Recently, we have used cryogenic droplet-vitrification

technique whereby approximately100 shoot tips from the potato cv 'Moonlight' have been experimentally processed. In New Zealand potatoes, damage caused by the disease Candidatus *Liberibacter solanacearum* (transmitted by *Bactericera cockerelli*, the tomato potato psyllid or TPP) has become a serious problem. There is an urgent need to eliminate this pathogen from potato seed. Cryotherapy was considered a suitable method. Initially, pathogen tests for *Liberibacter*, using semi-nested qPCR assays, were conducted. *In vitro* tissue cultures were established from *Liberibacter* infected plants. These cultures appeared symptomless and grew vigorously. Cultures that were retested by qPCR assays determined that the pathogen had been significantly reduced or eliminated. This experiment and assays have been repeated over two seasons and we conclude that this relatively simple method of establishing tissue cultured plantlets of *Liberibacter* infected cv 'Moonlight' potatoes results in the significant reduction or elimination of the disease.

### P31.006 Characterizations of various opine types *Agrobacterium* virulence on different plant species

*H.H. Hwang[1], E.T. Wu[1], S.C. Chang[2] and K.C. Tzeng*
*[1]Department of Life Sciences, National Chung-Hsing University, Taichung, Taiwan, China; [2]Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan, China*
*Email: hauhsuan@dragon.nchu.edu.tw*

*Agrobacterium tumefaciens* is a Gram negative bacteria and cause plants to produce crown gall disease due to the transfer, integration, and expression of oncogenes encoded by the T-DNA (transferred DNA) region of the Ti-plasmid (tumor-inducing plasmid). A set of transferred genes directs the production of bacterial nutrients, called opines, formed by condensation of an amino acid and a keto acid or a sugar. Transformed cells synthesize and secrete significant quantities of particular opines which are then utilized by *A. tumefaciens* as a carbon and sometimes nitrogen source. *A. tumefaciens* are usually classified on the basis of the opines they can catabolize. The most well studied strains are octopine, nopaline, agropine, succinamopine, and leucinopine. Because of the ability to transfer DNA between different kingdoms, the *A. tumefaciens* is also frequently used to generate genetic modified crops. Here, we examined the tumorgenesis and transient transformation efficiencies of five known and six uncharacterized wild-type *A. tumefaciens* strains on selected plant species. We have tested two to three plant species among ten different plant families, including *Brassicaceae, Asteraceae, Solanaceae, Apiaceae, Leguminosae, Cucurbitaceae*, and so on. Results shown in this study demonstrated that several uncharacterized wild-type *A. tumefaciens* strains showed comparable and/or higher transformation efficiencies than the known *A. tumefaciens* strains on several economical important crops. These data suggest further characterizations of these unknown wild-type *A. tumefaciens* strains may provide new *A. tumefaciens* strains to generate important genetic modified crops easier and faster.

### P31.007 Tzs is involved in *Agrobacterium* virulence and growth

*H.H. Hwang[2], Y.L. Lee[2], Y.H. Li[2], F.J. Yang[2], T.F. Cheng[2], Y.L. Tsai[1] and E.M. Lai[1]*
*[1]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan, China; [2]Department of Life Sciences, National Chung-Hsing University, Taichung, Taiwan, China*
*Email: hauhsuan@dragon.nchu.edu.tw*

*Agrobacterium tumefaciens* is an organism capable of trans-kingdom DNA transfer, transforming mainly plants but also other eukaryotic species, from fungi to human cells. Genetic transformation by *A. tumefaciens*, which in plants causes neoplastic growths called "crown gall", results from the transfer and integration of a specific DNA fragment (transferred DNA or T-DNA) from the bacterium into the plant genome. Here, we characterized a Ti-plasmid encoded gene, *tzs* (*trans*-zeatin synthesizing), that is responsible for the synthesis of a plant hormone cytokinin in *A. tumefaciens* when bacteria were induced by a phenolic compound acetosyringone (AS). To determine the role(s) of *tzs* in *A. tumefaciens* virulence, two *tzs* deletion mutants (Δ*tzs*-277 and Δ*tzs*-278) and three *tzs* frame-shift mutants were generated and characterized. High performance liquid chromatography (HPLC) analyses demonstrated the *tzs* deletion and frame-shift mutants produce no *trans*-zeatin under AS inductions. Both *tzs* deletion and frame-shift mutants reduce stable and transient transformation efficiency in *Arabidopsis* roots, suggesting that Tzs is likely involved in step(s) prior to T-DNA integrations. The expression of *tzs* on a plasmid rescued the phenotypes of frame-shift mutants in both *trans*-zeatin production and transformation efficiency. The exogenous applications of cytokinin during infections also restored the transient transformation efficiencies in the *tzs* mutants, suggesting that the cytokinin is responsible for the efficient transformation on *Arabidopsis* roots. The *tzs* mutants were able to enhance transformation efficiency on green pepper and cowpea, reduce transformation efficiency on white radish and other plant species. Interestingly, the *tzs* mutants are impaired in cell viability and/or growth in both AS-induced and infection conditions. Several *vir* gene promoter activities and Vir protein expression levels are increased in the *tzs* mutants. These data strongly suggest that Tzs, likely via synthesizing *trans*-zeatin at early stage(s) of infection process, is involved in the transformation efficiency of *A. tumefaciens* and may play different roles in different host plants.

**P31.008 Classification of *Ralstonia solanacearum* isolates from diseased Eucalyptus using phylotyping and DNA sequence analyses**

G.D. Carstensen, S.N. Venter, M.J. Wingfield and T.A. Coutinho
*Department of Microbiology & Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa*
*Email: gabrielle.carstensen@fabi.up.ac.za*

*Ralstonia solanacearum* is the causal agent of bacterial wilt on a wide range of plant species. This pathogen has a worldwide distribution and was first reported infecting *Eucalyptus* trees in Brazil. *R. solanacearum* is considered to represent a "species complex", thus requiring a classification scheme that is able to differentiate between isolates. Initially *R. solanacearum* isolates were classified into races and biovars depending on the host and the biochemical properties of the isolate. Fegan and Prior (Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex, pp. 449 – 461, 2005) developed a classification scheme, known as phylotyping, which is based on the use of a multiplex PCR. The results of the multiplex PCR group *R. solanacearum* isolates into one of four phylotypes that loosely link each isolate to a geographic origin. In this study, phylotyping was used on a collection of *R. solanacearum* isolates from diseased *Eucalyptus* plants showing symptoms of bacterial wilt. In addition, the endoglucanase (*egl*) gene was sequenced for each isolate as part of a multilocus sequence analysis (MLSA) approach. The multiplex PCR revealed that the isolates obtained from Brazil grouped into Phylotype II (Americas) whereas isolates obtained from China, Indonesia and Africa grouped into Phylotype I (Asia). The same result was obtained for the phylogenetic analysis of the *egl* gene sequences. Future research will include the phylotyping of a greater number of isolates from other geographical areas, ultimately providing insights into the distribution and diversity of *R. solanacearum* on *Eucalyptus* spp.

**P31.009 Colonisation patterns of a mCherry-tagged *Pectobacterium carotovorum* subsp. *brasiliense* strain in potato plants**

G.C. Kubheka, T.A. Coutinho and L.N. Moleleki
*Department of Microbiology and Plant Pathology, Forestry Agriculture and Biotechnology Institute (FABI) University of Pretoria, Lunnon road, Pretoria 0002, South Africa*
*Email: Lucy.moleleki@up.ac.za*

*Pectobacterium carotovorum* subsp. *brasiliense* (*Pcb*) is a newly identified member of the potato soft rot enterobacteriaceae (SRE). The pathogenesis of this pathogen is still poorly understood. In this study, a mCherry-*Pcb* tagged strain was generated to study *Pcb*-potato plant interactions. Prior to use, the tagged strain was evaluated for *in vitro* growth, plasmid stability and virulence on potato tubers and shown to be similar to the wild type. Four potato cultivars were evaluated for stem-based resistance against *Pcb*. Confocal laser scanning microscopy and *in vitro* viable cell counts showed that *Pcb* is able to penetrate roots of a susceptible potato cultivar as early as 12 hpi and migrate upwards into aerial stem parts. Due to the phenotypic differences observed between tolerant and susceptible cultivars, a comparison of *Pcb* colonisation patterns in these cultivars was undertaken. In the susceptible cultivar, *Pcb* cells colonised the xylem tissue forming 'biofilm-like' aggregates that led to occlusion of some of the vessels. On the contrary, in the tolerant cultivar, *Pcb* appeared as free swimming planktonic cells with no specific tissue localisation. This suggests that there are resistance mechanisms in the tolerant cultivar that limit aggregation of *Pcb in planta* and hence the lack of symptom development in this cultivar.

**P31.010 Fourier transform infrared microspectroscopy for rapid identification of gram-negative bacteria causal agent of bacterial leaf blight on cassava**

M. Sompong[1], K. Thumanu[2], R. Sungpueak[1], D. Athinuwat[3], S. Prathuangwong[4] and N. Buensanteai[1]
[1]*School of Crop Production Technology, Institute of Agriculture Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand;* [2]*Synchrotron Light Research Institute (public organization), Ministry of Science and Technology , Nakhon Ratchasima, 30000 Thailand;* [3]*Major of Organic Farming Management, Faculty of Science and Technology, Thammasat University, Pathumthani, 12121 Thailand;* [4]*Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900 Thailand*
*Email: Mathukorn_sompong@hotmail.com*

*Xanthomonas axonopodis* pv. *manihotis* is the most common phytopathogenic bacteria, causal agent of bacterial leaf blight (BLB) on cassava. In recent decades of commercial cassava production in Thailand, the cassava leaves has been colonized by other *Xanthomonas* pathovar. Therefore, in the present study, we developed a novel strategy for the rapid identification of *X. axonopodis* pv. *manihotis* based on Fourier transform infrared microspectroscopy (FTIR). Two reference strains of *X. axonopodis* pv. *manihotis* and *X. axonopodis* pv. *cassavae* were used in this study. The isolates were identified according to the guidelines of patogenicity test. The causal agents of BLB were further identified by 16S rDNA and sequencing standardized experimental protocol. FTIR spectral database containing more than 200 infrared spectra was investigated. Our results demonstrated that the high degree of reliability and strong potential of FTIR spectrum analysis for the rapid identification of causal agent of BLB suitable for use in routine disease diagnosis of BLB cassava.

**P31.011 Functional analysis of quorum sensing systems in *Pantoea ananatis***

*S. Sibanda[1,2], J. Theron[1] and T.A. Coutinho[1,2]*
*[1]Department of Microbiology and Plant Pathology, University of Pretoria, South Africa; [2]Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa*
*Email: teresa.coutinho@fabi.up.ac.za*

The plant pathogenic bacterium, *Pantoea ananatis* is believed to regulate its virulence by a collective group behavior called quorum sensing. An analysis of the genome sequence of the pathogen revealed three quorum sensing systems, namely, EanI/R, RhlI/R and LuxS. Analyses of specific virulence phenotypes regulated by these quorum sensing systems were investigated. The phenotypes examined included pathogenicity assays on onion seedlings, biofilm formation, exopolysaccharide production, motility and rhamnolipid synthesis. Mutants with defects in all or one of the three quorum sensing systems were used. It was shown that the virulence of *P. ananatis* on onions is regulated by quorum sensing. Furthermore, it was shown that the quorum sensing mutants, EanΔI/R,RhlΔI/R, ΔLuxS, as well as EanΔI/R and RhlΔI/R were deficient in biofilm formation and strain EanΔI/R was impaired in exopolysaccharides production. In *P. ananatis*, the synthesis of rhamnolipids and motility does not appear to be regulated by quorum sensing. The impaired phenotypes were restored in the complemented strains, EanΔI/R::EanI/R, RhlΔI/R::RhlI/R and ΔLuxS::LuxS. This study thus provides valuable insight into quorum sensing gene regulation in *P. ananatis.*

**P31.012 Unculturable state of *Ralstonia solanacearum* induced by low temperature in modified artificial soil microcosm**

*H.G. Kong[1], H.J. Joo[1], J.Y. Bae[2], E.J. Jung[2] and S.W. Lee[1,2]*
*[1]Department of Applied Biology, Dong-A University, Busan 604-714, Republic of Korea; [2]Department of Medical Bioscience, Dong-A University, Busan 604-714, Republic of Korea*
*Email: khgidea@naver.com*

Plant pathogen *Ralstonia solanacearum* has been known to enter into viable but nonculturable (VBNC) state at low temperature in water or soil microcosm. Here, physiology and gene expression of cold stress induced unculturable *R. solanacearum* SL341 strain were investigated over time at two different temperatures (25℃and 4℃) in modified artificial soil microcosm (mASM). The mASM contains the essential chemical components of soil except humic acid. Culturability and viability of *R. solanacearum* retained over a month at 25℃ in mASM. However, any detectable colony formation was not detected on the tested culture media from the mASM after 10 days at 4℃. There were no distinct differences of total DNA and protein contents of SL341 strain at two different temperatures. The Culturability of nonculturable *R. solanacearum* SL341 cell at 4℃ was not recovered by temperature upshifting or adding nutrient medium, but recovered by supplementation of catalase. To analyze gene expression of *R. solanacearum* in the VBNC cells, we isolated total RNA from bacterial cells maintained in mASM and performed reverse transcription-PCR. Expression of outer membrane porin proteins and *oxyR* genes were not different at two temperatures. This result suggested that the bacterial cells are viable but nonculturable at 4℃ in the ASM. Further extensive analysis of gene expression in unculturable *R. solanacearum* in mASM conditions are under investigation.

**P31.013 Bacterial leaf spot disease on kiwifruit cultivars 'Hayward' and 'Hort16A' caused by *Acidovorax valerianellae* in Korea**

*Y.S. Lee, K.H. Kim, H.S. Koh, J.E. Park, Y.J. Koh and J.S. Jung*
*Department of Biology and [1]Department of Plant Medicine, Sunchon National University, Suncheon 540-950, Republic of Korea*
*Email: jjung@sunchon.ac.kr*

A new bacterial leaf spot disease on kiwifruit cultivars 'Hayward' (*Actinidia deliciosa*) and 'Hort16A' (*A. chinensis*) was found during the rainy season of 2012 in Korea. Typical symptoms were characterized by dark brown irregular spots without halos on the leaves of kiwifruit. Bacterial strain isolated from the infected leaves were gram negative and formed 1-2 mm white colonies after 48 h of incubation on PS agar at 28 ℃. Pathogenicity tests were conducted on young kiwifruit leaves and typical spot symptoms were appeared on leaves after 3 days of artificial inoculation. The bacterium was successfully re-isolated from the diseased lesions, thus satisfying Koch's postulates. The 16S rDNA region of the isolates showed similarity of 99.3% (11/1492) with type strain of *Acidovorax valerianellae*, which cause black leaf spots of lamb's lettuce [*Valerianella locusta* (L.) Laterr.]. They shared 100% sequence identity with *A. valerianellae* strain U1 and strain 11-70 isolated from tea [*Camellia sinensis* (L.) Kuntze] leaves and watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], respectively. However, strain U1 and 11-70 did not induce any symptoms on kiwifruit leaves in pathogenicity tests. Biochemical and physiological tests, DNA-DNA hybridization and comparison of partial nucleotide sequences of *gyrB*, *gap*, *recA* and *rpoB* were performed to differentiate *A. valerianellae* strains.

**P31.014 First report of *Xanthomonas axonopodis* pv. *phaseoli* on Mungbean (*Vigna radiata*) in Iran**

*E. Osdaghi[1] and A. Sadr[2]*

[1]*Department of Plant Protection, Faculty of Agriculture, Ramin Agricultural and Natural Resources University, Ahwaz, Khuzestan, I.R. Iran;* [2]*Department of Animal Science, Faculty of Food and Animal Science, Ramin Agricultural and Natural Resources University, Ahwaz, Khuzestan, I.R. Iran*
*Email: osdaghi@yahoo.com*

During the summer of 2011 and 2012, several mungbean fields with suspected bacterial disease symptoms were recorded in the Southwest Iran (Khuzestan province: Shushtar, Dezfoul, Andimeshk, Mollasani and Gotvand areas). The symptoms included leaf blight and irregular necrotic spots surrounded by a thin chlorotic and/or water-soaked halo with incidence ranging from 80% to 90% in the surveyed fields. Diseased samples were collected from the fields in the Southwest Iran to verify bacterial infection. Leaf tissue was cut from the advanced portions of the necrotic areas, surface sterilized with household bleach (5.25% active sodium hypochlorite), and chopped up with a sterile scalpel in a droplet of water in a plastic Petri dish. Then, a loopful of the resulting suspension was streaked on Nutrient Agar (NA) medium (Audy *et al.,* 1994). Yellow, circular and raised bacterial colonies consistently appeared after 48 h of incubation at (conditions?); these colonies were subcultured, purified and identified using standard bacteriological techniques as described by Lak *et al.,* (2002). Five selected representative bacterial strains (Xapk1-5) and the previously identified *X. axonopodis* pv. *phaseoli* "Araxa1" strain (Osdaghi *et al.,* 2009) were subjected to biochemical and molecular identification. All yellow pigmented isolates were gram negative, rods, had a mucoid growth on yeast dextrose chalk agar and oxidative but not fermentative metabolism. All hydrolyzed gelatin, starch, esculin and casein, produced acid from arabinose, and hydrogen sulphide from cysteine. They were also positive for catalase and lecithinase. All isolates were negative for the presence of pectinase, oxidase, arginine dihydrolase and nitrate reductase. *X. axonopodis* pv. *phaseoli*–specific primers (X4c, 5'-GGC AAC ACC CGA TCC CTA AAC AGG-3′ and X4e, 5'-CGC CGG AAG CAC GAT CCT CGA AG-3′) were used in PCR to confirm the identity of isolates (Audy *et al.,* 1994). As expected, a 700-bp DNA fragment was amplified with the X4c/X4e primer pair. Pathogenicity test were carried out using 48 h old cultures of all the putative *Xapk* strains grown on NA. The inoculum was prepared in sterile distilled water at a concentration of about $1\times10^7$ cfu $ml^{-1}$ as described by Osdaghi *et al.,* (2009). Inoculation was done in triplicates by spraying 20 days old Mungbean plants to the point of runoff. Three plants inoculated with sterile distilled water only were used as negative controls. The inoculated plants were covered with transparent polythene bags for 24 h and incubated at 26℃ for 10 days in a greenhouse. Inoculated plants produced symptoms similar to those naturally observed on young leaves in the field. Small water-soaked spots started to appear 10-12 days after inoculation and continued to be yellow (Gilbertson and Maxwell, 1992). All of the isolates obtained in field surveys and identified as pathogenic on mungbean were *X. axonopodis* pv. *phaseoli*. To our knowledge, this is the first report of common bacterial blight caused by *X. axonopodis* pv. *phaseoli* on mungbean in Iran.

**P31.015 Functional and genetic analysis of Race 4 isolates of *Ralstonia solanacearum* causing bacterial wilt of Zingiberaceae plants**

A. Kumar, *T.P. Prameela*[1]*, R. S. Bhai*[1]*, M. Anandaraj*[1] *and B.A. Vinatzer*[2]
*Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012, India;* [1]*Division of Crop Protection, Indian Institute of Spices Research, Kozhikode-673012, Kerala, India;* [2]*551 Latham Hall (0390) Virginia Tech, Blacksburg, VA 24061, USA*
*Email: kumar@iari.res.in*

Functional and genetic analysis of Race 4 isolates of *Ralstonia solanacearum* causing bacterial wilt of Zingiberaceae family plants in India was conducted. The analysis revealed intra-racial diversity and dominance of Race 4/Biovar 3(R4/B3) isolates over Race 4/Biovar 4 (R4/B4) in India. A virulent lineage of R4/B3 isolate caused severe wilt incidence on Zingiberaceae plants and was found to spread across diverse geographical, host and seasonal boundaries that can be categorized as fast wilting isolate. The fast wilting isolate caused rapid wilt in ginger in 5-7 days and successfully infected several other closely and distantly related hosts such as turmeric (*Curcuma longa*); aromatic turmeric (*Curcuma aromatica*); black turmeric (*Curcuma caesia*); sand ginger (*Kaempferia galanga*); zedoary (*Curcuma zeodaria*); awapuhi (*Zingiber zerumbet*), galangal (*Alpinia galanga*); globba (*Globba* sp) & small cardamom *(Elettaria cardamomum*) in Zingiberaceae and tomato (*Solanum lycopersicon*) in Solanaceae family. The genotypic methods such as Phylotyping, Sequence analysis of 16SrDNA, 16-23S-intergenic spacer, & *rec*N gene and Multi Locus Sequence Typing (MLST) using *gdh*A, *adk*, *gyr*B, *pps*A, *gap*A, *egl*, *hrp*B, and *fli*C further confirmed that the fast wilting R4/B3 isolates were genetically identical as they shared most of the eleven loci analysed. The genetically distinct mild strain, identified as singletons, causing slow wilt was found to cause self-limiting wilt which belongs to certain R4/B3 isolates and R4/B4 strain. The high genetic homogeneity among the aggressive R4/B3 coupled with its rhizome borne nature further reinforces the hypothesis that the isolate has horizontally spread from bacterial wilt endemic location to other parts of India by latently infected seed rhizomes.

**P31.016 Reactions of Korean varieties of kiwifruits against old and new strains of *Pseudomonas syringae* pv. *actinidiae***

*G.H. Kim, C.S. Jeon, H.S. Koh[1], Y.S. Lee[1], J.S. Jung[1], Y.S. Cho[2], J.S. Hur[3] and Y.J. Koh*
*[1]Department of Plant Medicine and Department of Biology, College of Life Science and Natural Resources, Sunchon National University, Suncheon 540-900, Republic of Korea; [2]Fruit Research Institute of Jeonnam Agricultural Research and Extension Services, Wando 537-807, Republic of Korea; [3]Department of Environment Education, College of Education, Sunchon National University, Suncheon 540-900, Republic of Korea*
*Email: youngjin@sunchon.ac.kr*

The typical bacterial canker symptom developments were investigated on the leaves of Korean varieties of kiwifruits (*Actinidia* spp.) by artificial inoculation of various kinds of strains of *Pseudomonas syringae* pv. *actinidiae* (Psa). Bidan (*A. eriantha*), Chiak (*A. arguta*), Haegeum (*A. chinensis*), Haehyang (*A. chinensis*) and SKK10 (*A. chinensis*) which were recently released at farmers' fields in Korea were tested and their reactions were compared to those of the world-widely dominant varieties Hayward (*A. deliciosa*) and Hort16A (*A. chinensis*). Four strains of Psa were inoculated onto the leaves of seven varieties of kiwifruits on pots grown under greenhouse conditions and their reactions were evaluated 7 days after artificial wound inoculation.
Two old Korean strains CJW7 and KBE9 were isolated from Hayward in 1999 and Hort16A in 2008 and one newly emerging virulent strains ITK3 and SYS1 were isolated from Hort16A in Italy and Yellow King (*A. chinensis*) in Korea in 2011, respectively, which are responsible for the world widely destructive epidemics of bacterial canker on Hort16A in recent years. The dominant varieties Hayward and Hort16A and Chiak were susceptible to all the strains of Psa used in the study. Whereas Hayward was highly susceptible to all the strains, Hort16A were highly susceptible to two new strains but moderately susceptible to two old strains. Chiak were highly susceptible to 3 strains but moderately susceptible to CJW7, old Korean strain isolated from Hayward. SKK10 and Haehyang were more resistant than Hayward, Hort16A and Chiak to Psa. They showed moderately susceptible reactions to KBE9 and ITK3 but Haehyang showed hypersensitive reactions to CJW7 and SYS1 and SKK10 showed immune or hypersensitive reactions to CJW7 and SYS1, respectively. Haegeum and Bidan were highly resistant to all the strains of Psa. Haegeum showed hypersensitive reactions to two new strains but immune reactions to two old strains, and Bidan vice versa. The genetic backgrounds of highly resistant varieties Haegeum and Bidan are now under investigation for the possible utilization in the future breeding program as resistant gene sources to overcome Psa.

**P31.017 Lon is involved in negative regulation of *hrp* gene expression in *Xanthomonas oryzae* pv. *oryzae***

*S. Tsuge, Y. Kametani-Ikawa, A. Majima and A. Furutani*
*Laboratory of Plant pathology, Graduate School of Agriculture, Kyoto Prefectural University, Kyoto 606-8522, Japan*
*Email: y_kametani@kpu.ac.jp*

*Xanthomonas oryzae* pv. *oryzae* possesses *hrp* (hypersensitive reaction and pathogenicity) genes involved in composition of a type III secretion system (T3SS). The bacterium directly introduces effectors into plant cells through T3SS. HrpG (a putative response regulator of a two-component system) and HrpG-regulating HrpX (an AraC-type transcriptional regulator) are known as key regulators for *hrp* gene expression. Besides them, several *hrp* regulatory genes have been reported and a complicated regulatory network is predicted for the expression of *hrp* genes. To identify novel *hrp* regulatory genes, we generated a mutant with the *lux* operon controlled by the promoter of *hpa1*, which is one of the HrpX regulons, and a transposon was randomly inserted into the genome of the mutant. One of the mutants obtained showed higher bioluminescence than the parental strain under the *hrp*-inducing condition. Sequence analysis revealed that ATP-dependent serine protease (*lon*) is disrupted by the transposon insertion in the mutant. According to the semi-quantitative RT-PCR, transcripts of HrpX regulons were highly accumulated in the Lon deletion mutant compared with the wild type, although HrpG, HrpX and HrpA (another HrpG regulon) transcripts were at the similar levels between two strains. Western blot analysis revealed that accumulation of HrpX is significantly increased in the Lon mutant. These results suggest that Lon is involved in negative regulation of *hrp* gene expression by inhibiting accumulation of HrpX in *X. oryzae* pv. *oryzae*.

**P31.018 Seed infestation by bacteria causes disease symptoms on corn plant**

*S. Prathuangwong, S. Kasem and P. Khamkongkaew*
*Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand*
*Email: agrsupot@ku.ac.th*

The occurrence of bacterial disease of corn is estimably spreded by seed infestation. Use of diseases-free seeds and the available seed assays are essential for crop management. The 187 visually healthy seedlots of corn collected from different sources were analyzed to monitor the incidence of bacterial infestation using phenotypic and molecular identification. The randomly selected seeds were soaked overnight following standard protocols and aliquots of the extracts were plated onto a general agar medium. Potently bacterial colonies were tested for HR induction on either tobacco, tomato, or chilli plants. DNA extraction of only HR-inducing

strains was further conducted for species-specific PCR primers, sequencing, and polyclonal antibody analyses. The results revealed that the infestation of *Acidovorax avenae* subsp. *avenae*, *Pseudomonas putida*, and *Pantoea ananatis* were detected in 24, 15 and 7% of corn seed samples, respectively. The average colonies forming unit per seed with these 3 pathogens was found in a range of $10^3$ to $10^4$. Seed germination was not significantly different between infested and noninfested seed samples. Also, no visible symptom was observed on either seedling or adult plants grown from infested seeds. However, these bacteria demonstrated as virulent strains/primary inocula when they were inoculated onto seedling plants that exhibited clear symptoms of corn diseases similar to those natural infection. The study suggests the possibility of these diseases may play an important role in seed spread carrying bacterial pathogens. Their effects on seed quality and yield loss due to plant infection should be elucidated for effective management program.

**P31.019 Population typing of the causal agent of cassava bacterial blight in the Eastern plains of Colombia using two types of molecular markers**

*C.A. Trujillo, N. Arias, F. Gómez, L. Poulin, R. Koebnik and A.J. Bernal*
*Universidad de los Andes, Bogotá, Colombia*
*Email: ce-truji@uniandes.edu.co*

Molecular typing of pathogen populations is an important tool for the development of effective strategies for controls in the field. Diverse molecular markers have been used to characterize populations of the cassava bacterial pathogen *Xanthomonas axonopodis* pv. *manihotis* (*Xam*). Recently, diversity and population dynamics of *Xam* were estimated using AFLPs in the Caribbean and in the Eastern plains of Colombia. Populations in the Colombian Caribbean were more dynamic and diverse than populations from the Eastern plains. Eastern *Xam* isolates were mainly grouped in a common haplotype indicating that most of the isolates were not discriminated by AFLPs. To determine if there was encrypted diversity in the Eastern populations, the isolates were characterized using five loci of Variable Number Tandem Repeats (VNTRs), which are expected to be more discriminatory. Results obtained by both techniques were confronted to determine the discriminatory power of each technique in the study of *Xam* populations. VNTRs presented a high discriminatory power in the study of populations of pathogen that makes it interesting for its implementation for the surveillance pathogen. In addition, the time and cost of implementation of VNTRs were reduced in comparison to the use of AFLPs. This study shows that VNTR markers are ideal for the study of the dynamics of pathogen populations.

**P31.020 Deciphering the bacterial microbiome for HLB-affected citrus treated with antibiotics**

*M. Zhang[1,2,3], C.A. Powell[1], C. Yang[1,4] and Y. Duan[2]*
*[1]Indian River Research and Education Center, IFAS-UF, Fort Pierce, FL34945, USA; [2]USDA-ARS, US Horticultural Lab, Fort Pierce, FL34945,USA; [3]State Key Lab for Conservation and Utilization of Subtroical Agro-bioresources, Guangxi Univ., Nanning, Guangxi 530004, P. R. China; [4]Fujian Agricultural and Forestry Univ., Fuzhou 350002, P. R. China*
*Email: capowell@ufl.edu*

Huanglongbing (HLB), one of the most serious diseases of citrus worldwide, is caused by the fastidious bacterium (*Candidatus* Liberibacter) (Las), which is transmitted by a phloem-feeding insect (Asian citrus psyllid). To date, there is nowhere in the world where HLB is endemic that it is under adequate control, and wherever the disease does occur, it continues to increase in incidence and severity and causes great losses to the citrus industry. An ideal short-term control for the disease is to reduce the bacteria to levels that result in symptom remission after single courses of the thermo- or chemotherapy, either by acting directly on the bacteria or indirectly through induction of host defense compounds. We have evaluated the activities of the antibiotics gentamycin (Gm) and ampicillin (Amp) against Las bacterium using our optimized graft-based chemotherapy methods (soaking Las-infected lemon bud sticks in antibiotic solution followed by grafting the buds into uninfected grapefruit), and deciphered the bacterial microbiome in leaves from Las-free plants and plants grafted with buds from Las-infected plants treated with ampicillin, gentamicin or water by phylochip-based metagenomics (PhyloChip G3 array). The results showed that gentamicin had little effect on the '*Ca*. L. asiaticus' (Las) bacterium. However, ampicillin reduced the Las bacterium to undetectable levels (qtPCR) with no symptoms in graft-inoculated plants. A total of 7,407 bacterial OTUs in 53 phyla were detected out of 50,000+ known OTUs by PhyloChip G3 analysis from all combined treatments. Only 500 OTUs from 114 families were present in the Las-free grapefruit, including 24 OTUs from *Alcaligenaceae* with double fluorescent density (measure of total bacterial titer). However, 1,283 OTUs from 155 families were present in grapefruit grafted with Las-infected buds treated with water (control), including more OTUs from the families of *Comamonadaceae*, *Staphylococcaceae* and *Pseudonocardiaceae*. More than 4,679 OTUs from 218 families were detected in plants grafted with Las-infected buds treated with Gm, with OTUs in *P.* pseudomonas being especially abundant. Sixty-five families, such as *Pelobacteraceae,* had double fluorescence intensity in plants grafted with Gm-treated buds, when compared to the plants grafted with Amp-treated buds. Thirty-four families, such as *Nordellaceae,* doubled their fluorescence intensities in the Gm-treated plants when compared to the water control.

Only 700 OTUs from 132 families were recorded in the Amp-treatment, but no greatly different fluorescence intensity was observed in the families. Therefore, this study provides a comprehensive survey of the diversity and composition of leaf microbial communities in citrus treated by antibiotics.

**P31.021 Effect of drought stress on bioactive compounds and human pathogens survival on leafy vegetables**

*M. Alam, L. Mogren, S. Ahrné, G. Molin, P. Jensén, S. Boqvist, I. Vågsholm and B.W. Alsanius*
*Microbial Horticulture Laboratory, Department of Biosystems and Technology, Swedish University of Agriculture Sciences, P.O. Box 103, SE-23053 Alnarp, Sweden*
*Email: Mehboob.Alam@slu.se*

Fresh vegetables consumption increased worldwide. This may carry harmful human pathogens causing food illnesses, among these verotoxin producing *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica* or *Listeria monocytogenes*. Irrigation water with inferior hygienic quality is considered as one dominant route of transmission. Apart from different environmental factors, plant bioactive compounds may influence survival of human pathogens. We studied the interaction between concentrations of plant bioactive compounds in the plant tissue and persistence of culturable *E. coli* O157:H7 and *L. monocytogenes* on rocket salad, spinach and swiss chard leaves (grown in greenhouse) as affected by drought. Control irrigation was applied to plants by measuring percent volumetric water content (%vwc) of the growing media, so as to expose plants to drought stress. An attenuated strain of *E. coli* O157:H7 gfp+ and *L. monocytogenes* were mixed and sprayed with final irrigation water on these vegetables, at a density of log 7.6 CFU $ml^{-1}$ corresponding to an irrigation volume of 5 mm. Plants were harvested after 24 hours after irrigation with contaminated water, leaves were washed, and samples were serially diluted and spread on Luria-Bertani (LB) plates supplemented with L-arabinose and ampicillin for *E. coli* O157:H7 and Blood agar base supplemented with rifampicin for *L. monocytogenes* enumeration. *Enterobacteriaceae* and ambient microflora were monitored on violet red bile dextrose agar (VRBD) and Tryptic soy agar (TSA) plates, respectively. No differences were found in viable counts on VRBD and TSA. Culturable *E. coli* O157:H7 and *L. monocytogenes* decreased in numbers with increasing drought, with less counts on leaves exposed to drought. Concentrations on bioactive compounds and interactions between bioactive compounds and the introduced human pathogens will be displayed on the poster.

**P31.022 Genetic diversity of *Xanthomonas* from brassicas in Russia**

*A.N. Ignatov, E.I. Sheverdina, M.V. Sukhacheva, M.V. Egorova, K.P. Kornev, E.S. Mazurin and V. Karandashev*
*Center of Plant Quarantine, Moscow reg., Bykovo, Russia; Molecular Phytopathology Group, Center Bioengineering RAS, Moscow, Russia; Phytoengineering Ltd. Moscow reg., Russia*
*Email: an.ignatov@gmail.com*

Cultivated plants are infected by many bacteria of the genus *Xanthomonas pam.* (Dow.). The genus is divided into species, patovarianty serotypes and clonal groups (Alvarez et al., 1994; Vicente et al., 2002). Group of bacteria of the genus *Xanthomonas* differ in host plants and symptoms of disease. The pathogenic bacteria of the genus *Xanthomonas* were provided by the Institute of Phytopathology (B.Vyazemy, Moscow reg.) in the framework of the ISTC projects #1771 and #3431. *Xanthomonas* strains were studied by MLST / MLSA as described by Young et al. (2008) and Ignatov et al (2007) and by unpublished data of E. L. Schuenzel (FDWS-RU-USDA). Additionally, the gene fragments of protein of ice nucleation protein (inp) and intergenic fragment between two tandem copies of the tRNAala-tRNAglu repeat recommended by V.S. Zotov (2012) as marker for diverse bacteria. The result of study showed that strains of *Xanthomonas campestris* pv. *campestris*, isolated in Japan, USA, UK, Tanzania, Thailand, and Russia of vegetables (cabbage, cauliflower) belonged to a homogenous "epidemic" group for the majority of the studied genes. Strains isolated in Russia from oil brassicas were separated from the "epidemic" group. Some canola strains were identified as *X. raphani*, *X. hortorum* and *X. arboricola*. Some strains were similar to *X. campestris* on the sequence of the gene gyrB but close to *X. arboricola* by other genes. Previously, we found that a number of strains of *X. arboricola*, pathogenic for the cabbage, corn and sunflower isolated in the Moscow region and the Southern region of Russia had an identical gene cytP450 *X. campestris*, and suggested the presence of horizontal gene transfer between *X. campestris* and *X. arboricola*. Thus, we found further support for this hypothesis. Gene transfer can increase the adaptability of bacteria to new host plants. Only a few strains from oilseeds had inp gene in contrast to the strains from Brassica vegetables.

**P31.023 Detection of *Pseudomonas fuscovaginae* with LAMP**

*G.J. Ash, J.M. Lang, L.R. Triplett, B.J. Stodart, V. Verdier and J.E. Leach*
*EH Graham Centre for Agricultural Innovations (an alliance between Charles Sturt University and NSW DPI), Charles Sturt University, Locked Bag 588, Wagga Wagga 2678, NSW, Australia*
*Email: gash@csu.edu.au*

*Pseudomonas fuscovaginae* causes sheath brown rot of rice, which can also lead grain discolouration, panicle sterility and in severe cases, total yield loss. The disease is cosmopolitan and *P. fuscovaginae* often cohabits in mixed populations with other pathogens in the field. The symptoms can be confused with those produced by bacterial pathogens such as *Burholderia glumae, B. cepacia, Pantoea ananatis* and *Acidovorax avenae* subsp. *avenae.* Therefore a rapid diagnostic assay is required to differentiate *P. fuscovaginae* from these other pathogens and to help determine its distribution and importance. Loop-mediated isothermal amplification (LAMP) is a rapid and sensitive technique which has beenused widely as a diagnostic tool for plant, human and animal diseases, and which can be implemented in the field. We report on the use of genomic data to design a robust LAMP assay for the detection of *P. fuscovaginae*. LAMP primer design was based on conserved genomic regions identified using whole genome data from several isolates of *P. fuscovaginae* that represent the known extent of variability within the species. The specificity of this assay was established using *P. fuscovaginae* isolates from Africa, Asia, Australia and South America as well as a range of other common bacteria associated with rice.

**P31.025 Identification of proteins interacting with PdeR in *Xanthomonas oryzae* pv. *oryzae***

*H.Y. Li, F. Tian, H.M. Chen and C.Y. He*
*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China*
*Email: yousliyun@163.com*

Bis-(3'-5')-cyclic dimeric GMP (c-di-GMP) is a ubiquitous second messenger widely existing in bacteria, controlling pathogenicity, biofilm formation, motility and other biological functions. Diguanylate cyclases (DGCs) harboring GGDEF domain control the synthesis of c-di-GMP, while phosphodiesterases (PDEs) containing EAL or HD-GYP domain are responsible for its degradation. *Xanthomonas oryzae* pv. *oryzae* (Xoo), the causal agent of bacterial leaf blight of rice, is an important bacterial pathogen. The genome of Xoo strain PXO99$^{A}$ contains over 20 genes encoding such domains. Our lab recently has identified a response regulator PdeR from PXO99$^{A}$, containing GGDEF, EAL and REC domains. We showed PdeR possessed PDE activity, which was activated by its cognate histidine kinase PdeK. Deletion of *pdeR* or *pdeK* severely impaired the virulence of PXO99$^{A}$ on susceptible rice plants. In order to elucidate the signaling pathway involving PdeR, we carried out yeast two-hybrid screening assays to search for proteins interacting with PdeR at genomic level. Preliminary screening using PdeR as the bait has identified over 50 potential interactors. Some interesting ones include magnesium transporter (PXO_02891), general secretory pathway protein E (PXO_02675), and TonB-dependent outer membrane receptor (PXO_03611), etc. In addition, fluorescence microscopy has demonstrated that PdeR-GFP fusion protein was mainly localized at the poles of bacterial cells. These results implied that the function of PdeR might be involved in protein or ion translocation. Currently, we are working on confirming these interactions by far western blotting. Eventually, we want to find the real interactors of PdeR and understand their roles in the c-di-GMP signaling pathway.

**P31.026 Identification of *Acidovorax citrulli* using matrix assisted laser desorption Ionization time of flight mass spectrometry**

*J.J. Feng[1,2], G.M. Zhang[1,2], J.Q. Li[3], Y. Zheng[1,2], Y.N. Li[1,2], Y.H. Cheng[1,2] and Z.N. Chen[1]*
*[1]Shenzhen Academy of Inspection and Quarantine, Shenzhen, 518010, P. R. China; [2]Animal & Plant Inspection and Quarantine Technology Center, Shenzhen Entry-exit Inspection and Quarantine Bureau, Shenzhen, 518045, P. R. China; [3]Dept. Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: sccfjj@yahoo.com.cn*

Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) has proven to be a rapid and power tool for bacteria identification. Some databases used for this purpose lack enough reference profiles for *Acidovorax citrulli*, which has emerged as a serious threat to cucurbitaceous plant species worldwide, and also has been listed by EPPO and China as an official import plant quarantine pest. We have described for the first time the creation of profiles for MALDI-TOF Biotyper 2.0 database (Blruker Daltonics, Germany) and identification of *A. citrulli* from culture plates. Several sample preparation methods and matrices were investigated to improve mass spectra for the routine identification of *Acidovorax* species by MALDI-TOF MS. Using the optimized protocol, we constructed the database for 22 strains of *A. citrulli*, 4 strains of *A. avenae*, one strain each of *A. cattleyae* and *A. konjaci*. The identification of *A. citrulli* was conducted by matching these reference spectra contained in the database and calculating logarithmic final score value (log (score)), we also evaluated the capability and reproducibility of this analysis and establish an identification criteria for *A. citrulli* that log (score) is greater than 2.200. The 100% of the strains were correctly identified showing that MALDI-TOF MS assay is reliable for *A. citrulli* identification to the species level. Furthermore, a cluster analysis of mass spectra with the 22 strains of *A. citrulli* was performed, and the resulting dendrogram showed that there were at least two subgroups amongst global populations of the pathogen.

**P31.027 RFLP based characterization and comparative analysis of Spermidine/Putrescine ABC transporter gene of phytoplasma from Coconut, Sugarcane, Aster and Brinjal**

*R. Manimekalai, O.M. Roshna and A. Kumar*
*Senior Scientist, Molecular biology and Bioinformatics, Central Plantation Crops Research Institute, Kasaragod-671124, Kerala, India*
*Email: rmanimekalaiicar@gmail.com*

Root wilt disease (RWD) of coconut and grassy shoot diseases (SCGS) of sugarcane are phytoplasma associated diseases (16SrXI group). The present study aimed to characterize the phytoplasma spermidine / putrescine ABC transporter gene segments from coconut root wilt (16SrXI) and sugarcane grassy shoot (16SrXI) phytoplasma and their comparative analysis with aster yellows (16SrI) and brinjal little leaf (16SrVI) phytoplasma. We isolated full length *potC* gene of ATP-Binding Cassette (ABC) transporter system from RWD, SCGS, Aster yellows (AY) phytoplasma using two sets of primers PUTF3-R3 and PUTF4-R4 using PCR. We have done RFLP based gene characterization of *potC* gene. *In silico* and *In vitro* RFLP analysis has been performed. *In vitro* RFLP analysis includes 8 different restriction enzymes which gave appropriate differentiation among different phytoplasma. Virtual RFLP analysis has been carried out for the *potC* gene region from13 different phytoplasma species with 20 restriction enzymes using pDraw tool. Dendrogram was drawn from both *in vitro* and *in silico* RFLP data with NTSys software. In the dendrogram, coconut root wilt and sugarcane grassy shoot phytoplasmas were clustered together, Aster Yellow phytoplasma clustered with 16SrI group phytoplasmas and brinjal little leaf phytoplasma is separately placed. The dendrogram drawn from RFLP of *potC* gene follow 16SrRNA based taxonomic classification of phytoplasma. Thus *potC* can be considered as potential candidate for phylogenetic studies.

**P31.028 Identification of PXO_00403 from *Xanthomonas oryzae* pv. *oryzae* as a c-di-GMP receptor to regulate bacterial virulence**

*F.H. Yang, F. Tian, X.T. Li, S.S. Fan, H.M. Chen, M.S. Wu and C.Y. He*
*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China.*
*Email: fhyang1982@163.com*

*Xanthomonas oryzae* pv. *oryzae* (Xoo), the causal agent of bacterial leaf blight of rice, has been an important model to study bacterium-plant interactions. The genome of Xoo strain PXO99$^{A}$ contains over 20 genes encoding GGDEF, EAL, or HD-GYP domain proteins, which are potentially involved in the c-di-GMP signaling pathway. However, the roles they play and their biochemical features are largely unknown. Here, we characterized one of them PXO_00403, which possesses both GGDEF and EAL domains. Mutation of *PXO_00403* in PXO99$^{A}$ attenuated the bacterial ability to multiply in susceptible rice, and caused a delayed hypersensitive response (HR) on non-host tobacco. Both *in silico* analysis and enzyme assays indicated that the GGDEF and EAL domains of PXO_00403 were degenerate and inactive. In addition, isothermal titration calorimetry (ITC) demonstrated that PXO_00403 protein binds c-di-GMP with high affinity as a stoichiometry of 1:1 *in vitro*. A truncated protein PXO_00403GR without EAL domain showed no binding to c-di-GMP, suggesting the degenerate EAL domain is critical for the binding activity. Expression of PXO_00403GR in the mutant strain was not able to restore disease phenotype to wild-type level. These results suggested that c-di-GMP binding ability is required for the function of PXO_ 00403 *in vivo*.

**P31.029 Preliminary studies on potato blackleg disease in China**

*X.M. She[1,2] and Z.F. He[1]*
*[1]Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, P. R. China; [2]Guangdong Provincial Key Laboratory of High Technology for Plant Protection, Guangzhou 501640, P. R. China*
*Email: hezf@gdppri.com*

Potato (*Solanum tuberosum* L.) is one of important crops in China. In 2013, a bacterial disease of potato, blackleg and soft rot, was found in Guangdong Province, China，with an incidence about 20%. The diseased plant exhibited yellow, and the stem base blackened and rotted, which were similar to the symptoms of potato blackleg (stem rot) disease described by Pérombelon (1992). Twenty diseased plants with typical symptoms were collected from the potato fields. A total thirty bacterial strains were isolated from the vascular tissue of diseased plants on nutrient agar medium. The potato plants (cv. Favorita) inoculated with ten strains exhibited the same symptom as the diseased potato plants in fields. Using the universal bacterial 16S rDNA primer set 27f/1541R, approximately 1,400 bp-fragments were amplified from the ten strains, respectively. BLAST results showed that the sequences of the ten fragments were identical and had 100% identities with 16S rDNA of *Pectobacterium atrosepticum* CFBP 1526 (GenBank accession JN600332). Further, the 438-bp and 690-bp specific fragments were respectively amplified from all ten strains with the specific primers of *P. atrosepticum* Y45/Y46 and ECA1f/ECA2r. These results indicated that the pathogen causing potato blackleg and rot belonged to *P. atrosepticum*.

**P31.030 Five species of *Xanthomonas* associated with bacterial leaf spot symptoms in tomato from Tanzania**

*E.R. Mbega[1, 2], R.B. Mabagala[2], J. Adriko[1], O.S. Lund[1], E.G. Wulff[1] and C.N. Mortensen[1]*
*[1]Danish Seed Health Centre, Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Hoejbakkegaard, Allé 13, 2630 Taastrup, Denmark; [2]African Seed Health Centre, Department of Crop Science and Production, P.O.BOX 3005, Sokoine University of Agriculture, Morogoro, Tanzania*
*Email: mbegaernest@yahoo.co.uk*

From 2008 to 2010, leaf spot symptoms were observed on tomato (*Solanum lycopersicum* Mill.) plants growing in the northern, central and southern highland regions of Tanzania. Symptoms were dark, circular to irregular, water-soaked spots surrounded by chlorotic halos. A total of 136 yellow-pigmented Gram-negative bacteria were isolated from 117 symptomatic plants on nutrient agar. Loopfuls from 24-h-old bacterial cultures were suspended in 500 µl of sterile distilled water and 50 µl of the suspensions were printed on strips of 3MM Whatman chromatography paper. Isolates belonging to the genus *Xanthomonas* were subsequently identified by PCR amplification of a 402-bp fragment of the Xanthan synthesis pathway gene, *gumD* (primers: X-gumD-fw 5′ GGCCGCGAGTTCTACATGTTCAA and X-gumD- rv 5′CACGATGATGCGGATATCCAGCCACAA). Thirty of the 136 isolates reacted positively in *gumD* PCR. Pathogenicity of the 30 *gumD*-positive isolates was confirmed by spraying cell suspensions containing $10^8$ CFU/ml ($OD_{600}$=0.01) of each isolate on four 14-day-old tomato seedlings (cv. Tanya) and sweet pepper (*Capsicum annuum* L.) cv. Early-Calwonder in a growth chamber at 28±2℃ and maintained under humid conditions. Plants sprayed with *X. euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri* (2) strains NCPPB 2968, 422, 4321, and 881, respectively, served as positive controls. Plants sprayed with sterile distilled water alone served as negative control. The 30 tested isolates were pathogenic on tomato and pepper within 7 to 14 days and induced similar symptoms as those observed on tomato field plants and plants sprayed with reference strains of *Xanthomonas*. Symptoms were not observed on negative control plants. Yellow-pigmented colonies were reisolated from symptomatic plants and their identity confirmed with *GumD*-PCR. Based on partial sequencing of the *fyuA* gene using primers developed by Young et al. (4), all 30 isolates were subsequently grouped into five clusters of the genus *Xanthomonas*. With recent taxonomy of *Xanthomonas* (2,4), four of these clusters displayed more than 99% sequence identity to known species of *Xanthomonas*: *X. arboricola* EU498923 (18 isolates); *X. perforans* EU498944 (6 isolates), *X. vesicatoria* EU498876 (2 isolates), and *X. euvesicatoria* EU498912 (1 isolate). The remaining three isolates formed a fifth cluster displaying less than 94% sequence identity to any known sequence of *fyuA* (93% matching strains: *X. axonopodis* EU498914; *X. melonis* EU498918, and *X. cucurbitae* EU498891). Representative sequences for each of the five clusters of bacterial leaf spot (BLS) strains mentioned have been deposited in GenBank (Nos. JQ418487, JQ418488, JQ418489, JQ418490, and JQ418491, respectively). BLS of tomato plants and its economic impact has been reported in Tanzania (3). Different BLS causal agents have recently been reported from the Southwest Indian Ocean Region (1), however, corresponding information for Tanzania has been lacking. On the basis of *fyuA* sequences, this study reports four genotypes of BLS causal agents corresponding to known species of *Xanthomonas*. In addition, *Xanthomonas* isolates with a *fyuA* genotype not previously assigned to any known species has been identified as part of the BLS pathosystem in Tanzania.

**N31.001 Paulownia witches'-broom phytoplasma thymidylate kinase: cloning, prokaryotic expression and preparation of its polyantibody**

*C.S. Song, J.X. Hu, C.L. Lin, Z.G. Ren, X.S. Geng and G.Z. Tian*
*Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry/The Key Laboratory of Forest Protection, State Forestry Administration, Beijing 100091, P. R. China*
*Email: tiangz@caf.ac.cn*

Thymidylate kinase (TMK) catalyses the phosphorylation of dTMP to form dTDP in both the *de novo* and salvage pathways dTTP synthesis. In previous study, two homologues of thymidylate kinase genes were identified in onion yellows (OY) phytoplasma. TMK-b overexpressed in *Escherichia coli* was shown to have thymidylate kinase activity, while TMK-a not. In this study, to investigate the function of TMK-a, many copies of *tmk-a-1* ORFs (639 bp), *tmk-a-2* ORFs (627 bp) and *tmk* pseudogenes with sequence diversity were cloned from paulownia witches'-broom phytoplasma. All of them have three specific motifs that involved in NTP/NMP binding. All *tmk-a-1* ORFs have one specific sequence GTTCCTCAATTA but not in all *tmk-a-2* ORFs and there is no difference between *tmk-a-1* ORFs and *tmk-a-2* ORFs except the specific nucleotide residue site. To explore whether the TMK-a protein could be translated and play a part in paulownia witches'-broom phytoplasma, the polyHis-tagged TMK-a-1 fusion protein was over-expressed in *E. coli* BL21 (DE3) and purified by Ni-NTA column, and then the polyclonal antiserum to TMK-a-1 protein were obtained from rabbit by immunisation. The result suggested by western blotting that TMK-a-1 protein was expressed in paulownia witches'-broom phytoplasma. Prokyraotic expression and antibody preparation of TMK-a-2 protein are going on. Next step catalytic activities of both TMK-a-1 and TMK-a-2 will be determined.

**N31.002 Proteomic analysis reveals novel extracellular virulence-associated proteins and functions regulated by the diffusible signal factor (DSF) in *Xanthomonas oryzae* pv. *oryzicola***

*G. Qian, Y. Zhou, Y. Zhao, Z. Song, S. Wang, J. Fan, B. Hu and F.Q. Liu*
*College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China/Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, P. R. China*
*Email: fqliu20011@sina.com*

Quorum sensing (QS) in *Xanthomonas oryzae* pv. *oryzicola* (Xoc), the causal agent of bacterial leaf streak, is mediated by the diffusible signal factor (DSF). DSF-mediating QS has been shown to control virulence and a set of virulence-related functions, however, the expression profiles and functions of extracellular proteins controlled by DSF signal remain largely unclear. In the present study, 35 DSF-regulated extracellular proteins, whose functions include small-protein mediating QS, oxidative adaptation, macromolecule metabolism, cell structure, biosynthesis of small molecules, intermediary metabolism, cellular process, protein catabolism and hypothetical function, were identified by proteomics in Xoc. Of these, 15 protein encoding genes were in-frame deleted, and 4 of them, including three genes encoding type II secretion system (T2SS)-dependent proteins and one gene encoding a Ax21 (activator of XA21-mediated immunity)-like protein (a novel small-protein type QS signal) were determined to be required for full virulence in Xoc. The contributions of these four genes to important virulence-associated functions, including bacterial colonization, extracellular polysaccharide, cell motility, biofilm formation and anti-oxidative ability are presented. To our knowledge, our analysis is the first complete list of DSF-regulated extracellular proteins and functions in a *Xanthomonas* species. Our results show that DSF-type QS played critical roles in regulation of T2SS and Ax21-mediating QS, which sheds light on the role of DSF signaling in *Xanthomonas*.

## Concurrent Session 32-Plant Pathology Extension

### O32.001 Current status and prospective in crop disease management in China

*P. Yang*
*Division of Crop Pest Control, National Agro-technical Extension and Service Center, Ministry of Agriculture, Building No.20, Maizidian Street, Chaoyang District, Beijing 100125, P. R. China*
*Email: yangpy@agri.gov.cn*

Major crop diseases outbreak frequently and become one of the main threats to agricultural sustainability in China in the past two decades. Current practices in crop disease management are aiming at ensuring crop yields, improve farmers' income, and protect the environment. This presentation provides a glimpse of the history, national programs, and the other efforts undertaken in the implementation of integrated pest management in crop disease management for achieving the agricultural sustainability in China. Different extension approaches used were described and analyzed, and prospective on current issues and future challenges in the adoption of integrated pest management in crop disease management in China were put forward.

### O32.002 The role of the extension specialist in Michigan

*M.K. Hausbeck*
*Department of Plant, Soil and Microbial Sciences, 1066 Bogue Street, A286 PSSB, Michigan State University, East Lansing, MI 48824, USA*
*Email: hausbec1@msu.edu*

Michigan State University was established in 1855 as the first state agricultural land-grant institution in the United States. In Michigan, plant pathology extension operates via a network of campus-based extension specialists and county-based extension educators. Specialists on campus split their efforts between outreach and applied research. Educators located in the counties facilitate the flow of information between the state's agricultural clientele and campus specialists. Historically, campus-based specialists have been tenure-track professors who establish research programs focused on plant pathogen problems identified as priorities by agricultural stakeholders. As financial resources have become significantly reduced, the number of educators in the counties has decreased; the remaining county educators now work on a regional scale within the state. In the future, the number of full-time, tenure-track campus specialists may be reduced as resources devoted to extension become reduced. This is of concern as Michigan vegetable and greenhouse ornamental growers have recently encountered new and re-emerging plant pathogens that are destructive and threaten crop quality and yield. Examples include *Colletotrichum coccodes* on onion, *Pseudoperonospora cubensis* on cucurbits, and *Plasmopara obducens* on bedding impatiens. In each case, the current extension model in Michigan facilitated rapid identification of the pathogen threat and allowed for development, testing, and implementation of management strategies that preserved growers' livelihoods. By working closely with agricultural stakeholders and extension educators, the extension specialist can respond quickly to new and emerging pathogen problems and help to ensure the viability of Michigan agriculture.

### O32.003 Farmer field schools: An innovative approach to plant disease management in Pakistan

*I. Ahmad, M.R. Kazmi and S. Iftikhar*
*Chairman, Pakistan Agricultural Research Council, Sector G-5, Islamabad, Pakistan*
*Email: iftahmad@gmail.com*

Farmer Field School (FFS) approach has emerged as one of the successful way of educating small farmers in improved farm management. The basic philosophy is learning by doing. A collective of 25 growers meet every week or at intervals of important stages of crops in their fields for whole season along with a facilitator to learn improved crop management decision making. One session of FFS lasts 4-5 hours, covering crop issues, team building exercises and special topics. These activities are developed in a consultative workshop attended by all stakeholders (RND people, growers, private agribusiness traders etc) called Curriculum which covers tentative topics. The participant's competence is developed using this curriculum. The approach was first tested in Pakistan in early 90's in cotton in the backdrop of epidemic of *Cotton leaf curl virus* (CLCV) disease that came to be known as Vehari Model the world over. Later on the National IPM Program implemented cotton IPM through FFS. Recently FFS has been successfully implemented in developing the grower's capacity for Wheat Rust management & variety evaluation on the basis of varietal disease response, yield, grain quality, crop input requirements and other varietal characteristics. In last wheat season of 2012, 591 male and 64 female farmers were trained through 28 FFS. The growers practiced these newly acquired skills in Participatory Varietal Selection (PVS) conducted at each FFS. Now with the insurgence of new CLCV strain, FFS have been established to equip small cotton growers in Pakistan.

### O32.004 A county-based plant pathology program in California: a novel model for carrying out the extension mission

*S.T. Koike*
*University of California Cooperative Extension, Monterey County, 1432 Abbott Street, Salinas, CA 93901, USA*

*Email: stkoike@ucdavis.edu*

The Cooperative Extension service in the USA was established within the university system to develop and deliver research-based information to individuals and industries needing assistance and advice. However, this service that was designed to connect science-and-practice has been in steady decline for decades. In California, extension specialists appointed to university campuses today are down to 80% of the number hired in the mid-1980s. Extension academics assigned to counties are down to 36% of the mid-1980s roster. In the face of dwindling extension resources and changing clientele needs, a different approach for developing research-based plant pathology information for farmers was established in Monterey County. Through grants and industry support, a plant pathology diagnostic lab was set up by the county-based extension Farm Advisor in the Salinas Valley. The lab enables timely diagnoses to be provided to farmers and allied industry members. The lab facility also allows the county-based program to operate both independent and collaborative research efforts that require pathogen identification and storage, inoculum production, and other steps needed to investigate the etiology and epidemiology of diseases faced by local growers. Plant pathology procedures that traditionally were conducted on campuses were now being completed at a county facility. Following the 2006 outbreak of *E. coli* O157:H7 on spinach, the county plant pathology lab was further expanded so as to conduct research on field survival of foodborne bacterial pathogens. This county-based plant pathology program is now providing focused extension services to the cool season vegetable and strawberry industries in California's coastal region.

### O32.005 Mobile application for using citizen science to monitor incidence and severity of marri (*Corymbia calophylla) Quambalaria coyrecup* cankers in Australia

*G.E. Hardy, T.I. Burgess, T. Paap and C. Marbus*
*State Centre of Excellence for Climate Change, Woodland and Forest Heath, School of Veterinary and Life Sciences, Murdoch University, Murdoch, 6150, Western Australia*
*Email: g.hardy@murdoch.edu.au*

The incidence and severity of cankers caused by *Quambalaria coyrecup* in marri (*Corymbia calophylla*) has increased significantly since the early 1990s in the south-west of Western Australia. Marri is an iconic overstorey forest tree with an extensive range in a number of diverse forest ecosystems. It plays a major role as a food source, habitat tree and refugia for numerous vertebrate and invertebrate fauna including the endangered Carnaby's cockatoo (*Calyptorhynchus latirostris*), as well as being a key species for pollen and nectar for apiarists. Consequently, the impact of the pathogen on marri is causing increasing concern across the community for a wide range of reasons. We have developed a mobile marri application that works across mobile platforms (e.g. IPhone, Android and Windows) that can be used by interested members of the public, local government agencies, foresters and scientists to capture location (GPS), incidence and severity of cankers on trees, lodge photographs and other site information to a central server. The marri app also supplies detailed photographic information on what a canker looks like at different stages of development. Importantly, it also provides information on how to establish and monitor fungicide and other treatment trials that will be statistically robust and informative for scientific purposes. The development and use of the 'marri app' and its uses, together with strengths and weaknesses will be discussed.

### O32.006 Knowledge transfer towards sustainable agriculture

*M.P. Srivastava*
*Haryana Agricultural University, F-44, Tulip Garden, Sushant Lok-II, Gurgaon-122 011, India*
*Email: mpsrivastava28@gmail.com*

Plant diseases cause considerable losses in productivity. Though effective technology has been developed for several diseases, many are yet to reach the farmers. Until knowledge reaches them, losses cannot be prevented. To feed burgeoning population and achieve sustainability, it is imperative to reduce losses by transfer of unrivalled in-depth knowledge, and innovative technology leading to rural transformation towards food adequacy. Training, demonstration, field-days, awareness-drive, advisory, plant clinic/mobile clinic, print- and electronic media constituted basic tools of technology transfer. Training was focused on diagnosis of disease and their integrated management to minimize pesticide use, besides safe-use of pesticides, resistance management etc. Demonstration on improved plant protection practices were arranged to validate 'seeing-is-believing'. Field-days formed focal point for technology dissemination and confidence-building in neighboring farmers. Seed treatment and integrated management has become a common practice due to awareness-drive, continued inspiration and motivation. Diagnostic, advisory and pest alerts helped in mitigating losses while mobile clinics prevented flare-up of outbreaks. Such advices were frequently flashed from radio/satellite channels and handbills. Farmers had unlimited access to information through personal visits, correspondence and telephony. Alliance of extension personnel, researchers, and government machinery facilitated availability and use of resources/inputs. Leaflets/hand-outs, bulletins/question-answer series empowered farmers to keep diseases at bay. Amongst innovative publications, '*Plant Disease Warning'* was issued anticipating diseases outbreak to avert epiphytotics, *'Plant Protectionist'* was released

every month cautioning diseases likely to appear in the ensuing month and their prevention, while *'Plant Pathology Courier'* provided latest know-how on reducing losses, augmenting productivity and sustainability.

**P32.001 Mushroom cultivation for self-employment and rural food security among Adi tribal farmers of Arunachal Pradesh in India**

*P. Raja[1], J. Mohanty[1] and R. Saravanan[2]*
*[1]College of Horticulture and Forestry, CAU, Pasighat, India; [2]College of post graduate studies, CAU, Barapani, India*
*Email: prajachf@gmail.com*

Mushroom cultivation offer off seasonal income after paddy cultivation to the tribal farmers and their food and nutritional security among tribal people. The wild edible Oyster (*Pleurotus* sp.), Gill fungi (*Shizophyllum communae*), Tuber (*Tuber* sp.), Shiitake (*Lentinus edodus*) and Jews ear (*Auricularia* sp.) mushrooms were brought into commercial cultivation. By participatory approach program, the farmers are selected for the oyster mushroom cultivation and the cultivation techniques are demonstrated. Low cost model unit was constructed in the campus and the poor farmers, unemployed youth and women were converted into mushroom growers. A total of 339 farmers are benefitted by the pathology extension programme. Four farmers are having their own commercial production unit and the adoption rate was 1.17 per cent. From this programme, two self help groups has been created and actively involved in further dissemination of technology through participatory approach. We monitor the progressive farmers' production unit regularly and solve the practical problems in achieving high production in addition to recording the pest and diseases. We have succeeded in conversion of wildly available edible mushroom into commercial cultivation and facilitated to enhance substantial contributions to food security and rural development. With limited income generating possibilities, average income was increased (to INR 4, 430, 00) per family.

**P32.002 Selective autophagy gene *ATG20* is essential for host invasion and mediates infection-associated nuclear degeneration in *Colletotrichum orbiculare***

*C. Dong and Y. Kubo*
*Laboratory of Plant Pathology, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan*
*Email: achuang591@gmail.com*

The cucumber anthracnose fungus *Colletotrichum orbiculare* forms a specialized infection structure, appressorium, to breach the cucumber leaf surface and gain access to plant tissue. Appressorium development is controlled by cell cycle progression and mitosis is always followed by programmed cell death of conidium from which appressorium develops, but the mechanism by which it occurs remained to be studied. In yeast, nuclear breakdown requires a specific form of autophagy, known as piecemeal micro-autophagy of the nucleus (PMN), while non-selective macro-autophagy is required by infection-associated nuclear degeneration in the rice blast fungus *Magnaporthe oryzae*. We here investigated whether this process occurs in the cucumber anthracnose fungus. We performed a random insertional mutagenesis screen and identified *CoATG20*, a homolog of *ATG20* in yeast, which regulates membrane traffic and endosomal protein sorting and are essential for cytoplasm to vacuole targeting (CVT) and/or pexophagy in yeast, and Snx41 in *M. oryzae*, which is essential for conidiation and pexophagy in *M. oryzae*. The deletion mutant of *⊿coatg20* developed appressoria but showed a defect in pathogenicity during plant invasion. Importantly, the nuclear degeneration is significantly delayed in the conidium of *⊿coatg20*. These results indicated that selective autophagy gene *CoATG20* is required by pathogenicity during plant invasion and showed possible relationship between *Coatg20*-mediated selective autophagy and nuclear degeneration of conidium during cell development of *C. orbiculare*.

**P32.003 Sclerotium rot of cucumber caused by *Sclerotium rolfsii* in Korea**

*J.H. Kwon, S.L. Choi and C.Y. Cho*
*Gyeongsangnam-do Agricultural Research and Extension Services, Jinju 660-360, Republic of Korea*
*Email: kwon825@korea.kr*

Sclerotium rot of cucumber (*Cucumis sativus* L.) occurred at the experimental field of Gyeongsangnam-do Agricultural Research and Extension Services in July 2012. The infected fruits showed water-soaked and rot symptoms. White mycelial mats spread over lesions, and then sclerotia were formed on fruit and near soil line. The sclerotia were globoid in shape, 1-3 mm in size and white to brown in color. The optimum temperature for mycelial growth and sclerotia formation on PDA was 30℃ and the hyphal width was 4-8 μm. The typical clamp connections were observed in the hyphae of the fungus grown on PDA. On the basis of mycological characteristics and pathogenicity to host plants, this fungus was identified as *Sclerotium rolfsii* Saccardo. This is the first report of sclerotium rot on cucumber caused by *S. rolfsii* in Korea.

**P32.004 Plant Growth Promoting Rhizobacteria (PGPR) for the management of citrus canker of khasi mandarin oranges**

*P. Raja[1], A.K. Singh[1], P. Nallathambi[2] and S.R. Niranjana[3]*
*[1]Department of Plant Pathology, College of Horticulture and Forestry, Pasighat, Arunachal Pradesh; [2]Division of*

*Plant pathology, Indian Agricultural Research Institute, regional station, Ooty, Tamil Nadu; [3]Department of Studies in Biotechnology, University of Mysore, Mysore-570 006*
*Email: prajachf@gmail.com*

Khasi mandarin farming is the main source of income for more than 60 per cent of people of Arunachal Pradesh, India. The limiting factor in production and productivity is mainly due to the poor plant protection measure, negligence of orchard management, poor maintenance of seedling and trees etc. Commercial cultivation of khasi mandarin is seriously threatened by bacterial canker caused by *Xanthomonas axonopodis* pv *citri* and leaf miner in nursery crops, propagated plants and trees. Bacterial canker produces lesion, cankerous growth leading to premature drop of leaf. Larvae mines into the tender leaves in zig-zag manner and pave the way for citrus canker infection. Bacterial canker incidence was recorded up to 10- 26 per cent. To manage the leaf minor bacterial canker chemical and biological control methods are studied. It was observed that premonsoon spraying of dimethoate 0.05% and spraying of entomopathogenic nematode found to be effective to manage the citrus leaf minor and reduced the infestation by 65 per cent. The Spraying of Bordeaux mixture followed by Spraying of PGPR at 0.5% was effective against citrus canker and reduced the disease incidence by 70 percent compare to control.

**P32.005 The study on preservation and conidia culture of *Ustilaginoidea virens***

*H. He [1, 2], W. Sopone[2], J. Yuan[1], X. Yang[1], X. Chen[1], S. Wu[1], Q. Tai[1], L. Wang[1] and B. Natthiya[2, 3]*
*[1]Guizhou Institute of Plant Protection, Guizhou Academy of Agricultural Sciences, Guiyang 550006, P. R. China; [2]School of Crop Production Technology, Institute of Agriculture Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand; [3]Unité Mixte de Recherche Eco & Sols (Ecologie fonctionnelle & biogéochimie des Sols & des Agroécosystèmes), UMR 1222 INRA-IRD-SupAgro Montpellier, Place Pierre Viala, 34060 Montpellier cedex 01, France*
*Email: hhy200307@126.com*

False smut of rice (*Oryza sativa* L.) caused by *Ustilaginoidea virens* (Cooke) Takahashi (teleomorph *Villosiclava virens*) is one of the most common fungal disease of rice plant in China. The knowledge on preservation and conidia production methods of *U. virens* is limited. In the study, to find suitable preservation and conidia production methods, six different preservation methods were tested by checking at 3 month interval for the viability and sporulation, and 7 liquid media of different composition were tested by shaking culture at 150 rpm. Among six methods studied, the results showed that the periodic transfer and paraffin oil overlay were suitable methods, the fungus could survive more than 12 months. The conidia concentration went up to $7.25\times10^7$ conidia/ml in the PSB liquid medium after 9 days, indicated that PSB was the best medium for conidia production.

**N32.001 The correlation study on the sclerotia survival ability and its buried time and depth in soil**

*X.J. Li, P.H. Ren, X. Cao, H.Y. Bu, J. Zhao and H.Y. Zhou*
*Department of Agronomy, Innermonglia Agriculture University, Huhhot, Inner Mongolia Autonomous Region, 010018, P. R. China*
*Email: zhaojun02@hotmail.com*

*Sclerotinia sclerotiorum* (Lib.) de Bary is a ubiquitous pathogen with a host range of more than 400 plant species, such as sunflower, soybean, oilseed rape and various vegetables, etc. During its life cycle, the mycelium formed Sclerotia to survive in winter period and is functional as preliminary infection sources in the coming year. For the special structure of Sclerotia, there has report that it can be kept in soil for 8-10 years, however, the survive ability of Sclerotia after such long time in soil is still a question mark. In this work, we studied the correlation between the survival ability of sclerotia and its buried depth and time in soil within two years. Our data suggested that with buried time extended and buried depth increase, the survival ability of sclerotia showed decreased tendency, indicting the negative correlation between sclerotia survival ability and buried time and depth. Meanwhile, with buried time going on and buried depth increase, not only the total weight of tested sclerotia dropped dramatically, the sclerotia is also easy broken into small pieces. These results give us some hints that deep plough (deeper than 10 CM) and longer rotation with non-host crop (2 years) will control the severity of sunflower white mold to certain extent.

**N32.002 cAMP inhibites the sclerotia formation via targeting on $H_2O_2$ in necrotrophic fungi *Sclerotinia sclerotiorum***

*Y.G. Hou, H.Y. Bu, Y.J. Wang and J. Zhao*
*Agronomy Department of Inner Mongolia Agricultural University, Saihan District, Hohhot, Inner Mongolia Autonomous Region, 010018, P. R. China*
*Email: zhaojun02@hotmail.com*

Sclerotinia diseases lead to serious losses each year in both vegetable crops and plant oil crops. Sclerotia development is the pivotal stage of the disease cycle and it can be kept in soil and function as preliminary infection source in the coming year. Using *S. sclerotiorum* isolate X-8 collected from sunflower field in Inner mongolia region, we detected the decrease of $H_2O_2$ level at the initial stage of development of sclerotia. Adding $H_2O_2$ into PDA plate speed up the sclerotia formation and adding DPI, which blocked the formation of $H_2O_2$, sup-

pressed the formation of sclerotia, indicating the role of $H_2O_2$ during the formation of sclerotia. Adding cAMP inhibit the formation of sclerotia, meanwhile, dramatically inhibit $H_2O_2$ level; the enzymes activity of catalase, which converts the $H_2O_2$ into $H_2O$ and $CO_2$, was also suppressed by adding cAMP into plate. All these data indicat $H_2O_2$ is one of the target of cAMP during the inhibition of the formation of sclerotia.

## Concurrent Session 33-Plant Virus Diseases and Control

### O33.001 NbMIP1s, a group of J-domain proteins, are required for both *Tobacco mosaic virus* infection and plant innate immunity

*Y. Du, J. Zhao, T. Chen, Q. Liu, H. Zhang, Y. Wang and Y.L. Liu*
*School of Life Sciences, Tsinghua University, Beijing 100084, P. R. China*
*Email: yuleliu@mail.tsinghua.edu.cn*

*Tm-2*$^2$ is a CC-NBS-LRR class of resistance gene and confers durable extreme resistance against *Tomato mosaic virus* (ToMV) and *Tobacco mosaic virus* (TMV) by recognizing the presence of viral movement protein (MP). Here we report that NbMIP1s, a group of J-domain proteins, associate with tobamovirus MP, Tm-2$^2$ and SGT1. Suppression of *NbMIP1s* in *Nicotiana benthamiana* reduced the TMV movement and compromised *Tm-2*$^2$-mediated resistance against TMV and ToMV. Further, silencing of *NbMIP1s* reduced the steady-state protein levels of ToMV MP and Tm-2$^2$. Moreover, *NbMIP1s* are also required for plant resistance induced by other *R* genes and the nonhost pathogen *Pseudomonas syringae* pv. *tomato* (Pst) DC3000. In addition, we found that SGT1 also associates with Tm-2$^2$, and is required for *Tm-2*$^2$-mediated resistance against TMV by regulating the steady-state protein level of Tm-2$^2$. These results suggest that NbMIP1s function as co-chaperons to be required not only for virus infection but also for plant immunity.

### O33.002 Multiple resistance pathways elicited by TMV interacting with the N gene product

*P. Palukaitis, E. Baek and J.Y. Yoon*
*Horticultural Sciences, Seoul Women's University, Seoul, Republic of Korea*
*Email: scripath1@yahoo.co.uk*

Hypersensitive resistance to *Tobacco mosaic virus* (TMV) in tobacco is conferred by the *N* gene, which elicits the best characterized plant virus resistance response. Resistance involves multiple responses activated by phytohormones, although the responses activated by salicylic acid (SA) are the best characterized. At least three responses that inhibit the infection of TMV are activated by SA, through independent pathways: alternative oxidase (AOX), a mitochondrial enzyme involved in regulating reactive oxygen species; pathogenesis related (PR) proteins, synthesized in response to SA via the regulator NPR1; and RNA-dependent RNA polymerase 1, encoded by the gene *RDR1*. Another pathway, involving the synthesis of an inhibitor of virus replication (IVR), was shown to be independent of SA, as was the pathway involving the transcription factor (TF) ERF5. We found that ERF5 is upstream of and regulates IVR production. By contrast, expression of different *PR* genes is regulated by the TFs MYB1, WRKY and TGA. The kinetics of gene expression of *AOX1*, *PR1*, *RDR1* and *IVR* were examined in tobacco plants by real-time PCR, as were the kinetics of gene expression of *ERF5* and *MYB1*. The plants examined included wild-type *NN* tobacco, *nn* tobacco expressing mutants of the *N* gene that no longer restricted TMV to the inoculated leaf and *NN* tobacco silenced for expression of *ERF5*, *MYB1*, or both genes. The results indicate complex regulation of expression of the various defense genes, with ERF5 and MYB1 affecting the expression of each other's genes, even though they are in different pathways. A model for the effects of the TFs ERF5 and MYB1 on the expression of the defense effector genes (*AOX1*, *PR1*, *RDR1* and *IVR*) will be presented.

### O33.003 Pathogenicity of *Barley stripe mosaic virus* strains and interactions with the *Brachypodium Bsr1* resistance gene

*L.J. Yan*[1]*, Y. Cui*[1,2]*, M.Y. Lee*[2]*, M.H. Yu*[1]*, G.X. Wang*[1]*, X.J. Jin*[1]*, Y. Hu*[1]*, A. Kruse*[2]*, B. Kong*[2]*, Y.L. Zhang*[1,2]*, C.G. Han*[1]*, J.L. Yu*[1]*, Z.Y. Liu*[1]*, A.O. Jackson*[2] *and D.W. Li*[1]
[1]*State Key Laboratory of Agro-Biotechnology, China Agricultural University, Beijing 100193, P. R. China;* [2]*Department of Plant and Microbial Biology, University of California-Berkeley, Berkeley, CA 94720, USA*
*Email: Dawei.Li@cau.edu.cn*

*Barley stripe mosaic virus* (BSMV) causes a serious world-wide disease of barley and other cereals, yet dominant resistance (R) genes are not available for control. We have identified, mapped and cloned a novel R gene (*Bsr1*) from Bd3-1 and a nonfunctional allele (*bsr1*) from Bd21 in the model cereal *Brachypodium distachyon*. The *Bsr1* allele elicits resistance to the BSMV ND18 (ND) strain and several other strains, but not to the Norwich (NW) strain. Molecular genetic analyses reveal that *Bsr1* resistance is elicited by the ND18 triple gene block 1 (TGB1) movement protein, and that two residues at position 390 and 392 in the TGB1 helicase region are required for resistance. The $_{ND}$TGB1 and *Bsr1* proteins interact *in vitro*, whereas $_{NW}$TGB1 and *Bsr1* fail to interact, and infectivity studies show that the resistance phenotype correlates with the ability of TGB1 and Bsr1 to interact. A novel cell death assay developed in *Nicotiana benthamiana* and differential virulence of *B. distachyon* accessions are being used to define *TGB1*:*Bsr1* gene-for-gene resistance interactions, and to identify and characterize resistance pathways. The role of TGB1 in elicitation of *Bsr1* resistance, and analysis of functional and nonfunctional *Bsr1* alleles will be discussed.

**O33.004 Dissecting molecular network of virus-plant interactions: the complex roles of host factors**

*A. Wang*
*Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, Ontario, N5V 4T3, Canada*
*Email: Aiming.Wang@AGR.GC.CA*

Plant viruses are obligate intracellular parasites that infect many agriculturally important crops and cause severe losses each year. Genetic resistance is considered the most effective means to control these viruses. Unfortunately, resistant resources in nature are very rare. The development of novel antiviral strategies requires a better understanding of the complex molecular interplay between the host plant and the invading virus. Accumulated evidence suggests complex roles of host factors during virus infection processes. Some host factors play a role in defense against viruses, whereas many others are recruited by the viruses to improvise their replicase and/or transport complexes for genome multiplication and movement. In the past 10 years, our research has been focused on identifying host factors of potyviruses, the largest group of plant viruses and elucidating their functional roles in potyvirus infection. Several host factor genes have been identified that are essential for potyviral accumulation. In addition, we have generated mutant populations of several crop species such as peach and soybean and screening for specific host factor gene mutants resistant to relevant potyviruses.

**O33.005 Dynamic interactions between *Clover yellow vein virus* and resistance pea cultivars**

*I. Uyeda, S.H. Choi, G. Atsumi, Y. Hisa, and K.S. Nakahara*
*Graduate School of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan*
*Email: uyeda@general.hokudai.ac.jp*

Two genetically distinct recessive resistance genes (*cyv1* and *cyv2*) are known to confer resistance to *Clover yellow vein virus* (ClYVV) in pea. In pea carrying *cyv1*, the virus is restricted in single cells. On the other hands, the virus moves to cells around the infection site but viral movement is much slower in pea carrying *cyv2* than that in the susceptible pea lines. *cyv2* has recently been revealed to encode *eukaryotic initiation factor 4E*. And a single non-synonymous mutation in P1 cistron was found to be associated with the breaking cyv2 resistance. The resistance gene of *cyv1* has not been isolated yet. In this report, we showed P3 cistron was involved in breaking resistance of *cyv1*. P3 cistron is known to produce two proteins: P3 from the main open reading frame and P3N-PIPO which has the N-terminal part of P3 fused to amino acids encoded by a small ORF in the +2 reading frame. ClYVV isolate Cl-no30 was restricted in a single cell when the infectious cDNA was particle-bombarded to *cyv1* pea whereas isolate 90-1 Br2 overcame this resistance by infecting systemically. Chimera viruses were constructed between the two isolates and their infectivity on *cyv1* pea showed the breaking resistance was attributed to the P3 cistron. Mutational analysis of P3 cistron revealed both P3 and P3N-PIPO were involved in overcoming the resistance.

**O33.006 mRNA stability regulator and RNA virus genome**

*N. Kumakura, A. Takeda and Y. Watanabe*
*Department of Life Sciences, University of Tokyo, Komaba, Tokyo, 153-8902, Japan*
*Email: solan@bio.c.u-tokyo.ac.jp*

The machinery called RNA silencing is being well investigated. Host plants combat with plant viruses by producing short interfering RNAs (siRNAs) which would target sequences in viral genomes and inhibit their accumulation. The existence of 3'-5' exoribonuclease activities (also known as RNA exosome) in plant cells is also known, but yet not characterized in regards to possible inhibitory effects on RNA virus genome. Plant RNA viruses carry distinct 3' terminal structures in their genome RNAs. It is broadly accepted that such structures endow respective RNAs with specific identities to be recognized by viral replication machinery and to be distinguished from other cellular RNAs. To test whether the 3'-5' exoribonuclease activities have impacts on virus RNA accumulation, we established *Arabidopsis* lines where exosome components were conditionally knocked-down, and tested virus accumulation after infection. These plants were challenged with several kinds of virus with 3'-end structures of different types and evaluated for their possible susceptibility. The relationship between existence of exoribonuclease in host cells and robustness of viral RNA terminal structures would be discussed.

**O33.007 Engineering RNAi-based resistance to viruses, insects, and other pathogens**

*F. Qu*
*Department of Plant Pathology, The Ohio State University, 024 Selby Hall, 1680 Madison Ave, Wooster, OH 44691, USA*
*Email: qu.28@osu.edu*

RNA interfence (RNAi) is a highly conserved genome surveillance system operating in most eukaryotic organisms to guard the cells against the invasion or proliferation of molecular parasites including transposons, transgenes, as well as viruses. Studies in recent years have demonstrated that RNAi-based resistance engineering is highly effective at achieving resistance against not only viruses, but also nematodes, insects, and fungal pathogens. We applied this strategy to soybean plants and

observed robust resistance to multiple viruses with a single dsRNA-expressing transgene. Specifically, our transgene construct contained three short inverted repeats (IRs) with sequences of three soybean-infecting viruses (*Alfalfa mosaic virus, Bean pod mottle virus*, and *Soybean mosaic virus*). These IRs were assembled into a single transgene under control of the 35S promoter and terminator of *Cauliflower mosaic virus*. Three independent transgenic lines were obtained and all of them exhibited strong systemic resistance to the simultaneous infection of the three viruses in both greenhouse and field experiments. We are now testing the effectiveness of this strategy at controlling soybean aphids.

**O33.008 New and emerging Tospovirus diseases**

*M. Turina, P. Margaria and M. Ciuffo*
*Istituto di Virologia Vegetale, CNR- Torino, Strada delle Cacce 73, 10135 Torino, Italy*
*Email: m.turina@ivv.cnr.it*

Tospoviruses cause severe diseases in many crops all over the world. We will report on the identification and characterization of a number of putative new species from the Americas, Asia and Europe. A new lineage of Tospoviruses was established including Bean necrotic mosaic virus and Soybean vein necrosis-associated virus isolated in Brasil and USA respectively; Pepper necrotic spot virus is present in solanaceous hosts in Peru; Alstroemeria necrotic streak virus was isolated in 2010 in Colombia. Melon severe mosaic virus was also a recently characterized tospovirus species widespread on melon crops in Mexico. In Asia, Tomato necrotic ringspot virus from Thailand and Tomato zonate spot virus from China were respectively identified in 2011 and 2010. The tospovirus *Polygonum ringspot virus* (PolRSV), the only new species isolated in Europe, will be subject of specific attention because it was isolated from a common weed and because of the specificity of its vector, *Dictyothrips betae*, a new vector species for tospoviruses. Furthermore this virus has two interesting molecular features that are absent in other Tospoviruses: the small segment intergenic region displays a short stretch of AT reach repeats, with no possibility of internal pairing, as it is usually the case with all the other tospovirus species, implying a different mechanism for subgenomic transcription. Furthermore, an unreported RNA species resulting from the dimerization the S segment with a small deletion of circa 300 nt at the 5' end of the second RNA molecule accumulates during PolRSV infection of *Nicotiana benthamiana* and *N. clevelandii*.

**O33.009 RSV-derived vsiRNAs potent antiviral defense by rice AGOs**

*J. Wu [1, 2], Z. Yang [1], L. Zheng [1, 2], Y. Wang [1], H. Zheng [1], S. Ji [1], S. Zhao [1], L. Jin [1], W. Hong [1] and Y. Li [1]*
*[1]Peking-Yale Joint Center for Plant Molecular Genetics and Agrobiotechnology, National Laboratory of Protein Engineering and Plant Genetic Engineering, Peking-Tsinghua Center for Life Sciences, College of Life Sciences, Peking University, Beijing 100871, P. R. China; [2]Key Laboratory of Plant Virology of Fujian Province; Institute of Plant Virology, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, P. R. China*
*Email: liyi@pku.edu.cn*

Rice stripe disease (RSD), caused by *Rice stripe virus* (RSV), is a serious disease in temperate rice-growing areas. RSV, a typical member of the genus *Tenuivirus*, multiplies both in plants and invertebrate vectors, such as insects. The genome of RSV is composed of four negative-sense single-stranded RNA segments, named as RNA 1, 2, 3 and 4. RNA 1 has negative polarity, encoding a putative RNA-dependent RNA polymerase. The other three segments are associated with an unusual ambience encoding strategy, with both the viral-sense RNA (vRNA) and the viral complementary-sense RNA (vcRNA) having coding capacity.NS2 and NS3 encoded by vRNA2 and vRNA3 respectively are viral suppressors of RNA-silencing; vcRNA 2 encodes pC2 glycoprotein. vcRNA 3 encodes pC3, a viral NCP; RNA 4 encodes SP, a major non-structural protein that accumulates in infected plants, and pC4, a viral movement protein. Small RNA-mediated RNA silencing is a widespread antiviral or antibacterial mechanism in different organisms. Although the host and pathogen factors involved in this mode of host defense and pathogen counter-defense have been extensively investigated, much less is known about how a pathogen alters the small RNA metabolism in a host.To help fill this knowledge gap, we first used deep sequencing to characterize the small RNA profiles of RSV-infected rice plants. Our analyses showed that RSV vsiRNAs were derived almost equally from virion and complementary RNA strands and were mostly 20–22nt. Most vsiRNAs were produced within the coding sequences and 5' termini of the RSV genome. RSV vsiRNAs have a strong A/U bias at the first nucleotide and a U/C bias at the final one, suggesting preferential targeting of such sequences by rice DCL proteins. Furthermore, we have found that RSV-infection would enhance the accumulation of some rice miRNA* from conserved miRNA precursors and accumulation of phased siRNAs from a particular precursor. Furthermore, RSV-infection also induced the expression of some novel phased miRNAs from several conserved miRNA precursors. In addition, RSV-infection significantly elevated the expression of certain OsDCLs and OsAGOs. As a model plant in monocotyledons, rice encodes 19 AGO proteins, of which only four proteins (AGO1, AGO4, AGO5, AGO7 and AGO16) have been studied, with their functions revealed. Other OsAGOs have not been reported yet. Previous studies indicate that many OsAGOs display temporal and tissue-specific gene expression patterns.

Combined with deep sequencing and bioinformatics methods, we apply co-immunoprecipitation technology to research into the antiviral roles of some OsAGOs. We find that after infection with RSV, virus-derived vsiRNAs are enriched mostly in OsAGO1. It is well known that RNAi pathways and virus infection are connected closely. The plants use RNAi pathways to defend against virus. However, virus can also encode viral suppressors of RNA silencing (VSR) to counter host's antiviral activities and fulfill its infection. Through in-*vivo* and in-*vitro* co-immunoprecipitation we prove that CP protein of RSV interacts with one OsAGO protein. These results indicate that RSV CP protein maybe a suppressor of rice antiviral RNAi pathways. These findings are of great significance to further understand the pathogenesis mechanisms of RSV. This is the first report of the antiviral role of a rice AGO protein in the defense against a negative-strand RNA virus. Moreover, our research uncover new mechanisms and complexity of virus-host interactions that may have important implications for further studies on the evolution of cellular small RNA biogenesis that impact pathogen infection, pathogenesis, as well as organism development. These data uncover new mechanisms of virus-host interactions that affect host small RNA and viral vsiRNA metabolism.

**O33.010 Deep sequencing–based transcriptome profiling reveals comprehensive insights into the responses of *Nicotiana benthamiana* to infection with different isolates of *Beet necrotic yellow vein virus***

*H.Y. Fan, H.W. Sun, Y. Wang, Y.L. Zhang, X.B. Wang, D.W. Li, J.L. Yu and C.G. Han*
*Department of Plant Pathology and State Key Laboratory for Agro-biotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: huiyanfan@gmail.com*

*Beet necrotic yellow vein virus* (BNYVV) strains, contain four or five plus-sense single stranded RNAs, and are causal agents of rhizomania disease of sugar beet, which is widely distributed in most regions of the world. BNYVV can infect *Nicotiana benthamiana* systemically and causes severe or mild symptom based on different RNA components. The presence of RNA4 (BN34), but not RNA3 (BN3) is associated with severe symptoms in *N. benthamiana* such as curling and stunting. Here we used next-generation sequencing technologies to characterize the *N. benthamiana* transcriptome and to identify candidate genes for infection responses and resistance to BNYVV. A total of 3,016 differentially expressed transcripts (FDR＜0.05) were detected among three libraries of mock, BN3 and BN34 to provide a list of candidate genes that potentially are elicited as a response to viral infection. KEGG pathway analysis suggested that a large number of these genes are related to metabolic pathways and to photosynthesis-antenna proteins, which were significantly enriched only in BN34-infections. Our data also indicate that special expression modifications of genes involved in RNA silencing, ubiquitin-proteasome, cellulose synthesis and plant hormone gibberellin metabolism may be associated with severe symptoms elicited by BNYVV RNA4. This study expands our understanding of the genetic architecture of *N. benthamiana* and provides new insight into its complicated molecular and cellular responses to BNYVV infection.

**O33.011 Sequence-homology independent breakdown of transgenic resistance by super virus strains and the solution**

*Y.J. Kung[1], B.J. You[1,2], K.C. Chen[1], J.A.J. Raja[1] and S.D. Yeh[1]*
*[1]Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, China; [2]Current Address: Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung, Taiwan, China*
*Email: sdyeh@nchu.edu.tw*

*Papaya ringspot virus* (PRSV) limits the production of papaya worldwide. Underlying the mechanism of post-transcriptional gene silencing (PTGS), we generated CP-transgenic papaya lines with resistance to different strains of PRSV. However, during field trials, we noticed breakdown of CP-transgenic resistance by a PRSV super strain, designated PRSV 5-19, and also by an unrelated potyvirus *Papaya leaf-distortion mosaic virus* (PLDMV). The PRSV super strain 5-19 was also able to breakdown the transgenic resistance of the further developed PRSV-PLDMV double virus resistant papaya lines carrying a chimeric untranslatable construct comprising partial CP coding sequences of both PRSV and PLDMV. Recombinant analysis of the super strain 5-19 and the common strain YK indicated that a recombinant virus containing the gene silencing suppressor HC-Pro from the super strain 5-19 can break down the transgenic resistance in a transgene sequence-homology independent manner. Transient expression of PRSV YK and 5-19 HC-Pros in the leaves of *Nicotiana benthamiana* plants by agroinfiltration further confirmed that the HC-Pro of 5-19 has stronger capability for gene silencing suppression than that of YK. Comparison of YK and 5-19 HC-Pros revealed variations in five aa positions in the region responsible for gene silencing suppression and genome amplification. Consequently, to disarm the 5-19 super silencing suppressor, we generated transgenic papaya lines carrying an untranslatable 5-19 HC-Pro, and the transgenic lines conferred complete resistance to PRSV 5-19 and other geographic strains. This approach solves the problem of homology-independent breakdown of transgenic resistance.

**O33.012 Efficient artificial inoculation of *Rice black-streaked dwarf virus* to maize using the planthopper *Laodelphax striatellus***

*H.Q. Miao[1], D.P. Di[1], Y.G. Lu[1], M.G. Redinbaugh[2], L.Z. Tian and A.H. Zhang[1]*
*[1]Plant Protection Institute, IPM Centre, Hebei Acad. of Agri. and Forest. Sci., Baoding 071000, P. R. China; [2]USDA, ARS, CSWQRU and Department of Plant Pathology, The Ohio State University, OARDC, Wooster, OH 44691, USA*
*Email: miao5058345@163.com*

Maize rough dwarf caused by *Rice black-streaked dwarf virus* (RBSDV) is among the most important diseases of maize in China. Although deploying disease resistant hybrids would be the most effective and economic way to control the disease, identification and development of these hybrids has been limited by virus transmission rates that are too low for effective screening. An artificial inoculation technique for RBSDV was developed using *L. striatellus* as vector and wheat seedlings as the insect rearing and virus culture host. A planthopper colony was developed using wheat as an effective feeding and reproductive host. Subsequently, RBSDV-infected leafhoppers were obtained by allowing 3rd and 4th instar nymphs a 3 to 4 day acquisition access period on RBSDV-infected wheat. Planthoppers were then allowed to feed on healthy wheat for a 25 to 28 day latent period. Subsequently, the viruliferous leafhoppers were allowed a 3 day inoculation access period on maize seedlings (10 to 12 leafhoppers/plant in 60×40×58 cm cages). Plants of the susceptible hybrid Zhengdan 958 developed dwarfing and enation symptoms 10 to 14 days after being transplanted into a screenhouse. At tassling, 100% of plants were symptomatic with a disease index of 97.3 out of 100. The high efficiency of RBSDV transmission indicates this technique provides a reliable procedure to screen for RBSDV resistance in maize.

**O33.013 Virus diseases of kiwifruit**

*M.N. Pearson, A.G. Blouin, R.R. Chavan, C. Ratti, R. Biccheri and D. Cohen*
*School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand*
*Email: m.pearson@auckland.ac.nz*

Kiwifruit (*Actinidia* spp.) originates from China but is now grown as a commercial crop in many countries including China, Italy, New Zealand and Chile. The first definitive identification of a kiwifruit virus was *Apple stem grooving virus* in budwood imported from China and held in quarantine in New Zealand in 2003. Subsequent testing of budwood from the same consigment held in quarantine, as well as in germplasm in NZ and Italy, using a combination of biological properties, serology and sequencing, has identified 12 virus species. These represent a wide range of virus families and genera and include: common and widespread viruses such as *Alfalfa mosaic virus*, *Cucumber mosaic virus*, *Ribgrass mosaic virus* and *Turnip vein clearing virus*; known viruses with restricted host ranges such as *Apple stem grooving virus*, *Cherry leafroll virus*, *Cucumber necrosis virus* and *Pelargonium zonate spot virus*. In addition a citrivirus, two vitiviruses and a potexvirus have been fully sequenced, and appear to be new strains or species that may be specific to Actinidia. The identification and characterisation of viruses that infect kiwifruit is important so that measures can be developed to mitigate their spread via international movement of germplasm.

**O33.014 Viral complex detected in garlic by ELISA and RT-PCR and impact on yield and quality**

*O.G. García-Rodríguez, L. Pérez-Moreno, M.J. Navarro-León, M.D. Salas-Araiza, O.A. Martínez-Jaime, M.F. León-Galván and H.G. Núñez-Palenius*
*Agronomy Department, Life Sciences Division, Campus Irapuato-Salamanca, University of Guanajuato, Irapuato, Guanajuato, P. O. Box 311, Zip Code 36500, México*
*Email: luispm@ugto.mx*

The present study was aimed to: detect viruses in garlic plants viral complex by ELISA and confirmed by RT-PCR, and determine the effect on yield and quality. 12 garlic compounds were evaluated. There were analyzed: a) severity and incidence of virus diseases; b) bulb weight, bulb diameter and bulb height, c) yield and number of cloves by bulb. By means of ELISA five viruses were evaluated; LYSV, OYDV, SLV, IYSV and GCLV, at 71, 120 and 147 days after sowing. Confirmatory analysis was performed by RT-PCR. Serologically detected viruses were LYSV with 100, 100 and 100%; OYDV with 50, 75 and 77% GCLV with 100, 97 and 92%, SLV with 19, 30 and 0%, and finally, IYSV with 0, 3 and 22%. The viruses detected by RT-PCR were SLV 100%, IYSV 77.77%, 66.66%, LYSV and GCLV 55.55%. From all analyzed samples by RT-PCR, 30.7% were negative and 69.3% had a viral complex of three to four viruses. The viruses detected were in 55.55% of the samples as a complex of four viruses and 44.44% as of three viruses. Garlic compounds 4, 3 and 2 yielded 38.937, 38.771 and 38.175 t/ha.

**O33.015 Long-term retention of potyviruses within aphid vector: Implications for transmission of non-persistent plant viruses**

*A. Al-Mrabeh[1,2*], E. Hack[1], G. Cowan[2], A. Gatehouse[1] and L. Torrance[2]*
*[1]Newcastle University, School of Biology, Newcastle upon Tyne, NE1 7RU, England, UK; [2]Cell and Molecular Sciences, the James Hutton Institute, Dundee, DD2 5DA, Scotland, UK*

*Email: ahmad.al-mrabeh@ncl.ac.uk*

Viruses within the genus *Potyvirus* are transmitted by vector aphids non-persistently by a mechanism that is thought to involve short acquisition and retention times. The present study challenges the concept that these viruses are only retained within the aphid vector for short periods. Using an ultrasensitive nested polymerase chain reaction technique (N-RT-PCR), RNA of *Potato virus Y* (PVY) was detected in individual *Myzus persicae* for up to 14 days after they were given access to virus and then transferred daily to healthy plants. Virus RNA was detected in different parts of dissected aphids including the stylet, head and body. Similarly, RNA of *Potato virus A* (PVA) and a non-transmissible strain of PVY ($PVY^{C}$) was detected in aphids for up to 7 days following initial acquisition. However, PVA was detected in a smaller proportion of aphids than PVY. The N-RT-PCR technique was shown to detect virus at the attogram level and was approximately 100 times more sensitive than RT-PCR. To our knowledge, detection of PVA and transmissible and non-transmissible strains of PVY in aphids for 7 to 14 days is a novel finding. These results should stimulate a reassessment of the molecular mechanisms of uptake and retention of potyviruses and the determinants involved in aphid transmission of this important group of viruses. Importantly, this finding has relevance for the application of molecular diagnostic tools for forecasting aphid transmission to field-grown potato plants.

### O33.016 Promoter analysis of *Grapevine vein clearing virus* (GVCV)

*Y. Zhang, C. Angel, S. Valdes, W.P. Qiu and J. Schoelz*
*110 Waters Hall, University of Missouri-Columbia, Columbia, MO, 65211, USA*
*Email: YZWP9@mail.missouri.edu*

Grapevine vein clearing virus (GVCV) is a newly discovered DNA virus in grapevine. It is closely associated with grapevine vein clearing syndrome observed in vineyards in Missouri and surrounding states. The genome sequence of GVCV indicates that it belongs to the *Caulimoviridae*, a family of viruses that replicate by reverse transcription of a RNA intermediate. The type member of the *Caulimoviridae* is *Cauliflower mosaic virus* (CaMV), the virus that is the source of the 35S promoter. To identify the GVCV promoter, we cloned portions of the GVCV large intergenic region in front of a GFP gene present in the Agrobacterium binary vector pKYLX7. GFP expression was assessed by ELISA three days after agroinfiltration of *Nicotiana benthamiana* leaves. We found that the GVCV DNA segment between nucleotides 7,332 and 7,672 was sufficient to initiate transcription; in addition, the GVCV promoter activity was stronger than the CaMV 35S promoter. The DNA segment between nucleotides 7,332 and 501 can also initiate GFP expression; however the mechanism to get around with short ORFs (sORFs) in this region is still unclear, for which "ribosomal shunt" could be a possible explanation. 5' RACE and 3' RACE revealed that the GVCV transcription starts predominantly at nucleotide 7,571 and is terminated at7, 676bp, which would generate a terminally redundant RNA, a hallmark of the Caulimoviruses. The GVCV promoter analysis gives us some insights about how its genome is transcribed, and may be used as an alternative to the CaMV 35S promoter.

### P33.001 Characteristic and damage of WMV-G isolated from *Panax ginseng* in Korea

*W.K. Jung*
*Punggi Ginseng Experiment, Gyongbuk Provincial ATA, Yeongju, Republic of Korea*
*Email: jwonkwon@korea.kr*

Korean ginseng (*Panax ginseng* C.A. Meyer) is a perennial herbaceous plant belong to the family "Araliaceae" and its roots have been used as a herbal medicine for thousands of years. For the first time, mosaic, yellowing and vein banding symptoms were discovered on ginseng at Yeongju area in Kyongbuk province on June in 2006. The occurrence of these symptoms in 2006 was 4.4% and it was increased continuously up to 13.1% in 2010 by visual inspection. Average root weights of ginseng aged three and five years infected with virus were decreased to 48.5% and 42.4%, respectively. To say the conclusion first, virus isolated from ginseng was valued as variant of WMV on account of difference of pathogenicity for the host plant and serological response. Therefore, it was suggested that it was named by *Watermelon mosaic virus* Ginseng strain (WMV-G). WMV-G had the shape of filamentous rods having 720 to 780nm length through electron microscopic examination. In cells infected with WMV-G, potyvirus-characte- ristic inclusions of pinwheels, scrolls, laminated aggregates and cylindrical inclusion were present in the cytoplasm. In 35 indicator plant species including *Chenopodium amaranticolor* by mechanical inoculation, any virus symptoms were not developed. In ELISA test using 8 *Potyvirus* species including WMV, no positive reactions were obtained serologically with the WMV-G.Based on the positively reacted probes in DNA chip hybridization diagnosis, specific primers were designed and WMV-G was identical more than 90% with CP of WMV. Full nucleotide sequence of WMV-G was 10,042bp nucleotides in length including the poly(A) tract and contained large open reading frame(ORF) that started at nucleotide positions of 133 to 135 and ended at the position of 9769 to 9771 encoding a polyprotein of 3,212 amino acids. It had 93.3-96.3% sequence identity with 7 isolates of WMV from NCBI.

**P33.002 Effect of eggplant rootstocks on inhibiting infection to tomato seedlings with *Tomato yellow leaf curl virus***

*H. Osaki, K. Nomiyama, H. Sekiguchi, K. Tomioka and T. Takehara*
*Western Region Agricultural Research Center, National Agriculture & Food Research Organization, 6-12-1 Nishifukatsu, Fukuyama, Hiroshima 721-8514, Japan*
*Email: hideki@affrc.go.jp*

Yellow leaf curl disease, caused by *Tomato yellow leaf curl virus* (TYLCV), has occurred on tomato crops in Japan since 1996. Physical controls included spreading nets (mesh size; below 0.4 mm) on gaps of greenhouses to prevent the vector whitefly *Bemisia tabaci* from entering, and chemical controls are mainly carried out. Despite these control efforts, yellow leaf curl disease remains a significant problem on tomato production in Japan. To analyze TYLCV spread in tomato seedlings at the initial phase of infection, time-course detection of TYLCV by PCR was conducted in leaf and root tissues of tomato seedlings inoculated by whiteflies (*B. tabaci* B biotype). The results showed that TYLCV tended to be detected in root tissues one day earlier than in apical leaf tissues. Root tissue was considered to be an important site for TYLCV proliferation at the initial phase of infection. Influence of using eggplant, a non-host of TYLCV, as a rootstock was examined on inhibition of infection. Infection rates in tomato seedlings (cv. House Momotaro) grafted onto tomato rootstocks (cv. Chika) were 50 to 100%, whereas infection rates in tomato seedlings grafted onto eggplant rootstocks (cv. Daitaro) were 0 to 17%. It was suggested that usage of eggplant rootstock inhibited infection of TYLCV to tomato seedlings. These results also support the hypothesis that root tissue is an important site for TYLCV proliferation at the initial phase of infection. We will analyze mechanism of infection inhibition of TYLCV, and evaluate the practical utility of eggplant rootstock on tomato cultivation.

**P33.003 Development of multiple rapid immunofilter paper assay to simultaneously detect four major viruses of cucumber**

*H. Osaki, K. Nomiyama, H. Sekiguchi, K. Tomioka and T. Takehara*
*Western Region Agricultural Research Center, National Agriculture & Food Research Organization, 6-12-1 Nishifukatsu, Fukuyama, Hiroshima 721-8514, Japan*
*Email: hideki@affrc.go.jp*

On cucumber cultivation in Japan, mosaic diseases caused by *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus 2* (WMV2) and *Zucchini yellow mosaic virus* (ZYMV) are especially common. Spotted wilt disease caused by *Melon yellow spot virus* (MYSV) is now spreading across the western Japan. It is difficult even for experts to distinguish the four diseases by symptoms. Therefore, we tried to apply multiple rapid immunofilter paper assay (multi-RIPA) to simultaneous diagnosis for these virus diseases. Positions immobilized with four sensitized white latex particles on strips were evaluated for establishing multi-RIPA. For MYSV, WMV2 and ZYMV, no positive bands developed above 20 mm from the lower end of the strips. When bovine serum albumin (BSA) concentration in extraction buffer was doubled to 0.2% (w/v), a positive band for each virus developed in the multi-RIPA, regardless of the order that the four sensitized white latex particles were immobilized on the strip. The increase of BSA concentration also strengthened sensitivity of each RIPA. Besides, using mixture of commercially available red and blue sensitized latex solutions as a tracer, purple band appeared successfully on the strip. Furthermore, we were able to adjust the ratio of red and blue latexes to produce two purplish (reddish and bluish purple) bands; thus it was possible to differentiate positive bands of four viruses on the basis of color. It is important and convenient for inexpert users that diagnostic results be visually apparent. These findings are potentially helpful in adopting multi-RIPA for diagnoses of virus diseases on other plants.

**P33.004 A frameshift protein, P3N-afs, from P3 cistron has effect on cell-to-cell movement of *Clover yellow vein virus* in *Pisum sativum***

*S.H. Choi, Y. Hagiwara-Komoda, K.S. Nakahara, S. Naito and I. Uyeda*
*Graduate School of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan*
*Email: shsibs@res.agr.hokudai.ac.jp*

A member of *Potyvirus* including *Clover yellow vein virus* (ClYVV) codes 11 mature proteins containing a recently reported protein, P3N-PIPO. A small open reading frame, PIPO, in P3 that contains 60 codons and is translated as P3N-PIPOby fusing with the N-terminal part of P3 (P3N) possibly through -1 ribosomal frameshifting at $G_{1\text{-}2}A_{6\text{-}7}$ motif. It has been reported that P3N-PIPO is located on plasmodesmata and facilitates the intercellular movement of potyviruses. However, it is unknown that P3N-PIPO has effect on ClYVV movement in pea. Moreover, we found that a new P3 cistron product was produced possibly by +1 ribosomal frameshifting using a cell-free translation system and tentatively designated P3N-afs. In order to investigate their function in cell-to-cell movement, the GFP-tagged and frameshift deficient ClYVV mutant, pCl/P3ΔPIPO, was co-inoculated with *White clover mosaic virus* vector containing *P3N-afs* or *P3N-PIPO* to susceptible pea line, PI 250438. The virus movement was monitored with GFP fluorescence. Wild type of ClYVV systemically spreads toward vein from biolistically infected single cells, whereas the pCl/P3ΔPIPO failed. When P3N- PIPO

was expressed in trans with ClYVV and pCl/ P3ΔPIPO, ClYVV more rapidly spread into adjacent cells and pCl/P3ΔPIPO moved into adjacent cells. In contrast, P3N-afs expression in trans had no effect on cell-to-cell movement of ClYVV and pCl/P3ΔPIPO. However, expression of both P3N-afs and P3N-PIPO enabled ClYVV and pCl/P3ΔPIPO to form more large infection foci than those formed when only P3N-PIPO was expressed. These results suggested that P3N-PIPO of ClYVV was essential in cell-to-cell movement and P3N-afs facilitated the movement.

**P33.005 Virus and viroid diseases on fruit trees in Republic of Korea**

*H.R. Kim, I.S, Cho, I.S. Shin, K.H. Cho, S.H. Kim, K.H. Kim and H.S. Hwang*
*National Institute of Horticultural & Herbal Science, RDA, Suwon, Gyeonggi-do, 440-706, Republic of Korea*
*Email: Khelen@korea.kr*

Major fruit crop of Republic of Korea is apple, pear, grapevine, and peach. We surveyed the infections of virus and viroid diseases in commercial orchards of main area of 4 fruit crops for 4 years from 2009 to 2012 in Korea. RT-PCR was used to identify the presence of the virus and viroid pathogens. In Apple orchards, *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV), and *Apple scar skin viroid* (ASSVd) were detected. ASPV infection was highest at 24%, and the ASSVd infection was 2.3%. *Apple mosaic virus* (ApMV) was not detected in this investigation, but the occurrence has been already reported in Korea. ACLSV, ASGV, ASPV was also detected in pear orchards, and ASSVd was detected in fruits of bumpy shape. In grapevines, *Grapevine leafroll associated 1 virus* (GLRaV-1), *Grapevine leafroll associated 3 virus* (GLRaV-3), *Grapevine fleck virus* (GFkV), and *Grapevine yellow speckle viroid* (GYSVd) were detected. And *Prunus necrotic ringspot virus* (PNRSV), and ACLSV were occurred in peach trees. Especially, *Hop stunt viroid* (HSVd) was infected as highly ratio in grapevines and peaches. This survey results will be useful applied as basic information in national project for virus and viroid disease control.

**P33.006 Characterization of the *Grapevine virus A* AlkB motif and its involvement in plant viral infection**

*C. Dayan-Glick, L. Maslenin, Y. Iddan and M. Mawassi*
*The S. Tolkowsky Laboratory, Department of Plant Pathology, The Virology Unit, Agricultural Research Organization, the Volcani Center, Bet Dagan 50250, Israel*
*Email: kathy@volcani.agri.gov.il*

Grapevine is an economically important and one of the most widely grown fruit crops worldwide. Viral diseases constitute a major hindrance to the development and highly profitable production of viticulture. Rugose wood (RW) complex diseases cause serious damage to grapevine. The *Grapevine virus A* (GVA), genus *Vitivirus*, family *Betaflexiviridae*, is closely associated with RW complex diseases. The virus positive single-stranded RNA genome consists of five open reading frames (ORFs). ORF1 encodes replication-associated proteins, and protein with an AlkB motif. AlkB proteins have homologues in multicellular organisms, and involved in nucleic acids de-methylation mechanism. Yet, their specific role in viruses is unknown. In this research we have utilized GVA- and AlkB-mutated derived GVA clones to examine involvement of the AlkB domain in plant infection, as well as in suppression of RNA silencing. *Nicotiana benthamiana* plants agro-inoculated with some GVA variants with a mutated AlkB domain showed late symptoms development. In grapevine, we found that GVA variants with a mutated AlkB domain can inoculate plantlets, but cannot survive for a long time in mature plants. GVA encodes a small protein, p10, via ORF5 that exhibits RSS activity. In our research we found that expression of AlkB enhances p10 RSS activity. Using the yeast two hybrid system, we found that the p10 exhibits self interaction and, in addition, capable of interaction with AlkB protein.

**P33.007 Occurrence of *Blackcurrant reversion virus* in different *Ribes* species and elimination possibilities by the use of tissue culture**

*A. Gospodaryk[1,2], N. Pūpola[1], L. Lepse[3] and I. Moročko-Bičevska[1]*
*[1]Latvia State Institute of Fruit-Growing, Graudu str. 1, Dobele, LV-3701, Latvia; [2]ESC "Institute of Biology", National Taras Shevchenko University of Kyiv, Volodymyrska str. 64, Kyiv-601, Ukraine; [3]Pūre Horticulture Research Centre, Abavas str. 2, Pūre, LV-3124, Latvia*
*Email: alina.gospodaryk@lvai.lv*

Occurrence of *Blackcurrant reversion virus* (BRV), the causal agent of the reversion disease of black currant (*Ribes nigrum* L.) and of other *Ribes* species was investigated by RT-PCR among 30 different *Ribes* species and inter-species hybrids from three genetic resource collections in Latvia. In total, 29.9% of 147 collected samples were infected with BRV. BRV was detected in 48.5% of samples collected from *Ribes* plants with galls and distinctive symptoms of reversion disease. 24.6% of collected symptomless plants also were detected positive to BRV. Among different *Ribes* species *R. nigrum* and *R. rubrum* were the most infected with BRV– 59.1 % and 13.4 % of positive samples from total, respectively. Most of wild *Ribes* species and genotypes were without visible gall mite invasion, but some of genotypes expose symptoms of reversion disease and were positive in RT-PCR test. Meristem tip culture was used to test virus elimination possibilities *in vitro* from infected plants of

two *R. nigrum* cultivars 'Berdchanka' and 'Mara'. Explants of apical meristematic tissue of 0.5 to 1 mm size were cut and initiated in culture on the Murashige and Skoog (MS) medium with minimal modifications. After several passages on the multiplication media consiting of modified MS with Ribavirin plants were tested for BRV. All tested samples were BRV positive and attempts by combining tissue culture with Ribavirin to eliminate BRV from infected plants were not successful.

**P33.008 Visual tracking of plant virus infection dynamics using a reporter that activates anthocyanin biosynthesis**

*F. Martínez, L.C. Bedoya, D. Orzáez and J.A. Daròs*
*Instituto de Biología Molecular y Celular de Plantas (CSIC-Universidad Politécnica de Valencia), 46022 Valencia, Spain*
*Email: jadaros@ibmcp.upv.es*

Transcription factor Rosea1 (Ros1) from *Antirrhinum majus* is a 25.7 kDa protein involved in activation of anthocyanin biosynthetic genes. Anthocyanins are the compounds responsible of many of the bright colors of fruits and flowers in plants. We have explored the use of Ros1 as a visual reporter to follow the dynamics of viral infection throughout the host plant. We inserted Ros1 cDNA in two different positions in the genome of *Tobacco etch virus* (TEV; family *Potyviridae*) and we inoculated tobacco plants. Tissues showing symptoms turned bright red with an about two day delay. Red color of infected tissues was clearly visible through naked eyes in real time with no need of specialized instrumentation. RT-PCR analyses of RNA preparations from inoculated plants confirmed that only the red tissues from infected tobaccos contained the virus. Analysis of a series of Ros1-tagged TEV mutants showed a correlation between viral load and anthocyanin accumulation in infected tissues. Serial passages of TEV-Ros1 from plant to plant demonstrated the high stability of the reporter marker in the viral genome. We finally studied applicability of this reporter strategy to other virus-host plant combinations, obtaining good performance for *Turnip mosaic virus* (TuMV; family *Potyviridae*) infecting *Arabidopsis thaliana* and *Tobacco mosaic virus* (TMV; family *Virgaviridae*) infecting *Nicotiana benthamiana*. Ros1 is therefore an alternative to fluorescent proteins as a reporter marker to track virus infection and movement throughout the plant.

**P33.009 Potyviral P1 protein traffics to the nucleolus, associates with the host 60S ribosomal subunits and stimulates viral translation**

*F. Martínez and J.A. Daròs*
*Instituto de Biología Molecular y Celular de Plantas (CSIC-Universidad Politécnica de Valencia), 46022 Valencia, Spain*
*Email: jadaros@ibmcp.upv.es*

Genus *Potyvirus* comprises a large group of positive-strand RNA viruses infecting plants. The viral genome encodes a large polyprotein processed by three viral proteinases. P1 protein, the most amino-terminal product of the polyprotein, is an accessory factor stimulating viral genome amplification whose role during infection is mostly unknown. We infected plants with *Tobacco etch virus* (TEV; genus *Potyvirus*) recombinant clones in which P1 was tagged with a fluorescent protein to track its expression and subcellular localization or with an affinity tag to identify proteins forming complexes with P1 during infection. Results showed that TEV P1 exclusively accumulates in infected cells at an early stage of infection, and that the protein displays a dynamic subcellular localization trafficking in and out of the nucleolus during infection. Inside the nucleolus, P1 particularly targets the dense granular component. Consistently, we found functional nucleolar localization and nuclear export signals in TEV P1 sequence. Our results also indicated that TEV P1 physically interacts with the host translational machinery and specifically binds to the 60S ribosomal subunits during infection. *In vitro* translation assays of reporter proteins showed that TEV P1 stimulates protein translation, particularly when driven from the TEV internal ribosome entry site. These assays also showed that TEV helper-component proteinase (HC-Pro) inhibits protein translation. We propose that the coordinated action of potyviral proteins P1 and HC-Pro regulate protein translation in infected cells, stimulating viral translation and inhibiting host translation, respectively.

**P33.010 Possible groupings of naturally-occurring *Potato spindle tuber viroid* (PSTVd) isolates from the GenBank database**

*T.B. Kastalyeva and R.A. Owens*
*Molecular Plant Pathology Laboratory (USDA/ARS), Beltsville, MD 20705, USA; Russian Research Institute of Phytopathology VNIIF, B. Vyaziomy, Moscow Reg., 143050, Russia*
*Email: kastalyeva@vniif.ru*

Phylogenetic analysis of approximately 100 naturally-occurring isolates of PSTVd downloaded from the GenBank database indicates that these isolates may be divided into four groups based upon sequence differences located in the central and variable domains. Sequences of three known and one hypothetical isolate may be considered as ancestral to all other naturally-occurring PSTVd isolates. In the first group the ancestral sequence is the so-called "intermediate" or "type" strain of PSTVd (v01465). This isolate, as well as almost all others group members, contains 6 adenine residues at positions 118-123. A distinct subgroup within this group contains PSTVd isolates from solanaceous

ornamentals. The ancestral strain for the second group is Russian isolate Onega-Premier-94 (ef044304). All isolates from this group differ from the type strain by i) deletion of one of the six adenine residues at positions 118-123 and ii) replacement of a second adenine by uracil. Like the first group, this group also includes several isolates from solanaceous ornamentals. The third group of isolates with Russian isolate Bugry-95 (ef044303) as ancestor also lacks an adenine between positions 118-123; here, a second adenine has been replaced by cytosine. A majority of the isolates in the fourth and most divergent group of PSTVd isolates originated from New Zealand and Australia. In addition to the absence of two adenines residues between positions 118-123, these isolates also contain 11 other shared mutations plus another 7-9 mutations, most of which are present in a majority of isolates in this group.

**P33.011 Molecular analysis of badnavirus isolated from aucuba (*Aucuba japonica*) in Japan**

*M.S. Pinili and K.T. Natsuaki*
*Laboratory of Tropical Plant Protection, Department of International Agricultural Development, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo, 156-8502, Japan*
*Email: keiko@nodai.ac.jp*

Aucuba plants (*Aucuba japonica*), an ornamental shrub collected in mainland Japan showed various virus-like symptoms, from yellowish ring spots to vein-banding. Aucuba bacilliform virus (AuBV) has been reported in Japan; however no molecular analysis has been made so far. To identify the possible pathogen, detection by Polymerase Chain Reaction (PCR) with universal Badnavirus primer pairs for over 70 samples was conducted and high discrepancies on symptoms relative to badnavirus detection were observed among the collected samples. Majority of the samples with yellowish ring spots and vein-clearing were found positive to badnavirus as compared to unrecognizable advancing ring spots, chimeric types and healthy-looking plants. Since no sequences for badnavirus of aucuba are currently available in the GenBank, this study first revealed the high diversity (maximum 66 % nucleotide and amino acid sequence identities) of aucuba isolates (from two locations, Ueno and Ushiku) to *Sugarcane bacilliform virus* (SCBV) on the basis of the RT/RNase H gene. Although the possible role of transposable elements may affect the variegated expression of symptoms, the characterized badnavirus isolated from the typical yellow ring spots indicates novel badnavirus gene sequences. Since aucuba is vegetatively-propagated plant, this symptom-expressing badnavirus gene can be disseminated easily.

**P33.012 Protein interactions of *Nt*ERF5 in the *N* gene-resistance pathway of tobacco**

*J.Y. Yoon[1], E. Baek[1], M. Kim[1], S.K. Choi[2], K.H. Ryu[1] and P. Palukaitis[1]*
*[1]Department of Horticultural Sciences, Seoul Women's University, Seoul 139-774, Republic of Korea; [2]Virology Unit, Department of Horticultural Environment, NIHHS, RDA, Suwon 440-441, Republic of Korea*
*Email: yoonju@swu.ac.kr*

The tobacco *N* gene is one of better known plant resistance genes, conferring a hypersensitive reaction to *Tobacco mosaic virus* (TMV), largely through a salicylic acid (SA)-dependent resistance pathway. However, *Nt*ERF5 is a transcription factor (TF) also involved in the *N* gene-resistance mechanism to TMV, but in an SA-independent pathway. Previously, we showed that *Nt*ERF5 interacted with the TF *Nt*MYB1, which is known to be involved in the TMV (SA)-dependent resistance pathway. The interaction between NtERF5 and NtMYB1 was confirmed by bimolecular fluorescence complementation in *N. benthamina* using *Agrobacterium*-mediated transient expression. Interestingly, both *Nt*ERF5 and *Nt*MYB1 were distributed between the tonoplast, cytoplasm and nucleus. To confirm further the interaction *in vivo*, we used an *in situ* proximity ligation assay, immunostaining and protoplast assays, showing that *Nt*ERF5 interacted directly with *Nt*MYB1 in TMV-infected tissues. Using the yeast two hybrid (Y2H) and β-galactosidase assays, we determined that amino acids positions 170 to 209 of the *Nt*MYB1 protein were crucial for the interaction with *Nt*ERF5. In addition, we found that *Nt*ERF5 interacted with an inhibitor of virus replication (IVR) expressed during TMV resistance in tobacco plants containing the *N* gene. Using the Y2H and β-galactosidase assays, amino acid positions 85 to 185 in *Nt*ERF5 were important for the interaction with IVR. These results show that *Nt*ERF5 is associated with proteins in the *N* gene-mediated signaling pathways, both dependent and independent of SA, to defend against TMV infection in tobacco plants.

**P33.013 P3N-PIPO of *Clover yellow vein virus* exacerbated symptoms of pea infected with *White clover mosaic virus***

*H. Suzuki, Y. Hisa, S.H. Choi, K.S. Nakahara and I. Uyeda*
*Plant Breeding Science, Research Faculty of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan*
*Email: haruka.suzuki.c1ose2you@gmail.com*

Plant viral mixed infections of different genera can cause symptom exacerbations, resulting from facilitative interactions between viruses. The phenomenon is called synergism. Most of reports regarding the mechanism of viral synergism accompanied with the suppression of RNA silencing, a host anti-viral defense mechanism:

Viral RNA silencing suppressors (VSRs), such as helper component proteinase (HC-Pro) of potyvirus, play significant roles as viral factors when the exacerbations occur. Comparing with each single infection, *Clover yellow vein virus* (ClYVV), which is in the genus *potyvirus*, induced severer yellowing and stunt symptoms on pea infected with *White clover mosaic virus* (WClMV), which is in the genus *Potexvirus*. To confirm the significant viral factor for exacerbating them, we observed the symptoms on peas with using the WClMV vectors producing each of HC-Pro, P3 and P3N-PIPO proteins of ClYVV because we thought that these three proteins were possible to be involved in the virulence of ClYVV. The WClMV producing P3N-PIPO drastically exacerbated the symptoms of pea than did the infection of WClMV. The WClMV producing HC-Pro also showed severer symptoms to the lesser extent, suggesting that not only HC-Pro, which is the VSR of ClYVV, but P3N-PIPO can effect on the synergism. Our results imply the synergism accompanies with the other mechanism except the suppression of RNA silencing.

**P33.014 Genome sequence and construction of an infectious cDNA clone of Odontoglossum ringspot virus from orchid in Taiwan**

*S.C. Lee and Y.C. Chang*
*Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan, China*
*Email: shuchuanlee@ntu.edu.tw*

Two genetically distinct recessive resistance genes (*cyv1* and *cyv2*) are known to confer resistance to *Clover yellow vein virus* (ClYVV) in pea. In pea carrying *cyv1*, the virus is restricted in single cells. On the other hands, the virus moves to cells around the infection site but viral movement is much slower in pea carrying *cyv2* than that in the susceptible pea lines. *cyv2* has recently been revealed to encode eukaryotic initiation factor 4E. And a single non-synonymous mutation in P1 cistron was found to be associated with the breaking *cyv2* resistance. The resistance gene of *cyv1* has not been isolated yet. In this report, we showed P3 cistron was involved in breaking resistance of *cyv1*. P3 cistron is known to produce two proteins: P3 from the main open reading frame and P3N-PIPO which has the N-terminal part of P3 fused to amino acids encoded by a small ORF in the +2 reading frame. ClYVV isolate Cl-no30 was restricted in a single cell when the infectious cDNA was particle bombarded to *cyv1* pea whereas isolate 90-1 Br2 overcame this resistance by infecting systemically. Chimera viruses were constructed between the two isolates and their infectivity on *cyv1* pea showed the breaking resistance was attributed to the P3 cistron. Mutational analysis of P3 cistron revealed both P3 and P3N-PIPO were involved in overcoming the resistance.

**P33.015 The Occurrence and Distribution of Viruses on Garlic and Onion in Ethiopia**

*A. Abraham[1], K. Jemal[1,2], T. Feyissa[2], B. Abere[1], G. Berhanu[1, 2] and T. Holton[3]*
*[1]Biotechnology Laboratory, Holetta Agricultural Research Center, Ethiopian Institute of Agricultural Research; [2]Department of Biology, Addis Ababa University, Bioscience for Eastern and Centeral Africa/ International Livestock Research Institute, Nairobi, Kenya*
*Email: adaneabraham@yahoo.com*

Garlic (*Allium sativum*) and Onion (*Allium cepa*) are two important bulb vegetable grown in Ethiopia for which no information exists on the associated viruses Field surveys, serological and PCR-based tests were conducted in the year 2009 and 2012 to identify viruses occurring on these crops in the country. The survey indicated that leaf yellowing; yellow mosaic, stripes and stunting were the most common disease symptoms observed. The highest visually observed disease incidence in a garlic field was 93%, recorded in Oromia region. When 520 symptomatic and asymptomatic samples collected from 56 fields were tested by the double antibody sandwich enzyme linked immunosorbent assay for four common garlic viruses namely *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV) (Genus *Potyvirus* family *Potyviridae*) and *Garlic virus B* (GV-B) and Garlic virus C (GV-C) (genus *Allexivirus*, family, *Flexiviridae*), 119 samples (23%) were found to be infected with at least one virus. GV-B was the most frequent (17.7%), followed by OYDV (5.6%) and GV-C (4.8%). LYSV was detected only in seven samples. On onion fields, *Shallot virus X* is the most common followed by OYDV. When sequence of the 3' part of OYDV and GV-B isolates obtained from garlic were sequenced, they were very identical to those reported in literature suggesting that the same virus affects the crop in Ethiopia and elsewhere.

**P33.016 Host range and incidence of *European mountain ash ringspot-associated virus* in the Czech Republic and other European countries**

*L. Grimova, P. Rysanek and M. Zouhar*
*Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague, Czech Republic*
*Email: zouharmiloslav@seznam.cz*

Typical symptoms of *European mountain ash ringspot-associated virus* (EMARAV) infection are light rings, spots or variegation on *Sorbus aucuparia* leaves. In 2010, mountain ash trees showing ringspot symptoms were found around the Czech capital Prague. RT-PCR was performed and products of predicted lengths were obtained from all symptomatic plant samples. The partial EMARAV putative nucleoprotein gene fragments of

two Czech isolates were amplified and sequenced in both orientations. Comparisons of nucleotide and deduced amino acid sequences of the isolates with several sequences from GenBank showed identities ranging from 97 to 99% at the nucleotide level and 100% at the amino acid level. To our knowledge, this is the first proof of EMARAV occurrence in the Czech Republic. Successively, virus-free plants of *Sorbus*, *Amelanchier*, *Crataegus*, *Malus* and *Pyrus* species and varieties were inoculated by chip budding. In 2012, the 204 bp EMARAV-specific cDNA fragment was obtained from most tested species. So, the EMARAV host range was extended by new species and varieties of *Sorbus* and for the first time by *Malus*, *Pyrus*, *Amelanchier* and *Crataegus* species. Clear symptoms associated to EMARAV were exhibited by the some *Sorbus* and *Amelachier* species. Finally, a survey of infected trees was performed all over the Czech Republic. They were found in most localities checked, sometimes with a rather high prevalence suggesting that EMARAV may be rather widespread in this country. Limited survey was also done in other European countries. EMARAV was found in Slovakia and Italy, but not in Slovenia and on Corsica.

**P33.017 Application of RT-LAMP for direct detection of potato viruses in dormant tubers**

*K. Treder and A. Przewodowska*
*Plant Breeding and Acclimatization Institute - National Research Institute, Radzików, Department of Potato Protection and Seed Science in Bonin, 76-009 Bonin, Poland*
*Email: k.treder@ihar.edu.pla*

The virus indexing of seed tubers is important part of production of healthy seed tubers worldwide. Currently this is performed by the growing-on test. The procedure involves cutting eye cores from dormant tubers and growing them for several weeks in greenhouse to obtain offspring plants, tested by DAS-ELISA. This procedure is very reliable but time and cost-consuming. Thus there is a need for fast and sensitive test which can be used directly on dormant tubers. A loop-mediated isothermal amplification of DNA (LAMP) is a new diagnostic method allowing for fast, sensitive and specific detection of target DNA. When combined with reverse transcription (RT-LAMP) it allows for detection of pathogens, whose genomes are formed by RNA. RT-LAMP doesn't need specialized equipment and is well suited for high-throughput testing. Thus we developed optimized procedure for post-harvest detection of the most important potato viruses – PVY, PLRV and PVM by RT-LAMP.

**P33.018 Complete RNA2 nucleotide sequences of six distinct *Cherry leaf roll virus* isolates from New Zealand**

*E.N.Y. Woo and M.N. Pearson*
*School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*
*Email: ewoo040@aucklanduni.ac.nz*

The complete RNA2 nucleotide sequences of *Cherry leaf roll virus* (CLRV), a subgroup C nepovirus, originating from six different New Zealand host species, *Actinidia chinensis*, *Malus domestica*, *Ribes rubrum*, *Rubus idaeus*, *Rumex obtusifolius* and *Vaccinium darrowii*, were determined. RNA2 of the six CLRV isolates are 6361-6363 nucleotides (nt) in length. RNA2 genome has a single open reading frame, encodes one polyprotein with 1590 amino acids (aa) (molecular mass of 174.2-174.8 kDa) and comprises a 5´UTR, hypothetical peptide, MP, CP, 3´UTR and a poly-(A) tail. The entire genomic sequences of CLRV-Ribes, -Rubus and -Vaccinium isolates are highly similar to each other with 99.6-99.8% nt and 99.2-99.6% aa similarity whilst CLRV-Actinidia and -Rumex only have approximately 91% nt and 73% aa similarity to these isolates. Phylogenetic analysis of the 3´UTR revealed clustering of the isolates within two CLRV phylogenetic groups (B and C). CLRV-Rumex is found in Group B and is more similar to CLRV-Actinidia (Group C) than to CLRV-Malus, -Ribes, -Rubus and -Vaccinium (Group C). CLRV-Rumex shares 96% nt and 90% aa similarity with CLRV-Actinidia. Based on an analysis of RNA2 sequences, New Zealand CLRV isolates have 71.5-71.8% nt and 59.8-61.6% aa similarity to CLRV-Cherry from USA. These isolates were found to be more closely related to CLRV-Rhubarb from Germany with 88.6-92.1% nt and 74.7-89.2% aa similarity. Phylogenetic analysis established close associations between the six New Zealand CLRV isolates and members of subgroup C nepoviruses.

**P33.019 Ion-exchange membrane chromatography – a new tool for plant virus purification**

*A. Przewodowska, W. Przewodowski and K. Treder*
*Plant Breeding and Acclimatization Institute - National Research Institute, Radzików; Department of Potato Protection and Seed Science in Bonin, 76-009 Bonin, Poland*
*Email: a.przewodowska@ihar.edu.pl*

Currently routine detection of plant viruses is mostly performed by DAS-ELISA. This method requires good quality antibodies, specific for target virus. Thus pure virus preparation is a crucial for production of antibodies. Purification of viral particles usually consist of complex, long lasting procedures, involving several ultracentrifugation steps. The viral particles in final preparations often are broken due to mechanical damages and contaminated by co-purifying plant material. We report that instead of ultracentrifugation, an ion exchange membrane chromatography can be successfully used. This

method is based on binding of viral particles to charged groups located on the surface of large pore size membrane. Thus allows for fast purification in mild conditions. As a model of long, filamentous virus we used *Potato virus Y* (PVY) and as a model of small isometric virus – *Potato leafroll virus* (PLRV). Both viruses bound strongly to quaternary amine (QA) groups and eluted from the membrane as homogeneous and physically intact particles. Membrane ion exchange chromatography can be combined with other preparative virus purification methods allowing for purification of high quality viral preparations.

**P33.020 Antiviral resistance induced by chitooligosaccharides in tobacco**

*X.M. Zhao, H. Yin, W.X. Wang, H. Lu, Y.F. Chen and Y.G. Du*
*Liaoning Provincial Key laboratory of Carbohydrates, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, No. 457 Zhongshan Road, Shahekou District, Dalian, 116023, P. R. China*
*Email: zhaoxm@dicp.ac.cn*

Chitooligosaccharides applied by spraying protected tobacco plants against *Tobacco mosaic virus* (TMV). The maximum inhibition of TMV by chitooligosaccharides was observed at spraying 50 μg.ml$^{-1}$ chitooligosaccharides 24 h before inoculation. The tobacco antiviral resistance induced by chitooligosaccharides was connected with NO pathway. *mapk* (mitogen-activated protein kinase) was an important up-stream gene of PAL in the signal transduction path way induced by chitooligosaccharides, and also was a key gene in the TMV inhibition of tobacco signal transduction pathway. Chitooligosaccharides could induce expression of resistance genes in the gene-mediated signal pathway of tobacco such as *SGT1*, *RAR1*, *NtMEK2*, *SIPK*, *TIZZ*. Chitooligosaccharides could strongly inactivate activity of TMV particles in vitro. Chitooligosaccharides could inhibit assembly of TMV coat protein subunits. Treatment with chitooligosaccharides effectively inhibited TMV multiplication and long distance movement of TMV in tobacco plant. Chitooligosaccharides regulated expression of calreticulin (CRT) and decreased activity of tobacco pectin methlesterase which played an important role in TMV movement.

**P33.021 Complete sequence analysis reveals a new genotype of Brassica yellows virus infecting cabbage and radish in China**

*X.Y. Zhang, Z.J. Zhou, H.Y. Xiang, D.W. Li, J.L. Yu and C.G. Han*
*Department of Plant Pathology and State Key Laboratory for Agro-biotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: hanchenggui@cau.edu.cn*

Brassica yellows virus (BrYV) is a newly identified virus species that is closely related to but significantly different from *Turnip yellows virus* (TuYV). Two BrYV genotypes (BrYV-A and BrYV-B) have been identified that mainly differ in the 5'-terminal half of the genome. In our study, the complete nucleotide sequences of two BrYV isolates from radish and Chinese cabbage were determined. Sequence comparisons revealed that the radish and Chinese cabbage isolates belong to BrYV, but have several differences with the two previously described genotypes. The two isolates have 5678-nt genomes, compared with the 5666-nt BrYV-A and BrYV-B genotypes, and exhibit several differences in their genome sequences compared with the BrYV A and B genotypes. The 5' terminal ORFs of the radish and Chinese cabbage isolates displayed more divergence (86.8–93.7%) and the 3' terminal ORFs showed higher nucleotide sequence identities 96.5–99.3% than the BrYV-A and BrYV-B. Phylogenetic analysis also showed that the 5'-terminal ORF (P0, P1, P1-P2) encoded products of the three genotypes form three distinct subgroups and that they are more closely related each other and have significant differences from TuYV. Therefore we are proposing a new BrYV genotype to be designated BrYV-C.

**P33.022 Genomic sequence analysis of Beijing isolate of *Cucurbit chlorotic yellows virus***

*R.N. Zhang, Y.Y. Li, D.W. Li, J.L. Yu and C.G. Han*
*Department of Plant Pathology and State Key Laboratory for Agrobiotechnology, China Agirculture University, 100093 Beijing, P. R. China*
*Email: hanchenggui@cau.edu.cn*

*Cucurbit chlorotic yellows virus* (CCYV) is a new member of the genus *Crinivirus* within the family *Closteroviridae*, which causes major economic losses in cucurbit crops such as cucumber, melon and watermelon. In the mainland china, similar symptoms were observed in melon, watermelon, and cucumber plants grown in plastic houses of coastal cities since 2008, and Gu Qinsheng reported the detection of CCYV from the diseased plants in 2011. Complete RNA2 sequences of two CCYV isolates from Taiwan Erlun and Yilan were reported in Genbank. In this paper, full length genomic RNA sequence of CCYV Beijing isolate from cucumber (CCYV-BJ) was determined. CCYV-BJ RNA1 which harbors 4 ORFs is 8407 nt long with5' UTR and 3' UTR of 73 and 250 nt respectively. RNA2 is 8041 nt with 8 ORFs, its 5' UTR is 1034 nt, 3' UTR is 221 nt. Comparison between CCYV-BJ and CCYV Japan isolate (CCYV-JP) shows that they shared a nucleotide identity of 99.80% for RNA1. Nucleotide identities of ORF1a, ORF1b, ORF2 and ORF3 are 99.78%, 99.93%, 99.37% and 99.65%, andamino acid sequence identities of ORF1, ORF2 and ORF3 are 99.80%, 100% and 99.47%, respectively. CCYV-BJ shared a nucleotide identity of

99.79% for RNA2 with CCYV-JP, nucleotide identities of each ORFs are 100%, 99.94%, 100%, 99.94%, 100%, 99.87%, 99.72%, 99.42%, and their amino acid sequence identities are 100%, 100%, 100%, 100%, 100%, 100%, 99.37%, 98.59%, respectively. Interestingly, nucleotide sequence and amino acid sequence identity analysis of the 4 isolates shows that ORF1, ORF3 and ORF5 are most conservative among the 8 ORFs in RNA2, while ORF8 has more variation comparatively.

**P33.023 Discovery and characterization of a novel carlavirus infecting potatoes in China**

*Y.Y. Li, R.N. Zhang, H.Y. Xiang, H.M. Abouelnasr, D.W. Li[1], J.L. Yu[1], J.H. McBeath[2] and C.G. Han[1]*
*[1]State Key Laboratory for Agro-biotechnology and Department of Plant Pathology, China Agricultural University, Beijing 100193, P. R. China; [2]Plant Pathology and Biotechnology Laboratory, Agriculture and Forestry Experiment Station, University of Alaska Fairbanks, Fairbanks 99775, USA*
*Email: hanchenggui@cau.edu.cn*

A new carlavirus, tentatively named Potato virus H (PVH), was found on potato plants with mild symptoms in Hohhot, Inner Mongolia Autonomous Region, China. PVH was confirmed by genome sequencing, serological reactions, electron microscopy, and host index assays. The PVH particles were filamentous and slightly curved, with a modal length of 570 nm. Complete RNA genomic sequences of two isolates of PVH were determined using reverse transcription-PCR (RT-PCR) and the 5′ rapid amplification of cDNA ends (5' RACE) method. Sequence analysis revealed that PVH had the typical genomic organization of members of the genus Carlavirus, with a positive-sense single-stranded genome of 8410 nt. It shared coat protein (CP) and replicase amino acid sequence identities of 17.9–56.7% with those of reported carlaviruses. Phylogenetic analyses based on the amino acid sequences of replicase and CP showed that PVH formed a distinct branch, which was related only distantly to other carlaviruses. Western blotting assays showed that PVH was not related serologically to other potato carlaviruses (*Potato virus S*, *Potato virus M*, and *Potato latent virus*). PVH systemically infected *Nicotiana glutinosa* but not *N. tabacum*, *N. benthamiana*, or *Chenopodium quinoa*, which contrasted with other potato carlaviruses. These results support the classification of PVH as a novel species in the genus carlavirus. Preliminary results also indicated that a cysteine-rich protein encoded by the smallest ORF located in the 3' proximal region of the genome suppressed local RNA silencing and enhanced the pathogenicity of the recombinant PVX.

**P33.024 Identification of viruses infecting yam in China**

*C.W. Zou, J.R. Meng, Z.Q. Wang, L. Zhang and B.S. Chen*
*State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources and College of Life Science and Technology, Guangxi University, Nanning, 530004, P. R. China*
*Email: chenyaoj@gxu.edu.cn*

Yams (*Dioscorea* spp.) are used both as vegetables and as traditional herbal medicine in China. During 2010-2012, surveys of viral disease in yams were conducted in major jam production provinces of China. A total of 326 yam leaf samples collected during the surveys were analyzed by enzyme-linked immunosorbent assay (ELISA), immunocapture polymerase chain reaction (IC-PCR) and/or IC-reverse transcription-PCR (IC-RT- PCR) to identify the causal viral agents. Among known viruses that have been reported to infect yams, *Yam mild mosaic virus* (YMMV), *Japanese yam mosaic virus* (JYMV), *Chinese yam necrotic mosaic virus* (CYNMV), *Broad bean wilt virus 2* (BBWV-2), *Dioscorea bacilliform virus* (DBV) were identified. Complex symptoms from asymptomatic latent infection, typical mosaic, to sub-lethal were observed and double or multiple infections by viruses were common. To identify viruses that were less common or not reported on yam, the next generation sequencing technology was employed to analyze small RNA and transcriptome from the yams. Four known viruses (YMMV, JYMV, CYNMV, and BBWV-2) and two previously unreported viruses (carlavirus and potexvirus) were identified. Full-length genome sequences of these viruses were further verified by RT-PCR, RACE, and Sanger sequencing. Polyclonal antibodies against DBV, YMMV and Potexvirus and Monoclonal antibodies against JYMV, BBWV-2 and CYNMV were generated in rabbits and mouse, respectively. Geographical distribution and variability of the viruses infecting yams in China are currently under investigation.

**P33.025 Detection of *Cucumber green mottle mosaic virus* based on magnetic nanoparticles technology**

*X.L. Zhao[1], Q. Zhou[1], N. Sun[2] and C.L. Deng[1]*
*[1]Plant Laboratory of Beijing Inspection and Quarantine Testing Center, Beijing, 100026, P. R. China; [2]School of Biological Science & Medical Engineering, Southeast University, Nanjing 210096, Jiangsu Province, P. R. China*
*Email: dengcl@bjciq.gov.cn*

In this study, core-shell structural $Fe_3O_4/SiO_2$ composite magnetic nanoparticles were prepared, which could non-specifically absorb virus RNA from plant sample. A new method to detect *Cucumber green mottle mosaic virus* (CGMMV) was developed by using $Fe_3O_4/SiO_2$ composite magnetic nanoparticles extraction combined with Real-time RT-PCR assay. The repeatability and sensitivity of this method was compared with another

two methods including immunomagnetic extraction method and magnetic nanoparticles method through Real-time RT-PCR. The results indicated that all three methods had good repeatability. The method of magnetic nanoparticles extraction which could non-specifically absorb virion had the same sensitivity with that of immunomagnetic extraction method, and both of them could detect CGMMV from 1 ng infected leaves. The method of magnetic nanoparticles extraction which could non-specifically absorb virus RNA had the highest sensitivity. It could detect CGMMV from 10 pg infected leaves, indicating that the sensibility of this method was 100 times higher than another two methods. In total, the RNA extraction method using the $Fe_3O_4/SiO_2$ composite magnetic nanoparticles combined with Real-time RT-PCR is simple, rapid, sensitive and specific, and provides a new way for detection of CGMMV.

**P33.026 Study on expression in *Pichia pastoris* and antiserum preparation of *Prunus necrotic ringspot virus* CP gene**

*D. Chen*
*Inspection Technical Center of Zhongshan Entry-Exit Inspection & Quarantine Bureau, Zhongshan, Guangdong, 528403, P. R. China*
*Email: chendh@zs.gdciq.gov.cn*

The CP gene of *Prunus necrotic ringspot virus* (PNRSV) was obtained by RT-PCR and inserted into pGEM®-T vector to formed recombination pGEM®-T-PNRSV, which was then cloned in eukaryotic expression vector pPIC9K and transformed into *Pichia pastoris* strain GS115, the CP gene was expressed effectively after inducing with methanol. The PNRSV antiserum was got from rabbit immune with expressed CP in GS115 and the titer was $3.2\times10^4$ tested by ID-ELISA. The method by using of genetic technique to make PNRSV antiserum can thoroughly reduce the risk of the virus escaping from its propagation.

**P33.027 Induction of resistance in potato against *Potato virus Y* by systemic chemicals**

*S. Jaleel, F. Naz and S.M. Mughal*
*Department of Plant Pathology, PMAS, Arid Agriculture University Rawalpindi, 46300, Pakistan*
*Email: farahnaz@uaar.edu.pk*

For inducing SAR in potato plants against PVY four systemic chemicals viz. salicylic acid (0.20%), aspirin (2%), lopsine (2%) and *Pseudomonas flouorescens* (0.2%) were sprayed 24 hours before, after and at the time of inoculation of PVY in two potato varieties (Cardinal and Hermes) found susceptible during screening. Effects of these treatments were evaluated by determining changes in phenolic and mineral contents (Ca, K and Mg) of potato plants. Phenolic contents were increased in both varieties after spraying of systemic chemicals and mechanical inoculation of PVY as compared to untreated uninoculated control plants. Application of aspirin 24 hours before inoculation of PVY induced highest increase in phenolic contents (784.2%) as compared to other three. This increase in phenolic contents augments the resistance mechanism of potato plants against PVY. Reduction in calcium contents take place by spraying all chemicals at all sprays timings. Lowest reduction (10.99%) was brought about by *P. flouorescens*, when it was applied at the time of inoculation. Increases in potassium and magnesium contents occur in both varieties and the highest increase in potassium was found when *P. flouorescens* sprayed 24 hours before inoculation while, Salicylic acid induced higher Mg contents (56.485%) when applied 24 hours before inoculation of PVY. It can be manifested that application of systemic chemicals as foliar sprays are very effective in reducing PVY infection in potato by inducing systemic acquired resistance may be incorporated in disease management program against PVY.

**P33.028 Characterization of small interfering RNAs derived from maize infection by *Rice black-streaked dwarf virus***

*Y.Q. Li, T. Zhou and Z.F. Fan*
*Department of Plant Pathology, China Agricultural University, Beijing 100193, P. R. China*
*Email: fanzf@cau.edu.cn*

RNA silencing is a highly conserved defense mechanism in protection against parasites such as viruses, fungi, nematode and bacteria. Virus infection leads to accumulation of large amount of viral small interfering RNAs (vsiRNAs). The characterization of vsiRNAs has been well established in plant single-stranded RNA viruses and some DNA viruses, but little is known in plant dsRNA viruses. In this research, small RNA libraries were constructed with maize leaves infected by *Rice black-streaked dwarf virus* (RBSDV), a member of the genus *Fijivirus* in the family *Reoviridae* and the RBSDV-derived vsiRNAs were characterized by deep sequencing. The majority of siRNAs derived from RBSDV were 21 nt or 22 nt in length. Uridine (U) and adenosine (A) residues, in contrast to cytosine (C) and guanidine (G), are preferentially used in vsiRNAs derived from the ten genomic RNA segments of RBSDV. Analysis of vsiRNAs revealed the presence of multiple hotspots for small RNA production from the genomic sequence of RNA segment 4 and complementary strand of genomic RNA segments 7 and 10.

**P33.029 Occurrence and genetic characteristics of *Cotton leaf curl Multan virus* invading China**

*Z.G. Du[1], Y.F. Tang[1] and Z.F. He[1,2]*
*[1]Plant Protection Research Institute, Guangdong*

*Academy of Agricultural Sciences, Guangzhou 510640, P. R. China; [2]Guangdong Provincial Key Laboratory of High Technology for Plant Protection, Guangzhou 501640, P. R. China*
*Email: hezf@gdppri.com*

In the year 2006, China rose (*Hibiscus rosa-sinensis* L.) plants showing symptoms including leaf curling, yellowing, and vein swelling were found in Guangzhou, Guangdong province, China. A begomovirus exhibiting 96.1% nucleotide sequence identity with G62 isolate of *Cotton leaf curl Multan virus* (CLCuMV) was isolated from the diseased plants. In recent years, China rose leaf curl disease spreads quickly and becomes prevalent in many areas of Guangdong, Guangxi, Hainan, and Fujian provinces of China. In all cases tested by us, positive results were obtained when employing specific PCR primers to amplify CLCuMV using total DNA isolated from the diseased samples as templates. Complete nucleotide sequences of 23 CLCuMV isolates from different sites of China were determined. The sequences of the 23 CLCuMV isolates were 99% and 89%-100% identical to each other and to those of CLCuMV isolates from Pakistan. In contrast with the case reported in India, all the 23 isolates of CLCuMV were accompanied by a full-length DNAβ. Sequences of the DNAβwere more than 98% identical to each other and 83.2-99.3% identical to those associating with other isolates of CLCuMV. Our surveys conducted recently showed that CLCuMV could infect at least three other plant species in natural field conditions in China, namely *Abelmoschus esculentus*, *Malvaviscus arboreus* and cotton. These studies suggested that CLCuMV was endemic in China. CLCuMV invading China might originate from Pakistan. CLCuMV in China was genetically stable. Full-length DNAβwas maintained during intra- and inter-species transmission of CLCuMV in China.

### P33.030 Study of *Grapevine leafroll-associated viruses* in China

*G.Q. Pei, Y.F. Dong, Z.P. Zhang, X.D. Fan, F. Ren and G.J. Hu*
*National Center for Eliminating Viruses from Deciduous Fruit Tree, Research Institute of Pomology, Chinese Academy of Agriculture Sciences, Xingcheng, Liaoning, 125100, P. R. China*
*Email: hugj3114@163.com*

Two hundred Grapevine cultivars were surveyed for the prevalence of Grapevine leafroll disease (GLD). Eighty-two cultivars showed GLD symptoms, and the cultivars belong to *Vitis vinifera* L. had more cultivars, higher morbidity and more serious symptoms than *V. vinifera*-*V.labrusca*. No *V. labrusca* L. was found showing GLD symptoms in the vineyards. Fifty-eight samples collected from the vineyards were tested for *Grapevine leafroll-associated viruses* (GLRaVs) by ELISA and RT-PCR. The positive rate of GLRaV-1, 2, 3, 4, 5 and 7 was 20.7%, 17.2%, 62.1%, 3.4%, 5.2% and 15.5% respectively. The recovered fragments were cloned, sequenced and identified. The sequencing results have been submitted to GenBank. The evolutionary relationship of GLRaVs was conducted based on HSP70 encoding gene. Neighbor-Joining（NJ）phylogenetic trees were established, and GLRaV-1, 3 and 7, GLRaV-2, 4 and 5 were classified together in a cluster, respectively. Moreover, GLRaV-1 and 3 have closer genetic relation than GLRaV-7. And GLRaV-4 and 5 have closer genetic relation than GLRaV-2.

### P33.031 Seed transmission of *Tomato torrado virus* in *Physalis floridana*

*H. Pospieszny, N. Borodynko, B. Hasiów-Jaroszewska and N. Rymelska*
*Institute of Plant Protection–National Research Institute, Virology and Bacteriology Department, W. Węgorka 20, 60-318Poznań, Poland*
*Email: H.Pospieszny@iorpib.poznan.pl*

The goal of this research was to evaluate whether *Tomato torrado virus* (ToTV) can be transmitted through *Physalis floridana* seeds. In 2011 and 2012 *P. floridana* seedlings were inoculated mechanically with ToTV and were kept in greenhouse, at 20-25$^0$C for symptoms and fruit development. *P. floridana* plants with symptoms of mosaic and leaf malformation typical for ToTV and confirmed by RT-PCR were selected for seeds production. Harvested seeds before sowing were kept in refrigerator. Seeds were treated with 10% (wt/vol) trisodium phosphate to ensure that any viral infection that occurred was not simply the result of virus on the seed coat, but rather the result of embryonic infection. Treated seeds were sown individually in small pots with soil within 4 weeks of seed being harvested. *P. floridana* plants were grown in an greenhouse for at least 4 weeks before testing. In order to find out the presence of ToTV in seedlings, they were subjected to immunocapture (IC) real-time RT-PCR. Tissue of leaves from 10 plants (combined samples) were homogenized with buffer in plastic bags with Homex 6 (Bioreba). In 2011, total 320 pooled samples (3200 seedlings) were tested and 12 positives detected, indicating transmission rate of 0.38%. In 2012, for 3700 plants tested the rate of seed transmission was about 0.54. Positive samples were subjected to RT-PCR and sequencing. None of the plants that was positive for ToTV by IC/real-time RT-PCR or RT-PCR displayed any symptoms. This is the first report of ToTV seed transmission in *P. floridana.*

### P33.032 Characterization and complete genome sequence of *Potato virus S* from China

*H.M. Abouelnasr, Y.Y. Li, D.W. Li, J.L. Yu and C.G. Han*

*Department of Plant Pathology and State Key Laboratory for Agro-biotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: hesham_aboelnasr@yahoo.com*

*Potato virus S* (PVS, carlavirus) has been divided into two strains: *Chenopodium* non-systemic (Ordinary: $PVS^{O}$) and *Chenopodium* systemic (Andean: $PVS^{A}$ or $PVS^{CS}$) according to the reaction on *Chenopodium* spp. In the last decades, PVS has been recognized as major potato virus problems that have affected production in China. In this article, the complete genome sequences and molecular properties of Chinese PVS strains were determined for the first time. Three PVS strain ($PVS^{O/A}$) isolates were identified by host reactions, RT-PCR and western blotting. In *Chenopodium* spp. phenotypic screening of the Shandong ($PVS^{A}$-SD) and Heilongjiang $PVS^{A}$ ($PVS^{A}$-HL) isolates revealed localized infections followed by systemic infections, but the Yunnan $PVS^{O}$ isolate ($PVS^{O}$-YN) only exhibited localized infections. Subsequently, the complete genome sequence of $PVS^{O}$-YN isolate and partial sequences covering TGB1 (ORF3) to the 3' poly A terminus (2573 nts) of the $PVS^{A}$-HL isolate were determined. The $PVS^{O}$-YN genome contains six overlapping ORFs and consists of 8,488 nucleotides (nt), excluding the 3' poly (A) tail. $PVS^{O}$-YN shares nt sequence identities of 93-97% in all the ORFs of three O strain isolates ($Id4106^{O}$, $Leona^{O}$ and $WaDef^{O}$), whereas this isolate has lower (77-88%) identities with the two A strains, except for ORF2 where they share 95-97% identity. As reported previously, specific amino acid positions can be used to distinguish the PVS-A/O strains. Phylogenetic analyses based on nucleotide sequences also showed that $PVS^{O}$-YN clusters with the three Ordinary strain isolates, and that the $PVS^{A}$-HL isolate belongs to the Andean isolate clade. In addition, a breakpoint in recombination analysis was observed between $PVS^{O}$-YN isolate and the Ordinary strain isolate from America (Id4106-US).

### P33.033 SSR markers associated with the resistace genes to *Rice black-streaked dwarf virus* in maize

*D.P. Di, F. Yang, H.Q. Miao, Y.G. Lu, A.H. Zhang and L.Z. Tian*
*Institute of Plant Protection, IPM Centre, Hebei Academy of Agricultural and Forestry, No.437 Dongguan street, Baoding, 071000, P. R. China*
*Email: miao5058345@163.com*

Maize rough dwarf disease caused by *Rice black-streaked dwarf virus* (RBSDV) in China is transmitted by planthoppers. To develop resistant germplasm is the most economical, environmentally-sustainable and effective means to control the disease. In this paper $F_2$ population was constructed using the cross derived from a resistant and a susceptible parents (Qi319×Ye107) and the resistance levels of $F_2$ individuals were assessed with artificail inoculation RBSDV. The results showed that 17, 8, 11, 51 and 122 plants respectively fitted into a scale of 0-4, in which 0 and 4 represtented highly resistant and susceptible. Chi-square test demonstrated that the segregation ratio of phenotype was 15:1 (susceptible:resistant) or 9:6:1 (susceptible:moderate:resistant) in the $F_2$ population. Therefore the resistance gentic is controlled by multiple genes in maize and the duplicate or additive effects appeared in the interactions of genes. Then the $F_2$ individuals were further analyzed with the polymorphism markers which were obtained with bulked segregant analysis method and SSR-PCR technology, and also Chi-square test showed that phi051, umc1407, umc2053 and umc1432 exhibited segregation distortion significantly at the 0.05 and 0.01 level in susceptible individuals, which were identified as the potential markers linked to the resistant loci. 261 SSR primers involved ten chromosomes were selected from Maize GDB to construct genetic linkage map which consisted of 73 polymorphic SSR markers, spanning a genetic distance of 926.6 cM with an average interval of 14.0 cM between adjacent markers. Four potential resistant markers were preliminary mapped on chromosome 7 and 10.

### P33.034 Identification and characterization of new species of *Tospovirus* in China

*J.H. Dong, Y.Y. Yin, Q. Fang and Z.K. Zhang*
*Yunnan Key Laboratory of Agricultural Biotechnology, Biotechnology and Genetic Germplasm Institute, Yunnan Academy of Agricultural Sciences, Kunming, 650223, P. R. China*
*Email: zhongkai99@sina.com*

Tospoviruses cause significant losses of many economically important crops worldwide. In China, although the enveloped virion and serological assay confirmed the presences of tospovruses in the 1980's, molecular characterization of genome was not reported until *Tomato zonate spot virus* (TZSV) was reported in Yunnan in 2008. Since then, at least 10 tospovirus species have been discovered and confirmed in China. *Tomato spotted wilt virus* (TSWV), *Impatiens nectrotic spot virus*, Capsicum chlorosis virus (CaCV), *Groundnut yellow spot virus* (GYSV), Calla lily chlorotic spot virus (CCSV) , Hippeastrum chlorotic ringspot virus (HCRV), Macadamia leaf nectrotic spot–associated tospovirus were found in Yunnan. Mulberry vein banding virus was found in Guangxi and identified using RNA sequencing by Meng *et al.*. In Yunnan, we also found other three new species of *Tospovirus* affecting tomato and pepper. Comparison analysis of partial amino acid sequences of nucleocapsid (N) proteins with that of other tospoviruses showed these three new tospovirus species is phylogenetically close to members of *Watermelon silver mottle virus* serogroup. Five thrips species (*Frankliniella occidentalis* Pergande, *Thrips palmi* Karny, *T. tabaci* Lin-

deman, *F. intonsa* Morgan, *Ceratothripoides claratris* Shumsher), which can transmit tospoviruses, were found in China. However, except for TSWV and INSV, thrips-vectors of other tospoviruses in China are still under investigation. It is too difficult to control the tospovirus diseases under the lack of resistant crop varieties. Thus, farmers had to plant the low-value crops which are not the host of tospoviruses to substitute for economically important ones.

**P33.035 Distribution of predominant begomoviruses and *Bemisia tabaci* cryptic species in Yunnan province, Southwest China**

*J. Hu, W.H. Li, M. Ding, Y.D. Chen, J. Zhang and Z.K. Zhang*
*Key Lab of Southwestern Crop Gene Resources and Germplasm Innovation, Ministry of Agriculture, Yunnan Provincial Key Lab of Agricultural Biotechnology, Biotechnology and Germplasm Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming, 650223, P. R. China*
*Email: zhongkai99@sina.com*

Begomoviruses are plant viruses with circular, single-stranded DNA (ssDNA) and cause serve damage to many important economic crops worldwide. The whitefly, *Bemisia tabaci* is the only transmitter for begomoviruses. The *B. tabaci* is a cryptic species complex and composed of 28 species at least. Yunnan is one of concentrative distribution regions of begomoviruses that includes four different geographical areas of high incidence, the north Jinsha River Valley (NJR), the west Lancang River-Nu River Valleys (WLN), the mid south Yuanjiang River-Hong River Valleys (MSYH) and the south Lancang River Valleys (SLR). The field survey on begomoviruses and *B. tabaci* from cultivated crops was accomplished in the year, 2012. Results indicate in NJR, the *Tomato yellow leaf curl China virus* (TYLCCNV), *Tobacco curly shoot virus* (TbCSV), *Papaya leaf curl China virus* (PaLCuCNV) are the predominant begomovirus species, and the Middle East-Asia Minor 1 (commonly known as biotype B) is the predominant *B. tabaci* cryptic species, as well as the TbCSV, TYLCCNV, *Tomato yellow leaf curl Thailand virus* (TYLCTHV), PaLCuCNV and the Mediterranean (commonly known as biotype Q) in WLN, the TYLCCNV, TbCSV, PaLCuCNV, Mediterranean and Middle East-Asia Minor 1 in the MSYH, and the TbCSV, TYLCCNV and Mediterranean in SLR. A dominant indigenous *B. tabaci*, Asia I is found in all the four areas. Different *B. tabaci* cryptic species differs in the transmission capability to begomoviruses. Further studies should be conducted on the interaction between predominant begomoviruses and *B. tabaci* species to reveal the mechanisms of epidemics of begomoviruses diseases in Yunnan.

**P33.036 Characterization of Southern rice black-streaked dwarf virus in Vietnam**

*A.T. Hoang[1], H.M. Zhang[2], J. Yang[2], J. Xue [2,4], J. Li [2], J.P. Chen[2], E. Hébrard[3], G.H. Zhou[5], V.N. Vinh[1] and J.A. Cheng[6]*
*[1]Plant Protection Research Institute, Hanoi, Vietnam; [2]State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control, Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Sciences (ZAAS), Hangzhou 310021, P. R. China; [3]UMR 186 Résistance des Plantes aux Bioagresseurs, Institut de Recherche pour le Développement (IRD), 34394 Montpellier, Cedex 05, France; [4]College of Bio-Safety Science and Technology, Hunan Agricultural University, Changsha 410128, P. R. China; [5]Laboratory of Plant Virology, South China Agricultural University, Guangzhou 510642, P. R. China; [6] Institute of Entomology, Zhejiang University, Hangzhou 310029, P. R. China*
*Email: jpchen2001@yahoo.com.cn*

A novel dwarf disease first observed on rice in NgheAn Province in 2009 has spread rapidly to 35 provinces or cities of North and Central Vietnam. Infected rice plants were stunted with darker leaves, twisted leaf tips and split leaf margins. Later, white waxy enations that eventually turned black were observed on the underside of leaf blades, leaf sheaths, and culms. After rice harvest, the disease infected maize plants, which were stunted and dark green with small enations along the minor veins on the back of leaves. The disease agent has now been identified as Southern rice black-streaked dwarf virus (SRBSDV) recently reported from Southern China. Tubular structures and viroplasms containing crystalline arrayed spherical virions approximately 65 to 75 nm in diameter were observed under the electron microscope in ultrathin sections of infected plants. The virus was transmitted to rice and maize seedlings by the white-backed planthopper (*Sogatella furcimera*). RT-PCR confirmed the presence of SRBSDV in 477 plant samples from 29 provinces among 5 agroecological regions in Vietnam. *Rice black-streaked dwarf virus* was not detected in these samples. The genome of SRBSDV Vietnam isolate had 29115 nucleotides and was similar in organization to those of Chinese isolates and other group 2 fijiviruses. Partial sequences of RNA segments 4 and 10 from eleven isolates showed very low genetic divergence between isolates from Vietnam and China, suggesting a common origin. Phylogenetic analysis confirmed the placement of SRBSDV as a distinct virus within subgroup 2 of the genus *Fijivirus*, family *Reoviridae*.

**P33.037 First Report of a *Tospovirus* in Mulberry**

*J.R. Meng, P.P. Liu, C.W. Zou, Z.Q. Wang, Y.M. Liao, J.H. Cai, B.X. Qin and B.S. Chen*
*State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Agricul-*

*ture, College of Life Science and Technology, Guangxi University, Nanning, China, 530004;4.Institute of Plant Protection, Guangxi Academy of Agricultural Sciences, Nanning 530007, P. R. China*
*Email: chenyaoj@gxu.edu.cn*

Mulberry (*Morus alba* L.) is an economically important crop grown widely throughout Asia. Various virus-like symptoms including mosaics, vein banding, chlorotic ringspots had been observed and reported on mulberry trees in China and Japan for decades. In a recent (2010 to 2011) field survey in Guangxi Province, China, the incidence of virus-like diseases of mulberry ranged between 40 and 80%. A suspected member of the genus *Tospovirus* was found in the diseased mulberry tree sample pool via small RNA deep sequencing. A partial S-RNA segment of about 1000-bp was amplified from the tree showing vein banding symptom. This segment encompassed the open reading frame of the nucleocapsid (N) protein gene and the intergenic region. The coding region for the N protein was 831-bp and the deduced proteins of 277 amino acid residues shared up to only 74.4% identity to *Capsicum chlorosis virus*. Thus this virus may represent a new member of the *Tospovirus* genus, temporarily named Mulberry vein banding virus (MuVBV). To the best of our knowledge, this is the first report of a *Tospovirus* infecting *M. alba*. In a RT-PCR screening of 48 randomly selected mulberry samples suspected to be virus-infected, 32 were MuVBV-positive. Giving the high incidence and the high yield loss associated with *Tospovirus* and the presence of thrips, suspected vectors for the virus, MuVBV may represent a substantial threat to the silkworm industry in China.

**P33.038 Broad-spectrum virus resistance using DRIGS-mediated transitive silencing**

*L.C. Galvez[1], Z. Zhang[2] and A. Mitra[1]*
*[1]Department of Plant Pathology, University of Nebraska Lincoln; [2]Plant Transformation Core Facility, University of Missouri; Department of Plant Pathology, University of Nebraska Lincoln, Lincoln, NE 68583-0722, USA*
*Email: lcgalvez@huskers.unl.edu*

The RNA interference (RNAi) has been widely used to down regulate targeted transcipts and development of disease resistant plants. RNAi as a type of post-transcriptional gene silencing (PTGS) relies on the presence of dsRNA as an inducer for the entire silencing complex leading to the degradation/inactivation of the target mRNA. Here, we used a direct repeat induced gene silencing (DRIGS) vector as a tool for developing broad spectrum virus resistance in plants. Short fragments from different viruses were fused with a silencing locus in the DRIGS vector and transformed into *Arabidopsis*, *N. tabacum* and *N. benthamiana*. Virus inoculated transformed tobacco plants remain symptomless and no virus can be detected by ELISA. Virus specific siRNAs could also be readily detected in the transgenic plants. These preliminary results show that the system has the potential to provide broad spectrum resistance to multiple viruses simultaneously.

**P33.039 Study on mild strain cross protection between *Cucumber mosaic virus* subgroup Ⅰ and Ⅱ**

*Z.F. Tian, C. Luo, D. Dong and L.Y. Hou*
*Institute of Plant & Environment Protection, Beijing Academy of Agriculture & Forestry Sciences, Beijing 100097, P. R. China*
*Email: zhaofengtian2000@yahoo.com*

The symptoms between *Cucumber mosaic virus* subgroup I and II isolates present significant differences in tobacco. Most of the subgroup I virus isolates produce severe mosaic combined deformity symptoms in tobacco. However most of the subgroup II isolates produce mild symptoms, only showing mild mosaic or blade slightly yellow chlorosis. In order to understand the competitiveness and cross protection phenomenon between the two subgroups, cross-protection effects were compared in tobacco with two subgroup II isolates LM and LH producing mild mosaic symptom and slight yellow chlorosis as protection strain, and with subgroup I isolate SM producing severe mosaic combined deformity symptom as challenging strain. The results showed that mild strain LM with slight mosaic symptoms presented stronger protective effect than LH strain with slight yellow chlorosis in tobacco plant, the average disease index were 33.5 and 49.8 respectively for different challenging interval 3, 5, 7, 9 and 11d. The disease index significantly reduced when challenge interval time was up to 7d. So the minimum time interval was 7d for challenging inoculation after the protection inoculation. The study also found that the tobacco did not show protective effect when the protection and challenge strains were mixed to inoculate. And the disease symptoms got quite severe due to the competition of the two virus isolates, the average disease index reached 80.3 for the tobacco plant of mixed inoculation.

**P33.040 Plant-pathogenic viruses in insects associated to garlic crop**

*O.G. García-Rodríguez, L. Pérez-Moreno, M.J. Navarro-León, M.D. Salas-Araiza, O.A. Martínez-Jaime, M.F. León-Galván and H.G. Núñez-Palenius*
*Agronomy Department, Life Sciences Division, Campus Irapuato-Salamanca, University of Guanajuato, Irapuato, Guanajuato, P. O. Box 311, Zip Code 36500, México*
*Email: luispm@ugto.mx*

The garlic vegetative propagation system is the main way of viral transmission. The objective of the present study was to detect, by means of ELISA test, the presence of five viruses on garlic plant-collected insects.

The experiment was carried out during the Autumn-Winter (2008-2009) cycle. Insect samples were taken on three dates: 45, 110 and 140 days after garlic sowing. The insect species identification was done by using a Zeiss (30X) stereomicroscope and the O'Brien and Wilson (1985) and Mound and Kibby (1998) taxonomy keys. The serology test for the detection of virus was the DAS-ELISA technique. Coat-protein virus antibodies were applied for the potyvirus: *Leek yellow spot virus* (LYSV) and *Onion yellow dwarf virus* (OYDV); for the carlavirus: *Garlic common latent virus* (GCLV) and *Shallot latent virus* (SLV); and for the tospovirus: *Iris yellow spot virus* (IYSV). Of the total insect species analyzed, *Thrips tabaci* turned out to be positive on 18 samples for GCLV and 2 samples for IYSV, and *Collops quadrimaculatus* resulted positive for GCLV.

**P33.041 Selective regulation of cytoplasmic Hsp70 expression in *Nicotiana benthamiana* protoplasts during TMV infection**

*Z.R. Chen, Y.H. Wang, H.C. Liang and W.W Yu*
*Department of Horticulture, Tianjin Agricultural University, Tianjin 300384, P. R. China*
*Email: chenzr2000@yahoo.cn*

The 70kDa heat shock family of stress proteins (Hsp70s) chaperone plays a central role in multiple processes within cells, including protein translation, folding, intracellular trafficking, and degradation. Our previous studies demostrate the cytoplasmic Hsp70s can enhance the infection of *Nicotiana benthamiana* by diverse viruses. This protein is implicated in the replication and movement of diverse plant viruses. This study was designed to assess the impact of positive single strand RNA (-SSRNA) plant virus infection on host intracellular expression of Hsp70s. Experimental conditions were established wherein *N. benthamiana* protoplast cells undergo *Tobacco mosaic virus* infection cycle. Real-time quantitative polymerase chain reaction was utilized to determine levels of TMV viral mRNA and Hsp70s mRNA transcription at different times following viral infection. Western blot analysis was used to identify variations in levels of Hsp70 and viral coat protein expression duing the course of TMV infection. During the early phase of infection, TMV mRNA transcriptions were detected as early as 3-8 hr post viral infection (hpi). Cytoplasmic Hsp70 mRNA transcription was induced at the early times postinfection (4 hpi) before viral coat protein expression (8-10 hpi). Hsp70 mRNA transcription reached approximate levels of expression observed in infected cell lines by 8 to 12 hour prior to the virion assembly, and declined to steady state levels by 16 hr at end stages of the viral replicative cycle. These findings indicate that TMV infection leads to the selective modulation of Hsp70s transcription.

**P33.042 The development of a multiplex RT-PCR for the simultaneous detection of three viruses in pear plants**

*B. Yao[1,2], X. Ma[1], H. Zhu[1], N. Hong[1,2] and G. Wang[1,2]*
*[1]National Key Lab. of Agromicrobiology, Huazhong Agricultural University, Wuhan, Hubei 430070, P. R. China; [2]The Key Laboratory of Plant Pathology of Hubei Province, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, P. R. China*
*Email: whni@mail.hzau.edu.cn*

*Apple stem grooving virus* (ASGV), *Apple chlorotic leaf spot virus* (ACLSV) and *Apple stem pitting virus* (ASPV) usually occur as mixed infections in apple [*Malus domestica* (L.) Borkh] and pear (*Pyrus pyrifolia* L.), and dramatically reduce the plant growth and productivity. These three viruses belong to three genera *Trichovirus*, *Capillovirus* and *Foveavirus* in the family *Betaflexiviridae*. Multiplex multiplex reverse transcription PCR (mRT-PCR) is an effective routine diagnosis means for plant viruses. In this study, an mRT-PCR assay was developed for the simultaneous detection and differentiation of ASGV, ACLSV and ASPV. Primer pairs designed for each virus were tested in uniplex RT-PCR to determine their amplification efficiency and specificity against all three viruses. The virus-specific primer sets with high amplification efficiencies were selected for further evaluation in mRT-PCR. The concentration of each primer set, reagent and the anneal temperature for mRT-PCR was optimized. The detection of each target virus using a series of 10-fold dilutions of the cDNA showed that the mRT-PCR had the same sensitivity as each of the uniplex RT-PCR assays. The assay was evaluated using in-vitro cultured and field grown pear plants and fruits, and the total agreement between both detection methods was over 90%. All those results indicated that the mRT-PCR was sensitive and reliable for detecting these viruses in pear plants. The developed mRT-PCR provides a high efficient tool for the evaluation of the sanitary status of pear plants and propagation material and will improve the control of pear virus diseases.

**P33.043 Mixed infection of begomoviruses suppresses resistance in resistant plants**

*Q.J. Tang, C. Cheng and B.Y. Xie*
*Institute of Vegetables and Flowers, CAAS, No.12 Zhongguancun South St., Haidian District, Beijing, 100081, P. R. China*
*Email: tangqianjun@163.com*

Begomoviruses cause severe damage to important economical crops in China. Recombination of begomoviruses can dramatically impact evolution and epidemic of themselves. The recombination frequency of begomoviruses is increased significantly in plants infected by

mixed begomoviruses. The deployment of host-plant resistance is the one of the most effective strategies to control the disease caused by this kind viruses. However, little is known about what relation between evolution and mixed infection of begomoviruses in plants with resistant genes. In this study, two monopartite begomoviruses, *Tomato yellow leaf curl virus* and *Papaya leaf curl China virus*, were inoculated into tomato with Ty-1 resistant gene separately and together, respectively. The plants infected by alone of begomoviruses did not show disease symptoms, while the plants infected by mixed viruses were observed typical symptoms7 weeks post-inoculation. Moreover, our data show that recombination rates of begomoviruses in resistant plants and susceptible plants are the same by analysis of genome. Interestingly, the plants resistance was suppressed by infection of the mixed these two viruses. We are analyzing whether the recombination of these two viruses produces a novel virus to break resistance of host-plant.

### P33.044 Identification of *Strawberry vein banding virus* in strawberry in Beijing

*L. Chen[1], Q.X. Shang[1], X.Y. Chen[2] and Z.P. Liu[1]*
*[1]Department of Plant Protection and Beijing Key Laboratory of New Technology in Agricultural Application, College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, P. R. China; [2]Plant Protection Station of Beijing, Beijing 100029, P. R. China*
*Email: shangqiaoxia@bua.edu.cn*

Strawberry yield and fruit quality are greatly influenced by viral diseases especially aphid-borne viruses among which *Strawberry vein banding virus* (SVBV), *Strawberry mottle virus* (SMoV), *Strawberry mild yellow edge virus* (SMYEV) and *Strawberry crinkle virus* (SCrV) are the most important viruses in the world and has been reported in many countries. In our research, symptoms range from smaller and fewer leaves to severe dwarf and much more less runner were noticed in the strawberry field from the year of 2012. RT-PCR with reported specific primers was used to detect 4 aphid-borne viruses in samples with distinct symptoms. Then the RT-PCR products were sequenced and analyzed. Samples with severe symptoms could be detected only SVBV without other 3 viruses. SVBV is a member of the Caulimovirus and it was reported that SVBV is often symptomless in strawberry cultivars and usually induce more severe symptoms when mix infection occurs with *Strawberry crinkle virus* (SCrV). In our study, SVBV was identified in samples with severe symptoms without the combination with SCrV.

### P33.045 Detection of *Strawberry mild yellow edge virus* infecting strawberry by ELISA and RT-PCR

*Q.X. Shang, G.F. Cui, N. Zhu, Z.Y. Zhang*
*Department of Plant Protection and Beijing Key Laboratory of New Technology in Agricultural Application, College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, P. R. China*
*Email: shangqiaoxia@bua.edu.cn*

More than 20 viruses could infect cultivated strawberry plants, and may reduce vigor and yield. Virus diseases of strawberry spread widely in the world and occur wherever they are grown. A survey was carried out from the summer of 2010 by double antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA) using commercial antisera and other related products to detect 8 viruses infecting strawberry including *Strawberry mild yellow edge virus* (SMYEV), *Strawberry latent ring spot virus* (SLRSV), *Arabis mosaic virus* (ArMV), *Raspberry ring spot virus* (RpRSV)、*Tomato black ring virus* (ToBRV), *Tomato ring spot virus* (TRSV), *Tobacco necrosis virus* (ToNV) and *Tobacco streak virus* (TSV). Forty nine leave samples were collected from greenhouse growing strawberry plants in Beijing, china. Only 7 samples were detected containing SMYEV and then confirmed by RT-PCR using reported specific primers and protocol, however other 7 viruses mentioned above were not detected in the samples collected by DAS ELISA.

### P33.046 Identification of amino acid sequence motifs essential for RNA silencing suppression in P0 from an Inner Mongolia isolate of *Potato leaf roll virus*

*T. Zhuo, Y.Y. Li, H.Y. Xiang, Z.Y. Wu, X.B. Wang, Y. Wang, Y.L. Zhang, D.W. Li, J.L. Yu and C.G. Han*
*Department of Plant Pathology and State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: zhuotaotao@gmail.com*

The P0 protein of a Netherlandish isolate of *Potato leaf roll virus* ($P0^{PL\text{-}NL}$) has been reported to be a weak suppressor of RNA silencing. In contrast, a Chinese Inner Mongolian isolate PLRV P0 protein ($P0^{PL\text{-}IM}$) proved to be a strong RNA silencing suppressor in our studies. Five amino-acid substitutions in $P0^{PL\text{-}IM}$ with those corresponding to $P0^{PL\text{-}NL}$ did not affect the strong suppressor activity of $P0^{PL\text{-}IM}$, implying that the distinction between the two P0 proteins was independent of the differences between their amino acids. Strikingly, two $P0^{PL\text{-}IM}$ LP motif signature residues, L59/P60 and L76/P77, matched an F-box-like domain, but only L76/P77 contributed to the suppressor activity. In addition, W87G88 residues in the L76/P77 motif signature that are related to an F-box-like domain also played an essential role in suppression of GFP silencing. Interestingly, we found that a WG/GW-motif-like domain in $P0^{PL\text{-}IM}$, G139W140G141, contributes to suppressor activity. Additional experimental data revealed that $P0^{PL\text{-}IM}$ has a destabilizing effect on AGO1 protein accumula-

tion, but failed to interact with *Nicotiana benthamiana* SKP1 either in yeast or in plants.

**P33.047 Mechanisms of satellite RNA-mediated attenuation of viral disease symptoms in plants**

*W.X. Shen[1,2,3], P. Au[2], B.J. Shi[4], E.S. Dennis[2], H.S. Guo[5], C.Y. Zhou[1,3] and M.B. Wang[2]*
*[1]College of Plant Protection, Southwest University, Chongqing, 400715, P. R. China; [2]CSIRO Plant Industry, Canberra, ACT 2601, Australia; [3]National Citrus Virus Exclusion Center, Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing, 400712, P. R. China; [4]Department of Plant Science, Waite Institute, Adelaide University, Glen Osmond, SA, 5064, Australia; [5]Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, P. R. China*
*Email: wanxiashen136@yahoo.com.cn*

Viral satellite RNAs (satRNAs) are small non-coding RNAs that depend on their associated virus (helper virus) for replication and spread in plants. Some satRNAs exacerbate disease phenotypes by inducing their own symptoms in infected host plants, but the majority attenuates the symptoms caused by their helper viruses. How satRNAs modulate viral disease symptoms has been a longstanding question. In the current study we investigated how satRNAs attenuate helper virus-caused disease symptoms and provided a plausible model that involves satRNA-derived short interfering RNAs (siRNAs). Using Nicotiana plants and *Cucumber mosaic virus* (CMV) Y-satellite RNA (Y-Sat) as an experimental model, we demonstrate that satRNA infection releases the suppression of hpRNA-induced transgene silencing by the CMV-encoded RNA silencing suppressor 2b or tombusvirus-encoded RNA silencing suppressor P19. This is achieved by saturating P19 with satRNA-derived small interfering RNAs (siRNAs). We also demonstrate that satRNA infection minimizes the induction of miR168 by RNA silencing suppressors expressed from infecting viruses (CMV 2b) or from a transgene (HcPro of potyvirus), and this diminished miR168 induction is correlated with reduced disease phenotypes. Taken together, our results suggest that satRNAs attenuate viral symptoms by sequestering helper virus-encoded RNA silencing suppressors with satRNA-derived siRNAs, thereby preventing the suppressors from interfering with host microRNA function that is essential for plant development. Our findings have implications for symptom moderation by other subviral agents such as defective interfering viral RNAs that are associated with both plant and animal viruses.

**P33.048 Evaluation of low temperature treatment induced mutant of *Soybean mosaic virus* (SMV) for cross protection in soybean**

*W.R. Andayanie*
*Faculty of Agriculture Merdeka Madiun University, Serayu street, 63133, East Java, Indonesia*
*Email: wuye_andayanie@yahoo.com*

Early natural infection by *Soybean mosaic virus* (SMV) can reduce seed production in soybean. Cross protection enables the production of SMV free seed and is a mechanism which can signficantly reduce the impact of SMV. We proposed that attenuated isolates of SMV obtained by cold temperature treatments are able to produce SMV free seed in soybean [*Glycine max* (L.)] Merr. plants. We inoculate cotyledons with SMV infected plants, incubated these plants at low temperature, mechanically inoculated seedlings with virus subjected to cold and finally transplanted the inoculated seedlings into pot and then into the field. We examined plants with different symptoms. Serological assays, RT-PCR analysis, and electron micrography did not distinguish between the very mild mosaic symptom of attenuated isolate and the original virulent isolate. However the mutant of SMV did not give rise to local lesions on *Chenopodium amaranticolor*. Our results suggest that attenuated isolate of SMV is potentially useful for reducing the impact of SMV infection. When plants are inoculated with the attenuated isolate at 8 days after planting, symptoms of the disease do not develop.

**P33.049 Effects of coat protein on the pathogenesis of *Tobacco vein banding mosaic virus***

*A. Mou, B.H. Yang, R. Gao, J. Wang, C. Geng, H.T. Liu and X.D. Li*
*Laboratory of Plant Virology, Department of Plant Pathology, College of Plant Protection, Shandong Agricultural University, Tai'an, Shandong 271018, P. R. China*
*Email: xdongli@sdau.edu.cn*

The viral coat protein (CP) plays important role in the infection cycle. From the full-length cDNA clones for the HN39 isolate of *Tobacco vein banding mosaic virus* (TVBMV), twelve mutants with single amino acid (aa) mutation were analyzed for their effect on virus pathogenicity. Substitution of $Ser^{92}$ to Ala in TVBMV CP has no effect on the symptom of TVBMV, however, that to either Asp or His results in milder symptom and reduced accumulation level of virus. Substitutions of $Trp^{122}$ and $Arg^{192}$ to Ala or Glu, and $Lys^{225}$ to Glu eliminated the activity of CP in cell-to-cell movement, while substitutions of $Trp^{122}$ to Lys, $Arg^{192}$ to Cys, and $Lys^{225}$ to Ala or Cys only abolished the capacity of long-distance movement, not affecting its activity in cell-to-cell movement. These results indicate that aa $Ser^{92}$, $Trp^{122}$, $Arg^{192}$ and $Lys^{225}$ in the CP play different roles in the pathogenesis of TVBMV, and mutation of same sites to different aa may have different effects.

**P33.050 Molecular analysis for the multi-level translational mechanisms on the genome of *Tobacco bushy top virus***

*D. Wang, G. Wang and X. Yuan*
*Department of Plant Protection, Shandong Agricultural University, Daizong Road No.61, Taian, Shandong, 271018, P. R. China*
*Email: snowpeak77@163.com*

The polycistronic genome of positive-strand RNA viruses facilitates the translation of viral proteins through multi-level of strategy: 1) genomic or subgenomic RNA; 2) cap-dependent or cap-independent translation; 3) post-translational recoding. The genome of *Tobacco bushy top virus* (TBTV) has a 4152 nt of positive, single-strand RNA, which lacks 5' cap and 3' poly (A) tail. Based on software prediction and homology alignment, TBTV encodes 4 proteins (5'→3'): ORF1 and ORF2,which is the potential translational frame shift product of ORF1, are the functional proteins involved in the replication of genome; the highly overlapped ORF3 and ORF4, which are possibly expressed from a subgenomic RNA. The preliminary data suggested that BTE-like element located at the 3'-UTR participated in the cap-independent translation of TBTV genome (Wang *et al*, 2010). Our group made the further effort to identify the elements involved in the cap-independent translation of ORF1 as well as the post-translational recoding, which is responsible for the expression of ORF2. Double single-luciferase vectors were inoculated into *Arabidopsis* protoplasts to map the core regions directing the cap-independent translation. Meanwhile, antiserum of TBTV ORF1 were made and used to measure the translational frame shift ratio of ORF2/ORF1 in Wheat Germ Extract (WGE). Both levels of translational elements were then subjected to structural probing using in-line probing and SHAPE. All data are in update.

**P33.051 Development of a generic RT-PCR procedure suitable for detection of six potato viruses**

*W. Zhang[1], Y.J. Bai[1], Y.L. Gao[1], G.Q. Fan[1], S. Zhang[1] and X.Z. Nie[2]*
*[1]Virus-free Seedling Research Institute of Heilongjiang Academy of Agricultural Sciences, Harbin 150086, P. R. China; [2]Agriculture and Agri-Food Canada, Potato Research Centre, PO Box 20280, Fredericton, New Brunswick, Canada E3B 4Z7*
*Email: yanjubai@163.com*

Viral diseases are one of the limiting factors in potato production worldwide. In China, *Potato virus X* (PVX), *Potato virus Y* (PVY), *Potato virus S* (PVS), *Potato virus M* (PVM), *Potato virus A* (PVA) and *Potato leaf roll virus* (PLRV) are the most common and damaging viruses affecting the potato crops. For managing these viruses effectively and thus minimizing economic loss, reliable and efficient detection of the viruses is needed. Here we report a generic reverse transcription-polymerase chain reaction (RT-PCR) procedure that is suitable for detection of multiple viruses in potato. This procedure can accommodate uniplex, duplex, triplex, and six-plex RT-PCR assays targeting one to six viruses simultaneously. This procedure is reliable, cost-effective, and efficient, and thus can be used for large scale detection of potato viruses.

**P33.052 Responses of potato cultivars to PVY, PVX and PVS**

*G.Q. Fan[1], Y.J. Bai[1], Y.L. Gao[1], W. Zhang[1], S. Zhang[1] and X.Z. Nie[2]*
*[1]Virus-free Seedling Research Institute of Heilongjiang Academy of Agricultural Sciences, Harbin 150086, P. R. China; [2]Agriculture and Agri-Food Canada, Potato Research Centre, PO Box 20280, Fredericton, New Brunswick, Canada E3B 4Z7*
*Email:yanjubai@163.com*

The responses of ten potato cultivars to *Potato virus Y* (PVY), *Potato virus X* (PVX) and *Potato virus S* (PVS) were studied. PVY isolate used in this study belongs to the potato tuber necrosis strain of PVY ($PVY^{NTN}$). For the primary infection experiments, the virus groups of PVY, PVY and PVX, PVY and PVS had been respectively inoculated mechanically to ten potato cultivars. The plant symptoms were monitored on daily until harvest. Twenty two days after inoculation, all cultivars but "Kexin18" exhibited varied degrees of symptoms. "Kexin13" and "Youjin" developed mosaic and vein necrosis upon infection with all three viruses. Leaf drop and premature plant death occurred in "Huangmazi" infected with PVY and PVS, and "Zhong5", "Zaodabai" and "Favorita" infected with PVY and PVX. Interestingly, "Atlantic", "Zhong3" and "Kexin1" only exhibited mild symptoms ranging from near-symptomless to mild mosaic on all infection regimes. For the secondary infection experiments, the symptoms were generally more serious than the primary infections. Tuber symptoms were checked at the harvest and during the storage. Except "Youjin", which developed necrotic ringspots on PVY-infected tubers, no visual symptoms were observed on tubers from other cultivars or infected with other viruses/virus combinations. Potato yield and tuber size were measured at the harvest, and significant differrences were observed in different cultivars with different virus infection regimes. A yield reduction of 30-40% was found in most cultivars with different virus infections. The most noticeable yield reduction occurred in "Zhong5" infected with PVY and PVX reaching 73.5%. Meanwhile, the size of tubers infected with virus(es) were much smaller than the uninfected.

**P33.054 Screening protein factors in small brown planthopper that interact with RBSDV P10 and analyzing their function in the viral transmission process**

*Q.Q. Chen, Q.F. Xu, J.F. Zhang, S. Li and Y.J. Zhou*
*Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu, 210014, P.R. China*
*Email: yjzhou@jaas.ac.cn*

*Rice black-streaked dwarf virus* (RBSDV), an important pathogen in the genus *Fijivirus*, family *Reoviridae*, causes severe damage of crops including maize, rice and wheat. In recent years, rice black-streaked dwarf and maize rough dwarf diseases caused by RBSDV became a serious threat to China's food production. The virus is transmitted via *Laodelphax striatellus* (the small brown planthopper, SBPH) in a persistent, circulative manner. Until now, the molecular mechanism about how SBPH transmits RBSDV is unclear. RBSDV contains 10 segments (S1-S10) of linear dsRNA. The virion is double-layered particle. Studies have shown that the outer capsid of the virion plays a pivotal role in the process of virus transmission. In this study, the outer coat protein P10 encoded by RBSDV S10 was selected as bait to screen the SBPH cDNA library. 377 positive clones were acquired. The sequencing results show that these positive clones encode 13 proteins, including actin, tropomyosin, 26S proteasome non-ATPase regulatory subunit 8-like, 40S ribosomal protein SA-like, etc. The full-length of four genes encoding actin, tropomyosin, 26S proteasome non-ATPase regulatory subunit 8-like and 40S ribosomal protein SA-like were cloned. Yeast two-hybrid results show that the proteins encoded by the full-length genes could interact with P10 protein. Besides, the actin protein was prokaryotic expressed. And far western blot assay showed that actin could interact with virus particles. The results indicated that the proteins which interact with RBSDV P10 may play an important role during virus transmission process.

**P33.055 Systemic infectivity of *Beet necrotic yellow vein virus* RNA5 is dependent on its un-translated region rather than the open reading frame in a host-dependent manner**

*H.Y. Fan, Y. Wang, X.B. Wang, D.W. Li, J.L.Yu and C.G. Han*
*Department of Plant Pathology and State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: starrywheat@gmail.com*

*Beet necrotic yellow vein virus* (BNYVV), containing four or five plus-sense single stranded RNAs, belongs to the genus Benyvirus causing a worldwide sugar beet disease rhizomania. As a multipartite RNA virus, though the each RNA function has been studied in detail, little is known about fitness and infectivity of viral RNA5 in different hosts. In this study, Chinese BNYVV isolates was inoculated in systemic hosts *Nicotiana benthamiana* and *Beta macrocarpa*. RT-PCR and Northern blot showed that RNA5 could keep the higher level of accumulation and systemic infectivity in *B. macrocarpa*, but with high frequency of elimination in *N. benthamiana*. Expression of GUS gene in RNA4 and RNA5 derived replicons and recombinants comparative analysis revealed that the un-translated region (UTR) of RNA5 is a determinable domain to influence the RNA per se replication in protoplasts and systemic infection in *N. benthamiana*. Besides, deep sequencing analysis of small interfering RNAs (siRNAs) derived from BNYVV showed that majority of siRNAs full matching with RNA5 was significantly less and located on the homology region of RNA3, 4 and 5. These results indicate that the UTR of RNA5is probably more important than open reading frame (ORF) for its systemic infectivity in a host-dependent manner, and the low efficient infection of RNA5 in *N. benthamiana* may be caused both by a defect in virus RNA replication and the high efficiency targeting by siRNA from the homology region of other small RNAs, which consequently prevents the accumulation of viral RNA and affects long distance movement.

**P33.056 Oligonucleotide microarray detection of plant viruses and phytoplasmas at genus and groups level**

*Y.J. Zhang, H.Q. Mou, W.J. Zhao, G.F. Li, M.F. Li and S.F. Zhu*
*Chinese Academy of Inspection and Quarantine, Shuangqiao Middle Road, Chaoyang District, Beijing 100121, P. R. China*
*Email: zhushf@netchina.com.cn*

Plants are infected by a wide range of viruses and phytoplasmas. For avoiding introduce and spreading of dangerous pathogen, Chinese government has established a list of pest, including 14 phytoplasmas and 33 viruses. At the same time, the risk from emerging viruses and phytoplasmas has grown significantly due to a number of factors. Early and accurate pathogen diagnosis of these pathogens is essential for disease control. But the current detection technology such as ELISA and PCR can only be applied towards the detection of specific single or few viruses or phytoplasmas. They lack the capability to discover the new novel viruses and phytoplasmas that cause emerging infections. Therefore there is a need for high through-put and broad spectrum strategies for detection, identification and monitoring plant viruses and phytoplasmas. To address this concern, we have developed a new plant virus detection microarray detecting a wide range of plant viruses. The probe design protocol automatically searched for the conserved sequences of the virus genus to design a minimal number of conserved 70mer probes that accurately detect plant viruses at the genus level. We are also building an oligonucleotide microarray-based assay including

16S rRNA, *tuf*, 16/23S spacer and *imp* conserved regions, for identification of phytoplasmas at the groups' level. The use in conjunction with the probes of viruses and phytoplasmas will be an effective method for detection and identification of unknown viruses and phytoplasmas in the same samples using a single test.

### P33.057 Oligonucleotide microarray detection of viroids at genus level

*Y.J. Zhang[1], J. Yin[2], D.M. Jiang[3], Y.Y. Xin[1], F. Ding[4], Z.N. Deng[5], G.P. Wang[4], X.F. Ma[5], F. Li[5], G.F. Li[1], M.F. Li[1], S.F. Li[3] and S.F. Zhu[1]*
*[1]Chinese Academy of Inspection and Quarantine, Shuangqiao Middle Road, Chaoyang District, Beijing 100121, P.R. China; [2]School of Medicine and Medical Science, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland; [3]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; [4]College of Plant Science and Technology of Huazhong Agricultural University, Wuhan 430070, P. R. China; [5]Hunan Agricultural University, Changsha 410128, P.R. China*
*Email: zhushf020420@gmail.com*

A major challenge in agricultural industry is the development of techniques that can screen plant materials for viroid infection. The microarray technique shows promise in this regard, as their high throughput nature can potentially detect a range of viroids using a single test. In this paper, we present a microarray that can detect a wide spectrum of all the 8 reported viroid genera including 37 known plant viroid species. The array was constructed using an automated probe design protocol which generated a minimal number of probes to detect viroids at the genus level. The designed arrays showed a high specificity and sensitivity when tested with a set of standard virus samples. Microarray screening was applied on an infected citrus sample. Hop stunt viroid infection was identified as the major disease causing pathogen for the infected citrus sample.

### P33.058 Detection of RNA viruses in sweet potato by high-throughput sequencing

*Y.M. Liao, Z.Q. Wang, C.W. Zou, F.L. He, P. Li and B.S. Chen*
*State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Guangxi Univieristy, Nanning, 530005, P. R. China*
*Email: chenyaoj@gxu.edu.cn*

Sweet potato is the third important food crop in Guangxi province of China with an annual cultivation area of about 233 thousand hectares. Variety Red Girl is a famous local sweet potato grown in Fangchenggang city of Guangxi. However, severe viral diseases cause significant loss in yield and reduce the quality of the yam and as a result, lead to the decline of the variety. To identify the causal viral agents, a total of 51 samples with viral disease-like symptoms including leaf malformation, shrinking, curling, vein clearing, mosaic, and stunt were collected from the fields. Total RNA of was extracted from individual samples and then mixed together for high-throughput sequencing. As revealed by sequence homology, four RNA viruses including *Sweet potato chlorotic fleck virus* belonging to *Betaflexiviridae*, *Sweet potato feathery mottle virus*, *Sweet potato latent virus* and *Sweet potato virus G*, all belonging to. *Potyviridae*, were present in the samples. RT-PCR with specific primers confirmed the existence of such viruses in the pool of sweet potato samples.

### P33.059 Research on transgenic tobacco expressing p24 gene of *Grapevine leafroll associated virus 2*

*J. Li, M.J. Li, X.M. Li and Y.Q. Cheng*
*Department of Pomology/Laboratory of Stress Physiology and Molecular Biology for Tree Fruits, Key Laboratory of Beijing Municipality, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: Chengyuqin@cau.edu.cn*

The vector p-G2-p24 contained *Grapevine leafroll associated virus 2*（GLRaV-2）p24 protein gene was used to amplify sense/anti-sense strands (300 bp in size) and full sequence of p24 protein gene, respectively. PCR products of complete sequence of p24 protein gene were digested with *Sal*I /*Sac*I and cloned to the expression vector pSuper1300 to obtain recombinant vector pSuper1300-p24. The positive sense and anti-sense strands were separately inserted into the middle vector pBSint which contains an intron. The obtained recombinant middle vector pBSint-p24-F-R was digested with *Sal* I and *Sac*I, and the restriction products were cloned into the expression vector pSuper 1300 to construct the ihpRNA vector pSuper-p24-F-R. The pSuper1300-p24 and pSuper-p24-F-R were transformed into *Nicotiana benthamiana* mediated by *Agrobacterium tumefaciens*, respectively. RT-PCR and PCR testing showed that 17 and 38 transgenic plants were obtained, respectively. These results provided experimental materials for studying functions of p24 and testing the concept of pathogen-derived resistance against a closterovirus associated with grapevine leafroll disease.

### P33.060 Identification of virus species infecting watermelon and melon in Shanghai

*R. Zeng, L.H. Xu, J.P. Lu and F.M. Dai*
*Institute of Eco-Environment and Plant Protection, Shanghai Academy of Agricultural Sciences, No. 1000, Jinqi Road, Shanghai 201403, P. R. China*
*Email: fumingdai@163.com*

Watermelon and melon, as the most important economic crops in Shanghai, have production area of more than 16 000 hectares, which most of them grow in the plastic tunnels. Viral diseases occurred popularly and often accompanied with the mosaic, mottle, yellowing, crumpling symptoms. Virus identification using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Agdia, USA) and reverse transcription-polymerase chain reaction (RT-PCR), showed that Cucurbit choleric yellows virus (CCYV), *Cucumber mosaic virus* (CMV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV) were the virus species found on watermelon and melon in Shanghai. Among these infectious viruses, CCYV was the most widely distributed and important virus disease of watermelon and melon in Shanghai in recent years, and was detected in all the melons production bases, especially in fall. The virus was a new member of the genus *Crinivirus*, which was transmitted by whitefly and produced symptoms of chlorosis on the upper leaves and a bright yellow color on the lower leaves of melon in the field. CMV, SqMV, WMV-2 and ZYMV were detected in the watermelon and melon plants with mosaic or crumple leave in some bases. The virus species is still unknown and needs further identification for some other viral disease symptoms in the field.

**P33.061 Sequencing and phylogenetic analysis of *Potato virus Y* Liaoning isolate in China**

*F. Wang, M.N. An and Y.H. Wu*
*College of Plant Protection, Shenyang Agricultural University, Donglingxi road 120, Shenhe District, Shenyang 110866, P. R. China*
*Email: anmengnan@syau.edu.cn*

Complete genome sequence of *Potato virus Y* Liaoning isolate (PVY-LN) causing tobacco vein necrosis symptoms were isolated from Liaoning province in China. Infectious clone for PVY-LN were constructed and tested in tobacco variety Yunyan 87. Based on our sequencing results, genome sequences of PVY-LN were 9,714 nucleotides in length, excluding the 3′-terminal poly (A) tail. PVY-LN encodes a single long open reading frame (ORF) of polyprotein that is predicted to be cleaved into ten mature proteins by three viral proteases. No recombination can be predicted in PVY-LN sequences compared with that of the other PVY strains using Recombination Detection Programe V.4.16 (RDP4). Complete genome sequence comparison and phylogenetic analysis indicated that PVY-LN is closely related to PVY Necrosis strain ($PVY^N$).

**P33.062 Primary study on the virus elimination of grapevine by antiviral compound**

*G.J. Hu, Y.F. Dong, Z.P. Zhang, X.D. Fan, F. Ren and H.J. Zhu*
*National Center for Eliminating Viruses from Deciduous Fruit Tree, Research Institute of Pomology, Chinese Academy of Agriculture Sciences, Xingcheng, Liaoning 125100, P. R. China*
*Email: hugj3114@163.com*

Eight *in vitro* grapevine (*Vitis* Spp.) cultivars infected by *Grapevine spot virus* (GFkV), *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Grapevine virus A* (GVA), *Grapevine leafroll-associated virus*-1 (GLRaV-1), *Grapevine leafroll-associated virus*-2 (GLRaV-2), *Grapevine leafroll-associated virus*-3 (GLRaV-3) or *Grapevine leafroll-associated virus*-6 (GLRaV-6) were used as original materials. The virus elimination rates of *in vitro* grapevine treated by medical antiviral compound with four different concentrations, ribavirin injection, were analyzed. In the course of processing, the mortality rate of grapevine was higher, and nine regeneration plants from five species were obtained only from 25 mg/L ribavirin injection. Viruses in regenerated plants were detected by RT-PCR, and the results showed that only two regeneration plants of Victoria were still contained GRSPaV, and others were virus-free.

**P33.063 Complete genomic sequence analysis of *Wheat yellow mosaic virus* isolated from Zhouzhi in Shanxi Province**

*Z.Y. Zhang[1], C.J. Zhou[1], Y.F. Wu[2], D.W. Li[1], J.L.Yu[1] and C.G. Han[1]*
*[1]Department of Plant Pathology and State Key Laboratory for Agrobiotechnology, China Agriculture University, Beijing 100193, P. R. China; [2]Department of Plant Pathology, Northwest A&F University, Yangling 712100, P. R. China*
*Email: 13426015848@163.com*

Wheat samples from fields in Zhouzhi of Shanxi Province were investigated for *Wheat yellow mosaic virus* (WYMV), using RT-PCR and Western blotting. Subsequently, the complete genomic sequence of one Zhouzhi isolate (WYMV-ZZ) was determined and compared with six complete sequences of five Chinese isolates (WYMV-HC, WYMV-XQ, WYMV-ZZD, WYMV-YZ, WYMV-YA) and one Japanese isolate (WYMV-JPN). Sequence comparisons showed that WYMV-ZZ shared 96.6~97.3% and 95.1~97.3% nucleotide sequence identity for RNA1and RNA2, respectively, with the isolates mentioned above. At the amino acid level, WYMV-ZZ had 94.4~96.8% identity for RNA1, and 94.1~96.7% identity for RNA2, respectively, with the isolates mentioned above. Further phylogenetic analysis of seven different isolates based on RNA1 and RNA2 indicated that WYMV-ZZ was more closely related to WYMV-YA for RNA1 and more closely related to WYMV-ZMD for RNA2. Phylogenetic trees generated based on the NIa-Vpg region of RNA2 could make a

distinction between six Chinese isolates and WYMV-JPN. Moreover, a potential recombination event of WYMV-ZZ, which located in 2598~3334 nt of RNA1, may recombined with WYMV-JPN and WYMV-HC with a *P* value of $8.562 \times 10^{-05}$.

**P33.064 First report of Tomato spotted wilt tospovirus in gerbera and chysantemum in Venezuela**

*A. Mejías, A. Fernández, E. Rodríguez, K. Zambrano and E. Marys*
*Instituto Venezolano de Investigaciones Científicas, Centro de Microbiología y Biología Celular, Laboratorio de Biotecnología y Virología Vegetal, CP 20632, Caracas 1020-A, Venezuela*
*Email: eemarys@ivic.gob.ve*

In October 2012, greenhouse-grown gerbera and chrysanthemum hybrid plants with symptoms resembling those associated with tospoviruses, were observed in the Miranda State (Venezuela). Symptomatic plants with chlorotic rings and line patterns were sampled and tested by double-antibody sandwich (DAS)-ELISA using polyclonal antiserum against *Tomato spotted wilt virus* (TSWV). The virus was detected in 5 of 13 gerbera and in 4 of 10 chrysanthemum samples. The presence of TSWV in ELISA-positive plants was further confirmed by RT-PCR, using a pair of tospovirus universal primers specific for the L gene. A sequence analysis of the amplicons confirmed the authenticity of the virus isolated as TSWV. To our knowledge, this is the first report of TSWV infecting gerbera and chrysanthemum in Venezuela.

**P33.065 Bioinformatics analysis of MP gene sequence of *Cucumber green mottle mosaic virus* Shaoyang isolate**

*Y.D. Zhang[2], H.R. Zhao[2], Q. Zhou[1,2] and B.D. Gao[1,2]*
*[1]Hunan provincial university key lab for plant disease control and utilization, Changsha 410128, P. R. China; [2]Hunan Agricultural University, Changsha 410128, P. R. China*
*Email: bdgao@yahoo.com.cn*

The genome segment of MP (Movement Protein) of *Cucumber green mottle mosaic virus* of Hunan Shaoyang isolate was cloned by using the RT-PCR method, Sequencing and analysis. The result show that the MP had 795 nucleotides encoding a polypeptide of 264 amino acids with a molecular weight of 28.87kDa and an isoelectric point of 9.06, it was predicted that the protein as unstable by ProtParam. Compared with Liaoning isolates, the similarities of nucleotide and amino acid respectively are 99.6% and 98.9%. There are not highly coiled coil regions on CGMMV MP protein, but find transmembrane helices with hydrophobic and it is a potential protein interaction sites; phosphorylation sites uniform distribution in the whole of the polypeptide chain, there are five major B cell antigen epitope prediction sites; By searching the motif of Tobamo virus MP amino acids, and found that three conservative sections, and amino acids exist codon bias. Moreover, found a amidation site and a cAMP- and cGMP-dependent protein kinase phosphorylation site, which may involved in virus infection mechanism.

**P33.066 Sequencing and bioinformatics analysis of the nonstructural protein coding segment S6 of SRBSDV**

*Y.D. Zhang, J.Z. Zhu, Y. Cui, Q. Zhou and B.D. Gao*
*Hunan Provincial University Key Lab for Plant Disease Control and Utilization, Hunan Agricultural University, Changsha 410128, P. R. China*
*Email: bdgao@yahoo.com.cn*

The full-length cDNA of the genome segment (S6) of Southern rice black-streaked dwarf virus, and the complete sequence of Tongdao isolate was cloned, determined and analyzed from the aspect of bioinformatics. Results show that SRBSDV-HuNTD S6 had 2650 nucleotides, containing an open reading frame and encoding a polypeptide of 793 amino acids with a speculative molecular weight of 89.85kDa and an theoretical isoelectric point of 4.95,forecast this protein as unstable. Compared with other known viruses such as Guangdong isolates, Hubei, isolates and Yunnan isolates, the rates of homogeny of S6 segment were 97.8%,99.2% and 99.4% at the nucleotide level, 97.1%, 99.0% and 99.7% at the amino acid level, respectively. There are not transmembrane helices on SRBSDV SP6, but find a highly coiled coil regions with hydrophilic and it is a potential protein interaction sites. Glycosylation and phosphorylation sites in the whole polypeptide. There are seven main B cell antigen epitope prediction site. A SMC conserved domain and a leucine zipper structure can be found when comparing with conservative domain.

**P33.067 Dynamic modulation of host small RNA population profiles defining resistance or susceptibility in virus-infected wheat**

*A. Mitra and L. Galvez*
*University of Nebraska, Lincoln, NE 68583-0722, USA*
*Email: amitra1@unl.edu*

We have employed deep sequencing to evaluate the role of host small RNAs in virus-host interactions resulting in resistance or susceptibility. Using two wheat viruses in resistant and susceptible wheat cultivars at permissive and non-permissive temperatures, we have been able to identify a set of wheat small and micro-RNAs that determine host resistance against these viruses.

**P33.070 The ORF1 protein of the newly isolated *Sobemovirus Soybean yellow common mosaic virus* is involved in cell wall localization but not in gene silencing suppression**

*M. Nam[1], S.H. Cho[1], J.K. Kim[1], J. Hammond[2], and H.S. Lim[1]*
*[1]Department of Applied Biology, College of Agriculture and Life Sciences, Chungnam National University, Daejeon, 305-764, Republic of Korea; [2]USDA-ARS, FNPRU, Beltsville, MD, 20705,USA*
*Email: hyounlim@cnu.ac.kr*

*Soybean yellow common mosaic virus* (SYCMV; *Sobemovirus*) was recently identified as a newly emerging virus infecting soybean. In order to investigate the distribution of SYCMV in Korea, we performed a nationwide survey in 2012. Soybean tissue samples with virus-like symptoms were collected from 682 areas; among the collected tissue samples 17 were diagnosed as infected by SYCMV, and others were infected with different viruses. Among 17 SYCMV infected samples, five SYCMV strains were differentiated by their ORF1 sequences. Variation in ORF 1 sequence was detected at amino acid residues 123, 128, 133 and 146 in the C-terminal domain. The five ORF1 variants were cloned in binary vector pGD to investigate their possible silencing suppressor function and localization in the cell. Interestingly, in contrast to previous reports of the *Sobemovirus* ORF1 acting as a silencing suppressor, SYCMV ORF1 expressed by Agroinfiltration in *Nicotiana benthamiana* did not show any silencing suppressor function. However, GFP fused OFR1 produced similar pattern as TMV movement protein, localizing at cell wall with ER embedded small vesicles, which suggests function as a movement protein. However, there were no discernible differences among ORF1s from five variants. In this report, ORF1 of the newly isolated SYCMV was identified as a movement protein transporting virus from ER to cell walls and to what appear to be plasmodesmata. We can apply knowledge of the movement protein function of ORF1, aiding cell-to-cell movement of the virus, to development of SYCMV as a new VIGS vector for soybean.

**P33.071 Incidences of virus and phytoplasma diseases on potatoes in Yunnan province of China**

*J.H. Dong[1], D. Wang[1], X.X. Su[1], L.Z. Zhang [1], Z.K. Zhang[1], H.M. AbouElnasr[2], C.G. Han[2] and J.H. McBeath[3]*
*[1]Yunnan Key Laboratory of Agricultural Biotechnology, Institute of Biotechnology and Germplasm Resources, Yunnan Academy of Agricultural Sciences, Kunming, Yunnan, 650223, P.R. China; [2]Department of Plant Pathology and State Key Laboratory for Agro-biotechnology, China Agricultural University, Beijing, 100193, P. R. China; [3]Plant Pathology and Biotechnology Laboratory, University of Alaska Fairbanks, Fairbanks, AK 99775, USA*
*Email: dongjhn@126.com*

From 2010 through 2012, surveys for virus and phytoplasma disease were conducted on potatoes during the growing season in nine counties of Yunnan province, China. A total of 591 samples were collected from nine potato cultivars and tested for the presence of *Potato virus X* (PVX), *Potato virus Y* (PVY), *Potato virus A* (PVA), *Potato virus H* (PVH), *Potato virus M* (PVM), *Potato virus S* (PVS), *Potato leaf roll virus* (PLRV), *Tomato spotted wilt virus* (TSWV), and *Tobacco ringspot virus* (TRSV) using DAS-ELISA (Agdia, US), RT-PCR and western blot assays. Phytoplasmas were identified using nested-PCR with reported primer pairs, RFLP and sequencing techniques. Test results indicated that incidences of PVS, PVY, PLRV, PVA, PVX and PVM on potatoes were 20.36%, 8.48%, 8.01%, 7.26%, 5.09%, respectively. Similar to discoveries made in Heilongjiang, Inner Mongolia and Xinjiang provinces, mixed infections of viruses (up to four) and phytoplasmas (up to two) on single potato plants were prevalent. Since 2010, TSWV, PVH, TRSV, were also detected on potatoes in Yunnan. Although incidences of these emerging viruses were still quite low, among them, PVH, a new carlavirus discovered in 2010, was most commom. Among phytoplasmas, clover proliferation was most prevalent, aster yellows and *Candidatus* Phytoplasma fragariae, also an emerging pathogen in Yunnan were also common.

**P33.072 Elimination of shallot viruses through electro theraphy**

*R. Pangestuti, E. Sulistyaningsih, S. Hartono, R. Harimurti, J. Thomas, S. Harper and S. Subandiyah*
*Faculty of Agriculture, Universitas Gadjah Mada, Flora St. Bulaksumur Yogyakarta 55281, Indonesia*
*Email: ssubandiyah@yahoo.com*

Shallot is an important comodity used for dairy food spice and vegetable in Indonesia. There are several shallot production centers with 2-3 planting season a year using bulb seed for the propagation. Virus diseases of shallot reported in Indonesia were including *Onion yellow dwearf* (OYDV), *Shallot yellow stripe virus* (SYSV), *Leek yellow stripe* (LYSV) and *Shallot latent virus* (SLV) that have been spread widely through the bulb seeds. This study was conducted to eliminate virus contamination in the plants through microprapagation of meristematic tissue of stem, basal plate and pseudostem post electrotheraphy treatment planted in MS medium containing 5 mg/l ribafirin. The electrotheraphy of the explants was conducted at 0 mA, 5 mA/10 min and 15 mA/10 min. ELISA for OWDV and SYSV detection, transmission electron mycroscopy observation, and symptom development were conducted for the explants and the resulted plantlets. Therapy Efficence (TE) was calculated from the percenrage of via-

bility by the percentage of free virus planlets. Virus elimination was better at TE of 28.57 with 57.14% viability and 25% virus free at the combination treatments of meristematic stem tissue electrotheraphy at 5mA for 10 minutes compared to those at TE of 10 with 50% viability and 20% virus free respectively at the combination of meristematic stem tissue electrotheraphy at 15 mA for 10 min. Other explants at the same electrotheraphy treatments fail to get virus free plantlets.

**P33.073 Nucleocytoplasmic shuttling of capsid protein modulates systemic movement of *Tobacco necrosis virus A*$^{C}$ in *Nicotiana benthamiana***

*S. Niu, Y. Zhang, X. Zhang, Y. Zhang, X. Wang, C. Han, J. Yu and D. Li*
*State Key Laboratory of Agro-Biotechnology, College of Biological Sciences, China Agricultural University, Beijing 100193, P. R. China*
*Email: Dawei.Li@cau.edu.cn*

To date, the interaction of plant viruses and hosts has been studied extensively but still full of gaps in understanding when, how and where the virus package, cell-to-cell move, as well as systemically infect. For example, the capsid protein (CP) of TNV-A$^{C}$, a Chinese isolate of *Tobacco necrosis virus A*, has been proved to be essential for viral long-distance movement in *Nicotiana benthamiana*. Here, we further found that TNV-A$^{C}$ CP was localized both to the cytoplasm and the nucleus in infected plants and in transient expressed leaves. To examine the modulation of the subcellular distribution of CP on the pathogenesis of TNV-A$^{C}$, a series of TNV-A$^{C}$ mutants were constructed and analyzed in *N. benthamiana*. Our studies demonstrated that the CP harbored a N-terminal basic amino acids domain (aa 16-36) acted as a nuclear localization signal and a middle leucine-rich domain (aa 122-139) acted as a nuclear export signal, mediated by an importin α/β dependent pathway and CRM1 nuclear export pathway respectively. To investigate the functional role of nuclear and cytoplasmic distribution of CP, we enforced the subsellular relocalization of CP by fusing exogenous import or export signals to C-terminal of CP, and demonstrated that the nucleocytoplasmic distribution of CP played a crucial role in the systemic infection of TNV-A$^{C}$.

**N33.001 Germoplasm of farmers' varieties cleaning up cassava mosaic virus and morphological characterization**

*R. Sahondramalala and R.R. Delphin*
*Département de Biologie et Ecologie Végétale, Faculté des Sciences, Université d'Antananarivo, Madagascar*
*Email: hiraloso_86@yahoo.fr*

Cassava varieties appreciated by farmers are paticularly sensitive to cassava mosaic virus lead to yield drop even a risk of disappearance of these varieties much appreciated. Seven varieties are attacked by the virus: Tsidignaognaona, Anivorano, Mambole, Rangotrakoho, Miandrazaka, Valotao tsy miova and Ravoanjo stemming from prospecting are studied. These varieties are attacked by ACMV (*African cassava mosaic virus*) and SACMV (*South african cassava mosaic virus)*. Our goal is to make varieties healthy after soaking cuttings in warm water with 47 ℃ during 30 minutes. The effect of this treatment proved very beneficial for the plant on saving the specificity of studied varieties, on reducing the intensity of virus attack without eliminating the virus. The cleaning process provokes the going out of stems with systemic resistance, which allows the cleaning up. The treatment has contributed to elimination of exogenous pathogen germs, has speeded up the sprouting and has increased the vigour of seedlings on growing in high and in increasing diameter of noose (snare). As long as the virulence of CMD (Cassava Mosaic Disease) is weakened, the seedlings going out of the stem with systemic resistance are cleaned up and they do not mask their proper phenotypic characters, thus facilitating the morphological characterisation of each studied variety.

**N33.002 Sequence characteristic of miRNA effect on multi-viral resistance mediated by single amiRNA**

*Q.J. Han, Y.Z. Song, R.Z Sun, F. Jiang, C.X. Zhu and F.J. Wen*
*State Key Laboratory of Crop Biology, Shandong Key Laboratory of Crop Biology, Shandong Agricultural University, Tai'an, Shandong, 271018, P. R. China*
*Email: zhchx@sdau.edu.cn*

Aritificial microRNA (amiRNA) is gradually becoming a choice for viral defense in plants for its characteristics. We selected 9 amiRNA target sequences which respectively located in CI, NIa-VPg, NIb, CP genes depending on the sequence characteristics of natural miRNA, and with high similarity between the sequences of PVY$^{N}$ and TEV-SD1. We constructed 9 amiRNA plant expression vectors by replacing the functional sequences of miRNA 319a precursor with our selected amiRNA sequences. To study the effectiveness of gene silencing in amiRNA-mediated viral resistance, the constructs were introduced into tobacco plants. Northern blot assay verified that all 9 amiRNA plant expression vectors could express amiRNAs in plants successfully. Viral resistance analysis showed that these transgenic tobacco plants could inhibit viral infection of both PVY$^{N}$ and TEV-SD1 effectively, and the amiRNA targeting to CP gene showed higher silencing efficiency. Virus resistance in transgenic plants could be inherited stably through single copy of transgenic sequence. Northern blot assay demonstrated that the viral resistance should be attributed to the degradation of target RNA. Additionally, according to correlation analysis of amiRNA sequence characteristics with its activity, we concluded that the

secondary structure stability of the targeted sequence exhibited an insignificant impact on amiRNA activity; 1 to 4 bases mismatch between miRNA and target sequence could be allowable, especially which appeared in 3'-miRNA.

**N33.003 Production of marker-free and RSV-resistant transgenic rice using a twin T-DNA system and RNAi**

*Y.Y. Jiang, L. Sun, M.S. Jiang, Y.Z. Song, C.X. Zhu and F.J. Wen*
*State Key Laboratory of Crop Biology, Shandong Key Laboratory of Crop Biology, Shandong Agricultural University, Tai'an, 271018, P. R. China*
*Email: zhchx@sdau.edu.cn*

Twin T-DNA systems are a convenient and feasible strategy for distinguishing marker genes from genes of interest and creating selectable marker-free transgenic plants. The standard transformation plasmid pCAMBIA 1300 was modified into a binary vector that harbors two separate T-DNAs, one of which contains the hygromycin phosphotransferase (*hpt*) selectable gene. Using this binary vector, we constructed two vectors that expressed the inverted-repeat (IR) structures that target the 400-nucleotide middle regions of the *Rice stripe virus* (RSV) coat protein (CP) gene and the special-disease protein (SP) gene, respectively. Transgenic rice lines of cv. Lindao 10 were obtained through *Agrobacterium*-mediated transformation. In the PCR analysis, co-transformation frequencies of the primary transformants that harbor both the *hpt* selectable gene and the target gene (RSV CP or SP) were 46.67% and 43.75%, respectively. Approximately 8.72% of the $T_1$ transgenic plants lacked the marker gene (*hpt*), whereas 10.90% possessed the target gene. Resistance analysis of the $T_2$ plants revealed two CP and three SP lines harbored the homozygous target gene, whereas the *hpt* gene did not exhibit high RSV-resistance. Southern blot and northern blot analyses of the resistant transgenic plants confirmed the stable integration and expression of the target genes. Unlike the susceptible transgenic plants, lower levels of transgene transcripts and specific small interfering RNA were observed in resistant transgenic plants, which suggest that the RNAi induced viral resistance.

**N33.004 Establishment and application of TaqMan Real-time RT-PCR for detecting HSVd**

*X.L. Zhao[1], Q. Zhou[1], N. Sun[2] and C.L. Deng[1]*
*[1]Plant Laboratory of Beijing Inspection and Quarantine Testing Center, Beijing, 100026, P. R. China; [2]School of Biological Science & Medical Engineering, Southeast University, Nanjing 210096, Jiangsu Province, P. R. China*
*Email: dengcl@bjciq.gov.cn*

Two pairs of specific primers and TaqMan probe were designed according to the full-length sequence of HSVd for preparation of standard for recombinant plasmid. The standard curve was plotted and a TaqMan-based Real-time RT-PCR method was established and verified for specificity, sensitivity and reproducibility. 26 submitted samples were determined by the established method, and the results were compared with those by routine PCR method. The results indicated that the standard for recombinant plasmid was constructed correctly. The $C_T$ value of plotted standard curve showed good linearity to the log of copy number of template, with a $R^2$ value of 0.999. The specificity of the established method was high and there were no cross reaction with *Hop latent viroid, Grapevine yellow speckle viroid 1, Grapevine yellow speckle viroid 2* and *Peach latent mosaic viroid*. The sensitivity of the method was $1.0 \times 10^2$ copies/μL, and it's 10 fold sensitive than routine PCR. Both coefficients of variation were less than 3%, indicating a good reproducibility. The positive rate of submitted samples by the established method (96%) was significantly higher than that by routine PCR method (88%), and the result was accurate and reliable. Conclusion demonstrated that the established TaqMan-based Real-time RT-PCR method showed high specificity, sensitivity and reproducibility, which was suitable for rapid and quantitative detection of HSVd in practical samples.

**N33.005 Identification of the thrips-vector of Tospoviruses and host plants in Yunnan**

*J. Zhang, X. Zheng, T.T. Li, X.X. Su, L.Z. Zhang, J.H. Dong and Z.K. Zhang*
*Yunnan Key Laboratory of Agricultural Biotechnology, Biotechnology and Genetic Germplasm Institute, Yunnan Academy of Agricultural Sciences, Kunming 650223, P. R. China*
*Email: zhongkai99@sina.com*

The viruses from genus *Tospovirus* cause diseases that lead to significant losses in crop production and quality worldwide. The dominant species of *Tospovirus Tomato spotted wilt virus* (TSWV) and *Tomato zonate spot virus* (TZSV) were circulated alternatively in Yunnan province, which have been causing substantial yield losses in vegetable, tobacco and other cash crops in recent years. The objective of this study was to identify the species of the thrips-vector of Tospoviruses and host plants in agricultural ecosystem in Yunnan Province. The results showed that *Frankliniella occidentalis, F. intonsa, Thrips tabaci, T. palmi* were the main vectors of Tospoviruses, and more than 30 species of weeds and 20 species of crops such as *Galinsoga parviflora, Oenothera rosea, Ranunculus chinensis, Vicia faba, Solanum lycopersicum* were the host plants in Yunnan, respectively. The results would provide experimental foundation for controlling the prevalence of TZSV and its vector *F. occidentalis* and *T. palmi* effectively in Yunnan.

**N33.006 Synthesis and application of silica-coated magnetic particles on extraction of viral nucleic acid**

*N. Sun[1, 2], X.L. Zhao[2], C.L. Deng[2] and Q. Xia[1]*
*[1]School of Biological Science & Medical Engineering, Southeast University, Nanjing 210096, Jiangsu Province, P.R. China; [2]Plant Laboratory of Beijing Inspection and Quarantine Testing Center, Beijing 100026, P.R. China*
*Email: xiaq@seu.edu.cn*

To extract viral nucleic acid from plant tissues simply and fast, magnetic particles were synthesized and characterized. Silica-coated magnetic particles were synthesized by two-step methods: firstly, $Fe_3O_4$ magnetic nanoparticles were synthesized by thermal decomposition of iron (III) acetylacetonate in the presence of benzyl alcohol; secondly, silica-coated magnetic particles were synthesized by tetraethoxysilane (TEOS) hydrolysis at the alkaline condition. Silica-coated magnetic particles were characterized by transmission electron microscopy (TEM) and scanning electron microscope (SEM), and the size was determined by Dynamic light scattering. The adsorption capacity of nucleic acid was analyzed in the presence of guanidinium thiocyanate with and without ethanol by using salmon sperm DNA and rRNA extracted from cells of *E. coli* JM109. Viral nucleic acid was extracted from tomato leaf infected by *Tomato yellow leaf curl virus* (TYLCV) by using Silica-coated magnetic particles. The results of TEM and SEM indicated that the silica-coated magnetic particles were spheroid and identified core-shell structure. The silica-coated magnetic particles had a narrow hydrodynamic size distribution from 0.2 to 1 μm with an average about 0.5 μm. Adsorption capacity was up to 10.6 mg salmon sperm DNA or 7.69 mg rRNA per 1g silica-coated magnetic particles without ethanol, and if with one volume of ethanol, it was up to 144 mg of salmon sperm DNA. TYLCY DNA was extracted successfully from tomato leaf, and the limit of detection was 24 copies by reverse transcription quantitative polymerase chain reaction.

**N33.007 Complete sequence of an isolate of *Apple stem grooving virus* on apple**

*H. Chen, S.Y. Chen, T. Ye, L. Hao, Z.F. Fan, L.Y. Guo and T. Zhou*
*Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China*
*Email: taozhoucau@cau.edu.cn*

*Apple stem grooving virus* (ASGV) is one of the most important viruses infecting fruit trees worldwide. In this study, complete sequence of an isolate ASGV-T47 was determined with total RNA from *in vitro* apple shoots. Consisting of 6496 nucleotides in length, the genome shares the highest identity (97.6%) to an ASGVisolate from Japan (accession no. NC_0011749) and encodes three putative open reading frames (ORF) plus 5′ (1-36nt) and 3′ (6355-6496nt) ends. The nucleotide (nt) and deduced amino acid (aa) sequences of ASGV-T47 encoded ORF1, ORF2 and CP were compared to that of other ASGV isolates with full sequences available in GenBank. For ORF1, the identities were 79.2-97.5% and 85.6-98.2% at nt and aa levels, respectively. For ORF2, the identities were 84.2-97.2% and 92.2-96.9% at nt and aa levels, respectively. Amino acids alignment of ORF1 encoded by all ASGV isolates revealed two highly variable regions, R1( from amino acids (aa) 424 to 619) and R2 (aa 1,583 to 1,916).

**N33.008 Identification of a *Cucumber mosaic virus* isolate infecting maize in China**

*R. Wang[1], N. Wang[1], T. Ye[1], Y.C. Shi[2], K. Yuan[3], Z.F. Fan[1] and T. Zhou[1]*
*[1]Department of Plant Pathology, China Agricultural University, Beijing, 100193, P. R. China; [2]Beijing Plant Protection Station, Beijing, 100029, P. R. China; [3]Fanshan Station of Plant Protection, Beijing, 102488, P. R. China*
*Email: taozhoucau@cau.edu.cn*

A maize plant showing irregular mosaic stripe, mottle on the leaves was found in Beijing, China and the causal pathogen was identified to be *Cucumber mosaic virus* (CMV). Total RNA was extracted from symptomatic leaves of maize and screened by reverse transcription (RT)-PCR with degenerate primer pairs to detect poty- and cucumoviruses. A specific RT-PCR product (~650bp) was obtained only with the cucumovirus primers. The sap from leaves of the diseased maize plant could infect *Chenopodium amaranticolor* and showed local necrotic lesions on inoculated leaves. The representative single lesion was used as inoculum for three consecutive transfers, and CMV was isolated. This CMV isolate could systematically infect *Nicotiana tobacum, N. tobacum* cv. 'Xanthi-nc', *N. glutinosa, Solanum lycopersicum* and *Zea mays* L. (cv. Nongda 2238). The presence of CMV in the diseased maize plant was also confirmed by ELISA and Western blotting. The sap from leaves of the diseased maize plant reacted strongly to CMV serogroup I antibody (Agdia, USA) with I/H was 5.8. With Fny-CMV CP antiserum, specific bands were obtained from the total protein isolated from the leaves of the diseased maize plant, while there was no band from healthy samples. The isolate was named CMV-ZMBJ.

**N33.009 Gentiopicroside possesses strong anti-TMV activity**

*Y.D. Chen, X. Zheng, J.H. Dong and Z.K. Zhang*
*Yunnan Key Laboratory of Agricultural Biotechnology, Biotechnology and Genetic Germplasm Institute, Yunnan Academy of Agricultural Sciences, Kunming, 650223 P. R. China*

*Email: zhongkai99@sina.com*

Gentiopicroside (GE), a naturally occurring iridoid glycoside, has been developed into a novel traditional Chinese drug, and it was approved for the treatment of acute jaundice and chronic active hepatitis by SFDA. However, the inhibitory effect of GE on plant virus is unclear. In this study, we carried out two different application methods of GE, isolated from an extract of *Swertia cincta* Burk in Yunnan province of China, to examine its inhibitory effect on TMV. One way, one half-leaf of *Nicotiana glutinosa* was inoculated mechanically with TMV that pretreated by GE for 30 min, and the other half-leaf inoculated mechanically with TMV was as a positive control. The results showed that the number of local necrotic lesions on the GE-treated half-leaf was significantly less than that of the control half-leaf with an inhibitory rate of 51.2%, indicating that GE can inactivate TMV in vitro and suppress its infective activity to the host. Another way, one half-leaf of *N. glutinosa* was sprayed with GE, and the other half-leaf sprayed with control solution was as a control. Then, 6 h later, the whole leaf was inoculated mechanically with TMV. The results showed that the number of local necrotic lesions on the GE-treated half-leaf was significantly less than that of the control half-leaf with an inhibitory rate of 58.5%, indicating that GE can induce the resistance of host plant. These results suggest that GE possesses anti-TMV activity and that GE is a promising starting compound for the development of anti-TMV agents.

## Concurrent Session 34-Plant Virus Epidemiology

### O34.001 Circulative, nonpropagative transmission: a carefully tuned orchestra of virus, vector and host proteins

*S.M. Gray, M. Cilia, M.S. Bereman, S. Deblasio, J. Chavez, D. Smith, M.J. MacCoss and J.E. Bruce*
*USDA, ARS and Dept of Plant Pathology & Plant-Microbe Biology, Cornell University, Ithaca, NY, USA*
*Email: smg3@cornell.edu*

Nonpropagative, circulative plant viruses replicate in and associate with phloem tissue where they are available to sap sucking aphid vectors. Once ingested into the aphid, the virus must actively recognize and be transported across specific gut and salivary tissues. Multiple domains in two virus structural proteins mediate virion movement in phloem and insect tissues via physical interactions with a suite of aphid, symbiont and plant proteins that orchestrate these complex and interactive processes. Aphid vector competency is regulated by a multitude of aphid and symbiont genes operating in an additive fashion that ultimately give rise to clonal aphid populations disparate in their transmission efficiency phenotype. These genes encode proteins associated with cell binding, uptake, vesicle transport and immune responses, and some interact directly, or in complex, with virions. A more practical application is that several of the aphid proteins serve as robust biomarkers for vector competence and identify aphid populations that should be targeted for control. Tissue tropism of the virus in the plant is critical for virus transmission. Cytosolic forms of two virus proteins mediate the retention and movement of virus in phloem tissues. The identified host-virus interactome increased transmission efficiency by either influencing virus uptake directly or facilitating virus ingestion via an alteration of the phloem environment. The genes coding for the aphid and plant proteins interacting with virus and/or mediating phloem retention may be exploited as components of novel forms of precision virus management strategies that target and disrupt the molecular interactome.

### O34.002 Epidemiology of mite-borne *Wheat streak mosaic virus* in wheat in Australia: roles of infection sources and patterns of spread

*B.A. Coutts[1,2], M. Banovic[1], M.A. Kehoe[1,2], D.L. Severtson[1,2] and R.A.C. Jones[1,2]*
*[1]Department of Agriculture and Food, Locked Bag No.4, Bentley Delivery Centre, WA 6983, Australia; [2]School of Plant Biology, Faculty of Science, University of Western Australia, Stirling Highway, Crawley, WA 6009, Australia*
*Email: roger.jones@agric.wa.gov.au*

When cyclonic rainfall events coincide with hot summer or autumn conditions in the West Australian grainbelt, environmental conditions are conducive to rapid build-up of wheat curl mite (WCM; *Aceria tosichella*), the vector of *Wheat streak mosaic virus* (WSMV). If WSMV sources are also present and moist conditions continue, WCM then spreads the virus rapidly in volunteer cereals and some annual grasses. In rainfed wheat crops sown in late autumn, greatest WSMV spread and yield losses develop when internal WSMV infection sources occur within the crop. Such internal sources consist of (i) WSMV-infected volunteer cereal or grass plants that persist when the crop is planted, or (ii) WSMV-infected wheat seedlings that grow when WSMV-infected wheat seed stocks are sown. Different cereal and grass species differ in their relative importance as virus infection sources. WSMV spread was examined in three initially irrigated field experiments sown early to coincide with environmental conditions favoring WCM activity and dispersal. Wheat infector transplants served as internal or external virus infection sources. Internal sources simulated different levels of seed-borne infection (0.1-1%). WSMV spread only occurred when infector plants were also infested with WCM, and killing these plants with herbicide or leaving them alive did not alter the extent of virus spread. Spatial spread patterns were examined using the SADIE program. When WSMV spread from internal sources, infection was concentrated in patches centred on infector transplants, extent of infection depending on numbers of infector plants introduced. WSMV incidence declined rapidly with distance from adjacent external infection sources.

### O34.003 Occurrence and transmission characteristics of *Wheat dwarf virus*

*Y. Wang, Y. Liu and X. Wang*
*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R . China*
*Email: xfwang@ippcaas.cn*

*Wheat dwarf virus* (WDV) is a member of the genus *Mastrevirus*, *Geminiviridae*. In 2007, we reported firstly its occurrence in China. Since then, the samples collected from 13 provinces throughout China were identified as WDV positive by PCR and ELISA, suggesting a broad distribution of WDV in China. Plant susceptible varieties and high density of vector *Psammotettix alienus* (above 600 insets/ $m^2$) play a critical role in WDV epidemics. There are fewer studies about the interaction between WDV and its leafhopper vector. Following a 5-min acquisition access period (AAP) on diseased wheat plants and different inoculation period (IP) on healthy wheat plants, the digestive system and salivary glands were dissected, and then observed by iCLSM and

TEM. The results showed two pathways of WDV movement in its leafhopper vector: through the filter chamber to the salivary glands via hemocoel, and through the anterior and middle midgut to the salivary glands via hemocoel. WDV could spread to the salivary glands at a short time after AAP in the first pathway in which could last only several hours. The spreading time of WDV from anterior and middle midgut to the salivary glands was occurred later, but it could last for long time. When recombinant WDV capsid protein and nonstructural protein REP and REPA incubated with the alimentary canal of leafhopper, only recombinant capsid protein was retained. It is suggested that the capsid protein of WDV is a key factor for WDV retention in digestive system of leafhopper.

**O34.004 Direct and indirect effects of plant viruses in vector behavior and performance**

*A. Fereres*
*Insituto de Ciencias Agrarias, ICA, CSIC, Serrano 115 dpdo, Spain*
*Email: a.fereres@csic.es*

Plant pathogens can influence the behavior and fitness of their insect vectors, by two different ways: directly -mediated by the presence of the virus in the vector's body and indirectly -mediated by changes occurring in the plant as a consequence of infection. It has been proposed that plant viruses may modify the behavior of their insect vectors adapting to each type of virus-vector relationship in a way that transmission efficiency is optimized. Furthermore, there are different, that suggest that both plant viruses and vectors have evolved resulting in mutualistic relationships. We will report results of two different studies on the direct and indirect effects of virus infection on their insect vectors (aphids and whiteflies) that result in mutualistic beneficial interactions for both the virus and the vector. In the first study we analyzed virus-vector interactions between a non-circulative (CMV) and a circulative (CABYV) plant virus and its aphid vector – *Aphis gossypii* – in its shared host plant – cucumber. Our results show that the non-circulative virus induced a pull-push strategy and the circulative virus a pull-arrest strategy in its insect vector, which optimizes virus transmission efficiency. In the second study we will report on how the acquisition of the begomovirus *Tomato yellow leaf curl virus* (TYLCV) by the whitefly *Bemisia tabaci* directly alters its behavior. Our results show evidence that TYLCV directly manipulates the settling, probing and feeding behavior of its vector *B. tabaci* in a way that enhances virus transmission efficiency and spread. Furthermore, TYLCV-*B. tabaci* interactions are mutually beneficial to both the virus and its vector because *B. tabaci* feeds more efficiently after acquisition of TYLCV. Our research has clear implications in the epidemiology and management of the aphid and whitefly-transmitted viruses.

**O34.005 Genetic diversity of viruses associated with the mosaic disease of *Saccharum* inter-specific hybrids in China**

*Y.L. Zhang[1], Z.G. Xiong[1, 2], N.T. Yu[1, 2], Q.X. Huang[1], J.H. Wang[1], S.Z. Zhang[1] and Z.X. Liu[1]*
*[1]Key Laboratory of Biology and Genetic Resources of Tropical Crops, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Science, Haikou 571101, P. R. China; [2]School of Plant Sciences and BIO5 Institute, University of Arizona, Tucson, AZ, U.S.A.*
*Email: liuzhixin@itbb.org.cn*

Leaves with mosaic symptoms were collected from 44 sugarcane varieties in Southern China and analyzed by reverse-transcription polymerase chain reaction (RT-PCR). The coat protein (CP) gene fragments were cloned, sequenced, and identified as those of *Sugarcane mosaic virus* (SCMV), *Sorghum mosaic virus* (SrMV), or *Sugarcane streak mosaic virus* (SCSMV). Amongst 78 samples tested, 67 hybrid sugarcane samples were found to be infected by SrMV. In nine of these samples, SrMV sequences were sufficiently distinct from the known strains (92-94% nucleotide identities) to be considered as four new SrMV strains. Four hybrid sugarcane samples were also infected by SCSMV but only one sample was co-infected with both SrMV and SCSMV. The only two noble sugarcane samples analyzed were infected with a new SCMV strain (90.0 - 94.0% nucleotide identities to known SCMV isolates), however none of the hybrid sugarcane samples was found to be infected with SCMV. These results indicate that SrMV was the most common pathogen of mosaic disease in hybrid sugarcane and that co-infection of sugarcane viruses is uncommon in China. SrMV isolates in China can be classified into three groups based on the analysis of the CP genes sequenced in this study and previously reported CP genes.

**O34.006 Banana bunchy top and banana streak viruses in East and Southern Africa**

*J.L. Dale, A.P. James, J. Kubiriba, C. Changa, J.A. Mugini, L. Karanja, W. Tushemereirwe, M. Soko and R.M. Harding*
*Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, Queensland, 4001, Australia*
*Email: j.dale@qut.edu.au*

*Banana bunchy top virus* (BBTV), a persistently aphid transmitted virus, is the most devastating banana virus usually leading to 100% yield loss. It is widely but not universally present in countries in Asia and Africa as well as the South Pacific, including Australia. *Banana streak virus* (BSV), transmitted by mealybugs, is also widely distributed but without the devastating effects associated with BBTV. We surveyed for both these

viruses in Uganda, Kenya, Tanzania, Rwanda and Malawi. In Uganda, Kenya and Tanzania, only BSV was detected. The incidence was sporadic and low except in Uganda where there were a few extensive outbreaks. In contrast, BBTV was only detected in Malawi and Rwanda. In Malawi, BBTV was widespread and severely limited banana production. The situation in Rwanda was probably the most revealing. BBTV was found in a very few bananas in the highlands immediately adjacent to the road directly down to the Rift Valley. In the valley, the incidence of BBTV rose dramatically. The distribution of these two viruses appeared to be closely correlated with the distribution of their vectors. Both BSV and mealybugs were common in the highlands, particularly in the higher rainfall regions, while aphids were apparently not present. Conversely, in the Rift Valley of Malawi and Rwanda where the rainfall is significantly lower, aphids were prevalent as was BBTV with no evidence of mealybugs. The incidence of BBTV in the Rwanda highlands was probably due to human movement of infected plants rather than natural spread.

**O34.007 Water born plant viruses: detection, survival and transmission**

*M. Ravnikar, I. Gutierrez-Aguirre, N. Prezelj, P. Krambergar and N. Mehle*
*National Institute of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia*
*Email: maja.ravnikar@nib.si*

Widely used hydroponic systems and intensive irrigation in horticulture hold the potential for rapid and efficient spread of water-transmissible plant pathogens throughout the whole crop (Mehle and Ravnikar, Water Research 46(16) 2012). Although numerous plant viruses have been detected in aqueous environment, for many of them, the survival in water and the potential for direct transmission through irrigation water or nutrient solution are still unknown. Therefore we decided to explore whether the water can be a source of infection with relatively stable and contagious viruses/viroid, a serious threat to tomato and/or potato production We have experimentally confirmed that Pepino mosaic virus (PepMV) remained infective in water at room temperature for up to three weeks, *Potato virus Y* (PVY) for up to one week and *Potato spindle tuber viroid* (PSTVd) for at least up to five weeks and are efficiently transmitted through nutrient solutions in hydroponic cultivation. In irrigation waters, the viruses are usually present in concentrations lower than the detection limit of classical methods, but that may be sufficient to infect plants; therefore the usage of highly sensitive diagnostic methods like qPCR and ddPCR is necessary. In addition, the concentration step allowing the handling of larger water volumes improves water monitoring diagnostic scheme. The results of fast and efficient concentration of highly diluted viruses using Convective Interaction Media® (CIM) monolithic chromatographic supports, which have already been successfully applied to the concentration of several plant and human viruses will be presented.

**P34.001 Begomovirus: From weeds to economically important crops**

*A. Sahu, A. Marwal, R. Prajapat and R.K. Gaur*
*Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology and Science, Lakshmangarh, Sikar-332311, Rajasthan, India*
*Email: gaurrajarshi@hotmail.com*

Geniniviruses have circular single stranded DNA genomes and are responsible for infecting major crop as well as weeds worldwide. *Calotropis procera* is a common weed distributed in warmer parts of India. All parts of this are of immense medicinal value. Leaves are applied to cure pains and flowers in curing piles. During the survey of roadside weeds in Rajasthan in 2009 for begmovirus, typical chlorosis symptoms were observed (up to 100% of plants) in *C. procera.* Electron microscopic examination of field samples revealed few, typical geminate particles. To confirmed the presence of a begomovirus, polymerase chain reaction was performed by using the primers CLCV1 (virion-sense primer 5'-CCGTGCTGCTGCCCCCATTGTCCGCGTCAC-3'; annealing within the coat protein gene) and CLCV2 (complementary-sense primer 5'-CTGCCACAACCAT GGATTCACGCACAG GG-3'; annealing in the C2 gene), designed to sequences conserved among begomoviruses from the Indian subcontinent. An amplification product of approx 1 kbp was produced from all the symptomatic plant but not from non-symptomatic plant. The PCR product was cloned and sequence obtained showed the 70-95% identity with other begmovirus. Phylogenetic analysis of nucleotide sequence of the core of the coat protein of the virus isolate with the most related begomovirus or begomovirus-like sequences was performed using MEGA neighbour joining version 4.0 tool. This analysis shows 70-95% identity with other virus isolate. This weed may be the one of the main source in the transmission of the virus to other economic important crops in India and also may decrease the medicinal value of *Calotropis* sp. The further experiment regarding the full length clone for the virus is in progress. To the best of our knowledge this is the first report of begomovirus with in leaf yellowing disease in *C. procera.*

**P34.002 Virus surveys of *Vicia faba* crops in Canterbury, New Zealand 2011–12**

*J.D. Fletcher, H. Ziebell and R.M. MacDiarmid*
*The New Zealand Institute for Plant & Food Research Limited, Lincoln, New Zealand; Julius Kühn-Institut, Braunschweig, Germany*

*Email: john.fletcher@plantandfood.co.nz*

Broad bean (*Vicia faba* L.) is a vegetable crop grown the South Island of New Zealand. Recently there has been interest in growing related field bean or tick bean crops for both human and animal consumption. These crops can form a useful break crop for cereals, brassicas, pasture and other cropping systems. The last virus survey of *V. faba* was completed in 1991 when we detected *Soybean dwarf virus* (SDV) and *Beet western yellows virus* (BWYV), now classified as *Turnip yellows virus* (TuYV), which cause 'bean leafroll'; *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV); *Pea seed-borne mosaic* (PSbMV) and *Bean yellow mosaic virus* (BYMV). In 2011, 16 broad, faba and tick bean crops throughout Canterbury were surveyed for viruses known and not known to be present in New Zealand. Overall, virus incidences were low. Only TuYV appears to have become more widespread but with a similar incidence (0-7%). SDV, while being less widespread, had a higher incidence (0-25%). The incidences of other viruses seem similar to the previous survey: AMV (0-9%), PSbMV (0-3.5%), BYMV (0-5%), and CMV (not detected). *Broad bean wilt virus* 1 (BBWV-1), *Broad bean wilt virus* 2 (BBWV-2), and *Broad bean true mosaic* viruses not known to be present in NZ were not detected. It may be significant that we have detected *Red clover vein mosaic virus* (RCVMV) which has not been described for New Zealand before and found it to be present in over 50% of crops with incidences of up to 20%.

### P34.003 Ryegrass, a potential reservoir for *Wheat dwarf virus* and viruses associated with barley yellow dwarf disease

*E. Yazdkhasti, I. Eriksson, R.J. Hopkins and A. Kvarnheden*
*Department of Plant Biology and Forest Genetics, Uppsala BioCenter, Linnean Centre of Plant Biology in Uppsala, Swedish University of Agricultural Sciences, Box 7080, 750 07 Uppsala, Sweden*
*Email: elham.yazdkhasti@slu.se*

Viruses infecting cereals often have grasses as alternative hosts, which may act as virus reservoirs. In Sweden, *Wheat dwarf virus* (WDV; Family *Geminiviridae*; genus *Mastrevirus*) has for more than 100 years been an important pathogen on wheat, with periodic outbreaks causing significant yield losses. Also viruses belonging to different species of *Barley yellow dwarf virus*/*Cereal yellow dwarf virus* (BYDV/CYDV; Family *Luteoviridae*; Genera *Luteovirus* and *Polerovirus*) regularly affect wheat and other cereals. A predicted warmer climate will favour the insects, which act as virus vectors and will probably result in increased disease incidence. In our studies to identify important factors for the epidemiology of cereal-infecting viruses, we are investigating the potential role of ryegrass, grown as a perennial crop. Ryegrass samples from fields previously affected by WDV were tested by DAS-ELISA for infection by WDV, BYDV-PAV, BYDV-MAV and CYDV-RPV. Preliminary results show that WDV was present at a low frequency, and infection could also be confirmed by PCR. Nucleotide sequence analyses revealed that the ryegrass isolates were closely related to WDV isolates previously characterized in Sweden. Controlled inoculation experiments using leafhopper vectors were carried out to study the ability of WDV to infect different ryegrass cultivars and species. Surprisingly, just a few ryegrass samples from this experiment were found to be positive in DAS-ELISA while wheat plants exhibited high ELISA values. These samples will be analyzed further by real-time PCR to determine their susceptibility or resistance.

### P34.004 Artificial inoculation of TYLCV and physiological activity change on tomato plant

*J.H. Lee, C.G. Son, S.G. Park and H.S. Choi*[2]
[1]*Institute for Bioresources Research, Gyeongsangbuk-do Agricultural Research and Extension Services, Andong, 760-891, Republic of Korea;* [2]*National Institute of Agricultural Science and Technology, RDA, suwon 441-707, Republic of Korea*
*Email: ljh8888@korea.kr*

This research was performed for artificial inoculation of *Tomato leaf curl virus* (TYLCV) and physiological activity change on tomato plant after infection. TYLCV korean isolate was artificially infected by agro-inoculation and TYLCV DNA was introduced into host cells of shoot tips by pin-pricking method. A non-tumour-inducing strain of *Agrobacterium* (GV3101) was used in order to conform with biosafety regulations. Three to four weeks old tomatoes were used for agro-inoculation. The level of the bacterial inoculum was important to obtain maximum infection, with a high inoculum level ($0.5 \times 10^{10}$ cells/ml) resulting in up to 100% infection of a susceptible variety that was comparable with infection by insect transmission. TYLCV gene was detected by PCR (using appropriate detection primer) 10 days and virus symptoms (leaf curl and chlorosis) were appeared 15-20 days after artificial inoculation. After inoculation, the plant growth and fruit yield were decreased severely. The rate of photosynthesis of tomato leaves which is inoculated by TYLCV was sharply decreased (5-7 μmol $CO_2$ $m^{-2}$ $s^{-1}$) compare to that of normal leaves (13-15 μmol $CO_2$ $m^{-2}$ $s^{-1}$) after three to four weeks. However, the transpiration rate was no significant difference between inoculated and normal leaves.

### P34.005 Characterization of *Chrysanthemum stunt viroid* in Rajasthan, India

*R. Raizada and R.K. Gaur*
*Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology and Science,*

*Lakshmangarh, Sikar, Rajasthan, India*
*Email: richaraizada@hotmail.com*

During the survey of ornamental plants in India, the chrysanthemum shows stunting in growth and yellowing leaf spots. The total RNA/DNA was extracted to test the types of viral/phytoplasmal infection. The RNA/DNA was used for the RT-PCR/PCR by universal primer of the different viruses i.e potyvirus, begomovirus, luteovirus and phytoplasma, which shows the negative results. Further, semi-universal viroid primers: Pospi1-RE was used for the presence of viroids. The amplicon of approximately 200 bp was sequenced and submitted to the NCBI. The BLAST analysis of the sequence revealed 97% identity with *Chrysanthemum stunt viroid* (CSVd). To our knowledge, this is the first report in Rajasthan, India.

**P34.006 Molecular identification of major rice dwarf virus in Jiangxi province and analysis of the variation of coat protein gene**

*X.T. Sun, R.Q. Cui, H.H. He and C.L. Zhu*
*College of Agronomy, Jiangxi Agricultural University, Nanchang, 330045, Jiangxi Province, P. R. China*
*Email: cuiruqiang@sohu.com*

According to the nucleotide sequences of Southern rice black-streaked dwarf virus (SRBSDV) and *Rice black-streaked dwarf virus* (RBSDV) from GenBank, three primers were designed. By field survey, symptom observation, reverse-transcription polymerase chain reaction (RT-PCR) and sequencing techniques, the viral pathogen was identified. The S10 gene of SRBSDV isolate from Jiangxi was cloned and sequenced, and the obtained sequence, together with partial corresponding sequences available from GenBank, were used for molecular variation analysis by using some bioinformatics softwares. The results indicate that the viral pathogen of the rice dwarf disease in Nanchang city Jiangxi province was identified as SRBSDV. The S10 gene of SRBSDV isolate from Jiangxi was consisted of 1785nts, including 1674 nucleic acids of full length CP gene, encoding 557 amino acids. The CP gene nucleotide and amino acid sequences homology of SRBSDV Jiangxi isolate and other isolates in GenBank were between 97.5%-100% and 97.7%-100%, respectively. SRBSDV CP gene nucleotide sequences were highly conserved. No recombination event was detected. Phylogenetic analysis suggests that SRBSDV isolates were divided into two clusters, but there was no significant correlation between clusters and geographic origin or host. To our knowledge, this is the first report that SRBSDV isolates were divided into two clusters based on phylogenetic tree.

**P34.007 Simultaneous detection and survey of three rice viruses in Zhejiang province, China**

*W.Q. Wu[1,2], X.G. Guo[1,2], H.M. Zhang[1], J. Yang[1,2], M.F. Lv[1,3] and J.P. Chen[1]*
*[1]State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control; Key Laboratory of Plant Protection and Biotechnology, Ministry of Agriculture, P. R. China; Zhejiang Provincial Key Laboratory of Plant Virology, Institute of Virology and Biotechnology, Zhejiang Academy of Agricultural Science(ZAAS)s, Hangzhou 310021, P. R. China; [2]College of Chemistry and Life Science, Zhejiang Normal University, Jinhua 321004, P. R. China; [3]College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, P. R. China*
*Email: zhhengmu@tsinghua.org.cn*

*Rice black-streaked dwarf virus* (RBSDV; genus *Fijivirus*, family *Reoviridae*), *Rice stripe virus* (RSV; genus *Tenuivirus*), and Southern rice black-streaked dwarf virus (SRBSDV), a novel member of the second group of fijiviruses, have rapidly become prevalent and cause significant yield losses in some rice-growing areas of China. The three diseases often overlap to some degree and may even occur in the same fields, particularly in Eastern China. The similarity of symptoms and complex patterns of infection in fields make it difficult to detect and survey the viral pathogens. We now report a multiple RT-PCR method for detection of RSV, RBSDV, and SRBSDV. This is also the first report of simultaneous detection and survey of these three major viral diseases on rice plants. The method was used to detect the viruses in samples of rice, maize, small brown planthoppers, and white-backed planthoppers collected from different regions of China and Vietnam, showing that it is simple, rapid, and sensitive. The method will be useful for investigating a series of unsolved biological problems that should contribute to disease forecasting, monitoring and control. A survey of the three viruses throughout Zhejiang province, Eastern China, using this method showed that both RBSDV and RSV had continued to spread and that the newly-emerging virus, SRBSDV, was present in at least 27 counties or cities. More effort is needed to monitor and control the threat from these three viral diseases.

**P34.008 Occurrence and distribution of *Soybean mosaic virus* 11 strains in Korea**

*Y.H. Lee[1], S.T. Lim[1], Y.N. Yoon[1], M.G. Jeon[1], M.S. Choi[1], H.Y. Kim[1], B.K. Kang[1], H.T. Yun[1], H.T. Kim[1], J.M. Ko[1], S.H. Lee[2], K.W. Lee[2] and I.Y. Baek[1]*
*[1]National Institute of Crop Science, RDA, 20th Jeompiljaero, Miryang, 627-803, Republic of Korea; [2]Department of Agricultural Biology, Kyungpook National University, Daegu, 702-701, Republic of Korea*
*Email: sky3832@korea.kr*

*Soybean mosaic virus* is a prevalent pathogen that causes significant yield reduction in soybean worldwide. *Soybean mosaic virus* belongs to potyvirus and causes symptoms such as mild mosaic, mosaic and lethal necrosis. *Soybean mosaic virus* is seed-borne and is also transmitted by aphid. *Soybean mosaic virus* 11 strains, G1 to G7, G5H, G6H, G7H, and G7A was reported in Korea. We generally have used the system of Cho and Goodman's differential reaction for identification of *Soybean mosaic virus* strains. Recently, RT-PCR/RFLP analysis was reported for detection and identification of *Soybean mosaic virus* 5 strains, G2, G5, G5H, G7, and G7H by Kim et al. (2004). For the analysis, the amplified fragment of cylindrical inclusion (CI) gene was used for RT-PCR and RFLP profiles of the RT-PCR products were compared after restriction digestion with restriction endonucleases. In this study, we collected samples with viral symptoms of *Soybean mosaic virus* from 13 areas of 8 provinces. And then RT-PCR/RFLP analysis was conducted to identify strains of *Soybean mosaic virus*. As a result, the distribution of G5, G5H, G6 and G7H were identified as dominant. No-cutting and nonspecific reactions were supposed to be caused by a genetic mutation.

### P34.009 Occurrence of *Zuchini yellow mosaic virus* in cucurbit crops of Khyber-Pakhtunkhwa, Pakistan

*A. Ali[1, 2], A. Hussain[2], M. Ahmad[2], I. Naz[2], A. Bibi[2] and M. Junaid[2]*
*[1]Faculty of Agriculture, Laboratory of Plant Pathology, Utsunomiya University, Mine Campus, Japan; [2]Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan*
*Email: asadjp2005@yahoo.com*

High incidence of mosaic, mottling, chlorosis and deformation was revealed during survey of cucurbit fields in Khyber Pakhtunkhwa, Pakistan. ZYMV was found infecting all kind of cucurbits grown in KPK. Incidence in hilly areas was comparatively high when compared with the incidence in plains which could be attributed to the high population of aphid vectors, high crop density, cropping pattern and conducive environmental factors in hilly tracks. Among the seven districts surveyed, average percent incidence of ZYMV was recorded up to 54.7% in district Nowshera, followed by 46.4 % in district Peshawar, 44.1% in district Charsada, 42.2% in district Swabi and 38.6 % in district Mardan. In Swat and Dir districts average incidence ZYMV was recorded upto 40.1% and 29.8%, respectively. Among the different crops highest incidence in plain areas of NWFP was recorded in Pumpkin (59.5%) followed by 46.7% in Squash, 46.2% each in Luffa and Lageneria, 33.9% in Melon and 33.8% in Cucumber. In Northern hilly areas highest incidence of 45.4% was observed in pumpkin, followed by 38.1% in Cucumber, 33.5% in Luffa, 30.9% in Lageneria and 25.4% in Squash.

## Concurrent Session 35-Population Genetics and Evolutionary Biology of Plant Pathogens

### O35.001 Multilocus support for a Central Mexico center of origin for *Phytophthora infestans*

E.M. Goss, J.F. Tabima, D.R. Cooke, M. Cardenas, K. Myers, R. Oliva, S. Restrepo, W.E. Fry, G. Forbes and N. Grünwald
*Department of Plant Pathology and Emerging Pathogens Institute, University of Florida, PO Box 110680, Gainesville, Florida, USA*
*Email: emgoss@ufl.edu*

The late blight pathogen, *Phytophthora infestans*, threatens global food security and costs billions of US dollars in losses each year. There are two competing hypotheses on the evolutionary origin of the pathogen. One hypothesis argues that South America is the center of origin and the other that Central Mexico is the center of origin. Genetic data have been used to support each of these positions. We conducted a comprehensive evolutionary analysis of *P. infestans* in order to resolve the question of the origin of *P. infestans*. We sequenced four nuclear genes and genotyped 11 microsatellite loci in a global sample of *P. infestans* isolates and sister taxa in *Phytophthora* Clade 1c. Detailed Bayesian phylogeographic and coalescent analyses show decisive support for a Central American origin of this pathogen, which is congruent with the natural history of the pathogen in this area. Our analyses also generated an evolutionary hypothesis to explain the observed pattern of genetic variation in *P. infestans* in the Andes.

### O35.002 Population structure and pathotype diversity of the wheat blast pathogen *Magnaporthe oryzae* 25 years after its emergence in Brazil

P.C. Ceresini[1], J.L.N. Maciel[2], G.H.J. Kema[3] and B.A. McDonald[4]
[1]*UNESP – Universidade Estadual Paulista, Departamento de Fitossanidade, Engenharia Rural e Solos, Ilha Solteira, São Paulo, Brazil;* [2]*Embrapa Trigo, Brazil;* [3]*Wageningen UR – University and Research centre, Plant Research International, The Netherlands;* [4]*ETH Zurich– Swiss Federal Institute of Technology, Switzerland.* *FAPESP (São Paulo State Foundation for the Advancement of Science, grant 2013/10795-0), and CNPq (454543/2013-1) have jointly sponsored the attendance of PCC to the ICPP 2013*
*Email: paulo.ceresini@bio.feis.unesp.br*

Since its first report in Brazil in 1985, wheat blast, caused by *Magnaporthe oryzae* (anamorph: *Pyricularia oryzae*) has become increasingly important in South America where the disease is still spreading. We used 11 microsatellite loci to elucidate the population structure of the wheat blast pathogen in wheat fields in Central-Western, Southeastern and Southern Brazil. No subdivision was found among the wheat-infecting populations, consistent with high levels of gene flow across a large spatial scale. Although the clonal fraction was relatively high and the two mating type idiomorphs (*MAT1-1* and *MAT1-2*) were not at similar frequencies, the clone-corrected populations from Distrito Federal and Goiás, Minas Triangle and São Paulo were in gametic equilibrium. Based on these findings, we propose that populations of the wheat blast pathogen exhibit a mixed reproductive system in which sexual reproduction is followed by the local dispersal of clones. Seedling virulence assays with local wheat cultivars differentiated six pathotypes in the current population. Detached head virulence assays differentiated eight virulence groups on the same wheat cultivars. There was no correlation between seedling and head reactions.

### O35.003 The whole genome sequence of *Pseudomonas syringae* pv. *actinidiae*: insights into evolution, genome dynamics and pathogenicity

M.D. Templeton[1,2], F. Bertels[3], H. McCann[4,5], A. Lu[6], E.H.A. Rikkerink[1], B. Haubold[7], J. Rees-George[1], A.P. Gleave[1], M.T. Andersen[1], P.W. Wang[5], D.S. Guttman[5] and P.B. Rainey[4,7]
[1]*New Zealand Institute for Plant and Food Research Ltd, Auckland, New Zealand;* [2]*University of Auckland, Auckland, New Zealand;* [3]*Biozentrum, University of Basel and Swiss Institute of Bioinformatics, Basel, Switzerland;* [4]*New Zealand Institute for Advanced Study and Allan Wilson Centre, Massey University, Auckland, New Zealand;* [5]*Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto Canada;* [6]*New Zealand Institute for Plant and Food Research Ltd, Lincoln, New Zealand;* [7]*Max Planck Institute for Evolutionary Biology, Plön, Germany*
*Email: matt.templeton@plantandfood.co.nz*

Most crops were domesticated centuries - even millennia - ago, thus limiting opportunity to understand the emergence of disease. Kiwifruit is an exception: domestication began in the 1930s. Outbreaks of canker disease caused by *Pseudomonas syringae* pv. *actinidiae* (*Psa*) first recorded in the 1980s. Based on SNP analyses of two circularized and over 60 draft genomes, *Psa* is comprised of distinct clades exhibiting negligible within-clade diversity, consistent with a disease arising by independent samplings from a source population. Three clades correspond to their geographical source of isolation; a fourth, encompassing the *Psa*-V lineage responsible for the 2008 outbreak, is now globally distributed. *Psa* has an overall clonal population structure; however, genomes carry a marked signature of within-pathovar recombination. Removal of SNPs by recombination yields an uninformative (star-like) phylogeny consistent with diversification of *Psa*-V from a single clone within the last ten years. Most SNP polymor-

phisms reside within PsyrGI-6-like conjugative elements (ICEs) whose evolution is unlinked to the core genome. Five distinct ICEs have been identified in *Psa*-V isolates. *Psa*-V from New Zealand, Chile and Italy each have a difference ICE however all five ICEs are found in isolates from China. Our analyses capture a pathogen in the early stages of emergence from a predicted source population associated with wild *Actinidia* species.

**O35.004 The origins of aflatoxin chemotype diversity in *Aspergillus* populations**

*I. Carbone, B.W. Horn, R.A. Olarte, G.G. Moore, C.J. Worthington, J.T. Monacell, R. Singh, E.A. Stone, K. Hell, S.N. Chulze, G. Barros, G.C. Wright and M.K. Naik*
*Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695, USA*
*Email: icarbon@ncsu.edu*

Species in *Aspergillus* section *Flavi* commonly infect agricultural staples such as corn, peanuts, cottonseed, and tree nuts and produce an array of mycotoxins, the most potent of which are aflatoxins, which can be classified into B and G toxin chemotype classes. Experimental matings in the laboratory revealed that sexuality has the potential to generate novel toxin chemotypes via meiosis and crossing over, but the specific adaptive processes that create and maintain aflatoxin diversity in nature are unknown. During adaptation, specific toxin genotypes may be favored and swept to fixation or be subjected to drift and frequency-dependent selection. We found that the frequency of sexual reproduction in populations of these fungi is directly correlated with the magnitude of recombination in the aflatoxin gene cluster. Moreover, clonality maintains distinct aflatoxin chemotypes in populations, whereas sexuality generates novel toxin chemotypes but tends to equalize toxin differences in populations. Results from intra- and inter-specific matings indicate that genetic exchange within the aflatoxin gene cluster can occur via crossing over between divergent chemotype lineages. Furthermore, interspecific matings suggest that aflatoxin chemotype evolution can potentially transcend species boundaries. On a contemporary time scale, the frequency of genetic exchange may be driven by differences in fertility among individuals in populations and by local environmental conditions that may directly impact the relative proportion of asexual and sexual reproduction. Our work shows that a combination of population genetic processes may influence aflatoxin chemotype diversity in these agriculturally important fungi.

**O35.005 Experimental evolution in field populations of pathogenic fungi**

*J. Zhan[1,2] and B.A. McDonald[3]*
*[1]Key Lab for Biopesticide and Chemical Biology, Ministry of Education, Fujian Agriculture and Forestry University, Fuzhou, 350002, P. R. China; [2]Institute of Plant Virology, Fujian Agriculture and Forestry University, Fuzhou, 35002, P. R. China; [3]Institute of Integrative Biology, ETH Zurich, LFW, Zürich, CH-8092, Switzerland*
*Email: jiasui.zhan@fafu.edu.cn*

Field experimental evolution using mark-release-recapture approach provides a powerful way to test and verify the hypotheses and assumptions associated with evolution of plant pathogens. We used this approach to study the population genetics and evolutionary biology of cereal pathogens *Mycosphaerella graminicola, Phaeosphaerea nodorum* and *Rhynchosporium secalis*. We found evidence that increasing host heterogeneity by growing mixtures of different cultivars mitigated evolution of the pathogens. We also found evidence for differential selection between parasitic and saprophytic phases of life cycles in *P. nodorum* and *R. secalis*. The results on the contribution of partial resistance to the evolution of pathogens are polarized. Reduced evolution was found in the pathogen populations sampled from partial resistance hosts in *M. graminicola* and *P. nodorum* but not in *R. secalis*.

**O35.006 The evolution of plant virus host range: lessons from experimental evolution**

*S.F. Elena*
*Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Valencia, Spain*
*Email: sfelena@ibmcp.upv.es*

In the context of host range expansion, a virus can recurrently be submitted to the selection pressures of a new host or to the selection pressures of various alternating hosts, depending on the ecological conditions. We were interested in (1) understanding the different strategies of adaptation that can result from these two situations, and (2) analyzing the importance of historical contingencies in the context of adaptation to a new host. In a first experimental evolution phase, we started from an infective clone of *Tobacco etch virus* (TEV) and derived independent evolutionary lineages, transferred either on the same host at each transfer (four specialist evolutionary histories) or on alternative host (three generalist evolutionary histories). In a second experimental evolution phase, the lineages of the seven evolutionary histories were serially transferred on the same host. After each of two experimental evolution phases, we used a complete crossed design to evaluate the infectivity and the virulence of all the evolved virus lineages on the different hosts. These data allowed us to look for the signature of antagonistic pleiotropy and of specialisation after the first phase and we were also able to evaluate how the specificities of each evolutionary history faded

out during evolution on the same host. Moreover, we acquired the consensus sequences of the 70 evolved lineages after each phase. We analysed the host specificity and the history specificity of the mutations accumulated in each lineage, after each step and could establish the relationship between genotype and phenotype in some cases.

### O35.007 Population genetic analyses enhance the recognition of species boundaries in fungal pathogens

*N.A. van der Merwe[1], B.D.Wingfield[1], M.J. Wingfield[1] and E.T. Steenkamp[2]*
*Departments of Genetics[1] and Microbiology and Plant Pathology[2], Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa*
*Email: albe.vdmerwe@up.ac.za*

The operational criteria to define fungal species boundaries include, amongst others, morphological characteristics, sexual compatibility, and phylogenetic criteria such as exclusive ancestry. Although these criteria are useful for the majority of taxonomic purposes, they generally fail when cryptic species are considered. Cryptic species are those species that can be discriminated based on one or a few of a multitude of species recognition criteria, but they do not meet the requirements for a sufficiently large number of criteria to define them as species with certainty. We have thus investigated the use of population genetics to aid in species definition. Using population genetic criteria, a species can be defined as a cohort of individuals that collectively differ in their allele frequency compositions from other such cohorts. Even where all other recognition criteria fail, unique allele frequency distributions can provide convincing evidence of genetic isolation. However, such data should be considered within a polyphasic framework, which should include at least one other line of supporting evidence. As an example, we have shown that for the genus *Chrysoporthe*, phylogeography can provide strong supporting evidence for the recognition of distinct taxa among cryptic species. Although the availability of biological material for population studies may be problematic for most taxonomic studies, population genetic tools can be very useful in order to delimit species boundaries, at least in those instances where sufficient numbers of isolates can be obtained for this purpose.

### O35.008 The role of hybridization and gene exchange in the evolution of the *Gibberella fujikuroi* complex

*E.T. Steenkamp[1], N.A. van der Merwe[2], M.P.A. Coetzee[2], L. De Vos[2], Q.C. Santana[2], G. Fourie[1], D.A. Herron[1], S.H. Martin[3], M.J. Wingfield[1] and B.D. Wingfield[2]*
*[1]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; [2]Deparment of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa; [3]Department of Zoology, University of Cambridge, Cambridge, United Kingdom*
*Email: emma.steenkamp@fabi.up.ac.za*

The *Gibberella fujikuroi* complex represents a monophyletic assemblage that includes many pathogens of economically important plants and/or producers of highly toxic compounds that contaminate food and feed stocks. Our research focuses on the development of a detailed understanding of the evolution of this complex, with the ultimate goal of revealing the processes involved in speciation and the emergence of traits such as host-specificity and mycotoxin production. For this purpose we utilize a range of genetic and microbiological methods, as well as phylogenetic and genomic approaches. Our results have shown unexpectedly high levels of synteny among the genomes of representative species, although localized and gross chromosomal rearrangements were also observed, many of which apparentlycame about early during the evolution of the complex. These data also highlighted the possibility ofgene exchange among species because of the occurrence ofunique genes and genes that support highly conflicting phylogenies. In fact, the notion of hybridization in the *G. fujikuroi* complex is not novel as viable progeny can be obtained from inter-specific crosses for numerous species. Although such crosses appear to be associated with high levels of segregation ratio distortion that could potentially impact the fitness of progeny, the possibility cannot be excluded that some ancestral hybrids might have survived and shaped the evolutionary trajectories in this complex. The *G. fujikuroi* complex thus represents an excellent model within which to study not only the evolution of species and their traits, but also the processes driving the emergence of new pathogens.

### O35.009 Interspecific variability and analysis of genetic diversity of *Monilinia* spp. populations from stone fruit orchards, in Greece

*A.A. Papavasileiou[1], Y. Luo[2], T.J. Michailides[2] and G.S. Karaoglanidis[1]*
*[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 54124, Thessaloniki, Greece; [2]University of California, Kearney Agricultural Research and Extension Center, Parlier, CA, USA*
*Email: gkarao@agro.auth.gr*

An extensive sampling was conducted aiming to study the population structure of brown rot causal pathogens on four different stone-fruit hosts, from two distinct regions of Greece, during 2011 and 2012. In total, 1,434 isolates of *Monilinia* spp. were obtained and identified to species based on the size of a *cyt*b intron. *Monilinia*

*laxa* and *M. fructicola* were detected at frequencies of 59 and 41%, respectively. *M. fructicola* was more common on fruit while *M. laxa* occurred in equal frequency on blossoms and fruit. *M. fructicola* populations showed higher genetic diversity compared to *M. laxa* populations, analyzed with ISSR markers. For both species, the highest genetic diversity among hosts was in the population from apricot. The, levels of genetic variation were similar between populations of blossom and fruit for each species and the main variances were all from within rather than between populations for the respective regions, hosts and growth stages. Genetic distance (Nei's analysis) was smaller between peach and apricot populations and between cherry and plum populations of *M. fructicola.* The analysis of index of association (IA) showed the absence of sexual recombination for both species. Although this is the first report of *M. fructicola* in Greece, the wide dispersal and high genetic diversity of pathogen populations suggests that this pathogen has existed in Greece for a long period of time. The higher genetic diversity in populations obtained from Macedonia and apricots indicate that *M. fructicola* was probably introduced in Greece, through apricots in the region of Macedonia.

**O35.010 Population structure of conifer pathogens associated with the mountain pine beetles analyzed by Bayesian methods**

C.K.M. Tsui
*University of British Columbia, Vancouver, BC, V6T 1Z4, Canada*
*Email: clementsui@gmail.com*

The mountain pine beetle (MPB: *Dendroctonus ponderosae*) and its fungal associates have destroyed over 18 million hectare of pine forests in Western Canada since the last decade. We are using microsatellites and SNPs markers to characterize the genetic structure and diversity of *Leptographium longiclavatum*, one of the fungal associates during the MPB outbreak. Both distance and Bayesian inference methods detected three clusters of populations influenced by its geographical distribution. Approximate Bayesian computation, and the isolation-by-distance pattern provided evidence in the northward movement/ expansion of pathogens, consistent to the expansion pattern of its vector MPB. Mantel tests also indicated general concordance among the genetic structure of MPB and other fungal associates. The insights of genetic structure and migration pattern can provide data useful to management and monitoring of the MPB outbreak.

**O35.011 Genetic analysis of *Phytophthora infestans* populations reveals mechanisms of genetic variation in Northwestern China**

Y.E. Tian, J.L. Yin, J.P. Sun, Y.F. Ma, H.P. Yang, F. Zhang, J.L. Quan and W.X. Shan
*State Key Laboratory of Crop Stress Biology in Arid Areas and College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, P. R. China*
*Email: wxshan@nwsuaf.edu.cn*

Late blight caused by the oomycete pathogen *Phytophthora infestans* is the most devastating disease of potato in China, which frequently resulted in significant economic losses in the past years. To better understand the *P. infestans* population genetic structure in Northwestern China, 962 single-lesion isolates were purified from samples collected from 2009 to 2011 and were characterized for mating types, pathotypes, mtDNA haplotypes and SSR genotypes. The results showed that the distribution of *P. infestans* mating types changed significantly, from coexistence of both A1 and A2 in Gansu and Ningxia in 2009 to the dominance of A2 in 2010 and 2011. Among 74 selected isolates determined for virulence on a set of near-isogenic potato lines containing R1 to R11 resistance genes, four pathotypes were detected, with a dominant pathotype virulent to most of tested resistance genes. Three mtDNA haplotypes (Ia, IIa, IIb) were detected. The dominant isolates were Ia and was a few were IIa, while IIb was detected only in one isolate collected in Ningxia region in 2009. SSR genotyping analysis with eight microsatellite markers distinguished 962 isolates into 151 different genotypes, including five that were dominant. Isolates sharing the identical multilocus genotypes were detected in several distant regions in Gansu and Ningxia. Shared genotypes were also identified when compared with 306 isolates collected from Southwestern China in 2010 and 2011. The genetic analysis results provide presumptive evidence that migration could have occurred between regions and is likely the dominant driving factor for the population dynamics of *P. infestans* in China. Mutation may also contribute to the genetic variation while sexual reproduction of *P. infestans* was not detected in Northwestern China. The results have implications for the role of monitoring *P. infestans* populations and development of efficient disease control measures in China.

**O35.012 Origin and distribution of the eucalypt stem canker pathogen *Teratosphaeria zuluensis***

L. Jimu[1, 3], S. Chen[2], M.J. Wingfield[2], E. Mwenje[3] and J. Roux[2]
[1]*Forestry Department, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa;* [2]*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa;* [3]*Bindura University of Science Education (BUSE), P. Bag 1020, Bindura, Zimbabwe*
*Email: Luke.Jimu@fabi.up.ac.za*

*Teratosphaeria zuluensis* and the closely related *T. gauchensis* cause one of the most serious stem canker disease of plantation-grown eucalypts outside Australasia. The origins and patterns of distribution of these pathogens are not fully understood. In this study, we considered the origin and patterns of spread of *T. zuluensis*. We surveyed for *T. zuluensis* in Malawi and previously un-sampled areas of Mozambique, Uganda and Zambia. Simple sequence repeat (SSR) data from previous population genetic studies were combined with data from this study in order to obtain a better understanding of the pathogen's origin and distribution in Africa. Moderate to low genetic diversities recorded for Malawi, Mozambique and Zambia populations support the hypothesis that the pathogen was introduced into Africa, possibly from south-east Asia. Furthermore, the relatively high genetic diversity in Malawi suggests earlier or multiple introductions of the pathogen, compared to other countries in eastern and southern Africa. In Uganda, the clonal nature of the population suggests very recent introduction of *T. zuluensis*. The index of association further supports the dominance of clonal reproduction in the pathogen. High spatial population differentiation, coupled with variable genetic diversities suggests independent introductions, possibly via seed. Information on the origin and movement trends of *T. zuluensis* has important implications regarding the formulation of efficient disease quarantine systems and effective control of Teratosphaeria stem canker.

### O35.013 Population dynamics of *Aspergillus flavus* following biocontrol treatment of corn

*R.A. Olarte, B.W. Horn, C.J. Worthington, M.H. Lewis, R. Singh, R.W. Heiniger, P.S. Ojiambo and I. Carbone*
*Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695, USA*
*Email: raolarte@ncsu.edu*

*Aspergillus flavus* is a fungal pathogen of many agronomically important crops worldwide. We sampled *A. flavus* strains from a cornfield in Rocky Mount, North Carolina, over a period of two years. The field was planted in 2010 and plots were inoculated at tasselling with either AF36 or NRRL 21882 (=Afla-Guard) biocontrol strains, both of which are mating type *MAT1-2*. Subsequently, toxigenic strain NRRL 3357 (*MAT1-1*) was applied to all plots, including control plots not inoculated with biocontrol strains. Sclerotia were collected from infected corn ears at harvest and ninety single-ascospore isolates were obtained from ascocarps originating from plots treated with AF36 and NRRL 21882. In addition, eighty *A. flavus* isolates were collected from soil one month after planting (before biocontrol application) and one and two years after biocontrol application. PCR revealed grouping of isolates into three distinct mating-type classes: *MAT1-1, MAT1-2* and *MAT1-1/MAT1-2*. A significant proportion (54%) of isolates sampled prior to biocontrol treatments and 39% of isolates obtained from ascospores were heterokaryotic for mating type (*MAT1-1/MAT1-2*). The vertical transmission of *MAT1-1/MAT1-2* to progeny ascospore isolates suggests that heterokaryosis can be maintained in subsequent generations. The population genetic structure before and after the application of biocontrol treatments will be discussed. The potential for the biocontrol strain to undergo sexual reproduction and the degree of relatedness of the biocontrol strain to the predominant indigenous lineage may influence the long-term success of a biocontrol strain. These findings will be instrumental in the selection of strains for use in next-generation biocontrol strategies.

### O35.014 Displacement of the US-1 clonal lineage of *Phytophthora infestans* from potato in Kenya and Uganda

*A. Njoroge, G. Tusiime, G. Forbes and J. Yuen*
*Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, SE 750 07 Uppsala, Sweden*
*Email: Jonathan.Yuen@mykopat.slu.se*

*Phytophthora infestans*, the causal agent of potato late blight, was formerly present as an old clonal lineage (known as US-1) in much of sub-Saharan Africa. A collection from South Africa, Mozambique, Malawi, Tanzania, Burundi, Rwanda, Uganda, and Kenya, made in 2007 by researchers from South Africa, consisted primarily of the US-1 clonal lineage, although one lineage, designated KE-1, was found in two fields in Kenya. Our analysis of 260 samples from Kenya and 166 samples from Uganda collected in 2011 and 2012, using mtDNA haplotype and microsatellite markers, indicate that the US-1 clonal lineage (identified by the 1b mitochondrial DNA haplotype) cannot be found on potato in Kenya, indicating complete displacement of US-1 by newer genotypes characterized by the la mtDNA haplotype. In Uganda, collections from the eastern part of the country were also of the 1a haplotype, but in western Uganda, most fields sampled had the 1b haplotype (US-1), though the 1a haplotype was found in 8 fields, which is consistent with an on-going displacement process. The US-1 lineage was also found on tomato in Uganda, but there are differences between the potato US-1 and the tomato US-1 lineages, as well as differences between the new and the old lineages. These new clones of *Phytophthora infestans* are more aggressive than the older US-1 clonal lineage, since they have displaced this older population. Simulation of late blight epidemics using the model LB2004 indicated that these new lineages will cause increased difficulties in controlling potato late blight in this region.

**O35.015 High genetic variability within populations of the wheat pathogen *Mycosphaerella graminicola* in the Russian Federation**

S.B. Goodwin, M. Mustafina, M. Kabdulova and S.S. Sanin
*USDA-ARS, Department of Botany and Plant Pathology, 915 West State Street, Purdue University, West Lafayette, Indiana 47907-2054, USA*
*Email: Steve.Goodwin@ARS.USDA.gov*

*Mycosphaerella graminicola* (aka *Zymoseptoria tritici*) is the cause of septoria tritici blotch, one of the most important diseases of wheat in Russia and many other parts of the world. This pathogen typically has high genetic variability within populations in most wheat-growing regions worldwide, but little is known about those in the Russian Federation. To test the hypothesis that populations in different regions of the Russian Federation also contain high levels of genetic variability, 251 isolates collected from four regions were analyzed for mating type and for genetic variability at seven microsatellite or simple-sequence repeat (SSR) loci. Both mating types were found at relatively even frequencies in all regions and most local populations sampled. The SSR loci had from 2 to 7 alleles each, and gene diversities were very high, ranging from 0.38 to 0.47 depending on the region. AMOVA revealed that most (89%) of the genetic variability occurred within populations, with only 11% among them. The lowest genetic distance occurred between the Southern and Central regions while the greatest was between isolates from the Northwestern and Central populations. The high genetic diversity within all populations sampled and even frequency of both mating types indicates that sexual reproduction of this pathogen most likely occurs frequently and contributes to the high genotypic diversity seen in all populations sampled from the Russian Federation. These results support previous observations of the sexual stage on infected leaves from the same regions.

**O35.016 Point mutation of H249Y in SDHB gene conferring resistance to thifluzamide in *Rhizoctonia solani***

W.J. Mu, X.X. Ni, B.T. Li, D. Lin, C. Chen, S.L. Zhang, and X.L. Liu
*Department of Plant Pathology, College of Agriculture and Biotechnology, China Agricultural University, Beijing 100193, P. R. China*
*Email: muwenjun@126.com*

Rice sheath blight causing by *Rhizoctonia solani* is an important disease in China, which causes losses of yield and quality in rice worldwide. Thifluzamide developed by Dow Agroscience, is an effective novel succinate dehydrogenase inhibitor (SDHI) fungicide to control rice sheath blight. The resistant basis of thifluzamide in *Rhizoctonia solani* is unknown. The aim of this study was to explore the resistance molecular basis of *Rhizoctonia solani* to thifluzamide. Resistant mutants were obtained in field and in laboratory using UV-induction and thifluzamide-adaption. Subunits of succinate dehydrogenase including *sdh*A, *sdh*B, *sdh*C and *sdh*D genes were cloned in 13 resistant mutants and 4 sensitive isolates. Amino acid (AA) sequence of *sdh*A, *sdh*C and *sdh*D were identical in all resistant mutants and sensitive isolates. One AA substitute from histidine to tyrosine was found in all resistant mutants at position 249 in *sdh*B, compared to parent isolates. According to the genotype and susceptibility, the resistant mutants were subdivided into two types. Type I mutants were heterozygote with two alleles of H249 and Y249 in *sdh*B gene, which $EC_{50}$ values were 20 to 120 folds as their parent isolates. Type II mutants were homozygote with one allele of Y249 in *sdh*B gene, which $EC_{50}$ values were greater than 200 times. Based on this result, the rapid methods AS-PCR and CAPS were established to detect the mutants in the field.

**P35.001 Genetic diversity in *Groundnut bud necrosis virus* isolates infecting legumes in India**

M. Akram
*Division of Crop Protection, Indian Institute of Pulses Research, Kalyanpur, Kanpur 208024, India*
*Email: akram23859@gmail.com*

Surveys were conducted in five states of the country during 2009-2012 to know the status of *Groundnut bud necrosis virus* (GBNV) and to decipher the genetic diversity in its population associated with legume crops. The GBNV infection was confirmed in seven (mungbean, urdbean, cowpea, French bean, dolichos, soybean and pea) out of ten leguminous crops studied. GBNV incidence was in the range of 0-90% in different crops. In general, cowpea appeared to be more sensitive to GBNV as compared to mungbean and urdbean. GBNV infection in pea and in wild Vigna (*V. umbellata*, *V. hainiana*, *V. glabrescens*, *V. radiata* var. *sublobata*, *V. mungo* var. *mungo* and *V. radiata* var. *radiata*) was confirmed for the first time. Sequence analysis of *NP* gene of 22 GBNV isolates revealed 0-4% diversity in both nucleotide and amino acids levels, whereas diversity in *NSm* gene of 18 isolates was 0-8% at nucleotide level and 0-3% at amino acids levels. *NP* gene is more conserved than the *NSm* gene in all the GBNV isolates. Clustering of GBNV isolates in phylograms constructed based on *NSm* and *NP* genes sequences does not appear to be based on their place of origin and host plant species. This indicates that the genetic diversity exists in the GBNV populations, but could not be differentiated based on geographical location and host plant species. Biological properties of the isolates in sub-clusters need to be investigated in detail and compared to settle unequivocally the possible existence of variants or strain.

**P35.002 Geographic variation in *Leptosphaeria maculans* (phoma stem canker of oilseed rape) populations in the UK**

*Y.J. Huang, G.K. Mitrousia and B.D.L Fitt*
*School of Life and Medical Sciences, University of Hertfordshire, Hatfield, Herts, AL10 9AB, UK*
*Email: y.huang8@herts.ac.uk*

Major resistance (*R*) gene-mediated resistance against *Leptosphaeria maculans* is associated with a gene-for-gene interaction in which the product of a pathogen effector (*Avr*) gene is recognised by the product of a host *R* gene so that the pathogen is unable to infect the host. Therefore *R* gene-mediated resistance to *L. maculans* is effective only if the corresponding avirulent allele is predominant in the *L. maculans* population. To use *R* genes effectively, there is a need to monitor *Avr* alleles in *L. maculans* populations. *L. maculans* populations were sampled from ten sites in England by single pycnidial isolation from phoma leaf spots on leaves of cultivar Drakkar (no *R* gene). To determine the *Avr* alleles in each isolate, a set of differential cultivars/lines with known *R* genes was used. Eighty-nine isolates have been characterized by cotyledon inoculation. Results show that there were no differences between sites in proportions of avirulent alleles of *AvrLm7* and *AvrLm6* or virulent alleles of *AvrLm2*, *AvrLm3* and *AvrLm9*. The populations of *L. maculans* were 100% avirulent at *AvrLm6* and *AvrLm7* loci at all sites. This indicates that the corresponding resistance genes *Rlm6* and *Rlm7* are effective. By contrast, the populations of *L. maculans* were 100% virulent at *AvrLm2*, *AvrLm3* and *AvrLm9* loci. There were differences between sites in frequencies of avirulent alleles of *AvrLm1*, *AvrLm4* and *AvrLm5*. The frequency of *AvrLm1* varied from 0 to 45%, *AvrLm4* varied from 20 to 90% between the ten sites. Frequencies of *AvrLm5* were 100% in all except for two sites.

**P35.003 *Neonectria ditissima*, development of perithecia in culture**

*R.W.A. Scheper and E. Pauli*
*The New Zealand Institute for Plant & Food Research Ltd, Private Bag 1401, Havelock North, Hastings 4157, New Zealand*
*Email: reiny.scheper@plantandfood.co.nz*

Sexual reproduction of *Neonectria ditissima* (formerly *N. galligena*), the causal agent of European canker of apple, was investigated on three different culture media. To determine whether New Zealand isolates of *N. ditissima* were homothallic or heterothallic, ten single-ascospore isolates were chosen based on different morphological characteristics such as mycelium growth rate, colour, texture and sectoring frequency. The isolates were obtained from cankers on apple wood with mature perithecia that were collected in five different regions of the North Island and the South Island of New Zealand, between 2006 and 2010. Two randomly chosen isolates were crossed with all other isolates, and all isolates and an 11$^{th}$ isolate were crossed with themselves. The first mature perithecia with asci and ascospores were observed after 4½ weeks on malt extract agar with autoclaved apple wood pieces in the centre, under continuous near UV light at 18 ℃. Mature perithecia were also formed on water agar with autoclaved apple leaf pieces in the centre, under continuous fluorescent light at 22 ℃, after 13 weeks. No perithecia were observed after 5 months on glucose yeast agar under continuous fluorescent lighting at 20 ℃. Seven of the ten isolates produced fertile perithecia with viable ascospores when mated with a different isolate. Fertile perithecia were not produced in any of the 11 self crosses. The seven isolates that produced perithecia segregated into two arbitrarily designated mating types, with three isolates of one mating type and four of the other mating type.

**P35.004 Agressiviness of *Corynespora cassiicola* isolates from soybean in Brazil**

*A. Teramoto[1], C.C.P. Braz [1], R.A. Aguiar[1], R.M. Soares[2], M.C. Meyer[2] and M.G. Cunha[1]*
*[1]Phytosanitary Section, School of Agronomy, Federal University of Goiás, P. O. Box 131, 74001-970, Goiânia, Goiás State, Brazil; [2]Embrapa Soybean, Santo Antônio de Goiás, GO*
*Email:adritera@terra.com.br*

*Corynespora cassiicola* is the causal agent of target spot in soybean and frequently occurs in soybean crops in Brazilian Mid-Western regions, causing reduction of grain production. The objective of this study was to test the aggressiveness of sixty preserved isolates of *C. cassiicola* in two soybean cultivars M-SOY 7908 and BMX Potência RR, at greenhouse conditions. These isolates were preserved by Castellani method during one year. The experimental design was a factorial scheme with two cultivars versus sixty isolates of *C. cassiicola* with three replications. A four-liter pot with ten plants was considered one replication. The inoculation was done on thirty-day-old plants at V4 stage with a calibrated suspension in $10^4$ conidium/mL until the point of flowing. After that, plants were put on a humid chamber for 48 hours. The evaluation was done thirty days after inoculation, using a diagrammatic scale specific for target spot. Plants of cultivar M-SOY 7908 were at R4 stage and BMX Potência RR at R5.1 stage. Disease severity varied from 2% to 50% and cultivar BMX Potência RR presented the highest severity levels.

**P35.005 Genetic variation and global population dynamics of the *Leptosphaeria maculans* plant pathogen**

*S.H. Liban and W.G. Dilantha Fernando*
*Department of Plant Science, University of Manitoba,*

*66 Dafoe Road, Winnipeg, Manitoba, R3T 2N2, Canada*
*Email: s.h.liban@gmail.com*

The fungal pathogen *Leptosphaeria maculans* is the causal agent of phoma stem canker (aka blackleg) and is the most economically important disease affecting oilseed rape and Canola (*Brassica napus* L.). The fungus was first identified in the early 1900's on cabbage in Wisconsin, USA and following the breeding of oilseed rape as a major crop for human consumption and bio-fuel production, it has since spread to major canola growing regions through natural dispersal and trade. It is now found in most countries where Brassica spp. are cultivated and in the last four decades *L. maculans* has caused significant yield losses in Canada, Europe, and Australia. Moreover there are indications that *L. maculans* is an expanding species displacing the less aggressive *Leptosphaeria biglobosa* which has historically colonized oilseed rape crops. The risk of spread to non host countries (i.e. China) and growing yield losses due to rapid evolution and loss of avirulence genes has led to the examination of the genetic variation between and within different geographic regions. A set of microsatellites spread among the 76 sequenced supercontigs in the fungal genome were used to screen isolates collected from 7 different countries with emphasis on the Canadian population. A phylogenetic tree was generated to assess the evolutionary relationship between isolates. The pathogen population showed significant variation both within countries (i.e. Western and Central Canada) and between countries. Additionally the level of genetic variation shifted between countries and was greatly influenced by both *Brassica* spp. cultivation history and climate.

**P35.006 Non-synonymous SNP variety of the trehalase gene in Asian population of rice sheath blight pathogen**

*M. Arakawa, T. Ohmae, M. Shimaoka, K. Inagaki, P.C. Ceresini and B.A. McDonald*
*Faculty of Agriculture, Meijo University, Shiogamaguchi 1-501, Tempaku, Nagoya 468-8502, Japan*
*Email: aramasa@meijo-u.ac.jp*

The fungicide validamycin is widely applied to control rice sheath blight disease in Asian paddy fields. The trehalase of the causal pathogen of *Rhizoctonia solani* AG-1 IA, is the target enzyme for the validamycin as an antagonistic to the substrate trehalose. Low sensitive isolates to validamycin were recently reported in China and India. In this study, variations of the sensitivity to validamycin and DNA sequences of trehalase gene were investigated using 25 isolates of *R. solani* AG-1 IA in Japan, China, Korea, Taiwan and India. Eight isolates were regarded as a low sensitive that had shown less than 50 % inhibition with the 1.0 ppm treatment. PCR primers for the full-length DNA of trehalase gene in *R. solani* AG-1 IA, were designed by genomic databases of basidiamycota species and by conduct of the inverse PCR. The sequence of RT-PCR product indicated that the trehalase gene of *R. solani* AG-1 IA was encoded in 3,045 b of genomic DNA, 2,325 b of mRNA, 11 introns and the enzyme was putatively constructed with 774 amino acids. Twenty-four isolates showed heterozygotic genotypes with 92 sites of single nucleotide polymorphisms. Five SNPs lead non-synonymous changes compared with a consensus sequence of amino acids. Six isolates showed 1 or 2 non-synonymous SNPs among low sensitive 8 isolates. Furthermore, all 3 isolates with non-synonymous SNP (V/V568V/A) in Chinese population showed very low sensitivity to validamycin treatment. Gene pools relating fungicidal sensitivities seem to differ between geographic populations of *R. solani* AG-1 IA in Asia.

**P35.007 Genetic variability of *Phytophthora* × *serendipita* and pathogenicity on rhododendron**

*V.P. Kris, M. Martine and H. Kurt*
*Plant Sciences Unit, Institute for Agricultural and Fisheries Research (ILVO), Merelbeke, Belgium*
*Email: kris.vanpoucke@ilvo.vlaanderen.be*

We have developed an rDNA ITS real-time PCR method to discriminate between *P.* × *serendipita* hybrids and the parental species *P. cactorum* and *P. hedraiandra*. This technique, which is more sensitive than PCR-RFLP or sequencing analysis to identify hybrids, was applied to 184 isolates from 10 European countries that were previously designated *P. cactorum* or *P. hedraiandra* based on morphological characteristics. The results revealed that 49% of these isolates were hybrids. In most cases the hybrids carried the *cox*I of *P. hedraiandra*, in a few cases, *cox*I of *P. cactorum* was present, suggesting different hybridization events. At least four groups of hybrid isolates could be distinguished based on different *P. cactorum*/*P. hedraiandra* ITS copy number ratios. AFLP was used to study the genetic variability between isolates of *P. cactorum*, *P. hedraiandra* and representatives of the four hybrid groups. Most of the fragments were shared with one or both of the parental species. Some fragments were unique for *P. cactorum*, *P. hedraiandra* or the hybrids. The presence of differential fragments between certain hybrid isolates also suggests distinct hybridization events. Pathogenicity of *P. cactorum*, *P. hedraiandra* and their hybrids was tested using *Rhododendron* leaf inoculation assays. Some of the hybrids were more pathogenic than either of the parental species. These data indicate that *Phytophthora* × *serendipita* hybrids are more common than previously recognized and that several hybridization events between these species may have taken place. The higher pathogenicity may explain the recent increase in prevalence of *P.* × *serendipita* on *Rhododendron*.

**P35.008 Novel *Fusarium* species associated with diseased *Pinus* species in Colombia**

*D.A. Herron, M.J. Wingfield, S. Marincowitz, M. Kvas, B.D. Wingfield, C.A. Rodas and E.T. Steenkamp*
*Departments of Microbiology and Plant Pathology and Genetics, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa*
*Email: emma.steenkamp@fabi.up.ac.za*

*Fusarium* is a well-known genus that includes many species important, not only, in agriculture and medicine, but in forestry as well. A notable forestry example is the pitch canker fungus *Fusarium circinatum*, which causes severe damage to various *Pinus* species, worldwide. In this study, we explored the diversity of *Fusarium* species associated with diseased *Pinus patula, P. tecunumanii, P. kesiya* and *P. maximinoii* in Colombian plantations and nurseries. We specifically targeted plants displaying symptoms associated with *F. circinatum* infection; including stem cankers and branch die-back of plantation trees, and root and collar rot of seedlings. Fifty seven isolates, collected from the diseased tissue, were characterized using the DNA sequences for the genes encoding translation elongation factor 1-α and β-tubulin. Based on the combined data, the isolates were separated into 10 distinct lineages, one of which represents *F. circinatum*. For five of these lineages, we have also provided descriptions based on their morphology and DNA-based characters. These new species of *Fusarium* have tentatively been assigned the names *Fusarium fracticaudum prov.nom., F. parvisorum prov. nom., F. marasasianum prov. nom., F. pinemorale prov. nom* and *F. sororula prov. nom*. Pathogenicity tests on *P. patula* using representatives for each of these species showed that *F. parvisorum prov. nom* and *F. pinemorale prov. nom.* are pathogens with levels of pathogenicity similar to *F. circinatum*. These species thus pose a significant risk to forestry in Colombia and to other parts of the world.

**P35.009 Molecular basis of mating type switching in *Ceratocystis fimbriata***

*P.M. Wilken[1], E.T. Steenkamp[2], M.J. Wingfield[1], Z.W. de Beer[2] and B.D. Wingfield[1]*
*[1]Department of Genetics, [2]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria*
*Email: Brenda.Wingfield@fabi.up.ac.za*

Isolates of *Ceratocystis fimbriata* sensu stricto and its close relatives are typified by uni-directional mating type switching where strains switch from a self fertile to a self sterile condition. A fragment of the mating specific gene, *MAT1-2-1* is known to be deleted in self sterile isolates. The size of the deletion and whether other genes are involved has however, not been determined. By sequencing the genome of *Ceratocystis fimbriata*, we were able to consider this long standing and intriguing question. The aim of this study was to determine the extent of the *MAT1-2-1* gene deletion and whether other genes in the MAT locus are affected in the process. Interrogating the full genome sequence, we determined that self fertile strains of *C. fimbriata* have both the *MAT1-2-1* and the *MAT1-1* genes. The self-sterile isolates however, were missing the entire *MAT1-2-1* gene and one copy of a 230 base pair perfect repeat, which was found to flank this gene in self fertile isolates. The loss of fertility can be explained by the loss of the entire *MAT1-2-1* gene. The presence of an exact repeat on either side of this gene suggests that some form of recombination event is involved in the deletion process. The results of this study enable us to now consider the molecular mechanism involved in this unique switching event.

**P35.010 Characterization of dsRNA elements in *Rhizoctonia solani* AG1-IA isolates that causes rice sheath blight disease in Taiwan**

*T.C. Fang, S.J. Yang, S.H. Chen, Y.J. Lin, F.C. Hsiao , L.J. Jheng and L.C. Chen*
*Department of Plant Pathology, National Chung Hsing University, 250 Kuokuang Road, Taichung, Taiwan, 40227, China*
*Email: lcchen@dragon.nchu.edu.tw*

Rice sheath blight disease was caused by the pathogen of *Rhizoctonia solani* AG1-IA. According to previous studies, dsRNA mycovirus were found to be frequently associated with fungi. Some fragments of dsRNAs in *R. solani* diminished virulence, some enhanced virulence and others had no effect. The sizes of ten dsRNAs were found to be 1.5, 1.6, 1.7, 1.8, 2.0, 2.5, 3.0, 9.0, 10.0 and 14.0 kb. The 2.0 kb dsRNA were in fifteen isolates of *R. solani* AG-1 IA collected from Taiwan, but there was little similarity among dsRNA fragments from these isolates. The results of anastomosis test in *Rs1*7 containing 14.0 kb dsRNA and *Rs1*M-3 containing 2.0 kb dsRNA showed that the 2.0 kb dsRNA in *Rs1*M-3 could be transmitted through hyphal fusion. Although the amount of pigment and the number of sclerotia produced by six isolates containing the 2.0 kb dsRNA were less than the other two isolates without the 2.0 kb dsRNA, there were no difference at growth rate among those isolates. The molecular mass of the virus-like particle was approximately 60 to 70 kDa as resolved by SDS-PAGE. And the existing of dsRNA seemed to have nothing positively related with the disease severity of inoculation of test isolates and the phenomenon with the culture of test isolates. This study found the dsRNA are commonly associated with *R. solani* AG-1 IA, and it shown that the dsRNA elements could be transmitted and virus-like particles were presented in *R. solani* AG-1 IA.

**P35.011 Mitochondrial recombination suggests hybrid speciation within the *Gibberella fujikuroi* species complex**

*G. Fourie[1], N.A. van der Merwe[2], B.D. Wingfield[2], M.J. Wingfield[1] and E.T. Steenkamp[1]*
*Departments of Microbiology and Plant Pathology[1] and Genetics[2], Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa*
*Email: albe.vdmerwe@fabi.up.ac.za*

The *Gibberella fujikuroi* species complex (GFC) is a monophyletic assemblage that consists of many economically important pathogens. The complex is divided into three large clades, but the exact relationships among them remain unknown. Previous studies indicated that multi-gene phylogenies based on nuclear gene regions, as well as phylogenies from individual mitochondrion-encoded genes are incongruent. Thus, the aim of this study was to determine whether recombination could have contributed to the phylogenetic incongruence of mitochondrial genes. Nucleotide sequences of five mitochondrion-encoded genes from the well characterized biological species of the GFC were used to construct minimal ancestral recombination graphs (ARGs) in order to determine the likely number of historical recombination events. ARGs revealed extensive recombination in the sequences of some of the mitochondrial genes, while no recombination was detected for others. Mitochondrial recombination was also detected both within and between the clades of the GFC. The evidence for recombination between the mitochondrial genomes of these species could be linked to the life history of this species complex. Historical recombination between the clades further supports the hypothesis that the origins of the GFC could be associated with an ancient hybridization event, since recombination among mitochondrial genomes is common when parental leakage occurs during interspecific hybridization.

**P35.012 Cross infection of *Gibberella moniliformis* between sugarcane and rice reveals the host-pathogen horizontal transfer of pathogenic gene**

*Z. Lin[1,2], Y. Que[2], C.A. Powell[3] and M. Zhang[1,2]*
*[1]Guangxi University, Nanning, Guangxi, 530004, P. R. China; [2]Fujian Agri. and Forestry Univ., Fuzhou, Fujian 350002, P. R. China; [3]University of Florida, Fort Pierce, FL 34945, USA*
*Email: mqzhang@ufl.edu*

Sugarcane Pokkah Boeng disease and rice Bakanae disease, both of which caused by phytopathogenic *Gibberella moniliformis*, result in enormous losses of energy and food crop production, respectively. Morphological observation and rDNA-ITS sequences analysis revealed that *G. moniliformis* species were different in morphology, while their rDNA ITS sequences showed 96% identity. It should be stressed that, though they could cross infection both sugarcane and rice and thus lead to leaf rots or wilts respectively, there were significantly diverse symptoms in different hosts. From above, we hypothesis that the plant disease symptom is manifested by host-pathogen interaction during which host response to pathogen's aggression play a key role. It can be deduced that these two *G. moniliformis* species had a close relative relationship or even evolved from a common ancestor *G. moniliformis*. During the process of the strains infection to different hosts, they converted into a various pathogens and the pathogenic gene related host-pathogen interaction was evolved with distinct hosts to manifest host-specific adaptation and pathogenic variation. The possible explanation for pathogenic diversity is that the origin of pathogenicity gene in the *Fusarium* genome was acquired by horizontal transfer from plant host, which was different from house-keeping genome passed on by vertical transmission and changed across temporal and spatial variation. Further work should aim to sequence and analysis the whole-genome of *G. moniliformis* strain, ascertain and annotate the chromosome(s) and genes involved with pathogenicity, thereby put forward a more accurate evolution route of pathogenic gene.

**P35.013 An R package for genetic analysis of populations with mixed (clonal/sexual) reproduction**

*Z.N. Kamvar, J.F. Tabima and N.J. Grünwald*
*Horticultural Crops Research Laboratory, USDA Agricultural Research Service, Corvallis, OR 97330, USA*
*Email: grunwaln@science.oregonstate.edu*

We developed an R package for population genetic analysis. R is an open-source statistical programming language that has a large, well maintained repository of statistical packages. Poppr provides open-source, cross-platform tools for quick analysis of population genetic data enabling focus on data analysis and interpretation. While there are a plethora of packages for population genetic analysis, few are able to offer quick and easy analysis of populations with mixed reproductive modes. Poppr's main advantage is the ease of use and integration with other packages such as adegenet and vegan, including support for novel methods such as clone correction, multilocus genotype analysis, calculation of Bruvo's distance and the index of association.

**P35.014 Clonal structure of *Raffaelea quercivora* in galleries and mycangia on the *Platypus quercivorus***

*Y. Takahashi, N. Matsushita and T. Hogetsu*
*The University of Tokyo, 5-1-5, Kashiwanoha, Kashiwa, Chiba 277-8563, Japan*
*Email: ytakah@gmail.com*

*Raffaelea quercivora* is the pathogenic fungus causing

Japanese oak wilt. A female of a monogynous ambrosia beetle *Platypus quercivorus*, which loads the fungus in their mycangia on the pronotum, bores a gallery in an oak tree with her partner, reproduces offspring, and unloads the fungus onto the gallery wall from the mycangia. The offspring mature in the gallery, load the fungal pathogen and fly from the gallery to other healthy trees. To investigate unloading and loading modes of the fungus within the gallery, we developed polymorphic microsatellite markers of *R. quercivora*, and identified fungal genotypes in the gallery and mycangia of the beetle. From wall of five galleries in a dead *Quercus serrata* tree, small wood chips were sampled at 5 to 10 mm-intervals. Pronota were also sampled from five female adult beetles. Genotypes of *R. quercivora* isolates from the wood chips and pronota were identified using the microsatellite makers. Genotypic analysis showed that each gallery was patchily inhabited by five to 10 genotypes of *R. quercivora*, and mycangia of each beetle contained at least three genotypes. These results indicate that diverse *R. quercivora* genotypes are repeatedly unloaded from mycangia of a beetle female to the gallery wall, resulting in their patchy distribution in the wall, and that when her offspring leave the host tree, fungal clones proliferating in the wall are repeatedly loaded into mycangia of the grown-up beetles.

**P35.015 Species and trichothecene genotypes of the *Fusarium graminearum* complex causing head blight of wheat and ear/stalk rot of maize in Brazil**

*E.M. Del Ponte[1], T.J. Ward[2], D.J. Tessmann[3], P.R. Kuhnem[1], C.N. Silva[3], L.B. Gomes[1], P. Spolti[1] and C.P. Nicoli[1]*
*[1]UFRGS, Av. Bento Gonçalves, 7712, 91540-000, Porto Alegre, RS Brazil; [2]USDA-ARS 1815 N. University St., 61604, Peoria, IL, USA; [3]UEM, Av. Colombo 5790, 87020-900, Maringá, PR, Brazil*
*Email: emerson.delponte@ufrgs.br*

Species and trichothecene genotypes of the *F. graminearum* species complex in Brazilian wheat and maize were determined based on the analysis of a large number (n=1038) of isolates obtained between 2009 and 2011. The three populations studied were obtained from: 1) diseased heads from >150 wheat fields (n= 663); 2) maize kernels from fields at both central and southern regions (n=104); and 3) maize stubble showing perithecia obtained from 20 cultivated and non-cultivated fields (n=271). Multilocus genotyping was used to determine species identity and trichothecene genotypes for most isolates. For those from maize kernels, partial gene sequences of *TEF*, *tri3* and *tri12* were used. For the wheat population, it was found that *F. graminearum* with a 15-ADON genotype was dominant (83%), followed by *F. meridionale* with a NIV genotype (12.8%), *F. cortaderiae* with mostly NIV and a few 3-acetyl deoxynivalenol (3-ADON) (2.6%), *F. austroamericanum* with mostly 3-ADON and a few NIV (1.2%) and *F. asiaticum* with the NIV genotype (0.4%). Frequency of *F. meridionale* in wheat increased for lower latitudes. For the maize kernel population, *F. meridionale* was dominant (72%), followed by *F. graminearum* with the 15-ADON genotype (14.5%) and *F. cortaderiae* with the 3-ADON and NIV genotypes (13.5%). For the maize stubble population, *F. meridionale* was also dominant (50%), followed by *F. graminearum* with the 15-ADON genotype (30%) and *F. cortaderiae* with the NIV and 3-ADON genotypes (20%). For both maize populations, higher species diversity was found at higher latitude and elevation.

**P35.016 The potential of temperature gradient plates to measure temperature response in fungal pathogens**

*F. Newbery, M.W. Shaw, B.D.L. Fitt and P. Gladders*
*School of Agriculture, Policy and Development, University of Reading, Whiteknights, Reading, RG6 6AR, UK*
*Email: f.newbery@pgr.reading.ac.uk*

Temperature response curves have been studied for a wide variety of processes in an extensive number of organisms. The temperature response of growth in fungi, however, has rarely been studied across a wide range of temperatures. Temperature gradient plates, more commonly used to assess seed germination in angiosperms, are ideally suited to investigate fungal growth across a wide variety of temperatures. Results allow accurate estimations of $T_{min}$ and $T_{max}$, the minimum and maximum temperatures that support growth, and of $T_{opt}$, the temperature that supports the greatest growth rate. The generation of temperature response curves for a representative sample of individuals within a population also facilitates comparisons between individuals, as well as quantification of variation and heritability in the population's temperature response. With food security an important issue on the world agenda, prediction of the effects of fungal pathogens on our crops will play a vital role in plans for the future. Information from temperature response curves will be invaluable in the estimation of parameters in models that predict changes in the effects of fungal pathogens in response to climate change.

**P35.017 Characterization of *Rigidoporus* species from Rubber tree (*Hevea brasiliensis*) plantations in Nigeria**

*A.O. Oghenekaro, O. Miettinen, V. I. Omorusi, G. Evueh, M.A. Farid and F.O. Asiegbu*
*Department of Forest Sciences, University of Helsinki, P.O Box 27, 00014, Helsinki, Finland*
*Email: abbot.oghenekaro@helsinki.fi*

*Hevea brasiliensis* is basically valued for its natural rubber (latex); useful for the manufacture of a variety of industrial products. *Rigidoporus lignosus* (syn. *Rigi-*

*doporus microporus*) is the most destructive root pathogen of rubber trees in tropical and sub-tropical regions. It produces rhizormorphs which grow several meters in the soil to aid infection of the tree roots. This study was conducted using three gene regions; ITS, LSU and *tef*1 sequences and morphology to; characterize *R. lignosus* isolates from Nigeria and compare isolates from diverse geographic regions to get an insight on its phylogeny and phylogeography. Morphology of the Nigerian isolates was different from that of the South American isolates. Three clades; Africa, Asia and South America/Central America were formed in the phylogeny. Results of nLSU supported that of ITS, while the *tef*1 gene was able to show intra-specific variation within the clades. Isolates of *R. lignosus* from rubber plantations in Nigeria are genetically closely related. There is practically no geographic trend in the distribution of *Rigidoporus* species within West and Central Africa. The African and Asia isolates were more closely related than those from South and Central America. The *Rigidoporus* species in West and Central Africa could be a single species different from that of Asia and South/Central America. Intensive sampling from different countries could reveal if there are distinct species involved in the white rot disease of Rubber trees. This is the first recorded study of *R. lignosus* isolates from Nigeria based on molecular phylogeny.

**P35.018 Evolutionary history reveals two phylogenetically distinct species within *Puccinia graminis***

*A. Berlin, K. Dalman and J. Yuen*
*Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, S-750 07 Uppsala, Sweden*
*Email: anna.berlin@slu.se*

The cereal pathogen *Puccinia graminis* is considered to have high genetic diversity within the species. We hereby hypothesize that the different *formae speciales* of *P. graminis* have co-evolved with their various grass hosts. Elongation factor 1 alpha (EF-1α) and beta-tubulin (BT) genes, the internal transcribed spacer (ITS) region and mitochondrial cytochrome oxidase subunit I (COI) were sequenced from DNA isolated from *P. graminis* collected from cereal and wild grasses to infer the phylogeny of the fungus. The related species *Puccinia coronata*, collected from both oats and wild grasses, were used as a reference throughout the analysis. Coalescence analysis showed that the time to the most recent common ancestor (TMRCA) for *P. graminis* and *P. coronata* were in all models further away in time than TMRCA for the two *formae speciales*. Within the species *P. graminis*, two main clades were formed; one including samples collected from *Avena sativa*, *Avena fatua*, *Phleum pratense*, and the other including samples collected from *Triticum aestivum*, *Triticum monoccocum*, *Secale cereal* and *Hordeum vulgare*, suggesting that *P. graminis* is to be divided into two different taxa. However, samples collected from the weed host *Elytrigia repens*, did not show any clear pattern, the samples equally grouped with either of the two groups. The phylogeny of *P. graminis* was thus congruent with its respective grass hosts, which confirm a co-evolution with the host.

**P35.019 Genetic structure of five populations of the rice sheath blight pathogen *Rhizoctonia solani* AG-1 IA from provinces of Zhejiang, Anhui and Hubei**

*L. Wang[1], L.M. Liu[1], W.W. Huang [1,2], Z.L. Fan [1,2] and S.W. Huang [1,2]*
*[1]China National Rice Research Institute, Hangzhou 310006, P. R. China; [2]Agricultural College, Guangxi University, Nanning 530003, P. R. China*
*Email: hsw666@sohu.com*

Rice sheath blight (RSB), caused by *Rhizoctonia solani* Kühn, is one of the most destructive diseases worldwide. It can reduce rice yield by 10–30% generally, and up to 50% under highly favorable conditions. In recent years, the widespread cultivation of high-yielding cultivars with semi-dwarf and multi-tillers, and widely used farming measures of high crop densities and high rate of nitrogen fertilizer, aggravated the incidence and spread of sheath blight in China. To reveal the genetic background of the pathogen, ITS-5.8S rDNA sequencing approach was used to analysis seventy five isolates from the infected samples collected from Fu Yang (FY) in Zhejiang Province, Ji Xi (JX) and Chao Hu (CH) in Anhui Province, as well as Jing Zhou (JZ) and Xiao Gan (XG) in Hubei Province. The dominant pathogen was *R. solani* anastomosis group AG-1 IA. Twenty nine haplotypes were identified based on nucleotide variation. A high genetic diversity was observed among populations. *Fst* and *Nm* among populations indicated that the gene flow were frequent among populations. AMOVA revealed that most of genetic variation occurred within populations. No significant correlation was found between genetic distance and geographic distance. A "star like" pattern of interrelationship of haplotypes from *R. solani* AG-1 IA was found by network analysis. Neutral test for genetic structure showed that the populations were in Hardy-Weinberg equilibrium suggesting a high natural selection existed in the populations. Pathogen with a mixed reproductive mode would accelerate its evolution, more new genotypes could be generated by recombination and kept through asexual reproduction, which would cause high risk of fungicides resistance.

**P35.020 Population structure of *Phytophthora infestans* on potato from Heilongjiang province**

*M. Guo, S. Yang, F.X. Min, Y.F. Gao, F.F. Xu, X.D. Wang and D.Q. Lv*
*Virus-Free Seedling Research Institute, Heilongjiang*

*Academy Agricultural Sciences, No.368 Xue Fu Road, Harbin 150086, P. R. China*
*Email: guo_plum@yahoo.com*

A total of 155 isolates of *Phytophthora infestans* collected in 2010 from Heilongjiang Province were analyzed by their phenotype. Of these, 125 isolates (80.6%) were resistant to metalaxyl. All isolates were of A1 mating type. There were 24 physiological races identified among 95 isolates. Results showed the dominant races could be ranked as 1.3.4.7.8.10.11, with the frequencies of 29.5%. The virulence genes R3, R4 and R7 were most common, each with a frequency of 100%, followed by R1, R8, R10 and R11 with frequencies of 94.74%, 67.37%, 72.63% and 77.89%, respectively. R9 had the lowest frequency of 3%. According to SSR analysis by using two loci, Pi4B and Pi4G, total 3 different SSR genotypes (E-01, F-01, and F-06) were detected among 98 isolates, and E-01 was firstly reported in Heilongjiang. Statistic analysis of allele genes generated by two loci (Pi4B and Pi4G) indicated that diversity produced by the locus Pi4B were higher than that by Pi4G. And the SSR data showed that the genotypic ratio of SSR genotype F-01 was the predominant in Heilongjiang, of which accounted for 86.73%, and much higher than 12.25% and 1.02% of the genotypic ratio of SSR genotype E-01 and F-06, respectively. Genotype of the mitochondrial DNA (mtDNA) revealed that all isolates were determined as two haplotypes (Ia and IIa). Of which, the mtDNA haplotype IIa, accounting for 89.8%, was the major pattern in Heilongjiang province, and no Ib and IIb can be found in Heilongjiang.

### P35.021 Genotypic analysis of *Phytophthora infestans* on potato in some areas of China and Belgium

*S. Yang[1], M. Guo[1], J.L. Rolot[2], F.X. Min[1], V. Cesar[2], Y.F. Gao[1], F.F. Xu[1] and D.Q. Lv[1]*
*[1]Virus-Free Seedling Research Institute, Heilongjiang Academy Agricultural Sciences, No. 368 Xue Fu Road, Harbin 150086, P. R. China; [2]Walloon Agriculture Research Center, Rue du Serpont, 100B-6800 Libramont, Kingdom of Belgium*
*Email: guo_plum@yahoo.com*

A total of 117 isolates of *Phytophthora infestans* collected from five provinces which belong to the main potato production areas in China and 8 isolates from Belgium (provided by the Walloon Agricultural Research Center) were tested. The genetic structure of *P. infestans* isolates was analyzed based on microsatellite (SSR) and mitochondrial DNA (mtDNA). According to SSR analysis by using two loci, Pi4B and Pi4G, total 8 different SSR genotypes (B-01, B-02, B-03, D-05, E-01, F-01, F-06 and H-05) were detected among 125 isolates. And there are 3 different SSR genotypes (E-01, F-01, and F-06) were detected among 98 isolates from eight regions, and E-01 was firstly detected in Heilongjiang. Statistic analysis of allele genes generated by two loci (Pi4B and Pi4G) indicated that diversity produced by the locus Pi4B were higher than that by Pi4G. And the SSR data showed that the genotypic diversity of *P. infestans* populations from northern China is much lower than which from southern China and Belgium. Compared with Belgium, the geographical distribution shows significantly difference in China. In addition, genotype of the mitochondrial DNA (mtDNA) revealed that all isolates were determined as three haplotypes, Ia, IIa and IIb. There are no Ib haplotype can be detected which represent the old lineage. The genetic evidence shows that the populations of *P. infestans* in China are related to those of other countries occurred after the second worldwide migration of *P. infestans*.

### P35.022 SNP revealed ancestral haplotype and genetic diversity of *Puccinia striiformis* f. sp. *tritici* from Yunnan, China

*M.J. Li[1,2], W.Q. Chen[2], T.G. Liu[2], X.Y. Duan[2], Q.Z. Jia[3], Z. Xu[2], L. Gao[2], B. Liu[2] and T. Xu[2]*
*[1]Institute of Agricultural Environment and Resources, Yunnan Academy of Agricultural Sciences, Longtou Street, Panlong District, Kunming 650205, P. R. China; [2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P. R. China; [3]Institute of Plant Protection, Gansu Academy of Agricultural Sciences, Lanzhou 730070, P. R. China*
*Email: limingju1996@hotmail.com*

The population structure of *Puccinia striiformis* f. sp. *tritici* (*Pst*) by single nucleotides polymorphism (SNP) has not been researched previously, and Yunnan's population genetic structure is known little. After gathering 149 isolates from Yunnan, Guizhou, Sichuan, Shaanxi and Gansu, we analyzed population structure using SNP. DNA was extracted from urediospore, 13 pair of primers for 9 housekeeping genes were designed, and amplicons were examined for polymorphism. Three genes were found to contain 18 SNP loci, seven of which were phylogenetically informative. Twenty-six haplotypes were detected from the three concatenated genes in total. Three haplotypes account for 75%, shared among Yunnan and other provinces. Yunnan had 10 private haplotypes, one of it is ancestral haplotype. Gansu had 5 private haplotypes. Genetic diversity was great in Dehong, Kunming, Qujing, Shaanxi and Gansu populations. Dehong, Qujing and Gansu had larger population mutation rate. Kunming, Shaanxi and Gansu had larger recombination events. The variance was mainly from interior populations, accounted for 79.69%. Yunnan population has great genetic differentiation, coefficient of genetic differentiation Gst=0.12740. *Pst* exists migration among provinces, gene flow intensity Nm=5.03. Total neutrality test indicated that an excess of low frequency

polymorphisms of population and expansion or purifying selection. The results suggest that Yunnan is an origin of *Pst*, Gansu is another orgin of *Pst* in China.

**P35.023 The identification of the rice blast Avr-gene in Liaoning province in 2011**

*S.W. Wang[1], W.J. Zheng[2], J.M. Zhao[2], Y. Wang[1], B.H. Zhao[1] and Z.H. Liu[1]*
*[1]Shenyang Agricultural University, Shenyang, 110161, P. R. China; [2]Liaoning Academy of Agricultural Sciences, Shenyang, 110161, P. R. China*
*Email: zwj27@126.com*

Rice blast is one of the most serious fungus diseases of rice cultivation. *Magnaporthe grisea* has a strong ability of variation and virulent mutating, which always makes the rice varieties lose the resistance in 3-5 years after using in rice cultivation. The identification of *M. grisea* Avr-genes is helpful for us to understand the Avr-genes distribution in the physiological races; furthermore the results of the Avr-genes identification help us choose the rice varieties for rational distribution. The 8 Avr-genes CDS are used for the experiment to make primers, which have been cloned in recent years. The 26 *M. grisea* races of 6 groups are amplified by using PCR with the primers above, which races are prevalent in Liaoning province in 2011. The PCR products are tested by AGE (Agarose gel electrophoresis) and the PCR producting sequence analysis. The results show that the 26 *M. grisea* races don't contain 4 Avr-genes (PWL1, Avr-CO39, Avr-pia and Avr-pii). The other 4 Avr-genes (PWL2, Avr-pik, AvrPiz-t and Avr-pita) appear in *M. grisea* races; however, the results indicate that there are different levels of gene mutation of Avr-gene sequences appearing in different physiological races. Avr-pita genes have high-level genetic differences in *M. grisea*; on the contrary, the AvrPiz-t genes have low-level genetic differences.

**P35.024 rDNA-ITS sequence and SNP analysis of *Pseudoperonospora cubensis* of 12 cities in China**

*D. Miao[1], Z.J. Pu[2], B.Z. Ma[1], Y.J. Zhang[1], Z.W. Qin[1] and X.Y. Zhou[1]*
*[1]College of Agriculture, Northeast Agricultural University, No59 Mucai street, Xiangfang district, Harbin, 150030, P. R. China; [2]Graduate School of Science and Technology, Niigata University, 2-8050, Ikarashi, Niigata 950-2181, Japan*
*Email: yanjuzhang@yahoo.com.cn*

Cucumber downy mildew caused by *Pseudoperonospora cubensis* is one of the most common and destructive disease of cucumber. The oospores of *P. cubensis* were observed in 9 cities in China. To understand the genetic variation of *P. cubensis*, rDNA-ITS sequence and Single Nucleotide Polymorphism (SNP) analysis of 34 strains isolated from12 cities of China was conducted. The results showed basic radical complete sequence length of rDNA-ITS in *P. cubensis* was 802 bp. Besides, 866 SNP sites were observed in the rDNA-ITS sequence of 34 strains. The similarity of the rDNA-ITS sequence among 34 *P. cubensis* strains was from 47.87% to 100%, and average genetic distance was 1.231±0.200. Both the similarity and genetic distance showed a reduce tendency with the increase of geographic distance, implying a correlation between the infection source and geographic areas. Cluster analysis divided all tested strains into 3 groups: RG1, RG2 and RG3. RG1 included 17 strains from Beijing city and Qinghai, Ningxia, Shandong, Shanxi, and Hebei province, respectively; RG2 included 15 strains from Heilongjiang, Jilin and Liaoning province; and RG3 included 2 strains from Hangzhou city. The results indicated that *P. cubensis* differ from geographic area, which indirectly proofed that the primary infection source of cucumber downy mildew in cold area of China emerged locally rather than spread by monsoon.

**P35.025 Investigation and analysis of anthracnose on strawberry in Hubei province**

*Y.C. Han, F.Y. Xiang, X.G. Zeng, P. Zhang and Y.C. Gu*
*Hubei Academy of Agricultural Sciences, Institute of Industrial Crops, Wuhan 430064, P. R. China*
*Email: gych119@yahoo.com.cn*

Anthracnose is one of the major fungal diseases of strawberry in China. From July to September 2012, the anthracnose disease on strawberry in Hubei province was investigated. Incidence of anthracnose disease on strawberry cultivated varieties of Jingyao, Toyonoka, Sweet charlie, Hongyan and Akihime were investigated. The incidence of anthracnose on strawberry in Hubei province between 5.3%-69.1%; the average incidence of anthracnose were differed by cultivated varieties of strawberry. Toyonoka and Sweet charlie are considerably more resistance against anthracnose than Jingyao, Hongyan and Akihime. 18 strains were isolated from strawberry with anthracnose disease, The ITS sequence of these strains were cloned and sequenced. Blast and phylogenetic tree base on the ITS sequence show that all of the 18 isolates from Hubei province are *Colletotrichum gloeosporioides* Penz. The other two fungal *C. acutatum* and *C. fragariae* which can cause anthracnose disease on strawberry were not been isolated in this investigation.

**P35.026 Analysis of simple sequence repeats in the *Gaeumannomyces graminis* var. *tritici* genome and development of microsatellite markers**

*Y.X. Feng[1, 2], W. Li[1], H.Y. Sun[1], Y.Y. Deng[1], H.S. Yu[2] and H.G. Chen[1]*
*[1]Institute of Plant Protection, Jiangsu Academy of Ag-*

*ricultural Sciences, Nanjing 210014, P. R. China; [2]College of Life Sciences, Nanjing Agricultural University, Nanjing 210014, P. R. China*
*Email: huaigu@hotmail.com*

Understanding the genetic structure of *Gaeumannomyces graminis* var. *tritici* is essential for the establishment of efficient control strategies. Microsatellites or simple sequence repeats (SSRs) are important for the genome organization and a large source of molecular markers for population genetics. In this study, we examined the *G. graminis* var. *tritici* genome (1) to analysis its SSRs pattern; (2) to compare it with other plant pathogenic filamentous fungal species: *Magnaporthe oryzae* and *M. poae* and (3) to identify new polymorphic SSR markers for genetic diversity. In the *G. graminis* var. *tritici* genome, di-, tri-, tetra, penta and hexanucleotide repeats were more abundant than the same repeats in *M. oryzae* and *M. poae*. Thirty-nine out of 83 PCR-amplified di-, tri-, tetra, penta and hexanucleotide were polymorphic (47%) with *G. graminis* var. *tritici* isolates. The number of alleles varied from 2 to 6 and the He from 0.320 to 0.738. In conclusion, SSRs developed in this study were highly polymorphic, and our analysis indicated that the *G. graminis* var. *tritici* maybe is a species with high genetic diversity. The results provide a pioneering report for applications such as the assessment of population structure and genetic diversity of *G. graminis* var. *tritici*.

### P35.027 Genetic diversity and pathogenisity differentiation of *Sclerotinia sclerotiorum* on rapeseed (*Brassica napus* L.) in Anhui province of China

*D.F. Xu[1, 2], X.L. Li[1], P. Li[1], H.J. Zhang[1], M. Guo[1], Y.M. Pan[1] and Z.M. Gao[1]*
*[1]College of Plant Protection, Anhui Agricultural University, Hefei 230036, P. R. China*; *[2]Institute of Tobacco, Anhui Academy of Agricultural Sciences, Hefei 230031, P. R. China*
*Email: gaozhimou@126.com*

*Sclerotinia sclerotiorum* (Lib.) de Bary, one of the most devastating and cosmopolitan plant pathogens, can cause numerous diseases of over 400 species of plants worldwide. Rapeseed Sclerotinia rot caused by the fungus has been an important disease limiting seriously rapeseed production in Anhui, China. In this paper, Inter-simple sequence repeat-polymerase chain reaction (ISSR-PCR) was performed to investigate the genetic diversity of *S. sclerotiorum* isolates on rapeseed (*Brassica napus* L.) in Anhui , China. 12 random primers were selected to screen 47 individuals of *S. sclerotirum* isolates. As a result, a total of 136 reproducible ISSR fragments were obtained, of which 92.7% (125) were polymorphic, revealing high polymorphism among the isolates. Genetic similarity coefficients among all of the isolates ranged from 0.56 to 0.91. Nei's genetic diversity index (*h*) ranged from 0.099-0.378 and Shannon's information index ($I_s$) ranged from 0.172-0.562, which indicated a high level of genetic variation in *S. sclerotiorum* isolates from Anhui Province of China. Cluster analysis indicated that the Anhui isolates could be divided into seven groups according to the DNA fingerprints by using the unweighted pair-group method with arithmetic averages (UPGMA), which showed that there was partial correlation between the genetic polymorphisms and the pathogenicity of *S. sclerotiorum* strains, but no correlation between ISSR group and geographic origin. In addition, the results showed that both analysis assays were suitable for the evaluation of genetic polymorphisms among populations of the fungus.

### P35.028 Characterization of the phytoplasma species, causal agent of coconut lethal yellowing disease (CLYD) in Mozambique

*J. Bila, A. Mondjana, B. Samils and N. Hogberg*
*Universidade Eduardo Mondlane, Faculdade de Agronomia e Engenharia Florestal, Departamento Protecção Vegetal, Maputo, Mozambique*
*Email: jbilay@gmail.com; joao.bila@slu.se*

Coconut palm (*Cocos nucifera*) is an important perennial oil crop that supports the livelihood of most farmers in coastal areas of Mozambique, providing food, building materials and also conservation of the environment, as an agro-forestry crop. However, outbreaks of coconut lethal yellowing disease (CLYD) is now threatening the industry and the livelihood of over 2.0 million people in Mozambique. The causal agent of the disease in Mozambique is not yet known, however, based on preliminary sequencing it was hypothesised that it is more similar to that from Ghana samples rather than from Tanzania and Kenya. In the present study the potential phytoplasma species causing CLYD in Mozambique were characterized. More than one hundred representative samples from where the disease is present in the whole country were collected from diseased coconut trees during two growing seasons, in 2012. Additional samples were also collected in Tanzania. Extracted DNA was analyzed by sequencing 16S and secA genes, and sequences from GenBank were used as reference strains for phylogenic tree analysis. Mozambican strains were all clustered together with other Mozambican reference sequences from Genbank, close to some but not all Tanzanian references sequences, but far away from Ghanaian reference sequence from Genbank, which grouped together with other Tanzanian, Nigerian and Latin America sequences. The phylogenic analysis of all sequences is still in process, thus a robust picture is still to come. However, our results so far indicate an East African origin of the coconut lethal yellowing disease in Mozambique.

**P35.029 Analysis of *Puccinia striiformis* f. sp. *tritici* virulent population structure on two wheat cultivars in Tianshui, Gansu Province**

*G.M. Zhan, F.P. Wang, L.L. Huang and Z.S. Kang*
*State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling, Shaanxi, P. R. China*
*Email: kangzs@nwsuaf.edu.cn*

Strip rust disease, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most destructive fungal diseases on wheat worldwide, which severely threatens wheat production. The application of resistant varieties is thought to be the most important and efficient approach for prevention and control of wheat stripe rust. However, selection and deployment of cultivars significantly affect the genetic structure of *Pst* population, and genetic diversities of *Pst* on different wheat cultivars have not been thoroughly investigated. This study focused on virulence of *Pst* populations from different wheat varieties including Mingxian169, a high susceptible cultivar and Xiaoyan22 from 4 different over-summering zones in Tianshui. The main results are summarized as follows: 1) 198 isolates were identified as 13 races from 19 Chinese differentials, of which CYR32 and CYR33 were two dominant populations and their virulence ratios were 39.3%, 36.8%, respectively. The virulence ratio of other races is relatively low (< 10%). Since these races are all virulent to Suwon 11, they could be identified as both Hybrid46 pathogenic groups and Suwon 11 pathogenic groups. The virulent frequency of different isolates on the same Chinese differentials has little difference, all of which could not infect hosts including Zhong 4 Guinong22 and *T. spelta*, suggesting that cultivars carrying *Yr5, Yr10, Yr26* maintain effective resistance to *Pst* in Tianshui. 2) Nei's Gene diversity value and Shannon's information index of the populations were calculated on two different cultivars from four different over-summering zones by POPGENE version 1.32, and SAS was employed for ANOVA. The data indicated that the diversity of virulent populations in different areas has significant differences. The virulent diversity in Pingnan is most abundant, and subsequently Gangu, Yunshan and Fenghuang. Based on the ANOVA in different cultivars, Mingxian169 has more diversity of population virulence than that on Xiaoyan22. 3) VAT was used to calculate values of different populations including Nei's GD, Nei's Gst, KD, Rogers distance, MCD, Simpom differentiation, Shannon differentiation and Kosman differentiation. Thereafter, all the populations were compared to each other. The data demonstrated that the genetic distances of *Pst* populations between Pingnan and Yunshan, Pingnan and Fenghang are the farthest. Taken together, this study can provide a theoretical guide for integrated management of wheat stripe rust and effective deployment of resistance genes in *Pst* over-summering zones in China.

**N35.001 Population division of *Puccinia helianthi* Schw. and scar markers**

*L. Jing, X. Xu and J.L. Jing*
*College of Agronomy, Inner Mongolia Agricultural University, East Xinjian Street, Hohhot, 010019, P. R. China*
*Email: jinglan71@126.com*

This study was on the race identification of *Puccinia helianthi* Schw. in main production areas of sunflower in China. Through single urediospores isolation, we obtained 44 strains with 6 races identified according to the reaction of 9 international differential hosts which were named 300, 735, 310, 500, 737, and 724 by coded triplet system. The frequency of each race was 59%, 16%, 14%, 7%, 2%, and 2% respectively. Race 300 was used to inoculate 44 sunflower varieties from main production areas and 12 varieties are susceptible while 31 resistant. Among the susceptible ones, 15% are oil varieties, 39% are confectionery varieties, 31.6% are hybrid varieties, and 80% are conventional varieties. The genetic background diversity of the 44 isolates was analyzed using RAPD molecular marker. Genetic similarity coefficient of 44 isolates was 0.63~1.00, with average of 0.815. At the level of 0.74, 44 isolates could be divided into three RAPD groups, but the relations between RAPD groups and geographic distribution of isolates were not clear. Specific RAPD band was retrieved from PCR amplifying products with 13 primers. One primer (S8: ACGGATCCTG) was correlated to Race 300. The Specific polymorphic fragments were cloned and sequenced. One pair of primer was designed according to the sequences using SeqMan (Forward primer: 5'-CCT GAGGAAGACGGTATT-3', Reverse primer：5'-CCT GCATGCCATTGGTGTT-3'), for specific detection of Race 300.

## Concurrent Session 36-Postharvest Pathology

### O36.001 pH modulation of host environment a general mechanism modulating fungal attack in postharvest pathogen colonization

*D. Prusky*
*Department of Postharvest Science of Fresh Produce, ARO, Volcani Center, Bet Dagan 50250, Israel*
*Email: dovprusk@agri.gov.il*

Insidious fungal infections by postharvest pathogens remain quiescent during fruit growth until, at a particular phase during fruit ripening and senescence, the pathogens switch to the necrotrophic life style and cause decay. During ripening, fruits undergo physiological processes, such as activation of ethylene biosynthesis, cuticular changes, cell-wall loosening – changes that are accompanied by a decline of antifungal compounds, both preformed and inducible secondary metabolites. Pathogen infection of unripe host fruit will initiate defensive signal-transduction cascades culminating in accumulation of antifungal-proteins that limit fungal growth and development. In contrast, development of the same pathogens during fruit ripening and storage activates a substantially different signaling network that culminate in the secretion of pH modulating factors, that facilitates aggressive fungal colonization. This talk will focus on the fungal pH responses induced by the quiescent pathogens of postharvest diseases in unripe host fruits during the transformation to active necrotrophic pathogens. New genome scale experimental approaches have begun to delineate the complex and multiple networks of host and pathogen responses activated to maintain or to facilitate the transition from quiescence to necrotrophic life style.

### O36.002 Molecular mechanisms of a Rab/GTPase family gene *Bcsas1* in pathogenicity of *Botrytis cinerea*

*Z.Q. Zhang, G.Z. Qin, B.Q. Li and S.P. Tian*
*Key Laboratory of Plant Resources, Institute of Botany, the Chinese Academy of Sciences, Beijing 100093, P. R. China*
*Email: tsp@ibcas.ac.cn*

*Botrytis cinerea*, as a necrotrophic fungal phytopathogen, causes grey mould rot in more than 200 described host plant species, and is more destructive on soft and senescing tissues, resulting in serious economic losses in fruits and vegetables. As the largest branch of Ras superfamily, Rab proteins constitute a family of small GTPases required for vesicle docking and fusion, and play a central role in the secretory pathway. Although the previous reports suggested that some Rab/GTPase family genes were conservative in different organisms, their function showed difference to some extent in different organisms.To deeply explore the functions of Rab/GTPase family genes in filamentous fungi, we analysed the expression of 10 Rab family genes in *Botrytis cinerea* and disrupte a Rab/GTPase family gene *Bcsas1* using the method of homologous recombination and conducted a detailed analysis of the virulence and the secretory capability of the mutants compared with wild type. Our results indicated that disruption of *Bcsas1* resulted in striking changes in morphology. The mutants were markedly impaired apical extension as well as with compact colony and reduced sporulation, and exhibited impaired pathogenictiy on tomato fruit and lost infectious ability completely on apple fruis and tomato leaves. We found that the transport vesicles were greatly accumulated at the hyphal tip stained with FM4-64, and extracellular protein content was significantly reduced in *Bcsas1* mutant. The activities of cell wall degrade enzymes, polygalacturonase and xylanase showed to be lower in the mutant compared wild type strain. Moreover, the comparative analysis of secretome proved that the invalidation of *Bcsas1* significantly depressed the sectetion of polysaccharide hydrolases and protease. These results suggest that exogenous oxidative stress can regulate the expression of Rab/GTPase family genes, and *Bcsas1* plays a crucial role in development, protein secretion and pathogenicity of *Botrytis cinerea* causing grey mould rot in fruits and vegetables.

### O36.003 Genomic analysis of *Botrytis cinerea*: an essential tool for better understanding a complex pathogen

*J.A.L. van Kan*
*Wageningen University, Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*
*Email: jan.vankan@wur.nl*

The fungus *Botrytis cinerea* is an important pre- and post-harvest pathogen, causing very significant economic damage in dozens of crops worldwide. Despite extensive chemical control, and the development of biological control agents, yield and quality losses remain substantial. Some quantitative resistance loci have been identified in tomato and Arabidopsis, but the genes and mechanisms contributing to resistance remain to be characterized. In order to design a rational disease control strategy, it is crucial to increase our understanding of mechanisms that *B. cinerea* exploits to infect host plants. Obtaining a properly annotated genome sequence is instrumental to this understanding. The genomes of two *B. cinerea* strains, B05.10 and T4, were published (Amselem et al., 2011), but the coverage was low and the data contained errors. A second version was recently published (Staats and van Kan, 2012), for which the coverage and data quality were markedly higher. An effort to further improve the assembly and annotation is ongoing. I will present an overview of the current status

of *B. cinerea* genome sequencing and illustrate how genome information can be exploited to study fungal gene expression in different developmental stages and during host infection. Furthermore I will present preliminary results of an effort to sequence the genomes of ten other *Botrytis* species, all of which are specialized necrotrophic pathogens, specific for a single host species.

**O36.004 Exploring regulatory mechanisms of ambient pH on pathogenicity of postharvest fungal pathogens using proteomic approach**

*B.Q. Li and S.P. Tian*
*Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, P. R. China*
*Email: bqli@ibcas.ac.cn*

Postharvest fungal pathogens cause decay of fruits and vegetables, and lead to huge economic loss worldwide. The ambient pH, as one of the most important environmental parameters, has critical effect on the pathogenicity of fungal pathogens. In our study, we explored mechanisms of ambient pH regulating pathogenicity of postharvest pathogens, *Penicillium expansum* and *Botrytis cinerea*, mainly through proteomic approach. It was found that *P. expansum* showed lower germination rate under unfavorable pH value, such as 2 and 8. We identified 34 differential proteins under different pH treatments, and half of them were related to protein synthesis and folding, and down-regulated at pH 2 and 8. Accordingly, lower content of total soluble proteins and higher ratio of aggregated proteins were observed in those treatments. These findings indicated that ambient pH might affect *P. expansum* spore germination with regulating protein synthesis and folding as one of the main mechanisms. In *B. cinerea*, distinct differences in secretome were found between pH 4 and 6 treatments, and 47 differential spots were identified using MALDI-TOF/TOF. We found that more proteins related to proteolysis were induced at pH 4, whereas most of up-accumulated proteins were cell wall degrading enzymes at pH 6. Moreover, expressions of most of these proteins were regulated at the level of transcription. These findings indicated that *B. cinerea* can adjust protein profile of secretome responding to different ambient pH and provide evidence to deeply understand the complicated infecting mechanisms of *B. cinerea* on a wide range of plant hosts.

**O36.005 Pathogenicity of *Penicillium* spp. isolated from the apple and pear supply chains**

*L. Korsten, I. Scholtz, J.P. Louw and R. Jacobs*
*Department of Microbiology and Plant Pathology, University of Pretoria, Private Bag X20, Hatfield, Pretoria, South Africa, 0028*
*Email: lise.korsten@up.ac.za*

Several *Penicillium* spp. can cause spoilage on different fruit types. *Penicillium* is also well-known for its ability to effectively spread, attach to most surfaces and survive under cold storage conditions. In order to determine the presence, dominance and diversity of *Penicillium* species in the citrus and pear fruit export chains a *Penicillium* spp. presence and pathogen profile was established. Isolates were further characterised according to host specificity and virulence. Apple and pear cultivar trials were conducted to differentiate between *Penicillium* virulence and cultivar susceptibility. Of the *Penicillium* species isolated, *P. brevicompactum, P. commune, P. crustosum, P. expansum, P. griseofulvum* and *P. solitum* were commonly isolated and have previously been reported as pear pathogens. Well known citrus pathogens i.e. *P. italicum* and *P. digitatum* were similarly prevalent in the citrus chain. Apple inoculation studies revealed *P. expansum* as the most and *P. crustosum* as the second most virulent spp on the cultivars Royal Gala, Granny Smith, Golden Delicious, Topred, and Cripps Pink. On pears, varying results were obtained. *P. digitatum* expressed the highest virulence in Baurre Bosc, Baurre Hardy, and Sempre cultivars. The findings indicate that *P. digitatum* is not only highly virulent on citrus, but also on pears when simulating extended supply chain practices. The cold storage trails with apples revealed that only *P. expansum* was able to produce lesions. Results given in this paper provide an overview of Penicillium species presence, diversity, richness and pathogenicity in the pear and citrus fruit environment and export chains.

**O36.006 Anthracnose and stem-end rots of tropical and subtropical fruit- new names for old foes**

*K.R. Everett*
*The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169, Victoria Street West, Auckland 1142, New Zealand*
*Email: Kerry.Everett@plantandfood.co.nz*

Until relatively recently the naming of plant pathogenic fungi relied exclusively on morphological characteristics. Within the past decade, the use of molecular techniques to allow sequencing of various gene regions has revolutionized fungal systematics. In most cases DNA sequence analysis has aligned with conventional taxonomy, but not always. DNA analysis has simplified the identification of some closely related species that were difficult using conventional techniques, and has allowed some resolution of previously unresolved links between anamorphs and teleomorphs. Of concern to applied plant pathologists and biosecurity scientists is the propensity to use phylogeny trees based on DNA analysis of only one gene region to overenthusiastically 'split' fungal genera into an increasing number of species. An overview will be made of this issue with particular emphasis on *Botryosphaeria* and *Colletotrichum* species, and how the sensible use of sequence analysis can improve the

identification of fungi that cause anthracnose and stem-end rots in tropical and subtropical fruit.

### O36.007 Pre-harvest management strategies for post-harvest disease control in mango

*M. Kazmi[1], C.A. Akem[2], M. Weinert[2], A. Ghaffar[3], F.S. Fateh[4] and G. Poussio[4]*
*[1]Australian Centre for International Agricultural Research, Australia-Pakistan Agriculture Sector Linkages Program, Australian High Commission, Diplomatic Enclave No.1, Islamabad, Pakistan; [2]Department of Agriculture, Forestry and Fisheries, Queensland, Australia; [3]Mango Research Station, Ayuab Agriculture Research Institute, Punjab, Pakistan; [4]National Agricultural Research Center, Islamabad, Pakistan*
*Email: munawarkazmi@gmail.com*

Australia-Pakistan Agriculture Sector Linkage Program collaboration (2006 to 2013) is developing integrated crop management practices to enhance value chain outcomes for the mango industry in Pakistan and Australia. One component involves scaling up orchard management strategies which optimize nutrition, enhance constitutive resistance of mango fruit and reduce field disease inoculum as key underpinning the reduction of postharvest disease losses. The strategies include optimal tree nutrition, tree pruning and inoculum reduction and strategic use of field sprays with fungicides. This is coupled with a longer-term improvement of nursery stock, screening for cultivar resistance and selection of clean planting material as means of reducing stem end rot, anthracnose and (in Australia) dendritic spot. The research outcomes of crop management research from 2005-2010 are being demonstrated at different grower orchards in 25 integrated research block sites in both the Punjab and Sindh mango growing areas of Pakistan. The blocks have been established in the form of village or district clusters for easy management and to serve as demonstration blocks to adjacent or neighbouring farms. Pre harvest management protocols will be validated in the research blocks to fine-tune and assess their agronomic and disease reduction potential, and to foster grower ready adoption. The disease reduction risk and shelf-life potential of fruit from the blocks will be further tested in domestic and export market situations.

### O36.008 A genetic analysis of postharvest disease resistance in apple using a *Malus sieversii* mapping population

*M. Wisniewski, J. Norelli, S. Droby and C.T. Chao*
*USDA-ARS, 2217 Wiltshire Road, Kearneysville, WV 25430, USA*
*Email: michael.wisniewski@ars.usda.gov*

Blue mold of apple fruit, caused by *Penicillium expansum*, results in significant postharvest losses. While apple producers mainly use synthetic fungicides for decay control, biological approaches are being explored utilizing microbial antagonists, natural products, and physical treatments (heat, UV-C). One of the most effective and safest means to control plant diseases is the use of resistance cultivars. Blue mold resistance, however, is not a high priority in apple breeding programs due to the time required for seedlings to produce enough fruit to phenotype the trait and the absence of sources of resistance. Hence, identification of resistance would represent a significant accomplishment. *Malus sieversii* PI613981, collected from the wild in Kazakhstan, is resistant to blue mold. Fruit collected from a 'Royal Gala' X PI613981 mapping population (GMAL4593) in 2011 and 2012 were inoculated with *P. expansum* and evaluated for decay at 7 days post inoculation (dpi). There was a clear segregation of resistance with the mean radius of decay ranging from 0-11 mm among individual progeny. Lesion diameters observed on 101 genotypes evaluated for resistance in both years were significantly correlated ($r=0.44$, $P<0.001$). Kruskal-Wallis analysis (MapQTL® 6.0, Kyazma) indicated a significant association between blue mold resistance and DNA markers on linkage group 4. Transcriptomic analysis of individual and pooled susceptible and resistant genotypes utilizing RNA-Seq is in progress to identify candidate resistance genes that could be used for marker-assisted selection and to better understand the host-pathogen interaction. Additionally, at least 6 LysM effectors have been identified in the genome sequences of two strains of *P. expansum*.

### O36.009 Proteomic analysis to investigate the regulatory network of NADPH oxidase in *Botrytis cinerea*

*H. Li, Z. Zhang, S. Tian and G. Qin*
*Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, No.20 Nanxincun, Xiangshan, Haidian District, Beijing, 100093, P. R. China*
*Email: gzqin@ibcas.ac.cn*

Reactive oxygen species (ROS) produced by the Nicotinamide adenine dinucleotide (NADPH) oxidases in the plasma membrane of plant has been reported to be crucial for the defense reactions of plant against pathogen attack. In recent years, it was shown that some fungal pathogens also possess NADPH oxidases which are necessary for the infection processes. However, the mechanisms by which NADPH oxidases regulate the virulence of fungal pathogens remain largely unknown. We constructed a mutant of the regulatory subunit of NADPH oxidase, i.e. *bcnoxR* in *Botrytis cinerea* using a gene replacement approach and compared the protein expression profiles between *bcnoxR* mutant and wild-type. Through high-resolution, two-dimensional gel electrophoresis coupled with tandem mass spectrometry, more than seventy protein spots were identified whose expression was up-regulated or down-regulated in the

mutant. According to their function, these proteins were classified into several categories including metabolism, protein translation, protein degradation, intracellular signaling, detoxification and cell defense, and cytoskeleton. The effects of specific proteins in the infection processes of *B. cinerea* were investigated by construction of deletion mutants. Our study may contribute to the understanding of the regulatory network of NADPH oxidase complex in fungal infection.

**O36.010 Proteomic analysis in muskmelon fruits (*Cucumis melo* L.) treated with sodium silicate and challenged with *Trichothecium roseum***

*J.J. Wang and Y. Bi*
*College of Food Science and Engineering, Gansu Agricultural University, Lanzhou, 730070, P. R. China*
*Email: smkxwjj@163.com*

To obtain unique insights regarding the effect of induced resistance on postharvest sodium silicate (Si) treatments in muskmelons (cv. Yujinxiang) challenged with *T. roseum*, a complete proteome analysis was performed by using two dimensional gels electrophoresis (2-DE) followed by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). A total of 81 proteins were identified as significantly up- or down-regulated in response to Si induction and *T. roseum* inoculation in fruits. After functional categorization, these proteins were attributed to energy pathway, redox homeostasis, defense response, protein metabolism, transporter and cell structure. Among them, both 10 defense response and 5 redox homeostasis associated proteins were induced by Si induction and *T. roseum* inoculation fruit. In addition, 13 proteins were identified as the enzymes which catalyze the reactions of glycolysis and tricarboxylic acid cycle. These findings ascertain that defense response and redox homeostasis proteins, as well as enzymes associated with energy metabolism, were involved in induced resistance of muskmelon fruits by Si and challenged by *T. roseum*.

**O36.011 Oligochitosan induced defence reaction to postharvest decay in Jujube fruit**

*H.L. Guo, Q.Y. Zu, E. Zhu and Y. Song*
*College of Food Engineering and Biotechnology, Tianjin University of Science &Technology, Tianjin 300457, P. R. China*
*Email: ghl0628@163.com*

Black rot disease of lingwuchangzao jujube (Chinese Date), caused by *Alternaria alternate* were suppressed by oligochitosan (DP=3-9), reduced the disease incidences and lesion sizes compared to controls when spraying on jujube fruits with concentration of 50 $\mu g.ml^{-1}$ oligochitosan ,which had no effection on pathogen spore germination and mycelial growth in vitro. Compared with the water treatment, decline in lesion size and disease incidence rate in jujube fruit were more effectively after oligochitosan treated 1 to 3 days, and the best control effect was treated 1day before pathogen infection. When applied to jujube fruit under semi-commercial condition, the natural postharvest decay were inhibited both in 0 and 22℃. The isoenzyme analysis the activities of polyphenol oxidase (POD） significant changes and faster than the change of the SOD and PPO isoenzyme activities. The results suggest that the animal-derived oligochitosan induced host defensive resistance as exogenous inducer before pathogen infection and like all carbohydrate portion of fungi, which suppressed the occurred of natural disease.

**O36.012 The role of reactive oxygen species in ASM-induced disease resistance in 'Fuji' apples**

*Y.H. Ge , H.W. Deng, Y. Bi and Y.Y. Liu*
*College of Food Science and Engineering, Gansu Agricultural University, No 1 Yingmencun, Anning District, Lanzhou, 730070, P. R. China*
*Email: geyh1979@163.com*

Blue mould caused by *Penicillium expansum* is one of the most important postharvest diseases of apple. The present study was to evaluate how disease resistance in apples (cv. Fuji) was affected by the dipping of acibenzolar-S-methyl (ASM) and diphenylene iodonium (DPI), a NADPH oxidase specific inhibitor. Lesion diameter on the fruit inoculated with *Penicillium expansum* was significantly ($P \leq 0.05$) decreased by dipping with 0.1 g/L ASM. Decreased lesion development was associated with the accumulation of hydrogen peroxide ($H_2O_2$), release of superoxide anion ($O_2^-$), enhancement activities of NADPH oxidase (NOX), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT). Antioxidants content including ascorbic acid and reduce glutathione was also induced by ASM treatment. Compared with ASM treated fruit, fruit treated with DPI prior to ASM treatment exhibited higher lesion diameter. Moreover, DPI treatment inhibited ASM-induced $H_2O_2$ and $O_2^-$ accumulation, the increase of the activities of NOX, SOD, APX and content of ascorbic acid and reduced glutathione. These results suggest that pretreatment with DPI prevented accumulation of ROS induced by ASM and showed serious disease symptoms, which showed the important role of ROS in ASM-induced resistance in apple fruit.

**O36.013 Use of Lamiaceae essential oils to control postharvest rots on apples and peaches**

*D. Spadaro, J.G. Lopez-Reyes, A. Garibaldi and M.L. Gullino*
*AGROINNOVA, University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*

*Email: davide.spadaro@unito.it*

The efficacy of plant essential oils from basil, fennel, lavender, marjoram, oregano, peppermint, rosemary, sage, savory, thyme and wild mint was evaluated to control *Botrytis cinerea* and *Penicillium expansum* on apples cv Golden Delicious, Granny Smith, Red Chief and Royal Gala. The same essential oils were also tested on apricots (cv Kyoto and Tonda di Costigliole), nectarines (cv Big Top and Nectaross) and plums (cv Italia and TC Sun) to control *Monilinia laxa* and *Botrytis cinerea*. The volatile composition of the essential oils tested was determined by GC/MS analysis. After 15 and 30 days storage at low temperature, the rot diameter was measured. The treatments with essential oils from oregano, savory and thyme at 1% (v/v) showed a relevant efficacy in the apple cultivars tested, in apricots cv Tonda di Costigliole and plums cv Italia and TC Sun, however the same treatments were phytotoxic for the carposphere of nectarines cv Big Top and Nectaross. The treatments of 10% (v/v) essential oils were highly phytotoxic notwithstanding their efficacy against the pathogens tested. The essential oils containing as major components α-pinene, p-cymene, carvacrol, and thymol showed similar results, so their antimicrobial activity and the phytotoxicity produced could be based on the concentration of their principal compounds and their synergistic activity. The efficacy of the essential oil treatments on control of fungal pathogens in post-harvest depended on the fruit cultivar, composition and concentration of the essential oil applied and the length of storage.

### O36.014 Improving radio frequency treatment by immersing fruit in water to control brown rot in stone fruit

*M. Sisquella[1], C. Casals[2], I. Viñas[1], N. Lamarca[2], P. Picouet[3] and J. Usall[2]*
*[1]Food Technology Department, University of Lleida, XaRTA-Postharvest, 191 Rovira Roure, 25198-Lleida, Catalonia; [2]IRTA, XaRTA-Postharvest, 191 Rovira Roure, 25198-Lleida, Catalonia; [3]IRTA, Food Processing and Engineering, Finca Camps i Armet, 17121 Monells, Girona, Catalonia*
*Email: josep.usall@irta.cat*

Brown rot caused by *Monilinia* spp. is the most important postharvest disease of stone fruit. Currently, chemical fungicides are not allowed in the European Union to be applied to postharvest of stone fruit, which has increased the need to develop new alternatives controls. Radio frequency (RF) treatment at 27.12 MHz was studied to control brown rot in peaches and nectarines. From preliminary studies, a RF treatment for 18 min was selected to evaluate the effectiveness of the treatment to control *Monilinia* spp. in naturally infected fruit and fruit with different diameter. In general, high disease control was achieved in peaches, however, RF effectiveness was affected by fruit size and no brown rot control was observed in nectarines. In order to address these problems, RF treatment with fruit immersed in water was studied. RF treatment in fruit immersed in water at 20 ℃ for 9 min significantly reduced brown rot incidence in both peaches and nectarines and no significant differences in RF effectiveness were observed depending on fruit size. Moreover, the decrease in treatment time with increasing water temperature was also evaluated. Reduction of treatment time to 6 and 4.5 min was achieved by increasing water temperature at 35 and 40 ℃, respectively, to control brown rot without impair fruit quality in both, peaches and nectarines. In conclusion, RF treatment with fruit immersed in water at 40 ℃ for 4.5 min may provide a potential postharvest alternative treatment for brown rot control in peaches and nectarines.

### O36.015 Postharvest control of gray mold on blueberry based on critical growth stages and infection risk estimations

*B.A. Latorre, S.A. Rivera and J.P. Zoffoli*
*Pontificia Universidad Catolica de Chile, Vicuna Mackenna 4860, Santiago, Chile*
*Email: blatorre@uc.cl*

Gray mold (*Botrytis cinerea*) is an important postharvest disease of blueberries (*Vaccinium corymbosum*) in Chile, favored by the long (>15 days) transportation to reach the international markets. The aims of this research were to study the critical blueberry growth stages for postharvest gray mold control and to determine the infection risks on the basis of weather conditions. The critical stages for gray mold control were studied on blueberry 'Brigitta' and 'Duke' in two planting localities. Differential fungicide applications (0.5 g/L fenhexamid), performed between the early pink bud stage and mature fruit stage, showed that the best control of postharvest gray mold was obtained when fungicides were applied between the first blue fruit and mature fruit stages. The infection risks for *B. cinerea* infection were defined as >6 h of wetness and temperatures between 14 and 25 ℃. This algorithm to estimate the infection risks was studied in blueberry 'Brigitta', 'Duke' and 'Liberty' in four planting localities A significant correlation between the infection risk and gray mold prevalence in stored fruit was obtained ($r=0.96$, $P<0.0001$), suggesting that this algorithm could be used to optimize fungicide applications, but field validation remains to be determined. In conclusion, the mature fruit stage appears as the most critical stage for postharvest gray mold control if weather conditions, defined by this algorithm, occur.

### O36.016 The surface mycoflora of tomato fruits and their relationship to post-harvest disease develop-

**ment – can applications of Rhapsody *(Bacillus subtilis)* reduce disease?**

*Z.K. Punja, G. Rodriguez, A. Tirajoh and S. Formby*
*Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6, Canada*
*Email: punja@sfu.ca*

A survey of fungi causing post-harvest decay of greenhouse tomato fruits was conducted during 2010-2011. All isolates were inoculated onto tomato fruits to determine their pathogenicity. The fungus causing most disease at 21 ℃ was *Penicillium olsonii*, followed by *Rhizopus stolonifer, Botrytis cinerea, Alternaria alternata* and *Geotrichum candidum*. To determine the mycoflora on the surface of fruits, cotton swabs were gently rubbed on ripening fruit while still attached to the plant, at weekly intervals during June-September, 2011 and 2012. The fungi recovered from fruit surfaces were species of *Penicillium, Rhizopus, Alternaria/Cladosporium,* and *Aspergillus*. Colonies of *Botrytis* were recovered infrequently and *Geotrichum* not at all. Tomato plants in 2 commercial greenhouses were treated with Rhapsody (*Bacillus subtilis*) once a month during July-October, 2012 to determine effects on surface fungal populations and post-harvest disease. Disease incidence and severity were both significantly reduced on fruit treated with Rhapsody. Rhapsody-treated fruit had 0-5% fruit infection compared to up to 30% infection on untreated fruit at 21 ℃. A major source of inoculum of post-harvest fungi on fruit was stem and calyx tissues, which led to infection through the stem end. Rhapsody applications prevented spread of these fungi onto the fruit. Rhapsody applications made every 4 weeks are sufficient to maintain high populations of *Bacillus* on the fruit surface, resulting in significant post-harvest disease control on fresh market tomatoes.

**P36.001 Studies on seed-borne fungi in four rice cultivars in Paraguay**

*L. Viedma and N. Bogado*
*Department of Plant Pathology, National University of Itapúa, Faculty of Agricultural Sciences, Lorenzo Zacarías 255, Encarnación, Paraguay*
*Email*: *lviedmaq@gmail.com*

Rice *(Oryza sativa* L.) is an important staple food and cash crop of Paraguay, although only 90.000 hectares have been cultivated in the 2010/2011 crop season, this represents 100% of the crop expansion, related to 2003/2004 season growing area. The use of high quality seed ensures the proper establishment of seedlings, and is the base of this agribusiness. Little information is known on rice seed health testing in the country. The present research was undertaken to evaluate the seed health of four main rice cultivars in Paraguay. Forty rice seed samples of cultivars IRGA 417, IRGA 413, EPAGRI 113 and PUITA were collected from different rice growing area during the crop season 2009/2010. The occurrence of seed borne fungi on seeds was determined by standard blotter method as described by ISTA (1997). Among the field fungi, *Alternaria padwickii* was the most predominant pathogenic fungus in all cultivars evaluated. Others species such as *Fusarium* spp., *Drechslera oryzae, Curvularia lunatus* and *Pyricularia oryzae* have shown lower incidence on the four varieties. On the three storage fungi detected, the most prevalent fungus *were Penicillium* spp. and *Aspergillus flavus.*

**P36.002 Postharvest diseases diagnosis in papaya (*Carica papaya* L.) mango (*Mangifera indica* L.) and melon (*Cucumis melo* L.*)* in different fruit-growing areas in Paraguay**

*L. Viedma and M. Arriola*
*Department of Plant Pahology. National University of Itapúa, Faculty of Agricultural Sciences. Dr. Lorenzo Zacarías 255, Encarnación, Paraguay*
*Email: lviedmaq@gmail.com*

The postharvest fungal diseases of greatest incidence in papaya (*Carica papaya* L.), mango (*Mangifera indica* L.) and melon (*Cucumis melo* L.) in different fruit-growing areas in Paraguay were diagnosed. The fruits were collected from the market and sampled to identify and describe symptoms associated to rot and to isolate and identify their causal agents. The incidence was determined by the isolate of affected fruits and data were analyzed statistically. In mango and melon, the main diagnosed diseased were anthracnose caused by *Colletotrichum gloeosporioides* (Penz.). In papaya fruits were soft rot caused by *Rhizopus stonolifer* (Ehrenb.:Fr.), and anthracnose by *C. gloeosporioides* (Penz.).

**P36.003 Post-harvest diseases control by in-season crop protection**

*A. Goertz[1], G. Labourdette[2] and D. Steiger[3]*
*[1]Bayer CropScience AG, Research & Development, Alfred-Nobel-Str. 50, 40789 Monheim, Germany; [2]Bayer CropScience SAS, La Dargoire Research Center, 14-20 rue Pierre Baizet 69009 Lyon, France; [3]Bayer CropScience AG, Strategic Business Management, Alfred-Nobel-Str. 50, 40789 Monheim, Germany*
*Email: andreas.goertz@bayer.com*

Postharvest decays of fruits and vegetables mainly originate from latent fungal infections occurring in orchard and field. Such infections by fungal pathogens are common on a wide range of horticultural crops. Grape bunches are often quiescently infected by *Botrytis cinerea*. *Monilinia fructicola* and *B. cinerea* are described to establish latent infections in stone fruits. To preserve fruits and vegetables from problematic fungal diseases, the new fungicide fluopyram has been developed being particularly effective against fungal pathogens described

to cause latent infections. In stone fruits, pre-harvest applications of fluopyram at flowering, fruit growth and ripening stage decreased postharvest disease development of *Monilinia* spp.. After seven days of storage of apparently healthy bean pods in plastic bags, 96 % of pods harvested from fluopyram-treated plants were still marketable, whereas 36 % of untreated beans were now visually infested by *S. sclerotiorum*. Field experiments carried out on nectarines showed that about 50 % of the visually healthy fruits stored were colonized with *Monilinia* spp. after several days of storage. Under the same conditions, more than 85 % of the produce coming from fluopyram-protected orchard plots showed no fruit decay. This protection was also measured on crops with shorter shelf life (e.g. strawberries, lettuce, and cherries). The significant higher number of marketable fruits and vegetables at harvest and after storage in multiple crops indicates the potential of fluopyram to reduce the occurrence of latent fungal infections taking place in orchards and fields.

**P36.004 Control of postharvest green mold of citrus fruit by combining potassium sorbate dip treatments with brief exposures to high $CO_2$ or $O_2$**

*C. Montesinos-Herrero and L. Palou*
*Institut Valencià d'Investigacions Agràries, Ctra. Nàquera-Montcada km 4.5, 46113 Montcada, València, Spain*
*Email: palou_llu@gva.es*

Alternatives to synthetic fungicides are increasingly required by the citrus postharvest industry to reduce risks to human health and environment. Recent research showed that dip treatments with potassium sorbate (PS) and brief exposures to high $CO_2$ or $O_2$ atmospheres at curing temperatures effectively reduced citrus green mold caused by *Penicillium digitatum*. These treatments were combined in this work to check if individual effectiveness was enhanced. Fruit were artificially inoculated, dip-treated 24 hours later with water at 20°C (control) or 3% (w/v) PS at 62°C for 1 min air-dried and exposed to gaseous treatments in hermetic cabinets. 'Valencia' oranges were exposed to air (control) at 20 or 33°C, or 15 kPa $CO_2$ at 33°C for 24 h. After 7 and 14 days of incubation at 20°C, incidence reduction of green mold with respect to control fruit was 100 and 83%, respectively. 'Clemenules' and 'Ortanique' mandarins were exposed to air at 20 or 33°C, or 30kPa $O_2$ at 33°C for 24 or 48h. Green mold incidence after 7 and 14 days at 20°C was reduced by 100 and 100%, and 61 and 96%, respectively. After 14, 28, and 42 days at 5°C, green mold incidence on 'Clemenules' and 'Ortanique' mandarins treated with PS and 30 kPa $O_2$ for 48 h was reduced by 100, 96 and 68%, and 100, 97 and 79%, respectively. In all cases, disease incidence was lower for the combined treatments than for each individual treatment, and in many cases a synergistic effect was observed.

**P36.005 Postharvest application of potassium phosphites reduces blue mold on 'Packham's Triumph' pear**

*D. Correa, A. Bogo, J.S. Hipólito, A.C. Costa-Junior, F.R. Penter and R.T. Casa*
*Santa Catarina State University - UDESC. Crop Protection Graduate Program, Avenida Luiz de Camões, 2090, 88520-000, Lages/SC, Brazil*
*Email: amauribogo@udesc.br*

Blue mold (*Penicillium expansum*) is a common postharvest disease of pear (*Pyrus communis*). Applications of less hazardous substances to the environment, such as phosphite (phosphonic acid), is an alternative to the control of blue mold. The main goal of this study was to evaluate the efficiency of different doses of 0, 1, 2, 3, 4 and 5g of active ingredient per liter (a.i/L) of phosphite-K (40% $P_2O_5$ and 30% $K_2O$) for the control of blue mold on 'Packham's Triumph' pear in postharvest. Tests were designed in randomized blocks with six replications of 20 fruits. The variables incidence and severity were analysed by quadratic regression. Fruits were decontaminated with sodium hypochlorite (1%) for three minutes, washed with sterilized water, needle wounded (with a diameter and deepness of 1mm) in four equidistant points, dipped into treatments for 15 minutes, and then stored at 15-20°C. The fruits were submerged in suspensions contaminated with *P. expansum* (at the concentration of 1 x $10^4$ conidia.$mL^{-1}$) and the incidence (%) and severity (mm) were evaluated daily. Fruits of pear dipped in water with phosphite-K (4 g a.i/$L^{-1}$) or benomyl (150 mg a.i./$L^{-1}$) were less affected by the blue mold with reduction lesion size of 91,2%. Applications of phosphite-K or benomyl were the most effective treatments to control the disease.

**P36.006 Detection of multiple fungicide resistance in *Botrytis cinerea* from table grapevine berries**

*D. Errampalli and T. Barasubiye*
*Agriculture and Agri-Food Canada, Canada*
*Email: deena.errampalli@agr.gc.ca*

Botrytis bunch rot caused by *Botrytis cinerea*, Pers:Fr., is an economically important disease of grapes. Forty two single-spore isolates from four varieties of grapevine (*Vitis vinifera* L.) berries collected from Ontario and two reference isolates of *B. cinerea* B05.10 and BC-34R were identified based on colony morphology and spore size. Twenty two of these isolates were confirmed as *B. cinerea* based on the sequences from benomyl-A (*Ben-A)* of β-tubulin gene. The isolates were also tested for sensitivity to the benzimidazole (thiabendazole), the anilinopyrmidine (cyprodinil and penbotec), the phenylpyrrole (fludioxonil) and the dicarboximide (iprodione) classes of fungicides, on PDA media amended with a discriminatory concentration of 5 ug/ml of each of the fungicides. Sensitivity determinations to the fungicides

revealed a total of five phenotypes: each of the isolate was resistant to at least one fungicide; all were resistant to anilinopyramidines; and 3 isolates were resistant to all four chemical classes. As expected, cross resistance was observed between two of the anilinopyramidine fungicides, cyprodinil and penbotec. Pathogenicity test on grapevine cv. Thompson seedless revealed that all the isolates of *B. cinerea* were pathogenic on grapevine berries. The genotyping of the isolates showed that all benzimidazole resistant isolates carried the 198 Glu to Ala (E198A) mutation in *BenA* gene in β -tubulin and all dicarboximide resistant isolates carried 365 Ile to Ser (I365S) mutation in *BcOS1* gene that encodes histidine kinase. We report multiple fungicide resistance in *B. cinerea* isolates collected from grapevine berries.

**P36.007 The stem soft rot on lily caused by *Rhizopus stolonifer* in Korea**

*S.S. Hahm[1], G.W. Hong[2], B.R. Kim[1], K.S. Han[1], T.Y. Choi[2], Y.G. Nam[1] and S.H. Yu[3]*
*[1]Chungnam Agricultural Research and Extension Services, Yesan, Chungnam 340-861, Republic of Korea; [2]Taean Lily Expt. Station, Chungnam Agricultural Research and Extension Services, Taean 357-952, Republic of Korea; [3]Department of Applied Biology, College of Agriculture and Life Sciences, Chungnam National University, Daejeon, 305-764, Republic of Korea*
*Email: hahmsoo@korea.kr*

A Rhizopus stem soft rot of lily caused by *Rhizopus stolonifer* was observed in the field of Chungcheongnam-do Lily Experiment Station in Korea, 2012. The typical symptoms were initially water-soaked lesions on bottom stem and then rotten on leaf. The lesion rapidly expanded and the plant softened totally. Optimum temperature for mycelial growth on PDA was 25℃, grew vigorously the dark grayish brown mycelium and sporangia, sporangiophores, and stolons were formed on surface of the stem. This pathogen was observed the shape of sporangia, sporangiophores, spore and stolons with microscope on the basis of mycological characteristics, and analyzed sequences of ITS-rDNA (internal transcribed spacer region of ribosomal DNA). All isolates caused stem rot symptoms on the 'Sunshine' lily cultivar. On the basis of these results, stem rot fungi that occur in lily were identified as *R. stolonifer* (Ehrenberg ex. Fr.) Lind. This is the first report of stem soft rot on lily caused by *R. stolonifer* in Korea.

**P36.008 Anthracnose of pitaya fruits after harvest (*Hylocercus undatus* (Haw) Brit. & Rose) and its control**

*S. Sangchote and C. Kasee*
*Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, 10900, Thailand*
*Email: agrsrs@ku.ac.th*

Anthracnose of Pitaya fruits from four producing areas: Samut Sakhon, Nakorn Rachasima, and Chanthaburi was investigated. The causal pathogen was identified by using their morphological characteristics and molecular technique and it was *Colletotrichum capsici*. Infections of *C. capsici* were investigated from flower initiation, flower buds, and fruits at age 1-8 weeks. Infection of was the highest at 7 weeks before harvest at 5.8%. Pre harvest spray of prochloraz at the concentration of 400 ppm on the fruits at 10 days before harvest showed no reduction of this disease whereas, dipping the fruits in prochloraz at 400 ppm for 3 minutes reduced anthracnose incidence from 80.0 to 6.6%. Hot dipping in 200 ppm prochloraz solution at 53 ℃ for 1 minute was completely control of this disease with a residue of prochloraz at 0.01 ppm on the fruits.

**P36.009 Control of fungal pathogens causing stem end rot in mango Cv. Chaunsa (*samar bahisht*) with chemicals & plant extracts**

*A. Rehman[1], W. Alam[1], A.U. Malik[3], M. Saira[2], K. Riaz[1], K. Sumaira[1] and G.I. Johnson[4]*
*[1]Department of Plant Pathology, University of Agriculture Faisalabad, 38040-Pakistan; [2]Plant Pathological research Institute, AARI, Faisalabad, 38040-Pakistan; [3]Post-harvest Research and Training Center, University of Agriculture Faisalabad, 38040-Pakistan; [4]Horticulture for Development,P.O. Box 412,Jamison ACT 2614 Australia*
*Email: arb041@gmail.com*

Stem end rot of mango appears during storage, reduces mango shelf life and inflicts heavy losses to mango trade worldwide. Therefore, for devising disease control strategy, fully mature green mango fruits were collected from selected orchards of Punjab province in Pakistan and stored for four weeks at room temperature, 25 ±2 ℃ and cold storage, 10±2 ℃, followed by ripening at room temperature. Pathogens appearing during ripening were isolated and identified as *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Phomopsis mangiferae* and *A. alternata, Aspergillus niger, Rhizopus spp* and mixed type infection). The pathogens were artificially inoculated on fresh mango fruits and placed at room temperature for 21 days and in cold storage for 35 days. Maximum mean disease severity was observed with L. *theobromae* (39.23%), followed by *P. mangiferae* (36.96%) and *C. gloeosporioides* (34.03%) at ambient temperature, and by *P. mangiferae* (35.55%) followed by mix culture (34.65%) and *C. gloeosporioides* (27.03%) during cold storage. Consequently efficacy of different fungicides-pyraclostrobin + metiram (Cabriotop®), trifloxystrobin + tebuconazole (Nativo®), fludioxonil (Scholar®), carbendazim (Tecto®), azoxystrobin (Amistar®) and prochloraz (Sportak®) at 50, 100, 150, 200 and 250 μg/mL concentrations) and plant extracts (ethanolic and methanolic extracts of kasni (*Cichorium intybus)*, hermal

(*Peganum harmala*), clove (*Syzgium aromaticum*), moringa (*Moringa oleifera*), coriander, (*Coriandrum sativum*) and cinnamon (*Cinnamomum aromatic*) at different concentrations 5, 15, 25 and 50 µg/mL) were evaluated *in vitro* through the poisoned food technique. Results revealed that Cabriotop, Nativo, clove, hermal and moringa showed statistically significant antifungal activity against all the tested fungal pathogens.

**P36.010 Prevalence of post harvest rots and fungal pathogens associated with different cultivars of mango under Sindh Agro eco-conditions**

*A.M. Lodhi, M. Bhatti, R.N. Syed and M.A. Khanzada*
*Department of Plant Pathology, Sindh Agriculture University Tandojam, Pakistan-70060, Department of Plant Protection, Sindh Agriculture University Tandojam, Pakistan-70060.*
*Email: mubeenlodhi@gmail.com*

Pakistan is the fourth largest mango producer in the world. Mango orchards are cultivated on 0.16 million hectares and its total production was 1.75 million tonnes during 2011-12. Due to improper cultural and harvesting practices mango post-harvest rots not only reduce fruit quality and cause severe losses, they leave the fruit completely unmarketable. During the present investigation, fully ripe affected mango fruit of Sindhri, Chounsa, Anwar Rataul, Dusehri, Siroli, Began Pali, Sunera, Langra and Desi cultivars were collected from different local markets. Before isolation, symptoms associated with each variety were properly labeled and documented to distinguish the nature of symptoms on different cultivars and the pathogens associated with them. The results indicates that most of the cultivars were affected from stem end rot (*Lasiodiplodia theobromae*) and anthracnose rot (*Colletotrichum gloeosporioides)* followed by Alternaria rot (*Alternaria alternata*) and Rhizopus rot (*Rhizopous stolonifer*). Mango cultivar 'Sindhri' appeared more susceptible to stem end rot, whereas anthracnose rot was more prevalent on cv. 'Chounsa'.

**P36.012 Application of smart sensors for remote profiling of environmental conditions in nut stockpiles**

*S.C. Gouk[1], S. Kreidl[1] and D. Madge[2]*
*[1]Department of Primary Industries, Biosciences Research, 621 Burwood Highway, Knoxfield, Victoria 3180, Australia; [2]Department of Primary Industries, Biosciences Research, cnr Eleventh Street and Koorlong Ave, Irymple, Victoria 3498, Australia*
*Email: chin.gouk@dip.vic.gov.au*

Bulk quantities of low water activity produce such as nuts and grains are commonly stored in the field whilst waiting further processing. In-field storage may subject the produce to environmental conditions that lead to increased moisture contents, which may enhance microbial growth and spoilage. Real-time monitoring of environmental conditions in stored produce may aid decisions in scheduling processing. A sophisticated system for monitoring produce stockpile temperatures and relative humidity was designed and tested. An array of sixteen smart sensors that relay data through interconnecting cables to a single datalogger, was installed at four depths at four different positions within an almond stockpile up to 100m in length and 5 m in height. Weather and stockpile conditions were logged at five minute intervals over three months. The system was shown to meet the application requirements. The modular array of data cables and layout allowed flexibility for monitoring exterior and interior environmental conditions in stockpiles of various areas, heights, depths and lengths. The sensors and cables withstood pressure from a stockpile load of at least 5 m deep. Sensor performance was not affected when they were buried in a matrix of nuts, soil, grit, dust and debris. The system has enabled accurate measurement temperature and humidity within stockpile of various depths. Further features, applications of the system and profiling of environmental conditions are discussed.

**P36.013 Pathological and molecular characterization of post-harvest fungal pathogens of Mango**

*S. Iram and Q. Rashid*
*Department of Environmental Sciences, Fatima Jinnah Women University, The Mall, Rawalpindi, Pakistan*
*Email: iram.shazia@gmail.com*

Morphological, pathological and genetic characterization studies were carried out on the post-harvest disease causing fungi (*Lasiodiplodia theobromae, Colletotrichum gloeosporioides, Aspergillus niger* and *A. flavus)* of mango. Morphological studies confirmed the isolated pathogens on the basis of cultural and conidial characteristics. Aggressiveness of the fungi was tested through artificial inoculation under controlled conditions and all proved pathogenic with varying degree of aggressiveness in reference to control on both mango varieties (Sindhri and White Chounsa) with the exception of the isolates of *C. gloeosporioides* which produced no disease symptoms. The non-pathogenic behavior of C*olletotrichum* isolates was due to contamination because in present study it was not possible to revive them further. All pathological results were highly significant at $P<0.05$ through ANOVA analysis of variance. Calculated standard error means also varied with respect to difference in lesion area ($cm^2$) produced by the inoculated pathogens. Re-isolation of respective fungi was achieved with 100 percent success as verification of Koch's postulates. DNA was successfully extracted from isolates of *L. theobromea* (24), *C. gloeosporioides* (6) and *Aspergillus* species (28) and amplification was

done through ITS1 and ITS4 primers and the amplified fragments were productively digested with restriction enzymes (*Mbo*I, *Alu*I, *Eco*RI, *Hae*III, *Alu*I, *Taq*I) and good/considerable genetic variability was obtained among *L. theobromea, A. niger, A. flavus* isolates but *C. gloeosporioides* isolates did not show genetic variability.

**P36.014 Identification and characterization of post harvest fungal pathogens of mango from domestic markets of the Punjab in Pakistan**

*S. Iram, H. Meer, I. Ahmad , F.S. Fateh and M.R. Kazmi*
*PARC Institute of Advanced Studies in Agriculture, National Agricultural Research Center, Islamabad, Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi. Pakistan*
*Email: iram.shazia@gmail.com*

A systematic survey was conducted during October 2011 to assess the status of major postharvest diseases of mango fruit and losses due to these diseases in the local markets of the Punjab in Pakistan. Data on prevalence, incidence and severity was collected, and the associated pathogens isolated by the tissue segment method. General and specific media were used to isolate the fungi and then eventually, the frequency of recovery of each fungus was calculated x media and location. The present study found that anthracnose and stem end rot diseases were 100% prevalent in the markets of Punjab and that *Colletotrichum gloeosporioides, Lasiodiplodia theobromae*, *Alternaria alternata* and *Aspergillus niger* were the fungal pathogens involved in anthracnose, stem end rot, *Alternaria* rot and *Aspergillus* rot diseases, and these were the major postharvest diseases damaging the mango fruit. In this study, malt extract agar and V-8 agar were the best media for the growth of *L. theobromae* and *C. gloeosporioides*. Following on from the present investigation it would be possible to study the postharvest fungal pathogens of mango for their pathogenic behavior, fungicides resistance and genetic variability. All investigations will be very helpful for the future management of post-harvest diseases of mango.

**P36.015 Influence of antimicrobial agents on the extension of shelf-life of banana (*Musa sapientum* L.) fruits**

*M.C. Mbah and N.L. Aniekwe*
*Department of Crop Production and Landscape Management, Ebonyi State University, P.M.B. 053, Abakaliki, Ebonyi State, Nigeria*
*Email: mbahmat@fastmail.fm*

Experiments were conducted to study the influence of some antimicrobial agents on the extension of shelf-life of banana fruits (*Musa sapientum)*. The antimicrobial agents used in the study include, potassium permanganate ($KM_nO_4$), chlorine ($Cl_2$) sodium hypochlorite (NaOCl), soduim metabisulphite ($Na_2SO_3$) and neem-leaf (*Azadrichta indica*) extract (neemext). The experiments were laid out in a completely randomized design (CRD) with three replicates in each treatment. Results showed that potassium permanganate and neemext delayed ripening (Preclimacteric (green) phase). Ripening started on banana fruits treated with $KM_nO_4$ and neemext after 14 and 11 days respectively. While those banana fruits treated with NaOCl, $NaSO_3$, $Cl_2$ and control started ripening after 8, 8, 7, and 5 days respectively. The antimicrobial agents used in this study significantly extended the shelf-life of the banana fruits. Potassium permanganate and neemext extended the shelf–life of the banana fruits for a total of 21 and 18 days respectively while NaOCl, $Na_2SO_3$, $Cl_2$ had 16, 15, 15 days respectively. The control experiment had 10 days shelf-life. The results revealed the potential of $KM_nO_4$, neemext among other antimicrobial agents in the extension of the shelf –life of banana fruits which maybe very useful in our rural/ urban communities where there are no advanced technology for processing and preservation of banana fruits, thus enhancing food security.

**P36.016 A comparison of reactive oxygen species (ROS) metabolism in slices of potato tuber inoculated with *Fusarium sulphureum* and *F. sambucinum***

*G.H. Bao, Y. Bi, Y.C. Li, Z.M. Yang, Z.H. Kou and Y. Wang*
*College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, P. R. China*
*Email: 2857324911@qq.com*

Dry rot caused by *Fusarium* spp. is the most important postharvest diseases of potato tubers in China. In this study, the pathogenicity of two major strains of dry rot, *F. sulphureum* and *F. sambucinum*, was investigated, and the changes of ROS metabolism were assayed with inoculated slices of potato tuber (cv. Longshu No.3). The results showed that lesion diameters of slices and tubers inoculated with *F. sulphureum* were significantly larger than those inoculated with *F. sambucinum*. The cell membrane integrity quickly decreased in slices inoculated with *F. sulphureum*, and the content of malondialdehyde (MDA) remarkably increased. Hydrogen peroxide ($H_2O_2$) content reached its maxima on the first day (*F. sulphureum*) or second day (*F. sambucinum*) after inoculation, and a higher peak was found in slices inoculated with *F. sambucinum*. The peak of superoxide anion ($O_2^{\cdot-}$) appeared on the fourth (*F. sulphureum*) day or fifth day (*F. sambucinum*) after infection, and a lower production rate of $O_2^{\cdot-}$ was observed in slices inoculated with *F. sambucinum*. A higher activity of NADPH oxidase (NOX, EC1.6.3.1), superoxide dismutase (SOD, EC1.15.1.1) and glutathione reductase (GR, EC1.6.4.2), and a lower activity of catalase (CAT, EC1.11.1.6) were detached in slices inoculated with *F. sulphureum* during the whole experiment period. The slices inoculated with

*F. sulphureum* showed a higher peroxidase (POD, EC1.11.1.7) activity and a lower ascorbate peroxidase (APX, EC1.11.1.11) activity during later infection period. These findings suggest that *F. sulphureum* have a stronger pathogenicity correlated to the vast accumulation of ROS during interaction.

### P36.017 A comparison of inhibition effects of sodium silicate and corresponding pH on *Trichothecium roseum in vitro*

*L.L. Niu, X.D. Bai, Y. Bi, Y.C. Li and Y. Wang*
*College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, P. R. China*
*Email: niull@gsau.edu.cn*

*Trichothecium roseum* is one of the important postharvest pathogens. Sodium silicate (Si) and environmental pH have significant inhibition on the fungal growth. Spore germination, germ tube elongation and mycelial growth of *T. roseum* were significantly inhibited by different sodium silicate concentration (0-100mM) and corresponding pH (7.04-11.86). Inhibitory effect was concentration and pH dependent; however, sodium silicate showed much better than corresponding pH treatment. The inhibition rate of spore germination, germ tube elongation and mycelial growth were 25.5 fold, 7.3-fold and 100% inhibition in 100 mM sodium silicate treated which was higher than the corresponding pH respectively. Fluorescent microscopy showed obviously damage of the plasma membranes of *T. roseum* conidia treated with Si (100 mM) and pH (11.86) by propidium iodide (PI) staining. The leakage of protein and sugar were also significantly higher in Si-treated and pH-treated spores than that of control. Serious damage was observed in longer exposure periods to sodium silicate and pH. Similarly, ultrastructural observation showed that sodium silicate (100 mM) and pH (11.86) cause membrane retraction and invagination. And a greater alteration was observed in the conidia that exposed in Si and pH for long time. These results suggest that the excellent inhibitory effect of sodium silicate against *T. roseum* was correlated not only with pH, but also with silicon.

### P36.018 Induced resistance for control of dry rot of potato tubers with chemical elicitors

*Y.C. Li, Y. Bi, Y.H. Ge, Y. Wang and Y. Yin*
*College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, P. R. China*
*Email: biyang@gsau.edu.cn*

Dry rot of potato tubers caused by *Fusarium sulphureum* is one of the most important postharvest diseases restricting potato production and incidence of disease is up to 30% during storage in northwest of China. Primary control of pathogens is reported by postharvest application of fungicides, but public concerns over food safety require an investigation on the control methods with potentially less harmful to human health and the environment. The chemical elicitors treatments, including 0.25% chitosan, 100 mM sodium silicate, 100 mM β-aminonbutyric acid, 45 mM citric acid and 70 mM $K_2HPO_4$, significantly reduced the lesion diameter of potato tuber slices inoculated with *F. sulphureum*. The mechanism of induced resistance by these elicitors involved in ROS generation and antioxidative defense responses, increasing of the activity of defense enzymes such as peroxidase, polyphenoloxidase and phenylalanine ammonialyase and accumulation the contents of lignin, flavonoids and phenolics. Moreover, chitosan at 0.25% and sodium silicate at 100 mM also showed higher antifungal activities. These findings suggest that induced resistance by chemical elicitors could be a promising handling as a natural fungicide to partially substitute for the synthetic fungicides in potato tubers.

### P36.019 Cuticular wax of pear fruit during developmemt involved in the infection by *Alternaria alternata*

*Y.C. Li, Y. Yan, S.J. Chen, Y. Bi and Y.H. Ge*
*College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, P. R. China*
*Email: liyongcai@gsau.edu.cn*

Morphology and chemical composition of cuticular wax layers, represent the first site of contact with fungal pathogens, affect initial infection processes including conidial germination, appressoria and infection hyphae formation which are necessary for subsequent cuticular penetration of host surfaces. Dual role (prevention or facilitation of fungal invasion) of cuticular wax layers have been shown to be involved in the process of infection. *Alternaria alternata*, the causal agent of Alternaria rot of 'Pingguoli' pears (*Pyrus bretchneideri* Rehd cv. Pingguoli), could initially infect the fruit via the styles or peel of the fruit during the growing season and then remain in a latent state; also, the percentage of *A. alternata* colonization in the fruit peel was positively correlated with cuticle thickness. It was found that the attachment, growth and appressorium formation of *A. alternata* were inhibited by the wax of early period fruit development, the appressorium formation of *A. alternata* was induced by the wax of mature period fruit. *A. alternata* spores attached easily to fruit surfaces that had been dewaxed and this lead to better mycelium growth. Results of *in vitro* tests showed that conidial germination and mycelial growth of *A. alternata* could be inhibited by wax extracted from the fruit surface at different developmental stages. Studies are underway to elucidate the regulatory mode of the chemical composition or morphology of cuticular wax on infection behavior of *A. alternate* on 'Pingguoli' pears.

**P36.020 *Bacillus cereus* AR156 induces resistance against Anthracnose rot through priming of defense responses in loquat fruit**

*X.L. Wang, H.X. Liu, J. Wang, L. Wang, P. Jin and Y.H. Zheng*
*College of Food Science and Technology, Nanjing Agricultural University, Weigang No.1, Nanjing, 210095, Jiangsu province, P. R. China*
*Email: zhengyh@njau.edu.cn*

The biocontrol effects of *Bacillus cereus* AR156 on anthracnose rot caused by *Colletotrichum acutatum* in postharvest loquat fruit (*Eriobotrya japonica* Lindl. cv. Jiefangzhong) and the possible mechanisms were investigated. The results showed that *B. cereus* AR156 treatment resulted in significantly lower disease incidence and smaller lesion diameter compared with the control fruit. This treatment markedly enhanced activities of chitinase, β-1,3-glucanase, phenylalanine ammonia-lyase, peroxidase, polyphenoloxidase and promoted the accumulation of $H_2O_2$. Total phenolic content and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity were also significantly increased by *B. cereus* AR156 treatment. Fruit treated with *B. cereus* AR156 tended to maintain significant higher levels of sugars compared with the control fruit. Transcripts of three defense related genes were only significantly enhanced in fruit undergoing both *B. cereus* AR156 treatment and *C. acutatum* inoculation compared with those receiving either intervention alone. These results suggest that *B. cereus* AR156 can effectively inhibit anthracnose rot caused by *C. acutatum* and enhance antioxidant activity in loquat fruit through the priming of fruit defense responses.

**P36.021 Role of constitutive and induced defences in the differential resistance of some mango cultivars to anthracnose caused by *Colletotrichum gloeosporioides* and *C. acutatum***

*N.K.B. Adikaram, K.O.L.C. Karunanayake, G.D. Sinniah and C.L. Abayasekara*
*Department of Botany, University of Peradeniya, Peradeniya 20400, Sri Lanka*
*Email: n.k.b.adikaram@gmail.com*

Six mango (*Mangifera indica*) cultivars tested showed differential resistance to anthracnose caused by *Colletotrichum gloeosporioides* and *C. acutatum.* Resistance of unripe mangoes to anthracnose was previously shown to be due to antifungal resorcinols, gallotannins and chitinases. Unripe mangoes also respond to *C. gloeosporioides* challenge by localized generation of superoxide and $H_2O_2$ in early hours and enhanced PAL and chitinase activity. Autofluorescence was observed in challenged epidermal cells, indicating hypersensitive response. Two upregulated and 3 down regulated cDNA's were observed in fruits of resistant `Karutha Colomban' while there were seven down regulated and two down regulated genes in the susceptible 'Willard'. In general, induced defence responses were more prominent in 'Karutha Colomban' than the susceptible `Willard'. Constitutive gallotannin activity was generally high in the unripe fruit peel of all cultivars studied except in susceptible '*Willard*'. There was a significant negative correlation between the gallotannin level in the ripe fruit peel and the extent of anthracnose development. Concentration of 5-(12-*cis*-heptadecenyl) resorcinol was greater in cultivars more resistant to anthracnose and lower in more susceptible cultivars. The decline of resorcinols during ripening was faster in more the susceptible cultivar '*Willard*' compared to more resistant 'Karutha Colomban'. Cultivars, 'Gira' and 'Karutha Colomban' had significant antifungal activity in the non-aqueous phase of latex but lower chitinase activity in the aqueous phase. A higher chitinase activity was seen in the moderately resistant 'Rata' and 'Kohu'. The results indicated that both constitutive and induced defences contribute in different extents to the differential resistance of cultivars to anthracnose.

**P36.022 Postharvest myco-deterioration of watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai var. *Lanatus*) fruits**

*M.A. Abiala[1, 2], F.M. Ayandeko[1] and A.C. Odebode[1]*
*[1]Department of Botany, Faculty of Science, University of Ibadan, P. M. B. 128, Ibadan, Oyo State, Nigeria; [2]Department of Biological Sciences, Faculty of Natural Science, Ajayi Crowther University, Oyo State, Nigeria*
*Email: mos4me@gmail.com*

Postharvest pathogens most especially fungi are currently threatening storage of watermelon fruit in Nigeria and some other parts of Sub-saharan Africa. This study investigates postharvest fungal pathogens of watermelon fruit in Ibadan, Nigeria, with respect to their effects on chemical composition of watermelon fruit and the use of plant extracts (*Piper guineense, Xylopia aethiopica* and *Bambusa vulgaris* at 5%, 10% and 15% concentration levels) as phytofungicides against the fungal pathogens. Identification of the fungal pathogens after pathogenicity test re-affirm isolates identity as *Fusarium oxysporum, F. solani, Macrophomina phaseolina, F. verticilloides, Botryodiplodia theobromae, Aspergillus niger, A. flavus* and *Rhizoctonia solani.* All the fungal pathogens induced rot in the watermelon fruit after six days of inoculation. However, myco-deterioration activity of *M. phaseolina* was highly significant ($P \leq 0.05$) on chemical composition of watermelon fruit, followed by *F. solani* while other fungal pathogens were significantly ($p<0.05$) similar. The plant extracts significantly ($P \leq 0.05$) inhibited (*in vitro*) the mycelial growth of *F. solani, F. verticilloides* and *F. oxysporum* at concentration levels tested compared to other postharvest fungal pathogens. Antifungal effect of *X. aethiopica* and *B. vulgaris* significantly

(P≤0.05) varied in their phytofungicidal activities on *F. verticilloides, F. solani, F. oxysporum* and *B. theobromae*. The three plant extracts, most especially *P. guineense* were highly effective at 10% concentration level. This study showed the efficacy of the plant extracts on postharvest fungal pathogens, thus the plant extracts can be used as phytofungicides in the management of fungal pathogens and as preservative of watermelon fruits.

**P36.023 Characterization and safety analysis of one strain of *Fusarium asiaticum* causing postharvest disease of asparagus spears**

*P.K. Zhu, Y.W. Wang, L.Q. Wu, L. Huang , L.Y. Liu and L. Xu*
*School of Life Science, East China Normal University, 500 Dongchuan Road, Shanghai 200241, P. R. China*
*Email: lxu@bio.ecnu.edu.cn*

Asparagus spears are usually vulnerable to pathogenic microorganisms. In this study, one highly virulent fungus (designated EXAP-08) was isolated from the rotted asparagus spears in cold storage. Through morphological characteristics, ITS sequencing, and species specific molecular marker analysis, EXAP-08 was characterized as *Fusarium asiaticum*, which is one of the 9 phylogenetically subgroups of *F. graminearum* clade. Koch's postulates were checked through pathogenicity tests, indicating that EXAP-08 infection could cause reproducible rot symptoms similar to those observed on naturally infected asparagus spears. The optimum temperature for the growth of EXAP-08 was 25℃, and the optimal pH ranged from 5 to 8. Inoculation of EXAP-08 could additionally cause serious rot disease on a broad range of postharvest fruits and vegetables. Further identification confirmed that the EXAP-08 belongs to 3A-DON (3-acetyl-4-deoxynivalenol) chemotype, and it was found to produce the mycotoxin during the infection of plant, implying the potential risks of mycotoxin contamination in fresh crops infected by this pathogen. The expression of mycotoxin production related genes of this fungus could be regulated by environmental factors including temperature, light, and air composition, indicating the possible approaches to reducing the risks of the pathogen during postharvest storage. However, this emerging pathogen threatening edible safety of fresh postharvest crops should deserve particular quarantine inspection in the future.

**P36.024 Antifungal effect of nitric oxide on mycelium growth, sporulation and spore germination of the postharvest horticulture pathogen of *Botrytis cinerea***

*Q. Wang[1,2], Q. Wang[2], L.P. Gao[2], N. Yang[2] and M. Li[1]*
*[1]Anhui Province Key Lab of Agricultural Produce Quality Control, College of Plant Protection, Anhui Agricultural University, No.130 West Changjiang Road, Hefei, 230036, P. R. China; [2]Beijing Vegetable Research Center, Beijing Academy of Agriculture and Forestry Sciences, Banjingcun 2449, Haidian District, Beijing, 100097, P. R .China*
*Email: plantprotection2006@126.com*

Nitric oxide (NO) is an important signaling molecule, which has shown diverse physiological functions in plants. In this present study, using sodium nitroprusside (SNP) as a NO donor, the antifungal activity of NO against the growth of the postharvest horticulture pathogen *Botrytis cinerea* under *in vitro* conditions were evaluated. The results showed that all concentrations of SNP aqueous solution were found to produce an antifungal effect on spore germination, sporulation and mycelial growth of *B. cinerea*, with the most effective concentration for *B. cinerea* being 3 mmol/L. The contents of MDA, soluble carbohydrate and soluble protein increased rapidly for *B. cinerea* treated with 3mmol/L SNP aqueous solution. Furthermore, the result of spore staining with oxdiant sensitive probe 2,7-dichlorofluorescin (DCHF-DA) indicated that more cells at 3mmol/L SNP treatment were stained compared with the control. These results suggested that short-term exposure to a low concentration of NO was able to inhibit the subsequent growth of *B. cinerea*, which might be related with the damage of NO in high concentration to the cellular oxygen-elimination system, the enhancement of lipid peroxidation and the generation of ROS which subsequently causes severe oxidative damage to the process of spore germination of *B. cinerea*.

**P36.025 Effect of benzothiadiazole on disease resistance and related cell wall modification enzyme activity of harvested banana fruits**

*X.P. Li, L. Zhang, Z.W. Si, Y. Zou, S. Rao and W.X. Chen*
*State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources/Guangdong Provincial Key Laboratory for Postharvest Science and Technology, College of Horticulture, South China Agricultural University, Guangzhou 510642, P. R. China*
*Email: lxp280477@yahoo.cn*

The effects of benzothiadiazole (BTH) on induction of disease resistance in harvested banana fruits and the activities of several cell wall modification enzymes were investigated. Harvested banana fruits were soaked with 200 $\mu g \cdot ml^{-1}$ of BTH solution before being stored at (22±2) ℃. The disease incidence and six cell wall modification enzymes were monitored during the storage. The results showed that, compared with the control, BTH treatment significantly reduced the disease indices of banana fruits. Moreover, the activities of Polygalacturonases (PG), Pectate lyase (PL), Cellulase (CX), Xyloglucanase and Galactosidase relating to softening were found to be suppressed by BTH treatment, and thus the stability of cell-wall was hold. Therefore, it suggested

that the enhanced disease resistance in harvested banana by BTH treatment may result from the activation of the disease-related defense system.

**P36.026 Postharvest hot water dipping reduces decay by maintaining firmness of muskmelon (*Cucumis melo* L.) fruit**

*Y. Wang, L. Yuan, Y.H. Ge and Y. Bi*
*College of Food Science and Engineering, Gansu Agricultural University, Lanzhou 730070, P. R. China*
*Email: 99244392@qq.com*

Muskmelons (*Cucumis melo* L.) are major economic crops in northwestern of China, and postharvest decay of fruit is serious. In order to reduce postharvest decay and prolong the storage time, in this study, muskmelon fruit (cv. Yujinxiang) were dipped in hot water at 53℃ for 3 min to study the effect of hot water dipping (HWD) treatment on nature incidence, and the correlation among nature incidence, firmness and ethylene production. The results showed that postharvest HWD treatment effectively ($p<0.01$) reduced the natural incidence, 44.4 % lower than that in control fruit after 40 days of storage, maintained firmness which was 37.7 % higher than the control at 15 day after treatment, and inhibited the ethylene production, 59.1 % lower than control at 9 d of storage. And the data did show a high correlation between fruit firmness and nature incidence ($R2= 0.87$) or ethylene emission ($R2= 0.70$). The HWD treatment also noticeably reduced cellulose and pectin degradation by suppressing the activity of endo-1, 4-β-d-glucanase (EGase), β-glucosidase (β-Glu), polygalacturonase (PG) and pectinesterase (PME) of fruits, and promoted the accumulation of hydroxyproline-rich glycoproteins (HRGPs), lignin, suberin and callose. Furthermore, the HWD treatment could clean the surface of fruits, melt the epicuticular waxes, cover and seal the stomata. These results indicated that postharvest HWD treatment reduced nature incidence by maintaining the firmness in muskmelon fruit, and suggested that postharvest HWD treatment could reduce or/and substitute chemical fungicides to control postharvest decay in fruits.

**P36.027 Application of *Pichia anomola* strain SRF to control postharvest blue mold on apples under storage conditions**

*D.P. Zhang, W.C. Liu, D. Dong, C.G. Lu, H.L. Wu and D.W. Liu*
*Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Haidian District, Beijing, 100097, P. R. China.*
*Email: dreamstar26@126.com*

Fruit and vegetables are highly perishable products, especially during the postharvest phase. Blue mold caused by *Penicillium expansum* is one of the most destructive diseases of pears and apples, and it is accompanied by the production of patulin, a mycotoxin with immunosuppressive effects on humans. A yeast strain SRF isolated from apples (cv. Golden Delicious) in organic orchard was demonstrated to be *Pichia anomola* by sequence comparisons of 26S rDNA D1/D2 domains. The strain showed a strong activity against blue mould on apples. In apple juice media, the yeast at concentration of $10^8$, $10^7$ and $10^6$ cells $ml^{-1}$ inhibited the spore germination of *P. expansum* by 100%, and on wound-inoculated apples, controlled the blue mold decay also by 100%. Co-culturing *P. expansum in vitro* or *in vivo*, the inactivated cells had no significant effect on spore germination or germ tube elongation of the pathogen, however, the culture filtrate of the yeast showed inhibition activity on the germ tube elongation of the pathogen and this activity was lost at the present of the protein denaturants, suggesting that the production of secreted anti-fungus proteins. Under storage conditions, at 2℃ for 60 days, *P. anomola* SRF, used at $10^7$ cells $ml^{-1}$, reduced the blue decay incidence on apples (cv. Golden Delicious) from 20.5% (Control treatments) to 10.0%. Our preliminary study showed that the yeast *P. anomola* strain SRF had a potential to control postharvest blue mould decay on apples.

## Concurrent Session 37-Precision Agriculture and Plant Pathology

### O37.001 Sensing of diseases - prospects and limitations

*E.C. Oerke, A.K. Mahlein and U. Steiner*
*University of Bonn, INRES – Phytomedicine, Nussallee 9, D-53115 Bonn, Germany*
*Email: ec-oerke@uni-bonn.de*

The detection, identification and quantification of diseases at low levels are prerequisite for site-specific control of plant diseases. Sensors have to spot specific symptoms at early stages of epidemics in order to enable the operator to initiate effective disease control. Sensor technologies tested for their suitability and reliability in disease sensing include mechanical, optical (spectral reflectance, thermography, chlorophyll fluorescence), and (bio-) chemical (electronic nose) sensors. Despite of the pros and cons for the technical systems, the biological systems limit sensor use in (decision making for) disease control. As early symptoms are often inconspicuous, spatial resolution of sensors should be high at the plant level. Pathogens may produce first symptoms near the ground and subsequently spread to canopy top in relevant intensities. Sensors have to inspect the relevant lower plant parts, also in case upper parts interfere with the system. Polycyclic diseases caused by airborne pathogens require other sensing intervals than diseases from stationary pathogens. Pathogen spread and latent infections have to be considered by using additional safety distances in disease control. The huge amount of data produced by imaging systems has to be reduced to the essential features in order to facilitate rapid processing. These requirements affect the sensor type and the way it may be used (e.g. offline *vs*. online). In case the action threshold for disease control is zero, precision crop protection technologies may be used for more accurate disease forecasting and the sensing of inoculum (e.g. molecular diagnosis of airborne inoculum).

### O37.002 Precision fungicide application based on infrared sensor technique in orchard

*X.K. He*
*College of Science, China Agricultural University, P. R. China*
*Email: xiongkui@cau.edu.cn*

A new tractor mounted automatic target detecting orchard sprayer was designed to meet the demand of chemical fungi-control in orchards. This sprayer characterized high efficiency, saving fungicide and being friendly to environment. The techniques of automatic target detecting, electrostatic and air-assisted spraying were applied in this sprayer. Compared with conventional air-assisted orchard sprayer can save 30-50% pesticides, the electriferous droplets projected towards to the target by the assist of airstream can increase the penetrability and the deposit of fungicides on the trees, which can decrease 30% the loss of chemicals with automatic techniques combined with spraying techniques on this sprayer, when the detecting devices detect the target, automatic control system will activate spraying system to spray toward the target. Infrared sensor detecting technique has been adopted in target detecting orchard sprayer to discern targets and control the spraying system automatically. This new sprayer is to be commercialized easily for the low price of infrared sensor detector. Experimental results show that the new automatic target detecting orchard sprayer with an infrared sensor can save more than 50 to 75% fungicides, improving the utilization rate (above 55%) and control efficiency and decreasing significantly environment pollution caused by the chemical application.

### O37.003 Risk analysis for fungicide use in corn and wheat disease management

*P.A. Paul and L.V. Madden*
*Department of Plant Pathology, the Ohio State University, OARDC, Wooster, OH 44691 USA*
*Email: paul.661@osu.edu*

Fungicides are now an integral component of corn and wheat production systems in the U.S., not only because they are important for controlling diseases and minimizing losses, but also because some fungicides have physiological effects on both crops and as such may contribute to yield increases even when disease levels are relatively low. However, there is great uncertainty as to when and where fungicides will be most economically beneficial, since grain prices in the U.S. and yield responses are often not high enough to offset application cost. Consequently, producers are not only interested in the efficacy of the fungicide they apply, but also in the expected benefit of similar applications in future growing seasons. Case studies on the application of fungicides to manage Fusarium head blight of wheat and gray leaf spot of corn will be used to demonstrate how data from previous studies can be utilized to estimate the probability of future responses to fungicides and to assess the economic value of such responses. We will demonstrate and discuss the use of meta-analysis, a method of quantitative research synthesis, to estimate mean responses to fungicides and to analyze the risk of a grower losing money for a range of expected yield and disease responses, grain prices, and fungicide application costs. This type of quantitative risk analysis could be incorporated into precision agriculture programs to help guide the application of fungicides when they are most warranted and most likely to be economically beneficial.

**O37.004 China-blight — A web based DSS on potato late blight management in China**

*T.L. Hu, Y.X. Zhang and K.Q. Cao*
*College of Plant Protection, Agricultural University of Hebei, Baoding 071001, P. R. China*
*Email: tonglehu@yahoo.com*

Potato, the fourth important food crop in China, is planted mainly in 22 provinces, municipalities and autonomous regions. China has become the biggest potato production country in the world. Late blight caused by *Phytophthora infestans* de Bary is one of the most devastating disease of potato worldwide. Due to the shortage of resistance of cultivars in most cases, chemical control is still the main method to manage the disease right now. In order to improve the control efficiency, a web based DSS (Decision support system) on potato late blight management in China --- "China-blight" (www.china-blight.net) was developed since 2008. This DSS is composed of the three sub-systems of "Real-time distribution of potato late blight in China", "Infection risk of late blight pathogen based on MISP (Mass Infection and Sporulation Period) model and hourly weather data" and "A farm based simple DSS for the chemical control on potato late blight". Besides, knowledge information as well as services such as "IPM methods on late blight", "Fungicides database", "Other pests on potatoes", and "Questions & experiences exchange" were also included. At present, during potato growing season farmers from 11 provinces, municipalities and autonomous regions in northern China can receive a weekly newsletter sent by email to get decision support information or visit the web cite for daily updated information to help them for chemical control of late blight. In the future, more and more farmers from other region in China will be involved in the service.

**O37.005 Three models for fungicide dosage adjustment in vineyard**

*J.Z. Zhou[1], A.J. Landers[2] and X. He[3]*
*[1]Shenyang Research Institute of Chemical Industry, No. 8 Shenyang Dong Road, Tiexi District, Shenyang, Liaoning, 110021, P. R. China; [2]Cornell University, 312 Barton Lab., NYSAES, 630 West North St., Geneva; [3]China Agricultural University, No.2 Yuanmingyuan West Road, Haidian District, Beijing, China, 100193, P. R. China*
*Email: xiongkui@cau.edu.cn*

For fungicides are applied in vineyard, cautions should be exercised. It's common that too much fungicide liquid are sprayed resulting in huge pollution to the environment. Fungicides should be targeted at the canopy precisely. Considering the whole fungicide application system in vineyard, it's thought that the used traditional application method, particularly in early to mid-season application, caused large losses to the air and the ground. In this paper, three alternative models for fungicide dosage adjustment, Unit Canopy Row (UCR) (Australia), *DOSAVIÑA* (Spain and USA) and Fruit-Wall Area (FWA)(Germany and Belgium), were described, and the results in the trial were present. Results demonstrated that at early growth stage, deposits were not different from each other among DOSAVIÑA, LWA and UCR (application rate was 327, 281, 234 and 187 L/ha for the traditional, LWA, DOSAVIÑA, and UCR, respectively). Concerning the uniformity of distribution, UCR was the model chosen to apply at early growth stage, then the LWA model, from the safety point. At the middle growth stage, LWA and the traditional method still achieved similar results. LWA method achieved the best results in terms of deposits and uniformity of distribution. At the later growth stage, the traditional treatment achieved the highest deposits, while LWA and DOSAVIÑA achieved similar deposits. The results showed a high potential for reducing application rates using alternative models compared with the traditional method. Further biological efficacies need to perform to validate the dosage adjustment models.

**O37.006 Precision monitoring, quantification and management of bacterial blight under rice precision farming**

*M.B. Patil, N.L. Rajesh , B.G.M. Reddy, S.B. Gowdar, Guruprasad and Parameshwar*
*University of Agricultural Sciences, P.B.no.329, Raichur-584 102, Karnataka, India*
*Email: patilmb_65@yahoo.com*

The incidence and severity of bacterial leaf blight of rice (*Xanthomonas oryzae* pv. *oryzae* ) was assessed for its spatial and temporal variability in the rice precision farming experimental fields of Main Agricultural Research Station, Gangavathi under University of Agricultural Sciences, Raichur in south India during *Kharif* 2011 and 2012 as a part of Precision Farming Project activity funded by State Government. The 2.25 acre of experimental field was divided in to grids of 10m×10m size with total 90 grids and the grid wise disease data was recorded along with other parameters of soil fertility, crop response to biotic and abiotic stress and yield. GIS mapping was done for the data on the spatial and temporal variability after geo-referencing the each grid with GPS [Trimble's Geoexplorer ] of sub meter accuracy. Based on the quantification of the spatial and temporal variability the variable rate of application of anti bacterial management practices were taken up based on the prescription GIS maps. The blight incidence ranged from 5 % to 10% across grids spatially and 10 to 20% temporally across the seasons. The correlations between nutrient, disease and yield were worked out on grid basis. This study shows that, use of Precision Agriculture tools like GPS, GIS and variable rate technology in rice farming serves greatly the farmers in precision monitoring and management of blight disease and saves the cost and time while safeguarding rice ecosystem.

## Concurrent Session 38-Scientific Publications

### O38.001 Food security: birth of the ISPP Journal

*R. N. Strange[1] and P. Scott[2]*
*[1]Department of Biological Sciences, Birkbeck College, London University, Malet Street, London WC1E 7HX, United Kingdom; [2]CAB International, Wallingford OX10 8DE, United Kingdom*
*Email: r.strange@sbc.bbk.ac.uk*

Norman Borlaug, the Nobel Peace Prize Laureate was the inspiration of the ISPP Journal *Food Security: the science, sociology and economics of food production and access to food.* Fifteen years ago at ICPP 1998, which was held in Edinburgh, UK, he threw down the gauntlet to the ISPP by asking what the Society was doing about food security. A number of initiatives resulted, including the inception of a Task Force for Global Food Security (see the Plenary Session P2 on Tuesday 27th August and the associated evening session on Wednesday 28th August). A second spur to contemplating the possibility of starting a journal was the invitation to write a review for the Annual Review of Phytopathology with the title *Plant Disease: a threat to global food security* (Strange and Scott, 2005). The final spur came from someone who said to me, "Why don't you start a journal?" Peter canvassed the views of many eminent scientists whom he met in the course of his work for CABI. Meantime, I surveyed the literature and found that putting the two words "food" and "security" in a search engine turned up a large number of papers but, with few exceptions, they were thinly spread over a large number of journals. The rationale for a journal of food security was staring us in the face! And Springer agreed!

### O38.002 The contribution of a generalist plant pathology journal to dissemination and scientific awareness of the impact of plant disease

*M.J. Jeger*
*Division of Ecology and Evolution and Centre for Environmental Policy, Imperial College London, Silwood Park campus, Ascot SL5 7PY, United Kingdom*
*Email: m.jeger@imperial.ac.uk*

Plant Pathology is a curious amalgam of scientific disciplines brought together to deal with the phenomenon of plant disease. In terms of biological hierarchies, plant pathologists can operate as molecular biologists, plant physiologists, plant breeders, population biologists and epidemiologists; or in a broader sense as agriculturalists, horticulturalists, landscape managers and ecologists. The host plants range from tropical to temperate, annual to perennial, herbaceous to woody, open field to protected, monoculture to mixed cropping, and from managed to semi-natural environments. From the pathogen side, the organisms range from viruses and viroids to bacteria, oomycetes, fungi, nematodes, arthropod vectors of disease, and indeed parasitic plants. As a consequence it can be extraordinarily difficult to present a coherent body of knowledge with a shared motivation, methodology, terminology and vocabulary. Specialist journals exist representing the ranges in biological hierarchy, crops and cropping systems, and pathogen taxa noted above as relevant in plant pathology. The question is: what does a generalist plant pathology journal, covering all of the above dimensions, have to offer an intending author wishing the widest research exposure? Where is the desired impact to be seen? Traditionally most generalist plant pathology journals originated, sometimes directly, sometimes obliquely, in the context of a scientific society. Examples are the journals of the America Phytopathological Society and the British Society for Plant Pathology. Even here there are new journals that stress the different hierarchies of research, more comparable with specialist journals. Finally the continuing development of Open Access online journals will pose a challenge to the current mode of publishing for generalist plant pathology journals.

### O38.003 Adding value in journal publishing: Your paper is more than a paper at Springer

*Z. Bernhart*
*Springer Science + Business Media B.V., Van Godewijckstraat 30, 3311 GX Dordrecht, The Netherlands*
*Email: zuzana.bernhart@springer.com*

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peace of mind to enable you to focus on what is most important: your research.

**O38.004 Publishing scientific papers in the new world order**

*P.A. O'Brien*
*School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA 6150, Australia*
*Email: P.Obrien@murdoch.edu.au*

Scientific publications originally used as a means of communicating the results of research are increasingly used as an assessment of the quality of the research and the researcher. Pressure is placed on scientists to not only publish their results, but to publish in journals with high impact factors. At stake is tenure, promotion, and research funding. This is driven by government agencies who to see how their money is being spent and by universities who want their researchers to enhance the prestige of the institution so that it can become a top ranked institution. Competition for research funding has increased significantly. There are now 7.1 million researchers compared to 5.7 million in 2002. The geography of science has also changed with newer countries such as China, Korea and Brazil challenging the traditional powerhouse countries, Britain, France, Germany and the USA for scientific domination. All of this has had a direct impact on the publication process. We have seen more papers submitted to journals, with higher rejection rates sparking the genesis of a plethora of online journals, often with more relaxed standards. This paper looks at how the publishing of scientific papers will cope with changing developments in world science and with the application of new technologies to communication of scientific results.

**O38.005 Working successfully with APS journals**

*M.L. Gleason*[1] *and G.W. Sundin*[2]
[1]*Department of Plant Pathology and Microbiology, 351 Bessey Hall, Iowa State University, Ames, IA 5001, USA;*
[2]*Department of Plant, Soil, and Microbial Sciences, 578 Wilson Rd., Michigan State University, East Lansing, MI 48823, USA*
*Email: mgleason@iastate.edu; sundin@msu.edu*

An increasing percentage of manuscripts submitted to journals of the American Phytopathological Society (APS) - *Plant Disease, Phytopathology, Molecular Plant-Microbe Interactions,* and *Plant Health Progress* - originate from countries other than the United States. APS welcomes this trend because it contributes to making the journals truly international in scope. Some authors experience frustration during the submission process, however, due to misunderstandings concerning requirements for acceptance. Mark Gleason and George Sundin, the current Editors-in-Chief of *Plant Disease* and *Phytopathology*, respectively, will offer practical suggestions to help the review process run more smoothly for authors. Included in the presentation will be strategies for enhancing clarity of English writing, meeting requirements for experimental design of trials and statistical analysis of data sets, dealing with the need for repeating experiments, including sufficient detail so that methods can be replicated, working productively with Senior Editors and APS editorial staff, and consulting the online Instructions to Authors as well as Editors-in-Chief before submitting manuscripts. Our goal is to encourage and help authors to publish their work in APS journals.

**P38.001 E-mycelium-Plant pathology in the e-connected era**

*G.I. Johnson*
*Horticulture 4 Development, PO Box 412 Jamison ACT 21614, Australia*
*Email: greg.johnson@velocitynet.com.au*

As the 21st century advances, like some vast e-mycelium spawned in the 1990s, the global plant pathology community is becoming increasingly e-connected. Access to scientific literature, conference presentations and papers, diagnostic advice, photographs of disease symptoms, pathogens, molecular classification protocols, as well as a vast network of colleagues and expert (and not-so expert) opinions are just a click or two away. Aside from websites, opportunities to Skype, to Tweet, to Pinterest, to Scoop.it, to post on PestNet and report to Pro-Med, to sign-up for journal alerts and to friend on Facebook are easily accessible for most of us. How useful are tools for solving real problems in the plant pathology world? Are the traditional approaches to agricultural extension still relevant? What is the place now for training workshops and conferences like ICPP 2013? How can we make the best use of these e-nitiatives for ourselves and for training and extension? This poster shows the connectedness of plant pathology tweeters (top plant pathological tweeters have *c.* 2000 followers) and illustrates some of the current Plant Pathology/Plant Pathogen that ramify through our e-planet. See https://twitter.com/Plant_Pathogens, https://twitter.com/Food_Security and http://pinterest.com/plantpathogens/.

## Concurrent Session 39-Soil-borne Plant Diseases and Their Control

### O39.001 Predicting soil-borne diseases as a pro-active management tool

*A.K. Lees and J.L. Brierley*
*Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK*
*Email: Alison.Lees@hutton.ac.uk*

Real-time PCR diagnostic assays for the detection and quantification of many soil-borne pathogens and pests of potato have previously been developed and validated. However, factors including suitable field sampling strategies, delivery of advice to growers, timely throughput of results and cost need to be considered before quantitative diagnostic assays can be used effectively as a predictive tool for disease management. As a result, relatively few assays are currently being used on a large scale.

Epidemiological knowledge of individual diseases is also critical for accurately identifying relevant pathogen inoculum threshold levels, interpreting results and making control recommendations. Additionally, many environmental and agronomic parameters influence disease development and must be factored into any practical management advice. Successful adoption of predictive diagnostic tools to inform management decisions therefore relies on a truly integrated approach to delivery.

Specific examples of the varied progress and challenges in predicting potato diseases for the pro-active delivery of advice will be given, focusing on soil-borne pathogens of potato (e.g. *Spongospora subterranea*, *Rhizoctonia* and *Colletotrichum coccodes*). Research that aims to unravel the epidemiology of diseases, for example the relative importance of seed and soil-borne inoculum, inoculum thresholds and the effect of environmental parameters, whilst concurrently establishing the impact of control measures, will be highlighted. The translation of this knowledge into advice, the perceived and actual benefits in terms of disease reduction and the commercialisation of tests will be reviewed.

### O39.002 Metagenomics of the rhizosphere microbiome to identify disease suppressive bacteria

*R. Mendes[1], M. Kruijt[1], I. de Bruijn[1], E. Dekkers[1], M. van der Voort[1], J.H.M. Schneider[2], Y.M. Piceno[3], T.Z. DeSantis[3], G.L. Andersen[3], P.A.H.M. Bakker[4] and J.M. Raaijmakers[1]*
*[1]Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands; [2]Institute of Sugar Beet Research, Bergen op Zoom, The Netherlands; [3]Ecology Department, Lawrence Berkeley National Lab, Berkeley, California, USA; [4]Plant-Microbe Interactions, Department of Biology, Utrecht University, Utrecht, The Netherlands*
*Email: jos.raaijmakers@wur.nl*

Disease-suppressive soils are exceptional ecosystems in which beneficial microorganisms effectively guard plants against soil-borne pathogen infections. For most disease-suppressive soils, however, the microorganisms and mechanisms involved in disease suppression are not known. To identify such disease-suppressive microbes, PhyloChip-based metagenomics of the rhizosphere microbiome was performed and coupled with culture-dependent functional analyses to identify bacterial taxa and mechanisms involved in soil suppressiveness to the fungal root pathogen *Rhizoctonia solani*. The PhyloChip analyses led to the identification of more than 30,000 bacterial and archaeal taxa from soils with different levels of disease suppressiveness. The results of these analyses specifically pointed to the Proteobacteria, Firmicutes and Actinobacteria as the most dynamic groups associated with disease suppression. Although the richness of these bacterial taxa was not significantly different between suppressive and conducive soils, their relative abundance correlated well with the different levels of disease suppressiveness. Targeted isolation and functional analyses led to the identification of specific members of the γ-Proteobacteria with *in vitro* and *in situ* activity against *Rhizoctonia solani*. In conclusion, this study provides new insights into rhizobacterial diversity and fundamental mechanisms underlying multi-trophic interactions in natural disease suppressive soils.

### O39.003 Development of innovative approaches to control soil-borne fungal pathogens: resistance to *Fusarium oxysporum*

*Z. Hu[1], U. Parekh[2], N. Maruta[2], S. Poligoru[2] and J.R. Botella[2]*
*[1]Bioengineering College, Chongqing University, Chongqing, P. R. China; [2]Plant Genetic Engineering Laboratory, School of Agriculture and Food Sciences, University of Queensland, Brisbane 4072, Australia*
*Email: j.botella@uq.edu.au*

Collectively, "soil-borne plant pathogens" cause immense loses in agricultural production but due to their biological diversity it is almost unthinkable to devise a single strategy to confer broad spectrum resistance. Genetic engineering (GE) approaches can prove very useful in cases were natural resistance is not available or is available in non-compatible species. We have previously developed a technology named Host Derived-RNA interference (HD-RNAi) in which plant cells are used as delivery systems to induce an RNAi response in the pathogen. We applied HD-RNAi to nematodes and were able to prove specific silencing of the targeted gene in nematodes feeding on transgenic plants carrying HD-RNAi constructs. We have now adapted the technology to control fungal pathogens with promising results. Using HD-RNAi we have produced transgenic

Arabidopsis plants with increased resistance to the soil pathogen *Fusarium oxysporum*. We have targeted three different fungal genes that have conferred different degrees of protection. We will present proof that the targeted fungal genes are silenced in fungi feeding on the transgenic plants. In addition we will report the development of a novel methodology to control Fusarium by interfering with the plant's Jasmonic acid response pathway. Using our unique approach we have tested a number of different genetic constructs with some of them conferring 95%-100% Fusarium resistance in all transgenic lines analyzed. This new platform technology can be easily transferred to important crops such as banana and wheat to confer Fusarium resistance.

**O39.004 Integrated management of strawberry and tomato soilborne diseases**

*F.J. Louws*
*Director Center for Integrated Pest Management,1730 Varsity Drive, Suite 110, NCSU Centennial Campus, Raleigh, NC 27606, USA*
*Email: Frank_louws@ncsu.edu*

Integrated disease management of soilborne diseases requires an infrastructure of knowledge and experience rooted in a comprehensive understanding of the biology of the pathosystem within a farming systems framework. Knowledge and experience arises from well-funded programs at public and private institutions that enhance the science of plant pathology and farming systems research. Such programs are best informed by real-world issues through collaborative research and extension efforts that engage local extension personnel, growers and other key stakeholders. Collaborative efforts can lead to capacity to integrate historical knowledge and enable forward movement of relevant expertise, including effective use of novel high throughput diagnostic technologies. In North Carolina and surrounding Southeastern USA States, the loss of methyl bromide as a soil fumigant has afforded an opportunity to systematically explore IPM-tactics to manage soilborne diseases in strawberry and tomato production systems. The region-wide IPM research and extension program included: 1) tactic substitution that addressed short term needs of growers who sought non-ozone depleting fumigant alternatives; 2) tactic diversification efforts that focused on medium term alternatives including non-fumigant and IPM based tactics such as the use of tomato grafting technologies; and 3) tactic development that explored long-term goals to enhance microbial ecology and farming systems-based approaches to replace MeBr-dependent production systems including the development of organic or other non-fumigant-dependent systems. IPM research was accomplished in research plots and through farmer-driven on-farm-research efforts and information was translated through large scale field programs and region-wide outreach efforts. IPM programs developed were informed by new knowledge gained about the biology of each pathosystem, including the development of molecular tools and information about the diversity and dynamics of key pathogens. Major challenges in IPM of soilborne diseases remain but current and future advances properly integrated into production systems portend enhanced programs that will serve our growers and clientele well.

**O39.005 Use of molecular diagnostics for improved decision-making**

*A. McKay and K. Ophel-Keller*
*South Australian Research and Development Institute (SARDI), GPO Box 397, Adelaide, South Australia, Australia 5001*
*Email: kathy.ophelkeller@sa.gov.au*

Reliable prediction of the impact of soil-borne pathogens is an important first step in the uptake of integrated disease management strategies. DNA-based assays to quantify soil-borne pathogens have been developed over the past 10 years, with the ultimate aim of providing rapid tests for growers to make informed and timely decisions prior to planting a crop. A testing service has been available to cereal growers in Australia since 1997 (Ophel Keller *et al* 2008). The service is based on a representative soil sample from which DNA is extracted and quantitative PCR (QPCR) assays applied. The service now offers tests for 12 pathogens including nematodes (*Heterodera avenae*, *Pratylenchus neglectus* and *P. thornei*) and fungi (*Rhizoctonia solani*, *Gauemannomyces graminis* varieties, *Fusarium pseudograminearum* and *F. culmorum*). The value of the service is derived from linking the test result to a practical management decision for growers. A training program linked to a comprehensive manual on root disease management allows consultants to make this link. Research use of QPCR assays for soil organisms offers powerful insights into the impact of management strategies such as crop rotation and resistant varieties on pathogen inoculum levels. Australian researchers have adopted this technology as a routine method for assessing nematode resistance and tolerance for cereal breeding programs. QPCR technology is used to monitor the impact of rotation crops on pathogen levels in research trials. This information assists in development of management strategies, and may increase grower use of pre-plant testing to assess disease risk.

**O39.006 Multifunctional *Trichoderma* isolates and their application for disease bio-control and plant growth-promotion**

*Y.H. Tong[1], P.R. Harvey[2], L.J. Shun[1], Z.X. Jian[1]and Z.G. Zhi[1]*
*[1]Biology Institute of Shandong Academy of Sciences, No. 19, Keyuan Road, Jinan 250014, P. R. China; [2]CSIRO*

*Sustainable Agriculture National Research Flagship, CSIRO Ecosystem Sciences, Postal, PMB 2 Glen Osmond, South Australia, 5064, Australia*
*Email: yanght@keylab.net*

*Trichoderma* spp. have been successfully commercialised worldwide as bio-control inoculants for numerous plant diseases. Strains of *T. harzianum, T. atroviride, T. viride* and *T. asperellum* are those most commonly registered for disease suppression. Modes of action have been reported to include mycoparasitism, synthesis of anti-microbial metabolites, induced disease resistance and microbial competition. Many disease suppressive *Trichoderma* inoculants are multifunctional, also exhibiting plant growth-promoting (PGP) characteristics. *Trichoderma* isolates have been identified to produce phytohormones, mineralise organic phosphate (phytate) and increase availability of sparingly-soluble inorganic phosphate, potassium and micronutrients, thereby promoting plant growth. Recently, *Trichoderma* inoculants capable of degrading synthetic pesticides (organophosphates, organochlorines) and fungicides (carbendazim) have been identified. Consequently, these multifunctional inoculants have potential to enhance crop productivity and food safety, via decreased reliance on synthetic pesticides and bio-remediation of contaminated soils. Conidia are the primary inoculum source of most formulated products, although enhancing chlamydospore formation offers potential to increase the shelf-life of *Trichoderma* products. The majority of inoculants are produced by liquid-solid state fermentation and formulated as a wettable powder for application to seeds, foliage or directly to soil. Our research is applying molecular ecological and functional genomic approaches to define the rhizosphere competence of *Trichoderma* inoculants and the mechanisms by which they suppress disease, promote plant growth and degrade pesticides. Species- and strain- specific q-PCR have been used to quantify colonisation and soil persistence of inoculants, with non-target impacts on soil-borne fungal and bacterial communities assessed by T-RFLP analyses. Ongoing research is defining the spatial and temporal competence of *Trichoderma* strains and linking this with function. Integrating quantitative diagnostic (taxa-specific) and functional analyses will help define agro-ecological conditions that enhance *in situ* survival and activities of *Trichoderma* inoculants. These studies will contribute novel biological options and management strategies to enhance productivity and minimise environmental impacts of plant production.

### O39.007 Transgenic disease resistance – can this be used to control soil-borne pathogens?

D.B. Collinge
*Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871-Frederiksberg C, Denmark*
*Email: dbc@life.ku.dk*

Disease resistance is undoubtedly the most effective means for controlling plant diseases – when available. Unfortunately, effective natural sources of disease resistance are rarely available for necrotrophic pathogens. Soil-borne pathogens are often necrotrophic and it is therefore often difficult to combat the diseases they cause using resistance. Can transgenic disease resistance offer attractive approaches for controlling these difficult diseases? As far as I am aware, there are currently no success stories where transgenic disease resistance against fungal pathogens has been introduced into the field, let alone against soil-borne diseases. Is this entirely due to the caution of authorities given the negative public opinion that encompass transgenic technologies or is this because no effective solutions exist yet? Which biological approaches are the most promising? Are there prospects that they can provide effective control against soil-borne diseases? I will present the issues and discuss the biological prospects for transgenic disease control. In our own research, we are studying the mechanisms used by cereals to perceive and react against pathogen attack. Our efforts are concentrated on the NAC and CRK gene families of cereals which are transcription factors and cysteine-rich receptor-like protein kinases, respectively. Characterisation of specific members of these gene families has demonstrate that suppression of HvNAC6 but not HvNAC1 or HvNAC4 expression by RNAi results in increased susceptibility to *Blumeria graminis*. In contrast, RNAi studies with HvCRK1 resulted in increased resistance to *Blumeria*. These studies are being extended to other members of the gene families as well as to other pathogens of cereals.

### O39.008 Nematode suppression by endophyte-associated tall fescue

S. L. F. Meyer and A. P. Nyczepir
*Nematology Laboratory, United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Building 010A, Room 112, BARC-West, 10300 Baltimore Avenue, Beltsville, MD 20705 USA; USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA 31008, USA*
*Email: Susan.L.Meyer@ars.usda.gov*

Tall fescue is planted as a forage and turf grass and a postplant ground cover for reducing soil erosion. It withstands drought and is resistant to various pests, including some plant-parasitic nematodes. The presence of the endophytic fungus *Neotyphodium coenophialum* can increase tall fescue growth and survival and the ability to survive drought. However, some strains of *N. coenophialum* produce ergot alkaloids that cause fescue toxicosis in grazing animals. Endophyte-friendly tall fescue is associated with fungal isolates that provide the beneficial characteristics without producing the ergot alkaloids.

Some studies have indicated that root-derived compounds produced by tall fescue associated with the ergot alkaloid-producing endophyte may contribute to tall fescue resistance to nematodes. However, recent studies indicated that presence or absence of the endophyte status did not influence suppression of root-knot nematode reproduction on tall fescue. Consequently, planting of the endophyte-friendly tall fescue cv. Jesup (Max-Q) as a preplant ground cover was recently recommended as a sustainable approach for assisting in management of plant-parasitic nematodes on peach trees in the southeastern United States. While presence of the endophyte may not be essential for nematode management, the additional beneficial characteristics provided by the endophyte association may assist in providing enhanced vigor of the ground cover.

**O39.009 Soil microbial communities and their relation with soybean *Rhizoctonia* root rot**

*W.S. Shen[1, 2], H.H. Wei[2] and B. Liu[2]*
*[1]Department of Environmental Science and Engineering, Nanjing Normal University, No. 1 Wenyuan Road, Nanjing 210023, P. R. China; [2]Department of Plant Pathology and West Central Research and Education Center, University of Nebraska-Lincoln, 402 W State Farm Road, North Platte 69101, NE, United States*
*Email: wsshen@njnu.edu.cn; bliu5@unl.edu*

*Rhizoctonia* root rot, caused by *Rhizoctonia solani*, is a major soilborne disease of soybeans in Nebraska, the United States. The diseases cause seed rot and damping-off of soybean, and reduce the yield up to 50% or greater. Soil microbial communities play an importance role in suppressing soilborne plant pathogens. So far, little is known about the relationship among soil microbial communities and *Rhizoctonia* populations. Populations of soil total bacteria, *Pseudomonas*, total fungi, *Fusarium*, *Pythium*, *Rhizoctonia* and *Trichoderma* were characterized using culture-dependent (media culturing) and culture-independent (DGGE) methods from a soybean field in Nebraska. Results showed that the populations of soil total fungi and *Rhizoctonia* were significantly higher whereas beneficial *Pseudomonas* were significantly lower in soils with diseased plants and also on diseased soybean roots compared with the healthy soils and roots. There is no significant difference in *Pythium* and *Trichoderma* populations in diseased soils and healthy soils. Moreover, *Rhizoctonia* populations have a positive correlation with *Fusarium* populations and negative correlations with *Pseudomonas* populations. Soil microbial diversity using DGGE is in progress. This research provides important information for understanding the relationship between soilborne plant pathogens and soil microbial diversity, and the result will lead to more informed disease management of seed rot and damping-off of soybean.

**O39.010 Enhancement of Rhizoctonia-disease suppressive soils**

*J. Postma[1], M.T. Schilder[1] and B. Hanse[2]*
*[1]Plant Research International, Postbus 69, 6700 AB Wageningen, the Netherlands; [2]IRS, Postbus 32, 4600 AA Bergen op Zoom, the Netherlands*
*Email: joeke.postma@wur.nl*

*Rhizoctonia solani* is a soil-borne fungal pathogen, which causes worldwide serious losses in many different agricultural crops. *R. solani* AG2.2IIIB is an economically important problem in sugar beet with an estimated affected area of 70,000 ha in Europe (http://www.kwsbenelux.com). Enhancement of soil suppressiveness against damage caused by this pathogen would be a profitable strategy for farmers to control Rhizoctonia diseases without applying pesticides. Addition of compost or other forms of organic matter is often used to stimulate disease suppression of soil-borne pathogens, but this strategy is not reliable to control Rhizoctonia, since positive as well as negative results have been reported. Previous research has shown that three closely related species of *Lysobacter,* with the capacity to inhibit *Rhizoctonia* growth *in vitro*, were present in different *Rhizoctonia* suppressive soils. Therefore, we focussed on stimulation of these bacteria, which are known for their capacity to degrade various biomacromolecules. Repeated experiments in bioassays with sugar beet seedlings showed that chitin, yeast, as well as several animal by-products enhanced the indigenous *Lysobacter*-populations in the soil, as well as disease suppression of *R. solani* AG2.2IIIB. Feather meal and hoof meal, which were the cheapest products, were very effective and can also be applied as fertilizer. A first field experiment with chitin, feather meal and hoof meal applied during sowing, showed positive results on the yield of sugar beet. Efficacy testing in the field should be repeated. Although we detected a correlation between the *Lysobacter* populations and disease suppression, the mechanism of suppressiveness is not yet understood.

**O39.011 Identifying new sources of resistant in wheat germplasms for dryland crown rot caused by *Fusarium culmorum***

*G. Erginbas-Orakci, A.A. Dababat, A. Morgounov and H.J. Braun*
*CIMMYT/Global Wheat Program, Ankara-Turkey*
*Email: g.erginbas@cgiar.org*

The dryland Crown Rot (CR) caused by *Fusarium* species is among the most significant constraints facing wheat production especially in dryland areas and wheat monoculture cropping system. The most commonly reported causal pathogens are *F. culmorum*, *F. pseudograminearum* (formerly *F. graminearum* group 1). It was reported in West Asia, North Africa (Egypt, Tunisia, and

Morocco), USA, Canada, Australia, and Turkey. In Australia, it inflicts an annual loss of over $40 million USD and in the PNW-USA up to 35% of winter wheat yield. Extensive survey was carried out in 2000/2001 showed that *F. culmorum* was the dominant pathogen in Turkey and causes a yield losses of up to 43% in winter wheat. In rainfed wheat production system where cereal monoculture is extensively practiced rotation offers limited option to control the disease. Using resistance germplasm with high yielding potential and adapted under irrigated and non irrigated conditions is the main objective of the soil borne pathogens (SBP) program at CIMMYT-Turkey to control the disease. Therefore, screening of wheat germplasms for resistance to CR has been conducted by SBP program since 2001. A wide range and diverse germplasms from around the globe has been obtained through CIMMYT-Mexico and IWWIP (International Winter Wheat Improvement Program)-Turkey. Surveys, yield loss and agronomic studies were also conducted in colloboration with Ministry of Food, Agriculture and Livestock of Turkey. The SBP program aims to identify novel sources of resistance which are superior than globally known resistant cultivars as 2,49. Screening activities are being conducted under controlled growth room, greenhouse and heavily infested field conditions. A total of 25 wheat germlasms (20 spring wheat and 5 winter wheat) were performed equal/better than used checklines. In 2012, two international nurseries with high level of resistance to CR were distributed to collaborators representing different countries around the world. This promising valuable germplasm will be used by different breeding programs for germplasm enhancement and to limit negative effect of disease worldwide.

### O39.012 Natural biological suppression of soilborne diseases in southern Australian agricultural fields

*V.V.S.R. Gupta[1], D.K. Roget[1], D. Huberli[2], B. McLeod[2], J. Kirkegaard[3], A. McKay[4] and K. Ophel-Keller[4]*
*[1]CSIRO Ecosystem Sciences, Glen Osmond, SA 5064, Australia; [2]DAFWA, South Perth, WA, Australia; [3]CSIRO PI, Canberra, Australia; [4]SARDI Urrbrae, SA, Australia*
*Email: Gupta.Vadakattu@csiro.au*

Natural biological disease suppressiveness of soil is the ability of a soil to reduce disease severity even in the presence of a pathogen, its host plant and favourable climatic conditions. Disease suppression is considered as a function of both the activity and composition and of a diverse microbiota (microflora and microfauna) community. Although it is an inherent property of all biologically active soils, the level of suppression ability varies with edaphic and environmental variables. We measured the disease suppression potential (DSP) of surface (0-10 cm) soils collected from over 50 agricultural fields under continuous cropping and experimental sites in the Mediterranean region of southern Australia (Eyre peninsula and Mallee regions in South Australia, Western Australia and southern New South Wales) using a controlled environment based bioassay. The DSP index value for each soil was calculated (on a 0-1 scale where 1 represents higher DSP) based on reduction in disease when the soils were incubated with added carbon (*Rs*Suc) relative to disease incidence with added inoculum only. Disease incidence observations were also made in field experiments evaluating the effect of crop management practices and linked to estimates of DSP. Results indicated a wide range of suppressive activity and DSP values ranged from 0.2 to 0.95 and less than 10 soils showed DSP levels high enough (>0.55) to suppress disease to agronomically valuable levels. The majority of soils from Eyre peninsula region in SA and WA showed lower DSP values (<0.3). Both soil type and management had significant effect on the level of disease suppression potential. Results suggest that a multi-level bioassay (multiple C and pathogen levels) is required to measure DSP for soils varying in soil texture and chemical properties. Overall, in the rainfed cropping regions of southern Australia it is proposed that management practices that increased C inputs over a number of years would cause a change in the composition of the microbial community and enhance activities of specific microorganisms resulting in increased suppression of soilborne diseases such as Rhizoctonia bare patch.

### O39.013 Assessment of crop rotation in combination with cultivar resistance or a biofungicide for control of clubroot on canola

*G. Peng[1], D. Pageau[2], S.F. Hwang[3], R. Lahlali, R.K. Hynes, K. Anderson[4], S.E. Strelkov[5], M.R. McDonald[6], B.D. Gossen[1], T.K. Turkington[7], F.Q. Yu[1], K.C. Falk[1], S.M. Boyetchko[1] and L. McGregor[1]*
*[1]Saskatoon Research Centre, Agriculture and Agri-Food Canada (AAFC), 107 Science Place, Saskatoon, Saskatchewan S7N 0X2, Canada; [2]AAFC Research Farm, 1468, Saint-Cyrille St., Normandin, Quebec G8M 4K3, Canada; [3]Crop Diversification Centre North, Alberta Agriculture and Rural Development, 17507 Fort Road, Edmonton, Alberta T5Y 6H3, Canada; [4]Bayer CropScience, 295 Henderson Drive, Regina, Saskatchewan S4N 6C2, Canada; [5]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada; [6]Department of Plant Agriculture, University of Guelph, Guelph, Ontario N1G 2W1, Canada; [7] AAFC Research Centre, 6000 C & E Trail, Lacombe, Alberta, T4L 1W1, Canada*
*Email: gary.peng@agr.gc.ca*

Two field trials were conducted to assess the effect of crop rotation in combination with cultivar resistance or a biofungicide (*Bacillus subtilis*) seed treatment for control of clubroot on canola. In the first trial, a susceptible cultivar coated with a biofungicide formulation at $1 \times 10^5$

to $5\times10^6$ cfu/seed was seeded to plots with a 1-, 3- or 11-year break from a canola crop. In a second trial, susceptible (S), moderately susceptible (MS) and resistant (R) canola cultivars were seeded to plots with 0- to 4-year breaks from canola crops. All treatments were replicated four times and each trial was repeated once. None of the biofungicide seed treatments reduced clubroot impact regardless of crop-rotation practices, whereas the R cultivar consistently showed low clubroot severity and doubled the yield over that of the S cultivar. Plots with a 3-year break were 10-fold lower in inoculum loads than those with continuous canola. A 2-year or longer break alleviated clubroot impact on S and MS canola cultivars relative to no break or a 1-year break under high clubroot disease pressure. However, longer crop rotations alone will not reduce clubroot impact sufficiently for S or MS cultivars to reach their full yield potential. R cultivars, when combined a 2-year break, increased canola seed yield by about 25% relative to no break. We concluded that R canola cultivars used in a 3-year crop rotation (2-year break from previous canola) are the most effective strategy for control of clubroot under heavy infestation conditions.

**O39.014 New insights open the prospects for an effective control of powdery scab of potato, a disease of global importance**

*U. Merz, R. D. Gau and P. Brunner*
*Plant Pathology, IBZ, ETH Zurich, 8092 Zurich, Switzerland*
*Email: ueli.merz@usys.ethz.ch*

The disease powdery scab - lesions on tubers - is caused by the protozoan organism *Spongospora subterranea* f.sp. *subterranea* (*Sss*). The pathogen can also attack the roots and induce the production of galls. Both structures contain a powdery mass of sporosori. These resting structures are highly resistant to environmental stresses and enable the pathogen to survive for many years in contaminated soils. No effective control measures are available. Host resistance breeding will be a key component thus knowledge of the genetic diversity of the pathogen is needed. A combination of microsatellite and DNA sequence data derived from worldwide populations of *Sss* showed that South American populations were consistently more diverse compared to all other regions. Estimates of past and recent gene flow suggested that *Sss* was likely introduced from South America into Europe and from there, as a "bridgehead", further globally disseminated. The low global genetic diversity of *Sss* allows potato breeders to select for resistance which is likely to be durable. This was confirmed in a series of field trials in Europe over four years and at different locations with ten cultivars. The most resistant cultivar which originated from New Zealand performed best in all trials, even under high disease pressure. New introductions of *Sss* genotypes, particularly from South America, increase the potential of more aggressive inoculum, e.g. due to recombination. Thus strict quarantine measures for potato import need to be established, or must continue with strict enforcement to maintain the low global genetic diversity outside South America.

**O39.015 Advances in host plant resistance and identification of *Fusarium oxysporum* f. sp. *ciceris* disease resistance in chickpea genotypes**

*M. Sharma, T. Kiran Babu, P.M. Gaur, R. Ghosh, T. Rameshwar and S. Pande*
*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, AP, India*
*Email: mamta.sharma@cgiar.org*

Chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris*) is a major limiting factor in chickpea production worldwide. Host plant resistance is the best management method to control this disease. The aim of this work was to find sources of resistance to wilt disease and validate their stability across different environments. One-hundred and twenty three lines with wilt incidence <10% were selected from preliminary evaluation of 948 lines including germplasm and breeding lines from ICRISAT for wilt resistance in the sick plot. Sixty lines were selected for second round of evaluation (2005/06) and from those 57 lines were selected for third round of evaluation (2006/07). In order to validate resistance stability, a Chickpea Wilt Nursery was constituted with 27 lines (7 germplasm accessions, 19 breeding lines and a highly susceptible check) and further tested in multi-location experiment for wilt resistance at 9 locations in India for three years (2007/08 to 2009/10). Variability in wilt incidence due to genetic differences among the genotypes, among the environments, and that due to genotype × environment interaction was highly significant ($P < 0.001$). Genotype and genotype × environment (GGE) biplot analyses allowed the selection of three breeding lines (ICCV 05527, ICCV 05528 and ICCV 96818) and one germplasm accession (ICC 11322) with moderate level of disease resistance and stable performance across the environments. Genotype × Environment (G × E) interaction contributed 36.7% of total variation of the multi-environment evaluation, revealing instability of the phenotypic expression across environments. The identified resistant sources will be useful to chickpea disease resistance breeding programs.

**O39.016 Alternative hosts of soilborne pathogens of no-till maize in Kwazulu-Natal, South Africa**

*S.C. Lamprecht, Y.T. Tewoldemedhin and E. Retief*
*Agricultural Research Council-Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7599, South Africa*
*Email: lamprechts@arc.agric.za*

In KwaZulu-Natal province, soilborne diseases significantly reduce maize yields in irrigated systems where maize follows winter wheat. Important soilborne pathogens of maize identified in this cropping system, include *Fusarium boothii*, *F. graminearum*, *Phaeocytostroma ambiguum*, *Phialophora zeicola* and *Stenocarpella maydis*. The effect of rotation with different summer and winter crops on severity of soilborne diseases has been investigated in this province; however, the susceptibility of these crops to soilborne pathogens of maize has not been investigated in South Africa. Representative isolates of each pathogen were evaluated for the ability to incite disease on canola, black oat, soybean, stooling rye and wheat under glasshouse conditions. The plant growth medium (pasteurised mixture of equal amounts of soil, sand and perlite) was amended with sand-bran inoculum at a concentration of 0.05% (w/w) and seeds were sown one day after mixing the inoculum into soil. Canola was not susceptible to any of the test pathogens. *F. boothii* significantly reduced survival and growth of maize and stooling rye and increased root rot severity compared to the controls. *F. graminearum* significantly reduced survival and growth and caused root rot of maize, black oat, stooling rye and wheat. *P. ambiguum*, *P. zeicola* and *S. maydis* caused a very low level of root rot on soybean, stooling rye and wheat, but did not significantly reduce survival and growth. Results showed that black oat, stooling rye and wheat can be important alternative hosts of *F. boothii* and *F. graminearum* in rotation systems (double cropping) with maize.

### P39.001 Challenges in the management of *Sclerotinia sclerotiorum* in *Brassica*- an Indian perspective

*N. Mehta*
*Department of Plant Pathology, CCS Haryana Agricultural University, Hisar – 125004 Haryana, India*
*Email: nareshmehta282@gmail.com*

*Sclerotinia sclerotiorum* (Lib.) de Bary is a devastating plant pathogen due to its worldwide distribution, wide host range and the difficulties encountered in its management. *Sclerotinia* uses its ascospores as the primary inoculum and plant infection by mycelial growth or by contact between infected plants results from secondary infection. The effect of frequency and quantum of irrigation on the germination of sclerotia and development of apothecium in different soil types revealed least number of apothecia production in sandy soil whereas, sandy loam soil supported maximum number of apothecial production. Evaluation of plants extracts (50%) against this pathogen showed that *Bougainvillea spectabilis* and *Azadirachta indica* foliage extracts and *Allium sativum* (clove) were quite effective in checking mycelial growth and sclerotia formation. Soil amendment with dried leaves of *Bougainvillea spectabilis, Syzygium cuminii* and *Lowsonia inermis;* cakes/organic manures of poultry manures, *Syzygium cuminii* and *Azadirachta indica* seed powder were able to reduce the number of apothecia production, lesion length and disease intensity. Biological control studies revealed that isolates of *Trichoderma harzianum*-3, *T. harzianum*-4 and *Bacillus subtillis* to be potent in checking the linear growth and apothecia production in *in vitro* conditions. In screen house studies, *T. harzianum* was quite effective in reducing lesion length and disease intensity when applied simultaneously or seven days prior to the pathogen inoculation.

### P39.002 Integrated management of *southern blight* disease of carrot caused by *Sclerotium rolfsii*

*M.K.A. Bhuiyan and B.C. Sen*
*Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur -1706, Bangladesh*
*Email: kabhuiyan55@yahoo.com*

Attempts were made for the integrated management of Southern Blight disease of Carrot caused by *Sclerotium rolfsii* in pot and field experiment under inoculated condition through the integration of *Trichoderma harzianum* with fungicide and organic amendment. An antagonist *T. harzianum* isolate MYT-75, a fungicide Provax 200 and mustard oil cake 3% and a virulent isolate S-6 of *S. rolfsii* was selected through a series of preliminary experiments conducted during 2007 to 2010 and applied either alone or in combination in reducing the seedling mortality in the pot culture and field experiment. The highest 70.00% reduction of total seedling mortality was observed where the integration of wheat grain colonized *Trichoderma*, mustard oil cake amendment and Provax 200 treated carrot seeds were sown in the *S. rolfsii* inoculated pot soil ($T_8$). In the field, the highest 80% disease incidence was reduced under the treatment $T_8$ followed by $T_6$ and $T_5$. All the treatments had a positive influence of yield contributing components like root length, root diameters and individual root weight. Southern blight disease caused 67.47 % yield loss of carrot in the *S. rolfsii* inoculated plots ($T_2$) in comparison to un-inoculated plots ($T_1$). All the treatments either alone or in combinations significantly increased yield ranged from 50.45 to 180.07 % over control ($T_2$). The highest 180% increased yield was recorded in treatment $T_8$ followed by $T_6$ where *Trichoderma* and mustard oil cake were applied in combination. The highest 91.08% yield recovery was recorded in relation to the un-inoculated control $T_1$.

### P39.003 Efficacy of some plant extracts against *Rhizoctonia solani* on pea

*A.A. Al-Askar and Y.M. Rashad*
*Biology Department, Teachers College, King Saud University, Riaydh, Kingdom of Saudi Arabia*
*Email: aalaskara@ksu.edu.sa*

Antifungal activity of ethanol-water extracts of four medicinal plants, cinnamon (*Cinnamomum verum* Presl.)**,** anise (*Pimpinella anisum* L.), black seed (*Nigella sativa* L.) and clove (*Syzygium aromaticum* L. Merr. & Perry.) was investigated against pea (*Pisum sativum* L.) root-rot fungus *Rhizoctonia solani*. *In vitro* antifungal activity test showed a high growth inhibition at concentration (4%) of each plant extract. The highest antifungal activity was recorded for clove extract which causes complete growth inhibition at concentration of 1%. Efficacy of clove extract on disease incidence of *Rhizoctonia* root-rot of pea was investigated in the greenhouse pot experiment. Clove extract at concentration 4% as well as the chemical fungicide recorded highly significant increase in the percentage of survived plants (40 and 48%, respectively) and highly significant decrease in disease incidence.

**P39.004 Reducing Phytophthora fruit rot in eggplant and tomato fruits using rice straw and swine manure**

*R.T. Alberto*[1] *and S. Sanogo*[2]
[1]*Department of Crop Protection, College of Agriculture, Central Luzon State University, Munoz, Nueva Ecija, Philippines 3120;* [2]*Department of Entomology, Plant Pathology and Weed Science, College of Agricultural, Consumer and Environmental Sciences New Mexico State University, Las Cruces, NM 88003-800*
*Email: bertyx1275@hotmail.com*

This study was conducted in a field plot to determine the effects of rice straw and swine manure on the incidence and severity of Phytophthora fruit rot on eggplant and tomato fruits in Nueva Ecija, Philippines. Treatments were based on the presence or absence of rice straw in combination with swine manure applied at 0 kg/m$^2$, 0.5 kg/m$^2$, and 1 kg/m$^2$. Plots were artificially infested with *P. infestans*. Disease incidence and severity as well as marketable yields, were assessed at intervals. In both eggplant and tomato, lowest incidence and severity of Phytophthora fruit rot occurred in plots incorporated with rice straw (0.5 kg/m$^2$) + high swine manure (1 kg/m$^2$). The overall reduction on the incidence of the disease compared to plots without rice straw and swine manure was approximately 85% in eggplant and 72% in tomato. Similarly, the overall reduction on the severity in eggplant and tomato was 80% and 67%, respectively. Highest marketable yield was obtained in plants grown in plots incorporated with rice straw (0.5 kg/m$^2$) + high swine manure (1 kg/m$^2$). Other than the control, the incidence and severity were highest in eggplant and tomato plots where only rice straw was used as mulch.

**P39.005 Control of soilborne pathogens on vegetables by microorganisms isolated from compost and by microbial-fortified compost**

*M. Pugliese*[1,2]*, M.L. Gullino*[1,2] *and A. Garibaldi*[1]
[1]*Agroinnova – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy;* [2]*Disafa – University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy*
*Email: massimo.pugliese@unito.it*

Soilborne pathogens can cause serious damages to economically important crops in Italy and new strategies are requested for their control. The objectives of the present work were to investigate the effectiveness of microorganisms isolated from composts and of compost fortified with biological control agents to control soilborne pathogens on vegetables. A municipal compost showing a good suppressive activity was used as source of microorganisms. The same compost was also steamed and compared to non steamed. Both of them were inoculated with commercially available *Trichoderma* and with non-pathogenic *Fusarium* at 1, 2 and 4 g/l. A comercial peat substrate was used as control. The colonies isolated were tested in greenhouse against *Fusarium oxysporum* f. sp. *basilici*/basil, *Phytophthora nicotianae*/tomato, *Pythium ultimum*/cucumber and *Rhizoctonia solani*/bean. In the case of fortified compost, the tests were carried out also on the pathosystem *F. oxysporum* f. sp. *lactucae*/lettuce. Among the microorganisms isolated from suppressive compost, 28 showed a significant disease reduction of at least one of the pathogens tested, but none of the microorganisms was able to control all the pathogens in greenhouse trials. Compost suppressiveness was partially restored when compost was steam sterilized and biological control agents were applied at least at 2 g/l dosage. In conclusion, our results indicate that the selection of antagonists from suppressive composts and the addition of specific antagonists to compost represent new opportunities in disease management.

**P39.006 Quantification of *Rhizoctonia solani* AG2-2IIIB in agricultural field soils**

*B. Boine, J. Nechwatal and M. Zellner*
*Bavarian State Research Centre for Agriculture, Lange Point 10, 85354 Freising, Germany*
*Email: Michael.Zellner@LfL.bayern.de*

*Rhizoctonia solani* AG2-2IIIB is the causal agent of late crown and root rot in sugar beet (*Beta vulgaris* subsp. *vulgaris*). In a 4-year field study we analyzed the effect of different corn cultivars and agricultural practices on the soil inoculum density of *R. solani*. Moreover, we report on the evaluation of different on- and off-site monitoring systems to estimate soil inoculum potential of *R. solani*. After growing sugar beet in 2009 (with crop residues were either mulched or removed after harvest) and inoculation with *R. solani* of half the plots, the field trial consisted of a fully randomised block design with silage and grain corn variations in 2010 and 2011. In 2012 sugar beet was grown again on all plots. *R. solani* inoculum soil densities were monitored monthly

using root damage indices of corn and indicator plants (*Vicia faba*). In addition, soil samples were taken yearly after crops were harvested for a quantitative PCR (qPCR) test using quinoa (*Chenopodium quinoa*) seed baits. All tests clearly discriminated between inoculated and control plots, with the latter showing significantly lower disease indices in field and qPCR assays. Furthermore, it was revealed that repeated incorporation of organic matter in the soil enhances severity of sugar beet rots. Also, the qPCR data were correlating strongly with most inoculum soil densities. This demonstrates that a quinoa baiting of *R. solani* combined with qPCR techniques can measure fungal soil inoculum densities sufficiently. Hence, the established qPCR assay might be an alternative method to monitor soil-borne pathogens notoriously difficult to handle.

**P39.007 Distribution of *Ralstonia solanacearum*, the causing agent of bacterial wilt of vegetable sweet potato and its management in fields**

*Y.J. Chen, Y.S. Lin, C.Y. Lee, H.R. Pan and W.H. Chung*
*Dept. Plant Pathology, National Chung Hsin University, 250, Kuo kuang Rd., Taichung 402, Taiwan, China*
*Email: wenchung@nchu.edu.tw*

*Ralstonia solanacearum* (RS) is an important phytopathogen that causes wilting diseases on more than 200 plant species. In Taiwan, RS caused bacterial wilt (BW) and impaired 30 to 80% yield of vegetable sweet potato (VSP) last decade. RS was generally distributed in soil of VSP fields with $1.3\times10^2$ to $9.5\times10^5$ cfu/g soil and in VSP cutting seedlings with 2 to 98% isolation frequency. The severity of BW was closely related to RS-carried VSP cuttings (R=0.913); however, the severity of BW did not show significant correlation with the RS density in soil (R=0.086). Inoculation tests in greenhouse showed similar results to those in the field. Thus, the cuttings carried RS were more important inoculums source than the RS resides in soil. The distribution of RS in VSP stem indicated that the isolation frequency of RS was lower than 31% in terminal shoots or erect stems and 45 to 100% in creeping stem 8 wks after the cuttings were planted in infested soil ($10^6$ cfu/g soil). Results demonstrated that erect stem cuttings were not common source of RS. For confirming the efficacy of the erect stem cutting on control BW of VSP in the fields, the erect stem cuttings were collected from VSP field. Collected cuttings were tested with Bio-PCR and Tissue SM-1 Streaking Method (TSSM) examination before planting. Results showed that early RS detection in erect stems is an efficient method to replace the traditional cuttings on control of BW in VSP production.

**P39.008 Edaphic and environmental factors influence *Rhizoctonia solani* AG 8 inoculum level and disease impact**

*V.V.S.R. Gupta[1], A. McKay[3], K. Ophel-Keller[3], J. Kirkegaard[2], N. Wilhelm[3] and D.K. Roget[1]*
*[1]CSIRO Ecosystem Sciences, Glen Osmond, SA 5064, Australia; [2]CSIRO PI, Canberra, Australia; [3]CSARDI Urrbrae, SA. Australia*
*Email: Gupta.Vadakattu@csiro.au*

*Rhizoctonia solani* Kühn AG-8 causes seedling diseases in a wide range of cereal and legume crop plants. The disease causes significant economic losses in cereals across Southern Australia and Pacific Northwest in USA. *R. solani* fungus is known to grow on decomposing soil organic matter and produces hyphal networks in the surface soil. We measured changes in inoculum levels in surface soil during summer and in-crop as influenced by crop rotation and environmental factors in field experiments in South Australia and New South Wales. Results showed that non-cereal crop rotations substantially reduced the pathogen inoculum compared to wheat and cereal rye. Inoculum levels were lowest after canola and mustard. Inoculum build-up occurs throughout the growing season, e.g. crown root infection not only contributes to the inoculum build-up but also result in significant yield loss. The pathogen inoculum is concentrated near the surface of field soils (top 5 cm), especially in no-till systems. Soil disturbance at seeding influences the distribution of inoculum with depth. In the absence of host plants, regular summer rainfall reduced the pathogen DNA levels, whereas it increased during prolonged dry periods. Inoculum changes in summer are also related to the amount of decomposing organic residues (i.e. particulate organic matter) in soil. Higher soil microbial activity and catabolic diversity at sowing resulted in lower disease incidence even in the presence of higher inoculum. A better understanding of pathogen inoculum dynamics in-crop and non-crop period and soil biological activity is critical for developing management options that reduce the disease impact.

**P39.011 Fungal and oomycete species associated with soilborne diseases of soybean in South Africa**

*Y.T. Tewoldemedhin and S.C. Lamprecht*
*ARC-Plant Protection Research Institute, Private Bag X5017, Stellenbosch, 7599, Western Cape, South Africa*
*Email: tewoldemedhiny@arc.agric.za*

Previous research has shown that soilborne diseases of soybean can cause significant economic losses in most soybean growing areas of the world. In South Africa there is limited information available in this regard. Therefore, surveys were conducted during the 2010/11 and 2011/12 growing seasons in thirteen soybean production areas that represent three climatic regions (cool, moderate and warm) in six provinces of South Africa at three growth stages (two weeks after planting, flowering and physiological maturity or seed pod filling). The highest cotyledon, crown and root rot severities were

recorded at Bethlehem, Clocolan, Delmas, Kinross, Normandien, Vaalharts and Villiers. Over two seasons, twenty-eight genera and 71 fungal and oomycete species were isolated from diseased cotyledons, hypocotyls/ crowns and roots and identified based on morphology and DNA sequence analyses. The most frequently isolated genera included *Alternaria, Fusarium, Gliocladium, Macrophomina, Phoma, Diaporthe/Phomopsis* species complex, *Pythium, Rhizoctonia, Sclerotium* and *Trichoderma*. All of these were isolated from hypoctoyls/crowns and roots, and all except *Macrophomina* and *Sclerotium* from cotyledons. In addition, *Sclerotinia* was recovered from diseased soybean stems. Frequency of isolation for genera and species was affected by region, climate and time of sampling. Greater than 50% of all isolates obtained belonged to the genus *Fusarium* and *F. oxysporum* was the dominant species. Many of the fungi recorded in this study have been reported as important soilborne pathogens of soybean in other countries.

**P39.012 *Globoderas* in Poland – from nematode resistance of potato varieties to the distribution of pathotypes**

*A. Przetakiewicz*
*Plant Breeding and Acclimatization Institute–National Research Institute, Radzikow, 05-870 Blonie, Poland*
*Email: a.przetakiewicz@ihar.edu.pl*

*Globodera rostochiensis* and *G. pallida* belong to potato cyst nematodes (PCN) responsible for damage in cultivated solanaceous plants all over the world. In the European Union PCN have a status of quarantine organisms, instituted by EU Control Directive (68/465/EEC; CEC Council 1969). Cysts of *Globodera rostochiensis* were first found in Poland near Gdansk in 1946 (Wilski, 1956). Later, the regular regional surveys conducted since 1968 up to now have shown PCN to be widely distributed in Poland with the highest infestation throughout the Northwest. Currently, there are two pathotypes of *Globodera rostochiensis* reported in Poland Ro1 and Ro5 (Karnkowski, 2006; Przetakiewicz 2013). Membership of Poland in EU community and the opening of plant exchange market gives an opportunity of fields contamination by other pathotypes of PCN. Laboratory of Quarantine Organisms in Radzikow conducts research focussed on the assessment of resistance to five pathotypes of *G. rostochiensis* (Ro1-Ro5) and three pathotypes of *G. pallida* (Pa1-Pa3) of all polish potato varieties, breeding lines and clones according to EPPO recommendation (PM7/40). The other task we undertake is the identification of PCN pathotypes in soil samples coming from infested fields to prepare the information map of pathotypes distribution and resistance of potato varieties in highest degree mainly for farmers. As a quarantine laboratory we are additionally focused on detection of *Ralstonia solanacearum* in potato samples with the aid of molecular and biological methods.

**P39.013 Reaction of differential cultivars of potato to Polish isolates/pathotypes of *Synchytrium endobioticum* (Schilb.) Perc**

*J. Przetakiewicz*
*Plant Breeding and Acclimatization Institute – National Research Institute, Department of Plant Pathology, Laboratory of Quarantine Organisms, Radzikow, 05-870 Blonie, Poland*
*Email: j.przetakiewicz@ihar.edu.pl*

Polish isolates of *S. endobioticum* have been evaluated using Glynne-Lemmerzahl method. Virulence of isolates was compared with the most relevant European pathotypes [1 (D1), 2 (G1), 6 (O1), 8 (F1) and 18 (T1)] as well as with Polish local pathotypes of *S. endobioticum* [2(Ch1) and 3(M1)]. A set of differential cultivars of potato (Deodara, Tomensa, Eersteling, Producent, Combi, Saphir, Delcora, Miriam, Karolin and Ulme) (the EPPO Diagnostic protocol for *S. endobioticum* PM 7/28) and other commercial and/or differential cultivars (Asche Sämling, Bosman, Cekin, Chopin, Combi, Désirée, Gawin, Ibis, Irga, Legenda, Marylin, Sissi, Ślęza, Tamensa, Ulme i Zeisig) were infected. The virulence of pathotype 6(O1), 2(Ch1) and 3(M1) was identical when differential cultivars from EPPO were using. Cultivar Deodara, Tomensa, Eersteling, Producent and Combi were extremely susceptible, Delcora and Miriam were slightly susceptible as well as Saphir, Karolin and Ulme ware resistant to these pathotypes. Application of Asche Sämling and Désirée allowed for distinguishing those pathotypes. Ashe Sämling was extremely susceptible to pathotype 6(O1) and 3(M1) while slightly susceptible to pathotype 2(Ch1). Désirée was extremely susceptible to pathotype 2(Ch1) while slightly susceptible to pathotype 6(O1) and 3(M1). Application of all cultivars indicated for presence of at least 6 different pathotypes of *S. endobioticum* in Poland: pathotype 1(D1), 2(Ch1), 3(M1) and 18(T1). Winter sporangia of pathotype 1(D1) and 3(M1) were identified apart in the same old focus from 1965. Winter sporangia of pathotype 18(T1) were discovered as a new focus in 2008. Pathotype 2(Ch1) is presented as population of 2 different pathotypes.

**P39.014 Coinfection of *Plasmodiophora* and *Meloidogyne* on planting of cabbage in Karanganyar Central Java**

*Hadiwiyono*
*Departement of Agrotechnology, the University of Sebelas Maret (UNS), Jl. Ir. Sutami 36A Kentingan Surakarta Postal Code, 57726, Indonesia*
*Email: hadi_hpt@yahoo.com*

Cabbage (*Brassica oleracea* L.) is an important commodity in Karanganyar, Central Java. Since 1990's, the

local farmers face with a destructive disease called club root caused by *Plasmodiophora brassicae* Wor. In addition, it has been reported that Meloidogyne have been also found in the area, attacked destructively on some plants such as carrot and garlic. On the cabbage, it had been reported that Meloidogyne could infect it with no significant symptom. Based on an artificial co-inoculation with *Plasmodiophora* however, *Meloidogyne* could increase the disease severity of club root. This paper would like to report the field data about the co-infection of *P. brassicae* and Meloidogyne on cabbage in Karanganyar Central Java. At least it had been observed on 3 sub-districts (Pancot, Blumbang, and Gondosuli) with 10 sample plantings of cabbage for each sub-district to evaluate the co-infection. The observed variables were the disease severity of club root, population of Meloidogyne and yield of cabbage. Each planting of cabbage was sampled 30 plants to assess the disease severity and population of Meloidogyne. The disease severity was assessed by scoring method with 5 orders of score of root damage. Whereas the population of nematode was based on the composite sample of rhizosphere soil from 30 plants per plantings of cabbage. The results indicated that the population of Meloidogyne had positive linier correlation to the disease severity of club root of cabbage. The increase of Meloidogyne population could increase significantly the disease severity of club root.

**P39.015 Differential resistance to tuber-invading pathogens in somaclonal variants of Russet Burbank potato tubers linked to suberin production**

*T. Thangavel, R.S. Tegg and C. R. Wilson*
*Tasmanian Institute of Agriculture, University of Tasmania, New Town Research Laboratories, 13 St Johns' Avenue, Tasmania 7008, Australia*
*Email: tt8@utas.edu.au*

Enhanced resistance to common scab and powdery scab of potato were obtained in a somatic cell selection breeding programme. The mechanism of novel resistance was investigated by comparing parental cv. Russet Burbank to disease-resistant somaclones. Glasshouse and field experiments tested the susceptibility of the parent and somaclones to the diseases, common scab, powdery scab and black scurf caused by the soil-borne pathogens *Streptomyces scabiei*, *Spongospora subterranea* f. sp. *subterranea and Rhizoctonia solani*, respectively. Somaclones showed strongly enhanced resistance to common scab, but weaker resistance to powdery scab and black scurf compared to the parent cultivar. Histological (Fluorescent microscopy, compound microscope) and molecular methods (qRT-PCR) were used to identify the number and thickness of phellem (periderm) layers, degree of phellem suberisation and relative expression of three suberin synthetic genes (*CYP833A, StKCS6* and *POP_A*) from pathogen-treated and untreated control tubers. Tuber peridermal layer numbers and thickness, and phellem suberisation increased significantly ($P<0.05$) in response to the pathogen *S. scabiei*, with the increase greater in the resistant somaclones compared to the parental line. In response *to S. subterranea* and *R. solani* there were no changes in periderm layer numbers and thickness and only a small increase ($P>0.05$) in suberin production again with greater induced suberisation in somaclones than parent. This study suggests induced suberisation may be important mechanism for enhanced disease resistance in these somaclones, with the extent of induced subserisation associated with extent of resistance expression.

**P39.016 Preliminary validation in growers fields of soil DNA tests for 3 potato pathogens**

*T.J. Wiechel, N.S. Crump, A. McKay and K. Ophel Keller*
*Department of Primary Industries, Biosciences Research Division, Knoxfield, Victoria 3180 Australia, ViCSPA, Private Mail Bag 1, Healesville, Victoria 3777 Australia, South Australian Research and Development Institute (SARDI), Waite Campus, Urrbrae, SA Australia*
*Email: Kathy.Ophelkeller@sa.gov.au*

Soilborne pathogens are a significant threat to agriculture resulting in reduced crop yields and endangering food security. With an increasing land area affected by soilborne pathogens the ability to assess risk of disease development is of increased importance. DNA technology, based on quantitative PCR (qPCR) has been used to detect and quantify potato pathogens *Spongospora subterranea* (powdery scab), pathogenic *Streptomyces* species (common scab; based on thaxtominA gene) and *Rhizoctonia solani* anastomosis group (AG) 3 (black scurf). This study aims to validate quantitative tests for three major soilborne pathogens of potato to determine disease potential in potato fields. Over one growing season, soil samples (n=378) were collected from potato fields prior to planting and qPCR assays for *S subterranea*, pathogenic *Streptomyces* and *R solani* AG3 applied to DNA extracted from soil to determine the level of pathogen DNA present. Seed samples (n=71) of 100 tubers were similarly tested for the pathogens. At harvest, potato tubers were collected from tested fields (n=125) and visually scored for powdery and common scab and black scurf incidence. The percentage of detections of *S subterranea* was similar for both soil and seed samples. However, detection of pathogenic *Streptomyces* and *R. solani* AG3 were significantly greater on seed than in soils. The incidence of disease on tubers was similar to the level of detection of *R. solani* AG3 on seed, similar to detection of *S. subterranea* on both soils and seed and greater than both the additive effect of seed and soil for pathogenic *Streptomyces.*

**P39.017 *Bacillus* and *Streptomyces* spp. as potential biocontrol agents to control soil-borne pathogens of chickpea and sorghum**

*M. Sreevidya and S. Gopalakrishnan*
*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, 502 324, Andhra Pradesh, India*
*Email: vidya.meesala@gmail.com*

Chickpea and sorghum are mainly affected by biotic factors such as insect pests and pathogenic microorganisms. The main objective of present study is to isolate and identify antibiotics from culture filtrates of *Bacillus* and *Streptomyces* spp. against soil-borne fungal pathogens of chickpea (*Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium oxysporum* f. sp. *ciceri* causing collar rot, dry root rot and wilt) and sorghum (*Macrophomina phaseolina* causing charcoal rot). Bacteria and actinomycetes isolated from rhizosphere soils and herbal vermicompost were screened for their antagonistic potential against above mentioned fungal pathogens by dual culture assay. A total of 20 bacteria and 19 actinomycetes were selected for characterization of their plant growth promoting traits and biocontrol traits. In order to identify the antibiotic(s), cell free culture filtrates were partitioned against ethyl acetate and the resultant organic and aqueous phases were checked for their antagonistic potential. Organic phases of five bacterial isolates (VBI-4, VBI-19, VBI-23, SBI-23, and SBI-27) and four actinomycetes (SAI-13, SAI-29, VAI-7 and VAI-40) were found effective in controlling the growth of *S. rolfsii*, *R. bataticola* and *M. phaseolina*, whereas none of the aqueous phase samples were found effective. The bacterial isolates were identified as *Bacillus* spp. and the actinomycetes were identified as *Streptomyces* spp. in 16S rDNA analysis. The active metabolite(s) against fungal pathogens will be purified and chemical structure will be elucidated. The purified compound(s) will be tested for efficacy in greenhouse and field conditions against fungal pathogens.

**P39.018 Reaction of canola to inoculation with primary and secondary zoospores of *Plasmodiophora brassicae***

*M.R. McDonald, K. Sharma, B.D. Gossen, J. Feng, A. Deora and S.F. Hwang*
*Crop Divesificaton Centre North, Alberta Agriculture and Rural Development, Edmonton, Canada; Dept. of Plant Agriculture, Univ. of Guelph, Guelph, Canada; Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Canada*
*Email: mrmcdona@uoguelph.ca*

*Plasmodiophora brassicae* causes clubroot of canola (*Brassica napus*) and many other Brassica crops. Resting spores of the pathogen germinate and release primary zoospores that infect root hairs. Secondary zoospores are produced in root hairs, released, and then infect the root cortex. Studies were conducted to investigate the role of these two spore types on cortical infection and subsequent clubroot severity in canola cv. Zephyr. Plants were inoculated with resting spores (RS, as a source of primary zoospores) or secondary zoospores (SZ) of either a virulent (P3) or an avirulent (P6) pathotype, singly or with secondary zoospores of P3 added with resting spores of either P3 or P6. Percent area of the root cortex infected was assessed 10 days after inoculation and clubroot severity was assessed 42 days after inoculation. The pattern of response for cortical infection and severity were similar. Inoculation with RS-P6 (avirulent) resulted in almost no infection (0.1%) or severity (0%), but inoculation with SZ-P6 produced low levels of both infection (4%) and severity (31%). Inoculation with RS-P3 produced more infection (33% vs 12%) and higher severity (67% vs 100%) than SZ-P3. Adding SZ-P3 to RS-P3 did not increase cortical infection (34% vs 33%), but adding RS-P3 + SZ-P6 produced lower infection (18% vs 34%) and severity (84% vs 100%) than RS-P3 + SZ-P3. These results indicate that pathogen effectors act at the root hair infection stage and suppress (P3) or induce (P6) resistance in the host.

**P39.020 Aquatic ecology of zoosporic oomycetes**

*P. Kong and C. Hong*
*Virginia Tech, Hampton Roads Agricultural Research and Extension Center, Virginia Beach, VA 23455, USA*
*Email: pkong@vt.edu*

Zoosporic oomycetes including *Phytophthora* and *Pythium* attack hundreds of agriculturally and economically important plant species worldwide. The destructiveness of these pathogens is attributed partly to their multiple dispersal strategies. Some are soil borne plant pathogens, some are airborne, producing caducous sporangia, and others can be water borne by motile zoospores. Numerous species have been recovered from irrigation systems and natural waterways which are the most efficient means for the pathogen dispersal in nature. However, the aquatic ecology of zoosporic oomycetes is poorly understood which hampers the development of effective methods for control of disease spread. We recently investigated the effects of electrical conductivity (EC), dissolved oxygen (DO), pH and $CO_2$ on zoospore survival. All species tested survived a wide range of EC from 0.11 to 3.58 dS $m^{-1}$ although their survival rate decreased when EC was lower than 0.21 dS $m^{-1}$. Many species tolerated a broad range of DO from 0.3 to 11 ppm, but they were sensitive to higher DO levels. Zoosporic response to water pH was species-dependent. Dissolved $CO_2$ is a factor that can significantly reduce zoospore survival in water. Over 90% zoospores of test species were killed in 2 hours at 210 ppm of dissolved $CO_2$ or higher. Lower $CO_2$ concentrations were less effective but suppressed pathogen infectivity.

**P39.021 Biocontrol and plant growth promoting traits of *Streptomyces* spp. in chickpea and sorghum**

*G. Alekhya and S. Gopalakrishnan*
*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India*
*Email: alekhya.chinnu@gmail.com*

Chickpea and sorghum are two important crops grown in semi-arid tropics which are often encountered by many fungal pathogens resulting in reduced yield. In the present study, actinomycetes, isolated from herbal vermicompost and rhizosphere soils, were screened against pathogens of chickpea and sorghum. The pathogens studied include *Sclerotium rolfsii, Rhizoctonia bataticola* (three strains viz. RB-6, RB-24 and RB-115) and *Fusarium oxysporum* f. sp. *ciceri* (FOC) (causes collar rot, dry root rot and *Fusarium* wilt respectively in chickpea) and *Macrophomina phaseolina* (causes charcoal rot in sorghum, respectively). The plant growth promoting (PGP) traits of the actinomycetes were also studied. A total of 34 isolates of actinomycetes were studied from which 15 isolates were screened based on duel culture assay and PGP traits. Among the 15 isolates, 7 isolates (BCA-546, BCA-659, BCA-667, BCA-689, BCA-698, CAI-8 and CAI-133) were identified to be good biocontrol agents based on the secondary metabolite production bioassay. The PGP and biocontrol properties of the promising strains were carried out both *in vitro* and *in vivo* conditions, including the field trials, and promising results were obtained. The promising isolates were identified as *Streptomyces* spp. by 16S rDNA analysis. The metabolites of the most potential isolate will be further purified and identified.

**P39.022 Soil borne disease management of pulses in Bangladesh**

*A.H.M.M. Haque, A. Sarker, M.M. Kamal, M.M. Islam and M.G. Hossain*
*Pulses Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur-1701 and ICARDA, South Asia and China program, New Delhi, India*
*Email: mahfuzprc@gmail.com*

Foot and root rot is a complex soil borne disease of pulse crops in Bangladesh caused by plant pathogens i.e. *Sclerotium, Fusarium and Rhizoctonia*. Management of soil borne plant diseases are difficult and need for the integration of different approaches like cultural, early or late sowing/planting, deep plowing, crop rotation, use of resistant sources, incorporation of organic and inorganic residues, chemicals, biological control and use of antagonistic microorganisms are effective in controlling soil borne pathogens of pulses crop. Seed treatment with Provax -200 (Carboxin + Thirum) is also effective in reducing soil borne pathogens like *Sclerotium, Fusarium, Rhizoctonia*. Cultivation of tolerant or resistant varieties/ cultivars was found to be effective in managing foot ant root rot also wilt of lentil and chickpea. *Sclerotium rolfsii and Fusarium oxysporum* of lentil, chickpea and cowpea can be effectively controlled by chemicals. Biological agent *Trichoderma harzianum* was found to be effective in suppressing foot and root rot disease of lentil and chickpea. Integrated management of poultry refuse + Bavistin (Carbendazim 50%), Poultry refuse + Provax-200, Mustard oilcake +Provax-200 were found effective against soil borne fungal pathogens like *Sclerotium rolfsii* and *F. oxysporum*. Microbial antagonists are widely used for the bio-control of soil-borne fungal plant diseases. Rhizospheric bacteria and fungi have proved as excellent agents to control soil borne plant pathogens. Bacterial species of *Bacillus, Pseudomonas* as well as fungal species of *Trichoderma* have been showed capability in controlling soil borne fungal diseases.

**P39.023 Cultural practices on controlling ganoderma in Indonesia**

*A. Susanto, A.E. Prasetyo and H. Priwiratama*
*Indonesian Oil Palm Research Institute (IOPRI), Jalan Brigjen Katamso 51 Medan, Indonesia*
*Email: marihat_agus@yahoo.com*

Recently, basal stem rot disease of oil palm caused by *Ganoderma boninense* is the most destructive disease in oil palm plantation in South East Asia. *Ganoderma* could attack immature oil palm and incidence tend to increase with cycle of oil palm plantation. Symptoms appear earlier and heavier in the 3$^{th}$ and 4$^{th}$ generation of oil palm. Common strategies to control this soilborne plant pathogent are based on principles of soilborne disease epidemiology. Thus, reducing the number of early innoculant is the most effective strategy, and the reduced infection rate is complementary. The main source of *Ganoderma* innoculant are roots and trunks of infected palms. Therefore, sanitizing parts of plant which become source of innoculant is the best way to reduce *Ganoderma* attacks. Controlling *Ganoderma* innoculant is the key to reduce the incidence of basal stem rot. Researches of cultural practices consit of three series experiment, i.e. replanting through root and stem sanitation, surgery and mounding, replanting in immature palm by hole in hole tecnique. Replanting using root and stem sanitation could collect 7.25 ton roots/5 ha area. Cost to conduct this tecnique was Rp. 14.500.000, -/ha. No *Ganoderma* disease incidence has been observed yet after one year using this technique. The result from surgery and mounding technique experiment showed that surgery and mounding treatment could prolong bunch production of the infected palm. After two years application, only 1 palm collapse on surgery and mounding treatment, compared to 5 palms on control treatment. Replanting infected palm by hole in hole tecnique in immature stage was effectively decreasing

infection rate of *Ganoderma* disease. Obervation in the field after two year application of this tecnique, only 1 out of 563 palm was infected by *Ganoderma*. The research is on going to get more accurate data.

**P39.024 Influence of plant population and row width to the *Sclerotinia sclerotiorum* development on soybean**

*D.S. Jaccoud-Filho, F.F. Sartori, A.F. Fonseca, C.M. Vrisman, M.C. Pierre, C.R. Wutzki, M.A. Cantele, A. Berger-Neto, M.O. Manosso-Neto and A. Justino*
*Universidade Estadual de Ponta Grossa (State University of Ponta Grossa). Group of Applied Plant Pathology – DEFITO -Department of plant science and plant pathology, Avenida Carlos Calvacanti, 4748, CEP 84030-900, Ponta Grossa, PR, Brazil*
*Email: dj1002@uepg.br*

The "White Mold" (*Sclerotinia sclerotiorum*), has been responsible for losses in soybean crop areas in Brazil. The level of damage is related to the levels of cultivars susceptibility, climatic conditions and with the management strategies used in the crop production regions. In general, the average loss is 30%, but can reach more than 60% in some farms in Brazil. The chemical control has been the main strategy used, but despite the efficiency of some fungicides, they are expensive and present an environmental impact. Due to that, different strategies of management and control of the disease, more economic and environmental friendly has been searched. Amongst them, other strategies such as, the evaluation of plant population and row width in natural soyabean infected areas has been studied. In this project, a trial with 4 plant populations (150,000; 200,000; 250,000 and 300,000 plants) and row width (35, 45, 60 and 75 cm) was carried out in a natural infected field with 55 sclerotia/$m^2$. Based on the response surface results, the white mold incidence levels increase with the plant population and with the lowest row width. In the biggest plant population, the plants were highest, and presented the highest disease severity level. Also, the plant ramification levels increased with the row width reduction and population. The results of this research demonstrated the importance of the manipulation of plant population, row width and their influence in plant architecture in relation with the white mold management in soyabean crops.

**P39.025 Pathotype reaction in clubroot-resistant canola cultivars in Canada**

*A. Deora, B.D. Gossen and M.R. McDonald*
*Dept. of Plant Agriculture, University of Guelph, Guelph, Canada; Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Canada*
*Email: mrmcdona@uoguelph.ca*

Clubroot (*Plasmodiophora brassicae*) was reported first on canola (*Brassica napus*) in western Canada in 2003. Since then, several clubroot-resistant cultivars have been developed. The timing and expression of resistance to each of the major pathotypes (pathotypes 2, 3, 5, 6, Williams' system) in Canada was examined in four resistant cultivars. Root hair infection occurred at high levels in each resistant cultivar, but developed more slowly than in the susceptible control. Secondary infection and development in cortical cells was inhibited in each resistant cultivar; only a few bi-nucleated plasmodia were observed at 12 days after inoculation (DAI), and plasmodia were rarely observed at 18 and 24 DAI. In contrast, development in the susceptible cultivar had progressed to resting spores by 24 DAI. In addition, a dense ring of reactive oxygen species (ROS) accumulated in and around the endodermis of non-inoculated controls and inoculated plants of each of the resistant cultivars. However, the ROS ring disappeared rapidly in infected plants of the susceptible control. No specific points of ROS accumulation or lignification were observed in any of the resistant cultivars; which indicates that a hypersensitive response did not occur. Resistance to clubroot is generally pathotype specific, so the uniform response of the resistant cultivars to several pathotypes is one line of evidence indicating that the resistance in these cultivars is conditioned by a gene(s) from a single source. If so, this may pose a threat to the durability of this resistance for clubroot management on canola in this region.

**P39.026 Cost of resistance to *Plasmodiophora brassicae* when inoculum pressure is high**

*B.D. Gossen, M.R. McDonald, K.S. Harma, A. Deora and G. Peng*
*Dept. of Plant Agriculture, University of Guelph, Guelph, Canada; Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, Canada*
*Email: mrmcdona@uoguelph.ca*

Canola (*Brassica napus*) is a major crop in Canada, valued at over $15 billion (CDN) per year. Clubroot (*Plasmodiophora brassicae*) is spreading rapidly on canola, but use of clubroot-resistant cultivars provides effective disease reduction. However, several lines of evidence indicate that the yield of resistant cultivars of canola and other Brassicas is substantially reduced when inoculum pressure is high. In growth cabinet trials, biomass was reduced and plant development was delayed in plans of resistant cultivars inoculated with an avirulent pathotype, relative to non-inoculated controls. Similarly, growth and yield of resistant cultivars of canola and several Brassica vegetables in field trials were substantially lower at sites where inoculum pressure was high compared to nearby sites where inoculum pressure was low. In a crop rotation study that compared 1-, 3- and 11-yr breaks from canola at a heavily infested site, development of resistant canola cultivars was delayed

and yield was reduced by about 20% in the 1-yr break compared to the 3-yr break (severity in a susceptible cultivar was 100%, irrespective of cropping interval). Previous studies have shown that infection develops initially but does not persist in resistant canola cultivars, which indicates that resistance involves an active process of pathogen recognition and suppression. We conclude that there is a metabolic cost associated with expression of resistance when inoculum pressure is high that results in reduced plant size, delayed development, and reduced yield.

**P39.027 Infection by *Fusarium subglutinans* of *Pinus merkusii* seedlings and its biocontrol using *Trichoderma harzianum***

*S.M. Widyastuti, Harjono and M. Christita*
*Faculty of Forestry Gadjah Mada University Indonesia*
*Email: smwidyastuti@yahoo.com*

*Pinus merkusii* (tusam) is an Indonesian indigenous species with natural distribution in Aceh and North Sumatra. Damping-off disease, has become the main problem in the nursery. This disease is caused by the soil-borne pathogenic fungus *Fusarium subglutinans.* To develop an effective method of controlling this disease, the infection process of the fungus; the effectiveness of the biocontrol agent *Trichoderma harzianum* need to be addressed. The aims of this research were to understand (1) the infection process of *F. subglutinans*, (2) defense responses of tusam seedlings to infection by *F. subglutinans*, (3) the inhibition mechanism of *T. harzianum* on *F. subglutinans*. Methods used in this research included: (1) identification of the fungal pathogen, (2) pathogenicitiy test of *F. subglutinans*, (3) tissue staining (4) *in vitro* test of *T. harzianum*, and (5) *in planta* inoculation. The result showed that germinated spores of *F. subglutinans* were observed at two days post inoculation (dpi). Direct penetration of hyphae and hyphal penetration via stomatal aperture were found at three dpi. Hypersensitive response was detected at the stomatal aperture. Lignin and callose accumulation detected at three dpi. However, this host defense response was not effective in stopping fungal infection since *F. subglutinans* is a necrotrophic pathogen. *Trichoderma harzianum* GFP was able to delay the infection of *F. subglutinas in planta*.

**P39.028 Managing *Ganoderma* basal stem rot of oil palm: Innovative approach through endophytic microorganism application**

*S. Sundram, M. Sariah, A. S. Idris and O. Radziah*
*Malaysian Palm Oil Board, No 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia*
*Email: shamala@mpob.gov.my*

Basal stem rot of oil palm (*Elaeis guineensis* Jacq.) caused by *Ganoderma* spp. is the most devastating disease of oil palm in South East Asia. Endophytic microorganisms such as arbuscular mycorrhizal fungi (AMF) and endophytic bacteria (EB) has been previously described as potential biocontrol agents. The potential use of these endophytic microorganisms was investigated through antagonistic assessment against *Ganoderma* followed by *in vitro* compatibility between both endophytes, determination of biochemical responses and gene expression profile in pre-inoculated seedlings challenged with *G. boninense*, and finally field trial *via* seedling baiting technique. AMF (*Glomus intraradices* and *G. clarum*) and EB (*Pseudomonas aeruginosa* and *Burkholderia cepacia*) represent the endophytic microorganisms. Symbiotic interaction was observed between AMF species and EB with significant increase of germination and hyphal length of AMF spores. An interesting finding as these EB strains was never reported as potential mycorrhizal helper bacteria (MHB). Antagonistic effect of EB strains was also recorded through radial inhibition while scanning electron micrographs revealed severe morphological deformities such as shrivelling, flattening and shrinking of *G. boninense* hyphae in the presence EB strains. Production of POX, PPO, chitinase and β-1,3-glucanase during pre and post infection were enhanced in pre-inoculated seedlings and confirmed by the gene expression analysis. Field evaluation *via* seedling bait technique recorded reduced disease development. This is the first report of field seedling baiting technique to be successfully implemented in testing microbial pre-inoculation for disease suppression. Pre-inoculation with AMF and *P. aeruginosa* was most effective in reducing disease severity in oil palm.

**P39.029 Effect of mustard green manure and dried plant residue on chickpea wilt (*Fusarium oxysporum* f. sp*. ciceris*)**

*M. Abera*
*Department of Plant Sciences, College of Agriculture & Environmental Science, Bahirdar University, P.O. BOX 79, Bahirdar, Ethiopia*
*Email: merkuzabera@yahoo.com*

Pot experiments were carried out in the glass house at Amhara Regional Agriculture Research Institute (ARARI) Bahirdar, Ethiopia to evaluate the potential of *Brassica carinata* cultivars namely; Holleta-l, S-67 and Yellow Dodola. The effect of *Brassica carinata* (Ethiopian mustard) cultivars Holleta–1, S-67 and Yellow Dodola as green manure and Holleta–1 as dried plant residue on chickpea fusarium wilt (*Fusarium oxysporum* f. sp. *ciceris*) was studied. Six rates of green manure and dried plant residue (0, 20, 40, 60, 80 and 100g) each per kg of pathogen infested soil were used in the experiments. Infested soil without *B. carinata* cultivars amendment as a control and susceptible check variety JG-62 without amendment was used in the experiments. In the experiments, the treatments were arranged in randomized

complete block design in three replications and repeated twice. Data on seedling emergence, wilt incidence, fresh weight and dry shoot weight were collected. The amendments of infested soil with *B. carinata* cultivars green manure and dried plant residue reduced the incidence of chickpea fusarium wilt. The incorporation of the green manure Holleta-1, S-67 and Yellow Dodola at 20-100g/kg infested soil were effective in reducing wilt incidences on chickpea. However the incorporation of Yellow Dodola at 80 and 100g green manure per kg infested soil were the best combination in reducing significantly wilt incidence. The application of the dried plant residue at 20-100g/kg infested soil was effective in reducing wilt incidences on chickpea. However when applied dried plant residue at 60, 80 and 100g green manure per kg infested soil were better in reducing wilt incidence as compared to 20 and 40g/kg infested soil. The three cultivars green manure incorporated at different level of doses affected the influence of fusarium wilt on the fresh and dry shoot weight respectively. The use of Holleta-1 green manure at 20-100 g/kg infested soil significantly reduced disease incidence in the range of 20.0-33.3%. Green manure amendment S-67 significantly reduced disease incidence in the range of 26.7-53.3%. Yellow Dodola reduce disease incidence with 26.7-60%. The dried plant residue incorporated at different level influence fusarium wilt. The application of Holleta-1 dried plant residue at 20-100 g/kg infested soil reduced disease incidence in the range 20.0-26.7%. The results imply the potential of using *Brassica carinata* green manure and dried plant residue as cultural management components in chickpea fusarium wilt disease management.

**P39.030 Preliminary study on the *Pythium* root rot of garlic in Shandong province**

*B. Zhang, C.S. Li, J.S. Qi, Y.L. Zhang and L. Li*
*Plant Protection Institute, SAAS, No. 202 North Gongye Road, Licheng District, Jinan 250100, P. R. China*
*Email: zbo8341@163.com*

The *Pythium* root rot of garlic occurred in most garlic-growing areas of the Shandong province, causing considerable financial losses, and the disease has not been reported at home and abroad. The garlic was infected in the seedling stage and directly affected production. The pathogens cause plants dwarf, leaves bottom-up yellowed, root and basal part of stem discolored and rot. Fifty samples of root rot of garlic were collected from five counties in Shandong province. According to the Koch's postulates, the main pathogen was identified as *Pythium*, the isolated frequency was 46.78%, and 76 pathogens were obtained. Based on the morphological identification and ITS sequence alignment, the pathogens were identified as *Pythium sylvaticum*, *P. heterothallicum*, *P. paroecandrum* and *P. violae*. The biological studies suggested that the optimum temperature was 20-25℃ and the optimum pH was 6-7 for the mycelial growth of *Pythium*. In garlic field, quantity of *Pythium* changed with the temperature and humidity of soil. The 12 varieties of garlic from different areas were inoculated by 8 pathogens. The results showed that three varieties of garlic were resistant, two varieties of garlic were susceptible. In addition, the inhibitory effection of 8 fungicides on the mycelial growth of *Pythium* was investigated. Ovraclostrobin+metiram had exhibited the highest inhibitory action to *Pythium*, the median inhibitory concentration was 2.612 mg/L. Famoxadone+cymoxanil, Cymoxanil-mancozeb, Copper hydroxide and Mancozeb showed better. Screening of fungicides in the field, Ovraclostrobin-metiram and Copper calcium sulphate were better than others, their control efficiency were 91.86% and 81.46%, respectively.

**P39.031 Delayed leaf senescence of soybean caused by Rhizoctonia aerial blight in Japan and its chemical control**

*T. Takehara[1], H. Inoue[2] and H. Miyagawa[1]*
*[1]Western Region Agricultural Research Center, National Agriculture and Food Research Organization, Fukuyama, Hiroshima 721-8514, Japan; [2]Kyushu Okinawa Agricultural Research Center, National Agriculture & Food Research Organization, Koshi, Kumamoto 861-1192, Japan*
*Email: toshi@affrc.go.jp*

Rhizoctonia aerial blight of soybean caused by *Rhizoctonia solani* AG-1 sometimes become a serious problem in Japan. To investigate the relationship between the disease and the delayed leaf senescence (DLS, or green stem syndrome) of soybean which is also a problem in Japan, field inoculation tests were conducted over 2 years. Soybean was seeded in an experimental field in Hiroshima Prefecture in early- to mid-July. After flowering, plants were inoculated by scattering the pathogen cultured on mixture of wheat bran, rice chaff and rice straw. Five to 19 days afterward, Azoxystrobin wettable powder, Flutolanil wettable powder, or Validamycin A liquid concentrate was sprinkled on soybean foliage. In some plots, frequent overhead irrigation was performed from late August to early October to promote disease development. For each plant, disease level in mid-September, and DLS at time of harvest in October, was rated at index of 0-4. In inoculated and frequently irrigated plots without chemical application, disease and DLS occurred in high rate. Occurrence of disease and DLS was low in inoculated but non-irrigated plots. In plants with DLS index 4, the number of pods was notably low. Without chemical application, soybean yield decreased 12 to 35% by the disease compared with non-inoculated plots. DLS and yield loss by the disease was controlled by spray of the above-mentioned chemicals at pod-elongation period. From these results, it became clear that Rhizoctonia aerial blight of soybean is a

cause of DLS and that frequent rainfall promotes the disease development and the resulting DLS.

### P39.033 Crop rotation effect on *Fusarium oxysporum* Schlecht. and antagonists fungi

*M. Andreotti, R.A. Rojo, N. López, M.J. Beribe, R. Agamennoni, P. Baffoni, A.L. Gasoni, M.C. Martínez and V. Barrera*
*Instituto de Microbiología y Zoología Agrícola, Instituto Nacional de Tecnología Agropecuaria, Los Reseros y Las Cabañas, (1712) Castelar, Argentina*
*Email: gasoni@cnia.inta.gov.ar*

Crop rotation is utilized for controlling *Fusarium oxysporum*, causing basal rot of onion. The objectives were: 1) to determine the presence of *F. oxysporum* in 8 treatments; 2) To identify fungal genera with potential antagonistic ability against soil phytopathogens; 3) To evaluate the efficiency of the antagonistic agents through *in vitro* tests. Soil samples from different crop rotation combinations were analyzed during 2009, 2010 and 2011 periods: We used three different isolation methods: 1) Sterile seed baits; 2) Komada medium and 3) Serial dilutions. Fifteen combinations of dual tests were used with 20 experimental units for each treatment. Two measurements in each plate were repeated daily until 72hs: The radii of the pathogen colonies in the antagonistic colonies direction (R1) and the radii in the opposite direction were measured as an internal control (R2). The results obtained from different treatments were evaluated utilizing a mixed model for measurements repeated in time. Colonies of *F. oxysporum* were detected in 8, 7 and 4 treatments in 2009, 2010 and 2011 respectively. Antagonistic isolates were detected: *Trichoderma* spp., *Cladorrhinum* spp. and/or *Laetisaria* spp. *T. harzianum* and *L. arvalis* were efficient as antagonists against *Sclerotium* spp. and *Rhizoctonia solani*. *Cladorrhinum samala* and *C. bulbillosum* showed less efficiency on controlling them. *F. oxysporum* stopped the growth of *C. samala* and *C. bulbillosum* by forming an inhibition halo. It is necessary to determine the effect of the fungal antagonistic agents and rotations on the inoculum of *F. oxysporum* in the soil.

### P39.034 Pathogen identification of ginger stalk rot from Shandong province

*J.D. Yuan, Y.L. Zhang, J.S. Qi, B. Zhang, Z.T. Xu, L. Li and C.S. Li*
*Institute of Plant Protection, SAAS, No.202 North Industrial Road, Licheng District, Jinan, Shandong, 250100, P. R. China*
*Email: lics1011@sina.com*

In recent years, ginger stalk rot was a soil-borne fungi diseases caused by *Pythium* survived extended periods in naturally infested ginger rhizosphere soil. Ginger stalk rot occurred in most ginger-growing areas in Shandong Province, and caused varying degrees of destruction and economic loss. At present, the pathogens have not yet been clear, and the occurrence regularity and control methods were still in its infancy. 607 isolates from 56 ginger samples with rhizome rotting symptoms obtained in 2009-2010 from ginger fields from Pingdu, Tai'an, Anqiu, Changyi, Laiwu and Juxian areas, were identified as *Pythium* spp..The pathogenicity of the 96 strains typical pathogens of *Pythium* and Koch rule validation were made. The results showed that all of the pathogens had pathogenicity to ginger. The pathogens could be re-isolated from the infected ginger. The 14 *Pythium* taxons were identified as *P. myriotylum* Drechsler by morphological methods and nr DNA-ITS technology. The phylogenetic tree based on ITS sequence has showed that 14 *Pythium* taxons were divided into 3 subgroups: the strains from Py01, Py02, Py03, Py04, Py05, Py06, Py07, Py09, Py11, Py12, and Py13 were in a sub-group; the strains Py08 and Py10 in another sub-group; Py14 as a individual sub-group. This indicates that although all of the 14 *Pythium* were identified as *P. myriotylum*, but there were still some differences within species.

### P39.035 Identification of peanut *Pythium* root rot pathogen in Shandong province

*F. Qiao[1,2], B. Zhang[1], L. Li[1], J.S. Qi[1], Z.T. Xu[1], C.S. Li[1] and B.D. Li[2]*
*[1]Institute of Plant Protection, SAAS, No.202 North Industrial Road, Licheng District, Jinan, Shandong, 250100, P. R. China; [2]Qingdao Agricultural University , Qingdao, Shandong, 266109, P. R. China*
*Email: lics1011@sina.com*

During 2009 to 2010, samples of diseased peanut root were collected from Shandong Province of China. Ten isolates from different location of Shandong Province were obtained from diseased peanut root for pathogenicity tests. Inoculation on healthful peanut root with these isolates produced the same symptoms as naturally infected plants. The same strains were isolated from the inoculated peanut root. According to its characteristics of morphology and sequence analysis of the internal transcribed spacer region of the ribosomal DNA (ITS), the pathogens causing peanut root rot in Shandong province were identified as *Pythium myriotylum* Drechsler, *P. helicoides* Drechsler, *P. irregulare* Buisman and *P. ultimum* Trow. The four *Pythium* species grow best in the CMA, $VP_3$ and PDA from 5 kinds of medium. The optimum growing temperature range for the four *Pythium* species is between 25-30℃, and the optimum growing pH range for the four *Pythium* species is between 6-7.

**P39.036 Several pathogens causing ginger root diseases**

*H.H. Zhao, Z.F. Zhang and C. Liang*
*College of Agronomy and Plant Protection, Qingdao Agricultural University, No. 700 Changcheng Road, Chengyang District, Qingdao, 266109, P. R. China*
*Email: syliangchen@163.com*

*Zingiber officinale* Roscoe is an important plant as a condiment, herbal medicine and vegetable. But diseases always occur during the growing season. More threatened diseases on ginger root causing production reduction. In 2011 November, severe ginger root diseases happened in Qingdao. The diseased ginger roots exhibited complex symptoms, some were partially rot, some became red, brown or rusty in peel color, and some were colonized by mycelia on the surface. According to the Koch's postulates, pathogens were isolated and purified, subsequently morphological and molecular identification were carried out. Several pathogens were identified from this disease complex, including *Fusarium graminearum* Schwabe, *F. solani* (Mart.) Appel & Wollenw., *Rhizoctonia solani* J.G. Kühn, *Acremonium stromaticum* W. Gams & R.H. Stover. Additionally several *Pythium* species were also detected.

**P39.037 Identification of pathogenicity-related and functional genes in *Verticillium dahliae* by T-DNA insertional mutagenesis**

*S. Deng, C.Y. Wang, X. Zhang and L. Lin*
*Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, P. R. China*
*Email: linling@jaas.ac.cn*

In worldwide, many economically important crops are threatened by the vascular wilt caused by *Verticillium dahliae*. There are few resistant varieties and effective fungicides available, and after infection it will result in huge loss even complete crop failures. However, the molecular mechanism and the regulatory network of the pathogenicity are still little understood. In our study, more than 6000 transformants of *V. dahliae* were obtained by *Agrobacterium tumefaciens*-mediated transformation. One thousand of these random insertional mutants had been screened and the mutants which have defects or changes in pathogenicity and/or morphology were selected, including four mycelium morphological variation, two pigmentation traits, six mycelium growth reduction and nine pathogenicity defect or compromise. Recently, two pathogenicity-defective mutants were studied, and the disrupted genes will be cloned. We hope these mutants can tell us some interesting stories and shed new light on the molecular mechanism of the pathogencity of *V. dahliae*.

**P39.038 Differentiation of physiological races of *Fusarium oxysporum* f.sp. *niveum* in Jiangsu Province in China**

*H.Y. Sun, W. Li, Y.Y. Deng, and H.G. Chen*
*Institute of Plant Protection, Jiangsu Academy of Agriculture Sciences, Nanjing, 210014, P. R. China*
*E-mail: haiyansun@jaas.ac.cn*

*Fusarium oxysporum* f. sp. *niveum* (E.F.Sm.) W.C. Snyder & H.N. Han., the causal agent of Fusarium wilt of watermelon, is widespread in watermelon growing regions of the world, and frequently is the major factor limiting triploid (seedless) watermelon production. The promotion and use of resistant cultivars is the most economical and effective way to control the disease. Differences in virulence among isolates of *F. oxysporum* f. sp. *niveum* have long been recognized and isolates are subdivided into three races on the basis of virulence on watermelon cultivars that vary in their level of resistance. In order to investigate the differentiation of physiological races, vegetative compatibility groups of *Fusarium oxysporum* f. sp. *niveum* in Jiangsu province, and also the relation between them，fifteen isolates of *Fusarium oxysporum* f. sp. *niveum* were collected from different fields in Jiangsu province, physiological races of which were identified by inoculation on identified host(Sumi No1, Charleston Gray and Calhoun Gray) and vegetative compatibility of which were determined by the technology of nitrate reductase deficiency. Race 0, race 1 and race 2 were detected among 15 isolates, the frequencies of which were 6.7%, 73.3% and 20%, respectively. All isolates belong to one VCG, but each of them showed differences in the capacity to compatibility, which was not associated with Physiological races and locations. Isolates belonged to different races were vegetative compatible to each other.

**P39.039 Evaluation of potential risks of soils for Fusarium wilt of spinach using nitrate-nonutilizing mutants of the pathogen, *Fusarium oxysporum* f. sp. *spinaciae***

*H. Sekiguchi[1], T. Takehara[1], K. Tomioka[1], K. Nomiyama[1], H. Osaki[1], H. Miyagawa[1] and T. Shinano[2]*
*[1]Western Region Agricultural Research Center, National Agriculture & Food Research Organization, Fukuyama, 721-8514 Japan, [2]Hokkaido Agricultural Research Center, National Agriculture & Food Research Organization, Sapporo, 062-8555 Japan*
*Email: sekihiro@affrc.go.jp*

We are studying on control methods of soilborne plant diseases considering reduction of agricultural chemicals for environmental conservation. Application of soils having suppressive effects on the diseases seems to be one of the promising techniques. Close interactions of soil microbial communities with soil environments are recognized to influence occurrence of the diseases. But,

mechanisms of the influence as well as the interactions have not been completely clarified because there are no adequate means for their objective evaluation. In this study, we attempted to evaluate potential risks of soils for Fusarium wilt of spinach using a nitrate-nonutilizing mutant (*nit* mutant) of the pathogen, *Fusarium oxysporum* f. sp. *spinaciae*. Soil samples (50 g) collected from various areas in Japan was incubated at 28℃ after adding bud cells ($5\times10^5$ cells) of the *nit* mutant. Population density of the mutant was determined periodically using a selective medium containing chlorate, miconazole and pentachloronitrobenzene. The mutant formed clearly distinguishable colonies on the medium without disturbance from other diverse microorganisms, and the fluctuation of population density varied on the samples. From these results, we think that *nit* mutants are useful to evaluate potential risks of soils for soilborne plant diseases such as Fusarium wilt of spinach. It is expected that using these soils with different risks evaluated by this method, mechanisms of interactions between soil microbial communities and soil environment and their influence on occurrence of the disease may be efficiently analyzed.

### P39.040 Serological quantification of resting spores of *Olpidium virulentus* in lettuce roots and relationship between resting spore density in soil and severity of lettuce big-vein disease

*K. Nomiyama[1], H. Sekiguchi[1], T. Sasaya[2], K. Tomioka[1], H. Osaki[1], H. Miyagawa[1] and T. Takehara[1]*
*[1]Western Region Agricultural Research Center, National Agriculture & Food Research Organization, Fukuyama, 721-8514 Japan; [2]Kyushu Okinawa Agricultural Research Center, National Agriculture & Food Research Organization, Koshi, 861-1192 Japan*
*Email: sekihiro@affrc.go.jp*

*Olpidium virulentus* (Sahtiy.) Karling, a soilborne chytrid fungus, transmits *Miraforri lettuce big-vein virus*, the causal agent of lettuce big-vein disease. Since *O. virulentus* is an obligate parasite, it cannot be quantified by dilution plate technique. Nomiyama et al. (2013) already reported preparation of polyclonal antibody against resting spores of *O. virulentus*. This time, we used DAS-ELISA with the antibody to quantify the number of resting spores in infected roots and evaluated relationship between the spore density of *O. virulentus* in soil and severity of the disease. In DAS-ELISA, a calibration curve was prepared using the absorbance of purified resting spores pulverized with zirconia beads, and then infected root samples bead-beaten in the same manner were applied. Dry infected root powder in which resting spores had been counted by DAS-ELISA was added to autoclaved commercial nursery soil and the spore density was adjusted to 1, 10, $10^2$, $10^3$ and $10^4$ spores/g of soil. Thirty-day-old lettuce seedlings were transplanted in the soil and grown for 80 days. In the case of $10^4$ spores/g of soil, symptom began to appear in about 40 days after transplanting, and almost all plants showed symptom at 60 days after transplanting. As the spore density decreased, the onset of symptom delayed and the incidence reduced. All of tested plants remained disease-free at under 10 spores/g of soil. This finding can be used as an indicator in developing the soil diagnosis method for the disease.

### P39.041 Integrated management of bacterial wilt in the production of *Solanaceae* vegetables in Hainan

*J.R. Guo, Y.L. Pei, J. Peng, F.Y. Zeng and H.B. Long*
*Ministry of Agriculture Key Laboratory of Integrated Pest Management on Tropical Crops, Institute of Environmental and Plant Protection, Chinese Academy of Tropical Agricultural Sciences, 4 Xueyue Road, Haikou, 571101, Hainan, P. R. China*
*Email: guojianrong@hotmail.com*

Bacterial wilt caused by *Ralstonia solanacearum* (Rs) is currently the most serious disease in the production of *Solanaceae* vegetables. In recent years, primary studies on its occurrence, biodiversity, epidemiology and integrated management were done. More than 100 strains were isolated from eggplants, paprika or tomato plants at different locations of Hainan and their biodiversity was analyzed by using different DNA markers. High genetic biodiversity was shown not only among strains from different regions but also among those inside one field. Grafting was applied as a usual technique to effectively control bacterial wilt in eggplant production in Hainan since 2005, using Dulubamu (*Solanum torvum*) as rootstock. In order to find new rootstocks and varieties with high resistance to Rs, totally 14 different eggplant rootstocks and 15 tomato varieties was first evaluated in green house by artificial inoculation. The resistance of these rootstocks was further investigated under field conditions. Two rootstock varieties were better than Dulubamu and showed a good application potential. To find some useful biocontrol resources, an initial isolation and screening of endophytes and antagonistic strains from plant tissues or root rhizospheres has been started. Until now, more than 20 antagonistic strains were isolated and their effects on Rs will be further evaluated. In addition, the impact of some agronomic measures such as watering, fertilizing and rotating techniques on the epidemics of Rs was investigated. It is demonstrated that integrated management is important guarantee for the effective and sustainable control of bacterial wilt.

### P39.042 Molecular detection and markers of avirulent genes in *Phytophthora sojae*

*J.Z. Wen, L. Sun, Q.M. Chen and L.M. Zhao*
*Department of Plant Protection, Agricultural College, Northeast Agricultural University, Harbin, 150030, P. R.*

*China*
*Email: jzhwen2000@yahoo.com.cn*

AFLP molecular marker of avirulent gene *Avr1c*, which sequence was not published on Genbank, was studied in this experiment. The specific bands of avirulent gene *Avr1c*, amplified by primers of EGC 5´-GACTGCGTA CCAATTCGC / MAT 5´-GATGAGTCCTGAGTAAA T, were cloned and sequenced, and primers designed according to the sequence were test on effectiveness and specificity. The result showed that primers *F*: 5'-AGCC AGCAGAGAGTAGAA and *R*: 5'-ATTAGGGCAATA TCATA can successfully and specifically amplify a 600 bp fragment from *Phytophthora sojae* including avirulent *Avr1c*. According to Genbank sequence table of *Avr1a*, *Avr1k* and *Avr3a*, avirulent genes of *P. sojae*, specific primers were filtered out respectively for the three genes by using primer design method. 86 strains of *P. sojae* which have been identified virulence on the three differential soybean varieties were detected by PCR on the basis of optimized reaction system and amplification conditions. A specific detection system was established for detecting *Avr1a*, *Avr1k* and *Avr3a*, avirulent genes of *P. sojae*. The results of molecular identification and inoculation identification were compared and analyzed. And then amplified true and false positive bands were recovered respectively and cloning sequenced and compared with the original sequences of the three avirulent genes, to verify if *Avr1a*, *Avr1k* and *Avr3a* were suitable to be detected by the method of molecular markers. All the specific primers screened out could amplify a band with length of about 550 bp. The coincidence rate of molecular and inoculation identification for the three avirulent genes were *Avr1a*-45.3%, *Avr1k*-84.9% and *Avr3a*-97.7%. The true positive bands of three avirulent genes had over 97 percent of consistency in sequence with the original one. The false positive bands of *Avr1a* were about 80 percent, and *Avr1k* and *Avr3a* were less than 30 percent of consistency with their original sequences. The detection system established by using the primers filtered out by utilizing the gene sequence of *Avr1a*, *Avr1k* and *Avr3a* can be used to detect *Avr3a* rapidly, but not be suitable for detection of *Avr1a*, whether fit for detection of *Avr1k* needed further study.

### P39.043 The complete genomic sequence of a novel mycovirus from *Rhizoctonia solani* AG-1 IA strain B275

*L. Zheng, M.L. Zhang, X. Cao and E.X. Zhou*
*Department of Plant Pathology, South China Agricultural University, Guangzhou, Guangdong 510642, P. R. China*
*Email: exzhou@scau.edu.cn*

The basidiomycete fungus *Rhizoctonia solani* Kühn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk] is a notorious soil-borne plant pathogen and exhibits a wide host range that include rice, maize, potato, peanut, soybean, alfalfa, vegetables, ornamental plants, forest and fruit trees. Mycoviruses commonly occur in the major taxonomic groups of filamentous fungi, yeasts, and oomycetes. Most of the characterized mycoviruses contain double-stranded RNA (dsRNA) genomes. A novel mycovirus, *Rhizoctonia solani* dsRNA virus 1 (RsRV1) was isolated from *R. solani* AG-1 IA strain B275, the causal agent of rice sheath blight, and the complete genome of RsRV1 was sequenced and analyzed. It is composed of two dsRNA genome segments 2379 bp and 1811 bp in length, which were referred to as RsRV1-1 and RsRV1-2, respectively. RsRV1-1 contains a single open reading frame (ORF1), which has a conserved RNA-dependent RNA polymerase (RdRp) domain, whereas RsRV1-2 contains a single ORF2 which might encode a multifunctional protein. The genome organization of RsRV1 is similar to members of the family *Partitiviridae*. However, phylogenetic analysis indicated that RsRV1 formed a distinct clade together with three other unclassified viruses, suggesting that RsRV1 may belong to a new family of dsRNA mycoviruses. This is the first report of the full-length nucleotide sequence of a novel dsRNA mycovirus RsRV1 infecting *R. solani* AG-1 IA strain B275.

### P39.044 Quantification of *Verticillium dahliae* microsclerotia in soil using wet-sieving and qRT-PCR

*F. Wei[1], W.J. Shang[1], L.J. Ma[1], J.R. Yang[1], X.M. Xu[1,2] and X.P. Hu[1]*
*[1]State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Tai Cheng Road 3, Yangling, Shaanxi 712100, P. R. China; [2]Plant Pathology, East Malling Research, New Road, East Malling, Kent ME19 6BJ, UK*
*Email: malijie1016@163.com*

*Verticillium dahliae* Kleb. is a soilborne plant pathogen which infects more than 200 plant species. Microsclerotia, the main survival structures of *V. dahliae* and primary causal inoculum agent of host plants, may remain viable in soil more than 10 years with the lack of hosts. The quantification of microsclerotia of *V. dahliae* is the base for monitoring and prediction of Verticillium wilt. The number of microsclerotia in soil is usually estimated by colonies recovered from plating of soil samples in semi-selective medium and microscopic analysis. But it takes 6 to 8 weeks to complete. Hence, an alternative method to test 20 g soil samples using wet-sieving extraction of microsclerotia followed by quantification real-time PCR (qRT-PCR) assay was developed. The standard curve for quantification of *V. dahliae* microsclerotia was established using 10-fold dilutions for DNA of 10 000 microsclerotia. A high correlation was observed in regression analysis ($R^2$=0.93) between qRT-PCR results and soil plating in a range of field soils with different microsclerotia densities. Tests with naturally

infested soils showed that the new method is repeatable and sensitive (0.5 microsclerotium/g soil), and can be finished within 1 day. With this method, 66 soil samples from two disease nurseries were tested. Results indicated that the dencity of 40.9±18.1 microsclerotia /g dry soil caused 94.2% wilt incidence on Jimian 11 (*Gossypium hirsutum*) (susceptible to *V.dahliae*) and 61.4% on Zhongzhimian 2 (*G. hirsutum*) (resistant to *V.dahliae*), but about 8.7±2.1 microsclerotia /g dry soil caused 92.8% and 24.4% on both cultivars, respectively.

**P39.045 Identification of resistance genes to *Pythium* species in soybean cultivars by association analysis**

*L. Wei[1,4], Y.G. Xue[1], W.W. Wang[2], L.J. Liu[1], W.B. Li[3], D.Y. Yu[4], D. Nan[1], H.X. Liang[1] and B.G. Lou[5]*
*[1]Soybean Research Institute, Heilongjiang Academy of Agricultural Sciences, Harbin 150086; [2]Agricultural Products Quality and Safety Research Institute, Heilongjang Academy of Agricultural Sciences, Harbin 150086; [3]Northeast Agrivultural University, Harbin 150030; [4]Nanjing Agricultural university, Nanjing 210095; [5]Institute of Biotechnology, Zhejiang University, Hangzhou 310029, P. R. China*
*Email: nkyssbd@126.com*

By using 150 soybean varieties with different ecotype as material, and 4 *Pythium* species as inoculation pathogens, the cultivar resistances were identified through measuring the root rot level. 256 SSR markers were used to analysis the population structure and linkage disequilibrium. A soybean resistance association analysis to 4 *Pythium* species was run with GLM procedure from software TASSEL, and a T test on associated markers with significant difference was carried on, to uncover associated loci conferring effects. The result showed: there is a comprehensive linkage disequilibrium existed; the collinear locus D′declined quickly, which made it fit for LD analysis; tested cultivars were divided into seven subgroups by the structural analysis of SSR data. There were 25 markers obtained by association analysis, which were connected with the resistance to 4 *Pythium* species, respectively; through T test and resistance identifying, 12 association loci with significant difference, confer major effect values to the tested cultivars. The loci with significant difference highly matched the cultivars' resistant testing results; especially the ones with high reliability in association analysis show the same on T test, such as the loci associated with *P. aphanidermatum* resistance, Satt191-1226bp, Satt584 -1, Satt584-2, and those with *P. irregular* resistance, Satt602-1, Satt042-4.

**P39.046 The influence of rhizosphere environment material from three crops on pathogens of Fusarium wilt and Verticillium wilt**

*X.D. Lu[1], P. Kharbanda[2], W.L. Jin[1], J.M. Dong[1], L.H. Guo[1] and H.H. Tian[3]*
*[1]Shihezi Vegetable Research Institute in XinJiang, P. R. China; [2]The Alberta Senior Research Council in Plant Pathology, Alberta T6T1HI, Canada; [3]Shouyangshan D&C Mamagement Committee in Changle, Shandong Province, P. R. China*
*Email: lxdcom@yahoo.com.cn*

Soil-borne diseases of Cotton, Fusarium wilt (CF) and Verticillium wilt (CV) exist widely in Xinjiang area. Also, on Processing tomato, Fusarium wilt (TF) and Verticillium wilt (TV), and on peppers, Fusarium wilt (PF) and Verticillium wilt (PV) are commonly found in this area. We determined the effects of root exudates from aforementioned crop seedlings on soil-borne pathogens of CF, TF, PF and CV, TV, PV. Our results show that the root exudates of all these three crops can promote the growth of CF, TF, PF pathogens, and all of them show a positive linear correlation. In terms of degree of influence of root exudates on TF and PF pathogen growth cotton root exudates (CRE) > pepper root exudates (PRE) > processing tomato root exudates (TRE) > sterile water (ck). CRE and PRE could promote the TV pathogen growth and all show straight line equation trends. However, PRE also promoted the growth of PV and CV pathogens but showed a complicated polynomial equation trend. We applied 70% thiophanate-methyl WP (TPh), 4% pyrimidine nucleoside antibiotic AS (PN) and 20% Complex ammonia copper zinc (AC), Chlorothalonil WP (Ctl), and Myclobutanil WP (Mbl) as exogenous fungal inhibitors and were tested at four dilutions: 500, 1000, 2000, and 3000 times. The result showed that TPh diluted up to 3000 times, had strong inhibition on TF and TV pathogen growth. Interestingly, the PN antibiotic diluted 500 times had a stronger inhibitory effect than by diluting 1000 times on TF and TV pathogen growth. Complex ACZ diluted 500 times had a stronger inhibitory effect than when diluted further on TF and TV pathogen growth; Ctl and Mbl had inhibitory effect only when diluted by 500 times.

**P39.047 Effect of the fertilization in improving the physical and health quality of corm and cormels of gladiolus in Mexico**

*E. González-Pérez[1], O.J. Ayala-Garay[2] and M.J. Yáñez-Morales[3]*
*[1]Instituto Nacional de Investigaciones Forestales, Agrícolas and Pecuarias. INIFAP. Campo Experimental Bajío. Km 6.5 Carretera Celaya-San Miguel de Allende, Celaya, Guanajuato. C.P. 38110. México; [2]Instituto de Recursos Naturales; [3]Instituto de Fitosanidad, Colegio de Postgraduados. Campus Montecillo. Km. 36.5 Carr. Mexico-Texcoco. C.P. 56230 Montecillo, Texcoco, Edo. de Mexico. Mexico*
*Email: yanezmj@colpos.mx*

In Puebla State, gladiolus (*Gladiolus grandiflorus* Hort.)

production has asexual propagation problems mainly due to *Fusarium oxysporum* f. sp. *gladiolus*. The aim of this study was to evaluate the effect of different fertilization doses (FD) to improve some physical and health qualities in gladiolus corms and cormels and to protect them from soilborne fungi. During 2009 the corms of two varieties were generated at four different FD that included the regional one (81 N-24 P-171 K-23 Mg-37.2 Ca). For each FD physical quality was assessed in 240 corms (fresh weight, number of cormels generated and their total weight) and in their cormels (size in mm and total weight). Cormel ś health was analyzed in 160 disinfested tissue pieces that were plated in PDA with or without splitting. Percentage of identified fungus incidence was recorded. As a result, some corm ś physical qualities were characteristic of varieties; and only in 31%, FD2 in Borrega Roja generated the highest number of cormels. FD1 and FD2 were the best for total weight in both varieties. In cormels, the size 6-8 mm registered the highest weight in the same FD. The best cormel's health was with FD2. In both varieties the lowest percentage of isolated *Fusarium* colonies was 17.5% in external tissue and 0.0% in internal tissue, while the control had 70 and 30% respectively of *F. oxysporum*. Thus, the effect of FD2 was in generating a better physical and health quality of corms and cormels and unlike the regional FD, FD2 showed a balanced interaction of N and had the addition of B and S.

**P39.048 Good crop rotation practise prevents clubroot but not fungal root and stem base diseases in turnip and oilseed rape**

*M.L.T. Rastas, S.M.H. Latvala and A.O. Hannukkala*
*MTT AgriFood Research Finland, Plant Production Research, Planta, 31600 Jokioinen, Finland*
*Email: marika.rastas@mtt.fi*

In a field survey conducted in Finland in 2007-2009 turnip and oilseed rape (*Brassica rapa* subsp. *oleifera* and *B. napus* subsp. *oleifera*) fields were observed for plant pathogens and information about the cultivation techniques used in the fields was collected. Most of the farmers practiced crop rotation and in 70 % of the field plots there was at least five years break between the brassica crops. This seems to prevent the severe disease incidences of clubroot (*Plasmodiophora brassicae*). On average, clubroot was found in 25 % of the fields and in most of the fields less than 10% of the plants were infected. In the fields with continuous brassica cultivation or only one year break between brassica crops, the clubroot incidence was 50 % and a significantly higher proportion of plants was infected. However, for fungal soil borne pathogens causing root and stem base rots (*Rhizoctonia solani* and *Fusarium* spp.) crop rotation had no significant effect. Fungal root damages were found in 98 % of the fields. Only a minor increase in proportion of root rot incidence was observed with frequent brassica cultivation. Crop rotation had no effect on the stem base rot incidence. Crop rotation is not a sufficient method in maintaining the healthy root system in oilseed crops and more information is needed to control the fungal damages.

**P39.049 Fungal root damages in turnip rape in Finland**

*M.L.T. Rastas, S.M.H. Latvala and A.O. Hannukkala*
*MTT AgriFood Research Finland, Plant Production Research, Planta, 31600 Jokioinen, Finland*
*Email: marika.rastas@mtt.fi*

A high occurrence of the root damages in turnip and oilseed rape (*Brassica rapa* subsp. *oleifera* and *B. napus* subsp. *oleifera*) was recorded in a field survey conducted in Finland in 2007-2009. In nearly all of the 470 surveyed turnip and oilseed fields, plants suffering from severe root damages were found. In a similar survey in the 1980s damages of this kind were not observed, so the frequent occurrence of the root damages is relatively a new problem in the oilseed crops in Finland. The symptoms resembled brown girdling root rot (caused by *Rhizoctonia solani*): damaged roots were darkened throughout the tap root or were severely deformed with deep cavities. Even though the occurrence was high, the disease incidence in the fields was moderate: Only 15% of the fields had more than 30 % of the plants with root damages. The root damages are likely caused by plant pathogenic fungi. *R. solani* and *Fusarium* spp. were isolated frequently from the dark lesions in the roots. Insect damage was also observed in the roots but there was no correlation between the insect damage and the dark coloration.

**P39.050 A study on harmless comprehensive treatments of *Phytophthora capsici***

*Q. Zhang, Y. Zhang, C.J. Ma and Y.G. Li*
*Institute of Plant Protect, SAAS; Shanxi Seed Station; Shanxi Plant Protection Station, Taiyuan, Shanxi, China, 030032, P. R. China*
*Email: zhangqi54@126.com*

The *Phytophthora capsici* was a disease which often damaged pepper growth. It was not easy to find at primary infected stage. It was difficult to control on occurring and unstable for chemical control. For the point of ecology, biodiversity and no-harm product, the study was carried for 8 years. The field investigation of diseased plants under different ecological areas, soil texture and water quality was carried and more than 1000 soil samples for 3 years was collected. Analysis on *P. capsici* population number dynamic distribution, germs paired type and transmission routes were taken. Techniques of "interplanting maize in pepper field forming shading, barrier and rhizosphere effect" and "drag soil putting into sowing point" were developed and used,

which solved the instability of *P. capsici* control. The rate of diseased plant was less than 10% and it also controlled some other related diseases. These realized one control more treatment and a significant economic benefit of pepper production.

**P39.051 *Spongospora subterranea* causes three potato diseases; tuber lesions, root galls and root malfunction**

*R.E. Falloon and U. Merz*
*The New Zealand Institute for Plant & Food Research Limited, PB 4704, Christchurch 8140, New Zealand*
*Email: richard.falloon@plantandfood.co.nz*

The cercozoan pathogen *Spongospora subterranea* f. sp. *subterranea* causes powdery scab of potato tubers (modified stems), which is a quality-limiting and economically important disease. Lesions on tubers contain many sporosori, the perennation stage of the pathogen. The pathogen also causes hypertrophic growths (galls) on roots, which are small (<3 mm), initially white but later brown, and occur throughout root systems of plants growing in *S. subterranea*-infested soil. Root galls become filled with sporosori of the pathogen. Both of these diseases have long been recognized as important in powdery scab epidemiology. Lesions on seed tubers are responsible for widespread (including intercontinental) dissemination of the pathogen. Root galls cause build-up of inoculum in cropping soils. The significance of a third disease caused by *S. subterranea* has been recognized more recently. Zoospores released from resting spores infect roots of young potato plants at early stages of plant growth, and multiple cycles of zoospores are produced from zoosporangia developing in root epidermis cells. This development of the pathogen has been shown to coincide with disrupted uptake of water and plant nutrients, and results in reduced plant productivity, including diminished tuber yields. Root malfunction potentially poses severe problems for potato production, as pesticide control is unlikely to be economic. Furthermore, potato cultivars resistant to tuber powdery scab can develop severe root galling and root malfunction from *S. subterranea* infection. This third disease is causing severe problems for intensive potato production in New Zealand and Australia, and probably in other countries.

**P39.052 Glyphosate increases take-all inoculum in the rhizomes of *Elytrigia repens*, an alternative host of *Gaeumannomyces graminis* var. *tritici***

*S.F. Chng, M.G. Cromey, S. Keenan, R.C. Butler and R. Warren*
*The New Zealand Institute for Plant & Food Research Limited, Private Bag 4704, Christchurch 8140, New Zealand*
*Email: Soonie.Chng@plantandfood.co.nz*

Take-all, caused by *Gaeumannomyces graminis* var. *tritici* (*Ggt*), is an important root disease of wheat. *Ggt* relies on susceptible hosts and their residues for multiplication and survival. *Elytrigia repens*, a common grass weed in New Zealand wheat fields, is an alternative host of *Ggt*. While *E. repens* can be effectively controlled by the herbicide glyphosate, *Ggt* can remain viable and further colonise dead or dying rhizomes and roots, providing sources of infection for subsequent wheat crops. *Microdochium bolleyi* is commonly isolated from wheat roots and has been known to provide some control to *Ggt* in pot bioassays. A pot experiment investigated the colonisation of senescing rhizomes of *E. repens* treated with *M. bolleyi* by *Ggt*, following glyphosate treatment. Plants were destructively harvested at weeks 2, 4, 8, 12 and 16 from the time of planting. *M. bolleyi* (8% v/v) was applied at week 2 or week 8, while glyphosate (6 L $ha^{-1}$; a.i. 360 g $L^{-1}$) was applied at week 4. At each harvest, the extent of lesions on the rhizomes, dry weights (80˚C overnight) and *Ggt* DNA concentrations (qPCR) in the rhizomes were determined. Plants treated with glyphosate developed more lesions (20-40%), had reduced dry weights over time (100-200 mg), and increased *Ggt* DNA concentrations (10-15 folds, $p<0.001$) regardless of the presence of *M. bolleyi*. These results suggest that glyphosate is likely to hasten decomposition of *E. repens*, but may lead to increased *Ggt* inoculum concentrations in the dying rhizomes, and exacerbate take-all in subsequent wheat crops.

**P39.053 Screening and application of rapid detection method of banana wilt disease**

*S.P. Huang, T.X. Guo, Q.L. Li and J.Y. Mo*
*Institute of Plant Protection, Guangxi Academy of Agricultural Sciences, No.174 East University Road, Xixiangtang District, Nanning, Guangxi, 530007, P. R. China*
*Email: hsp@gxaas.net*

Banana wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* is a typical soil-borne fungal disease causing vascular damage and resulting in whole plant death. Specificity and sensitivity of 11 PCR primers specific for *F. oxysporum* f. sp. *cubense* were assessed, and four primers with high specificity and sensitivity were obtained. One primer named FocTR4 could detect *F. oxysporum* f.sp. *cubense* tropical race 4 and the other three primers named W106, W1805 and W2987 could detect *F.* spp., *F. oxysporum* f.sp. *cubense* tropical race 1, and race 4, respectively. Ten banana plant tissue samples including healthy and infected parts were collected, and total DNA of samples was extracted by the CTAB method, followed by PCR-specific amplification. The detection results were consistent with that of field observations of infected or non-infected. The PCR specific detection method can be used to quickly and effectively uncover *F. oxysporum* f. sp. *cubense*, which allows

management of pathogen spread.

**P39.054 Detection and quantification of *Corynespora cassiicola* in soil using real-time quantitative PCR**

*W. Gao, W.L. Wang, Y.J. Hao, C.Y. Liu, Y. Wang and J.F. Huo*
*Tianjin Plant Protection Institute, Tianjin, 300381, P. R. China*
*Email: gaowei5277@163.com*

Corynespora leaf spot was caused by *Corynespora cassiicola*, an important plant pathogenic fungus with a wide range of hosts. Based on the investigation datas in fields of 28 Provinces in China from 2008 to 2012, we found that *C. cassiicola* has become one of the most widely distributed and devastating cucumber diseases. *C. cassiicola* is an important soil-borne plant pathogen. The pathogen is difficult to identify and quantify in soil using conventional methods. It is different to control it by routine control methods, such as chemical fungicides and biocontrol. A rapid and reliable method to detect and quantify the fungus in soil before cultivating is an effective measure to inhibit pathogen transmission in continuous cropping greenhouse. In this study, we developed an SYBR Green-based quantitative real-time polymerase chain reaction assay for specific, sensitive detection and quantification of *C. cassiicola* in soil samples. Based on the differences in internal transcribed spacer (ITS) sequence of *C. cassiicola* with other fungal pathogens, a specific primer pair CIR5/CIF5, which only gave a single amplification of 259 bp from *C. cassiicola*, was developed. It could detect *C. cassiicola* at quantities as low as 10 fg of purified pathogen DNA in conventional PCR. The pathogen in soil could be detected by real-time quantitative PCR, and the sensitivity was down to *C. cassiicola* DNA of 1 conidia in 1 g soil in artificially infested soil samples. This real-time PCR provide a sensitive detection and measurement tool to quantify *C. cassiicola* in soil. It could assist in prevention and control of Corynespora spot leaf.

**P39.055 Controlling of canola clubroot with Cyazofamid and Fluazinam**

*L. Ren, K.R. Chen, F. Liu, L. Xu and X.P. Fang*
*Oil Crops Research Institute, Chinese Academy of Agricultural Sciences; Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Wuhan 430062, P. R. China*
*Email: xpfang2008@163.com*

Clubroot caused by *Plasmodiophora brassicae* is a severe threat to canola (*Brassica napus*) production in the Changjiang River in China, and a serious disease on crucifer vegetable crops in China. In this study, two field trials were carried out during 2011-2012 to assess the efficacy of Cyazofamid and Fluazinam on the control of canola clubroot. In trail I, 10% Cyazofamid SC were applied as nursery bed disinfection 3 days before seed sowing at a concentration of 5 mg a.i. $L^{-1}$. The liquid volume was about 400 ml $m^{-2}$. Plants were irrigated with water (control) or 2000 dilution of 75% Chlorothalonil WP at a ratio of 100 ml per plant after seedling transplant. At harvest time, clubroot incidences and canola yields were investigated. Results showed that control efficiency was above 90% to rapeseed seedling clubroot by nursery bed disinfection. Cyazofamid disinfection combination with or without roots irrigation using Chlorothalonil after transplant reduced the severity of plants clubroot by 67.6% and 57.1%, respectively, and significantly increased the canola yield by 57.5% and 48.4% respectively, compared with the control. In trial II, seeds coated with 10% Cyazofamid SC and 50% Fluazinam SC were directly planted. Clubroot incidences were investigated 2 months after planting. Results showed that Fluazinam coating at a ratio of 0.3 ml formulation per gram seed (PGS) and Cyazofamid coating at a ratio of 0.2 ml formulation PGS were effective in reducing the clubroot incidence by 75.3-95.3% and 35.2-49.8% compared with the control, respectively.

**P39.056 Genes regulating microsclerotium germination of *Verticillium dahliae***

*W.J. Shang, F. Wei, D.F. Hu, T. Cheng, C.S. Wang and X.P. Hu*
*State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest Agriculture and Forestry University, Tai Cheng Road 3, Yangling 712100, P. R. China*
*Email: xphu@nwsuaf.edu.cn*

Verticillium wilt caused by *Verticillium dahliae* Kleb. is a soilborne wilt disease of many crops all over the world and is difficult to control. Microsclerotia are the main resting structure of *V. dahliae* in soil and can survive for more than 10 years whether associated with or without debris. They are composed of compact masses of thick-walled, pigmented cells which originate from swollen, septate hyphae by a process of budding. The quantity of viable microsclerotium determines the severity of Verticillium wilt, e.g. on cotton and on strawberry. Surveys in the past 5 decades showed that the diseased area of Verticillium wilt of cotton kept expanding. In 2012, diseased area of Verticillium wilt of cotton was about 3000,000 acres in China. Current control measures, including chemical control, biofumigation, bio-control, crop rotation, failed to control this kind of disease. Easton and Nagle (1986) claimed that Verticillium wilt was efficient controlled through cropping to a green pea-sudangrass rotation. The root exudates of pea-sudangrass might stimulate the germination of micrsoclerotia in the soil, caused the reduction of microsclerotium quantity. Hence, one key strategy in controlling *Verticillium* wilt is to reduce the size of viable

microsclerotium population through forcing germination. Molecular mechanisms regulating microsclerotium germination would be beneficial to construct a new control strategy in Verticillium wilt management. Transcriptome analysis of dormancy and germinating microsclerotia showed that 1283 genes were differentially expressed. Several of highly expressed genes might associated with microsclerotium germination, including G-protein coupled receptor (GPCRs), esterase/lipase, cyclopentanone 1,2-monooxygenase (CPMO), fatty acid hydroxylase, mannose-6-phosphate isomerase, glucan 1,3-beta-glucosidase. All of these genes during microsclerotium germination were involved in the sugar metabolism, fat metabolism, sulfur metabolism, protein transport, and transcriptional regulation as occurred in the germination of plant seeds.

### P39.057 Effects of *Trichoderma harzianum* LTR-2 and electron treatments on the seed germination of winter wheat Florian

*X.Q. Wu[1], F.H. Rögner[2], M. Mehlstäubl[2], J.S. Li[1], K. Guo[1], Z. Li[1] and H.T. Yang[1]*
*[1]Shandong Provincial Key Laboratory for Applied Microorganism, Shandong Academy of Sciences, No. 19 Keyuan Road, Lixia District, Jinan, 250014, P. R. China; [2]Fraunhofer Institute for Electron Beam and Plasma Technology FEP, Winterbergstraße 28, Dresden, 01277, Germany*
*Email: xq_wu2008@163.com*

*Trichoderma* spp. inoculation and electron treatment are two validated bio-control methods, and the combination of both can be used on grain production, e.g. winter wheat. *T. harzianum* LTR-2, which has been registered, could inhibit pathogens and improve performance on variety of plants; the electron treatment has been proved to be an eco-friendly and effective way to reduce the risk of pathogen on wheat. Winter wheat Florian which were obtained from Fraunhofer FEP of Germany, germinated under four treatment conditions: no-treatment (as control), T-treatment (seeds immersed in LTR-2 suspension, which contained 5 million spores per gram, for 15 minutes), E-treatment (seeds under the condition of 105 kV acceleration voltage and 170 mA beam current, which carried out in Germany), T&E-treatment (combined T-treatment and E-treatment). The data showed that LTR-2 could significantly increase root length ($P \leq 0.05$), raw weight ($P \leq 0.05$) and dry weight ($P \leq 0.05$), and insignificantly improve the germination rate on the 4$^{th}$ day. Electron beam has insignificantly improved the germination rate, while significantly reduced root length ($P \leq 0.05$), raw weight ($P \leq 0.05$) and dry weight ($P \leq 0.01$) on the 4$^{th}$ day. The combination of two methods had made a positive balance, which were significant improvement of root length ($P \leq 0.05$), raw weight ($P \leq 0.01$) and dry weight ($P \leq 0.01$), and insignificant improvement of germination rate on the 4$^{th}$ day. *T. harzianum* was known to affect plant growth through interaction with phytohormone synthesis or transport in the plant, which could cover the damage caused by electron beam during the germinating period. The field tests of wheat disease (Wheat rust and Powdery mildew) and wheat development are underway.

### P39.058 Infection process of *Rhizoctonia solani* in potato and the relationship with resistance

*X.Y. Zhang and Z. Yu*
*College of Agronomy, Inner Mongolia Agricultural University, Huhhot 010019, P. R. China*
*Email: zxy2000@126.com*

Stem canker and black scurf by *Rhizoctonia solani* is one of the most severe disease affecting potato production worldwide. It is serious in the growing areas of potato in Inner Mongolia. The pathogen can cause stem canker symptom, characterized by brown and black lesions on the stems and stolons. Towards the end of the growing season, sclerotia of *R. solani* commonly referred to as black scurf, develop on the progeny tubers. In order to find the related factors with resistance and reveal the resistant mechanism, which are the basis of resistance breeding, the infection process of *R. solani* was observed for finding the difference of resistant and susceptible potato cultivars by optical and electron microscope after different time of underground stem inoculated by *R. solani*. The results showed that the initial infection time was 8h, the susceptible cultivars of potato were infected earlier and the mycelium extended faster. The pathogen can infect from cuticle cracks, lenticels, cell gaps, wounds, and a small amount of mycelia can directly penetrate the cuticle into. Then the mycelia spread in cell gap and intercellular. Susceptible cultivars had larger and more lenticels, thinner cuticle, more cuticle cracks. The infection structure, a few loose infection cushions and a large number of appressoria were observed, and the shape of appressoria were lobate, spherical, trident form, foot shape and irregular, infection pegs were formed from the appressoria. Susceptible cultivars had more infection structures than the resistant ones.

### P39.059 Thiamethoxam contributes to difenoconazole in controlling the take-all disease of wheat

*Y.Y. Deng, H.Y. Sun, W. Li and H.G. Chen*
*Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, No.48 Zhongling Street, Xuanwu District, Nanjing, 210014, P. R. China*
*Email:dengyy100@sohu.com*

Difenoconazole is widely used as seed treatment to control the take-all disease of wheat, and thiamethoxam, a neonicotinoid insecticide, is also applied on wheat seed in practice to control wheat aphid. It is reported that

thiamethoxam can promote plant vigor and induce plant defense response. In greenhouse experiments, the influences of thiamethoxam on both the control effectiveness of difenoconazole on wheat take-all and the impact of difenoconazole on the root growth of wheat were tested. When difenoconazole was combined with thiamethoxam as seed coating in conventional dose, its control effectiveness on take-all disease was promoted, with the ratio of infected roots notably lower than difenoconazole applied alone. Difenoconazole suppressed the root growth of wheat, but the suppression was greatly reduced when it was applied in combination with thiamethoxam. However, no significant inhibitory effect on take-all disease and promoting effect on root growth was found with thiamethoxam in this work.

**P39.060 Pathogen identification of bacterial fruit blotch of watermelon from Shandong Province**

*Y.L. Zhang, C.S. Li, J.S. Qi, B. Zhang, Z.T. Xu and L. Li*
*Institute of Plant Protection, SAAS, No.202 North Industrial Road, Licheng District, Jinan, Shandong, 250100, P. R. China*
*Email: yueligaoxing@163.com*

In recent years, watermelon has been attacked by a bacterial disease called bacterial fruit blotch (BFB) in some planting areas (including Linshu, Chanle and Zhangqiu) of Shandong Province. This disease mainly harms the leaves and the fruit of watermelon, causing a large number of lesion and fruit to decay and makes a serious threat to the production of watermelon and resulting in large economic losses since 2010. Fifteen strains were isolated from diseased fruits and leaves of watermelon from Linshu, Chanle and Zhangqiu. Inoculation on young and nearly ripe watermelon with these isolates produced the same symptoms as naturally infected plants. The same strains were isolated from the inoculated watermelon. The symptoms of this disease were described in some research papers. Based on stain reactions, their morphology, cultural characteristics, physiological and biochemical properties of the pathogens, and the comparison of these characteristics with the concerned parts in (Bergey′s Manual of Determinative Bacteriology) 9th ed, all tested isolates were identified as *Acidovorax avenae* subsp. *citrulli* Willems et al. 1992 (*Pseudoumonas pseudoalcaligens* subsp. *citrulli* Schaad et al. 1978). Inoculations with these strains on leaves of cucumber and melon produced the same symptoms. Bacterial fruit blotch of watermelon has been holding as aquarantine pest in China because of its distribution in some regions of China.

**P39.065 Alternative treatments to methyl bromide for growing tomatoes in Azapa Valley-Chile**

*G.F. Sepúlveda-Chavera, R. Salvatierra-Martínez and M. Rodríguez Molina*
*Universidad de Tarapacá. Av. General Velázquez 1775, Casilla 6 – D, Arica, Chile*
*Email: gsepulve@uta.cl*

Methyl bromide has been the most effective soil fumigant used for tomatoes cultivation in Azapa Valley-Chile. However, due to its prohibitions are necessary assessment products that replace the short term. We evaluated two fumigants (1,3 dichloropropene + chloropicrin and Metam sodium), a rootstock (Maxifort®) and a biological product Bafex® (*Bacillus subtilis*) for growing tomatoes in soil infected with nematodes and *Fusarium oxysporum*. The study was conducted in the field using 6 treatments with a Latin square design (6 x 6). Metam sodium (Nemasol®) and the commercial mixture 1,3-dichloropropene + chloropicrin (Anacelone®) were the most effective in reducing populations of fungi and plant parasitic nematodes. Maxifort®/Naomi® and *B. subtilis* treatments show not reductions populations and Maxifort® use may increased populations up to 32 times. The major production of tomatoes fruit was achieved with metam sodium (Nemasol®) at 120 and 80 $cc/m^{-2}$ followed by Maxifort®/Naomi®. Moreover, in *B. subtilis*, Maxifort®/Naomi® and control the fruit size and productions were statistically equal ($p<0.05$) to 1,3-dichloropropene + chloropicrin (Anacelone®), which may suggest toxicity of this product. *B. subtilis* showed the best quality fruit and higher yields to 1,3-dichloropropene + chloropicrin (Anacelone®). However, high nematode attack, demonstrating the ability biostimulant of this bacterium and its possible use in an integrated management. Furthermore study demonstrates that the use of rootstocks invigorating as Maxifort® increases parasitic nematode populations.

**P39.066 Biological control of *Sclerotium rolfsii*, the causal agent of seedling blight of groundnut (*Arachis hypogaea* L.)**

*M.N. Reddy, M. Saraswathi and C.H. Sireesha*
*Department of Microbiology, S V University, Tirpati-517502, (A.P), India*
*Email: mopuri_nr@yahoo.com*

There are very few cost-effective and eco-friendly control measures available for many soil-borne plant pathogens. The present study is aimed at screening of some potential *Trichoderma* isolates against *Sclerotium rolfsii*, the causal agent of seedling blight of groundnut (*Arachis hypogaea* L.). Twelve isolates of *Trichoderma* ($T_1$-$T_{12}$), isolated from soils and rhizosphere soil samples of groundnut plants, collected from the fields of major groundnut growing geographical areas of the State of Andhra Pradesh, India were tested for antagonistic activity against *S. rolfsii*, by using dual culture method and also by bioassay of culture filtrates. Of the twelve isolates, significant inhibition of the test pathogen was observed with the isolate $T_{6,}$ as compared to the others. The effect

of non-volatile substances on test pathogen was studied. With the increase in the concentration of culture filtrate, there was an increase in the mycelia growth inhibition, in terms of colony diameter. Culture filtrates also adversely affected the production and also germination of the sclerotia significantly. The per cent disease incidence/ inhibition was recorded at 30 days after sowing in the $T_6$ treatment to 10 day old seedlings in pot culture studies. The *Trichoderma* isolate was significantly superior over the control in reducing the disease incidence, maximum shoot and root length, dry weight of the plants and roots compared to control, followed by un-inoculated and inoculated controls, respectively. The studies were also conducted/tested in the field, in 'mini - plot' experiments and obtained significant results and hence attempted the biocontrol awareness camps to the farmers to recommend the method.

**P39.067 Management of black scurf of potato with effective microbes, biological potassium fertilizer (bpf), and *Trichoderma harzianum***

*F. Naz[1], C.A. Rauf[1], I. Ahmad[2] and A. Riaz[1]*
*[1]Department of Plant Pathology, PMAS, Arid Agriculture University Rawalpindi, 46300, Pakistan; [2]Pakistan Agriculture Research Council, Islamabad Pakistan*
*Email: farahnaz@uaar.edu.pk*

The efficacy of soil application with microbial preparations viz. *Trichoderma harzianum***,** effective microbe (EM) culture and biological potassium fertilizer (BPF) was evaluated for the management of soil-borne inoculum of black scurf of potato by sowing cv. Desiree. Soil application with three dosages of culture suspension of *Trichoderma harzianum***,** effective microbe (EM) culture and biological potassium fertilizer (BPF) were applied in the soil to know the efficacy of these treatments in reducing the disease. Soil application with *T. harzianum* at the time of sowing followed by two and three dosages at 20 days intervals gave significant protection to eyes with EGI of 30.55%, SK 24.07%, SCI 36.10%, StCI 30.60%, BSDI 26.43%, and YR of 35.09% against the fungus which ultimately contributed to better crop stand and increased yield as compared to inoculated control and rest of the treatments. Soil application with *T. harzianum* gave significant protection to eyes, sprouts and stolons, against soil-borne inoculum of the fungus which ultimately contributed to better crop stand and increased yield as compared to inoculated control and rest of the treatments.

**P39.068 *In vitro* and *in vivo* antioomycete activity of *Pseudomonas fluorescens* against *Pythium aphanidermatun*, causal agent of papaya collar and root rots in Côte d'Ivoire**

*N.B.C. Koffi and H. Atta Diallo*
*University Nangui Abrogoua (ex-University of Abobo-Adjamé), 02 BP 801 Abidjan 02, Abidjan, Côte d'Ivoire*
*Email: hortensediallo@live.fr*

*In vitro* and *in vivo* tests were conducted to evaluate the effect of *Pseudomonas fluorescens* on *Pythium aphanidermatum*, causal agent of papaya (*Carica papaya* L.) collar and root rots. Four discs of 5 mm in diameter each of *P. fluorescens* grown on PDA media in Petri dishes were placed 3 cm a part around a 10 mm in diameter *P. aphanidermatum* disc. Plates were observed for the presence of inhibition zones. These zones were then observed under a light microscope. Results indicate antifmicrobial activity of *P. fluorescens* against *P. aphanidermatum.* Mycelia in the inhibition zones were altered and could not grow on new culture media. In the *in vivo* assays, four treatments were made: (prior bacteria-treated plants + bacteria + pathogen), (untreated plants + bacteria + pathogen), Ti (untreated plants + pathogen) and Ts (healthy control). Plants were grown in pots. Over a period of 12 weeks, symptoms were observed. Application of known bacterial solutions reduced disease incidence from 76% to 18%. This study shows the efficacy of *P. fluorescens* as a biopesticide against *P. aphanidermatum*. Further experiments are needed to improve the technology.

**P39.069 *Plasmodiophora brassicae,* the cause of clubroot of rapeseed and vegetable brassicas is widespread in Polish agricultural soils**

*M. Jedryczka[1], M. Korbas[2], J. Kaczmarek[1], E. Jajor[2] and J. Niemann[3]*
*[1]Institute of Plant Genetics, Polish Academy of Sciences, 34 Strzeszynska, 60-479 Poznan, Poland; [2]National Research Institute of Plant Protection, 20 Wegorka, 60-318 Poznan, Poland; [3]Department of Genetics and Plant Breeding, University of Life Sciences, 11 Dojazd, 61-632 Poznan, Poland*
*Email: niemann@up.poznan.pl*

Vegetable brassicas and oilseed rape belonging to *Brassicaceae* family are popular crops in Poland. One of major concerns of *Brassica* growers is clubroot, caused by the protist *Plasmodiophora brassicae*. The aim of this work was to study the incidence of *P. brassicae* in randomly collected samples of agricultural soils in Poland. The collection was done from 900 fields in 320 out of 380 districts of Poland. Each sample was collected at several sites per field. The presence of *P. brassicae* was studied under greenhouse conditions with the bioassay and in laboratory using PCR-based detection. In a bioassay seedlings of *Brassica rapa* ssp. *pekinensis* and the Polish variety of oilseed rape Monolit were used as bait plants. Clubs were formed on roots of plantlets grown in soils contaminated with the pathogen. Molecular detection was highly correlated with the result of the biotest. Samples originating from over one third of studied

districts were contaminated with *P. brassicae* at least once. Most samples containing *P. brassicae* were obtained from the provinces located in north-west (Pomerania), north-east (Varmia) and south-west (Silesia and Opole) regions of Poland. The pathogen was also found in soil samples collected from regions considered as free from clubroot (Great Poland, Carpathian Foothills). It was found that clubroot is widespread in Polish agricultural soils.

**P39.070 Characterization of *Fusarium* spp. associated with sugar beet crown and root rot in China**

*S. Cao, W.J. Wang and X.H. Wu*
*Department of Plant Pathology, China Agricultural University(CAU), No.2 Yuanmingyuan West Road, Haidian District, Beijing 100193, P. R. China*
*Email: wuxuehong@cau.edu.cn*

Sugar beet (*Beta vulgaris* L.) is grown worldwide as the second largest sugar crop. Sugar beet crown and root rot caused by *Fusarium* spp. is an economically serious disease. Diseased sugar beet roots with symptoms of crown and root rot were collected from main growing areas, such as Heilongjiang Province, Inner Mongolia Autonomous Region, Xinjiang Uygur Autonomous Region, Hebei Province, Beijing Municipality, and Liaoning Province in China over two years. A total of 83 *Fusarium* strains were isolated from the diseased root tissues. These isolates were preliminarily identified as *Fusarium oxysporum*, *F. solani*, *F. equiseti*, *F. proliferatum* and *F. redolens* based on morphological traits, such as characteristics of fungal colonies on potato dextrose agar (PDA) and Carnation Leaf-Piece Agar (CLA), colony growth rate, morphological characteristics and size of conidium, conidiophore and chlamydospore. Furthermore, the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) and translation elongation factor 1-α(EF1-α) gene region of the all isolates were amplified with primers ITS1 and ITS4, EF1 and EF2, respectively. The resulting sequences were analyzed, and compared with morphological and pathological characteristics. Pathogenicity tests on *Beta vulgaris* 'HI0305' revealed that the five *Fusarium* species were all virulent on sugar beet roots. This study showed that at least five species of *Fusarium* can cause crown and root rot symptoms on sugar beet in China.

**P39.071 Characterization of *Rhizoctonia* spp. associated with sugar beet crown and root rot in China**

*C. Zhao, W.J. Wang and X.H. Wu*
*Department of Plant Pathology, China Agricultural University(CAU), No.2 Yuanmingyuan West Road, Haidian District, Beijing 100193, P. R. China*
*Email: wuxuehong@cau.edu.cn*

Sugar beet (*Beta vulgaris* L.) is grown worldwide as the second largest sugar crop. Sugar beet crown and root rot is an economically serious disease mainly caused by *Rhizoctonia solani* AG-2-2 and AG 4. Diseased sugar beet roots with symptoms of crown and root rot were collected from main growing areas, such as Heilongjiang Province, Inner Mongolia Autonomous Region, Xinjiang Uygur Autonomous Region, Hebei Province, Jilin Province, Gansu Province, Beijing Municipality, and Shanxi Province in China over three years. Of 58 strains isolated from the diseased root tissues, 53 were *Rhizoctonia solani* and 5 were Binucleate *Rhizoctonia* based on morphological traits, including characteristics of fungal colonies on potato dextrose agar (PDA), number of nuclei per hyphal cell stained with 4'-6-diamidino-2-phenylindole (DAPI), and anastomosis group(AG) of all isolates determined on glass slides against the tester strains. Molecular characterization of these *R. solani* isolates revealed that, of 54 isolates, 32 were AG2-2 IIIB, 10 were AG4HG-I, 8 were AG4HG-II, 1 was AG2-2IV, and 3 were unidentified *Rhizoctonia* spp., by sequencing the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) amplified from genomic DNA with primers ITS1 and ITS4. Pathogenicity tests on *Beta vulgaris* 'HI0305' revealed that *R. solani* AG2-2 and AG4 isolates were virulent on sugar beet roots. This study showed that at least three AGs of *Rhizoctonia* can cause crown and root rot symptoms on sugar beet in China.

**N039.001 Screening and identification of potential antagonistic rhizobacteria for biocontrol of tomato major soil-borne phytopathogens**

*J.Q. Qiao, Y.Z. Liu, X.J. Liang, Y.F. Nie and Y.F. Liu*
*Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, P. R. China*
*Email: junqingqiao@hotmail.com*

Protected Tomato has developed rapidly in China，due to its progressiveness and high efficiency. The tomato wilt caused by soil-borne *Ralstonia solanacearum* or *Fusarium oxysporum* is a disease widely distributed in greenhouse and is difficult to control by chemical pesticide. Biological control is now increasingly considered as an alternative treatment because of consumer demand for year-round production of fresh vegetables with reduced or no pesticide residues. In this study, a total of 302 strains were isolated from 31 plant rhizosphere soil samples in Jiangsu province. 10 isolates with ability of colonization in tomato rhizosphere were found to be antagonistic to at least one of the two pathogens, *R. solanacearum* and *F. oxysporum* excellently (diameter of the inhibition zone ≥10 mm). Plant MS agar plate result shown that some of strains can promote the growth of the tomato plant. In the meantime, plant hormones (IAA and GA3) were checked in some strains by the HPLC technique. The highest content of IAA and GA3 is about to 5.082 and 14.75 mg/ml, respectively. Then, 10 isolates

with strong antagonistic activities and colonization were subjected to a preliminary Physiological and biochemical characterization and molecular methods, and grouped into these clusters, *Bacillus amyloliquefaciens* (5 isolates), *B. subtilis* (1 isolates), and *Pseudomonas putida* (4 isolates). These strains are an important potential biocontrol agent bank and would be investigated deeply in future.

**N039.002 The controlling effect of biologic soil amendment agent on continuous planting disease of cucumber**

*Y.J. Hao, C.Y. Liu, Y. Wang, J.F. Huo, W. Gao and W.L. Wang*
*Tianjin Institue of Plant Protection 17 kilo Jinjing Road, Xiqing District ,Tianjin 300381, P. R. China*
*Email: yjhao1112@yahoo.com.cn*

Continuous planting resulted in the enrichment of soil borne diseases, and seriously influenced the yield and quality of cucumber in greenhouse. Biologic soil amendment agent (BSAA) were composed of organic compost, inorganic matter, organic coated with antagonistic microorganisms. In this study, BSAA 3%(w/w) was applied to the continuous cropped cucumbers (6 seasons) by means of pot and greenhouse trials. Results indicated that BSAA could promote seedling growth and reduce the pathogen propagulation in the rhizosphere soil and disease incidence. BSAA could control Fusarium wilt of cucumber by 63.67% to 84.85% in pot trial, as well as by 62.5% to 74.3% in greenhouse trial. The disease incidence could be controlled by 80.71% and the yield per plant could be increased by 27% when BSAA accompanied with soil sterilization. The microbial population in rhizosphere of cucumber were increased remarkably when tested at 30, 60, 90 days after the application of BSAA. Numbers of isolated bacteria and actinomycetes varied strikingly. The number of isolated fungi was increased slightly, but beneficial strains were increased to a higher degree. The activities of peroxidase (POD), ploy phenol oxidase (PPO) and root TTC were increased. The contents of binary phenols in cucumber plant were obviously higher.

## Concurrent Session 40-Taxonomy of Plant Pathogenic Bacteria

### O40.001 Differential imperatives of classification, nomenclature and identification leading to taxonomic confusion

*C. T. Bull*
*USDA/ARS Salinas CA 93901 USA*
*Email: carolee.bull@ars.usda.gov*

Taxonomy consists of three intertwined but distinct disciplines: classification, nomenclature and identification. Bacterial taxonomy is currently driven by the need to understand relationships among microorganisms, and thus the imperatives of nomenclature and identification are sometimes overlooked.

Nomenclature is codified by rules, whereas classification and identification are conducted according to the scientific method and peer review. Names of taxa at the subspecies level and above are regulated by the International Code of Nomenclature of Bacteria ("the Code"). Pathovar names are regulated by the International Standards for Naming Pathovars of Phytopathogenic Bacteria ("the Standards"). However, there is significant disconnect between the Code and the Standards. The pathovar designation provided continuity between names of plant pathogens published before 1980 and those causing differentiable diseases that could not be distinguished from other species by phenotypic characters. The intent of the Standards was to conserve the names for potential future reinstatement. Classification methods and bacterial species concepts have advanced and their application to plant pathogenic bacteria has resulted both in the elevation of pathovars to species and/or subspecies and in the transfer of pathovars from one species to another. Confusion has resulted when the intent of the Standards has not been followed or pathovar names are not formally designated when they are transferred to new species. Because the Code does not require that the Standards be followed, plant pathologists are responsible for insuring the continuity of the names of important plant pathogens by following the intent of the Standards.

### O40.002 What new technologies bring to challenge of bacterial classification: unraveling evolution and ecology of plant pathogenic bacteria

*M. Fischer-Le Saux*
*INRA, French National Institute for Agricultural Research, UMR1345 IRHS, 42 rue Georges Morel, BP60057, 49071 Beaucouze cedex, France; AgroCampus-Ouest, UMR1345 Institut de Recherche en Horticulture et Semences, Angers, F-49000, France; UMR1345 Institut de Recherche en Horticulture et Semences, SFR4207 QUASAV, PRES L'UNAM, Université d'Angers, Angers F-49000, France*
*Email: marion.le-saux@angers.inra.fr*

Plant health management of bacterial diseases relies on the capability to reliably detect and identify plant pathogenic bacteria. Taxonomy provides classification schemes, unambiguous names and identification tools to scientists and other stakeholders. Analyses of 16S rDNA sequences for higher taxa classification and phylogenetic studies, and DNA-DNA hybridizations (DDH) for species delineation, have been the gold standards for prokaryotic classification. However, the advent of sequencing technologies during the last 15 years has had a deep impact on taxonomic studies. Multilocus sequence analysis targeting housekeeping genes offers opportunity to deeply examine phylogenetic relationships between closely related species, and it has been shown that accurate species delineation can be achieve through this approach. At intraspecies level, multilocus sequence typing provides analytical methods to discriminate strains and study population structures. The development of Next Generation Sequencing technologies has brought genomic-based approaches a step further by providing a huge amount a whole genome data. Average Nucleotide Identity between two genomes and other parameters have been proposed as alternatives to the cumbersome DDH for prokaryotic species circumscription. Species-specific set of genes can be inferred using comparative genomics, sometimes leading to the identification of unexpected species-specific phenotypic traits. Coupling phylogenetic data based on core genome with repertoire mining of accessory genes has provided insights into pathovar classification and evolutionary scenarii of patho-adaptation have been proposed. Genomic data allow deciphering adaptive steps and ecological features that drive diversification of bacteria and emergence of plant pathogens and thus contribute to the on-going debate about bacterial species definition.

### O40.003 Taxonomy of bacterial plant pathogens from genera in the *Enterobacteriaceae* not commonly thought to include plant pathogens

*T.A. Coutinho and S.N. Venter*
*Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20, Pretoria 0028, South Africa*
*Email: Teresa.Coutinho@up.ac.za*

The *Enterobacteriaceae* is a large family of Gram negative bacteria that include saprophytes, human and plant pathogens. Of the 52 recognized genera, twelve contain plant pathogens. Genera such as *Dickeya*, *Erwinia* and *Pectobacterium* include only plant pathogenic species. The majority of the other genera include species from a diverse range of ecological niches and many, such as *Salmonella*, are not commonly thought to include plant pathogens. These species may be considered, in most

cases, to be opportunistic pathogens. This means that the bacteria reside either epi- or endophytically on/in the host, and disease symptoms will only develop when the plant is susceptible and environmental conditions favourable. The plant under ideal growing conditions is immune or resistant to infection and this complicates fulfilling Koch's postulates. However, despite this obstacle a number of species within this family have been shown to be successful plant pathogens. Today, the standard approach in the identification of members of the *Enterobacteriaceae* is the use of 16S rRNA sequencing followed by sequencing of relevant housekeeping genes. The selection of these genes is important and is typically determined when performing multilocus sequence analysis for the genera. It is our belief that in the future a phylogenomics approach will assist in the identification of novel species.

**O40.004 *Pseudomonas cannabina* pv. *alisalensis* has two lineages with genetic, phenotypic and pathogenic diversity**

*F. Takahashi and Y. Takikawa*
*Graduate School of Agriculture and Graduate School of Science and Technology, Shizuoka University, 836 Ohya, Shizuoka, 422-8529, Japan*
*Email: abytaki@ipc.shizuoka.ac.jp*

An outbreak of bacterial blight disease of crucifer plants caused by *Pseudomonas cannabina* pv. *alisalensis* (PCA) has been recorded recently in Japan by us (Takahashi et al. 2013). 65 strains of PCA isolated in Japan and USA were subjected for taxonomic analysis in detail. They were divided into two groups by utilization of four carbon sources. Group A consisted of 11 strains including 10 Japanese isolates and ICMP4326, and group B consisted of 53 new Japanese isolates of the recent outbreak and ICMP 15200$^{PT}$. On rep-PCR (BOX-PCR), an amplified band of ca. 1.3 kb was specifically detected in group B strains. Although the strains of group A and group B shared more than 99 % similarity on the sequence analyses of 4 housekeeping genes (*gap1, gltA, gyrB* and *rpoD*) and 4 *hrp* genes (*hrpS, hrpA, hrpZ* and *hrpB*), two SNPs (single nucleotide polymorphisms), that is specific to group A or group B, were found in *hrpA* and *hrpZ* genes. Group-specific primers were designed to divide the strains into group A and group B easily. The distinction based on these primers coincided well with the results of carbon source utilization tests and BOX-PCR. Moreover, in inoculations on eight plants (turnip, cauliflower, cabbage, broccoli, broccolini, radish, cucumber and tomato), differences of symptoms were observed between group A and group B strains. This is the first report of the phenotypic and pathogenic diversity between the two genetic lineages of PCA.

**O40.005 Diversity of *Xanthomonas hortorum* pv. *carotae*, the agent of bacterial leaf blight of carrot and the need for a new pathotype strain**

*M.A. Jacques, E. Dupas and K. Durand*
*INRA, French National Institute for Agricultural Research, UMR1345 IRHS, 42 rue Georges Morel, BP60057, 49071 Beaucouze cedex, France; AgroCampus-Ouest, UMR1345 Institut de Recherche en Horticulture et Semences, Angers, F-49000, France; UMR1345 Institut de Recherche en Horticulture et Semences, SFR4207 QUASAV, PRES L'UNAM, Université d'Angers, Angers F-49000, France*
*Email: marie-agnes.jacques@angers.inra.fr*

*Xanthomonads* are plant-associated bacteria responsible for diseases of considerable economic importance. While these bacteria spread by aerosols and mechanical means like infected pruning tools, the movement of contaminated seeds and other propagation material generally represents the main inoculum source for diseases due to xanthomonads. Plant health management of these diseases through sanitation practices and plant resistance mainly depends on reliable detection and identification tools. The genus *Xanthomonas* has been subject to numerous taxonomical and phylogenetic studies, but its taxonomy is still under active debate. Some of the relationships between pathovars and species have not been thoroughly clarified. Strains formerly part of one pathovar are now found in two different species, some pathovars need pathotype strains and others were left with unsuitable pathotypes. Bacterial leaf blight of carrot (*Daucus carota* L. subsp. *sativus*) is caused by *Xanthomonas hortorum* pv. *carotae* (*Xhc*), a seed-borne pathogen with a worldwide distribution. The pathotype for this pathovar was reported to be unsuitable in 1991. In order to test plant genetic material for resistance to this pathogen, we study the genetic diversity of strains within this pathovar. A large collection of *Xhc* strains and *Xhc* look-alikes isolated from carrots was established. A multilocus sequence analysis (MLSA) targeting six housekeeping genes revealed that *Xhc* strains cluster in two close genetic lineages sharing a common ancestor. Pathogenicity of strains representative of these lineages was confirmed. The low polymorphism revealed by these genes does not allow a thorough typing of strains. In contrast, a multilocus variable number of tandem repeats analysis (MLVA) yielded a large genetic diversity among strains of this pathovar that is useful for molecular epidemiology study of this disease. In contrast with *Xhc* strains, look-alikes split in various parts of the *Xanthomonas* phylogenetic tree. These strains are not pathogenic on carrots but most generally induce hypersensitive reaction on tobacco and possess a *hrp*-Type Three Secretion System. This polyphasic analysis of Xhc and look-alikes offer the opportunity to designate a neopathotype strain for *Xhc*, which will help future studies of strains belonging to this pathovar.

**P40.001 Classification of *Pseudomonas syringae* strains isolated from bacterial leaf spot of onions**

*M. Tsuji and Y. Takikawa*
*Graduate School of Agriculture and Graduate School of Science and Technology, Shizuoka University, 836 Ohya, Shizuoka, 422-8529, Japan*
*Email: abytaki@ipc.shizuoka.ac.jp*

Bacterial leaf spot of onions (BLSO) was first recorded in Japan by Goto in1972 and the pathogen was considered as a pathovar of *Pseudomonas syringae*, but it has not been taxonomically investigated in detail. In 2012, a disease suspected as BLSO re-emerged in Shizuoka, Japan. A pathogenic bacterium was isolated from the infected onions and suggested to be BLSO agent through preliminary examinations. The strains isolated in 1969, 1986, 1987 and 2012 were compared with the causal agent of bacteriosis of leeks (*P. syringae* pv. *porri*) that shows similar symptoms with BLSO. Rep-PCR distinguished the BLSO agent and pv. *porri*. The sequence analysis of housekeeping genes and *hrp* genes revealed that the BLSO agent and pv. *porri* formed independent clusters. In bacteriological characteristics, difference was observed in utilization of erythritol, DL-homoserine, glutaric acid and others between the BLSO agent and pv. *porri*. In pathogenicity tests, welsh onion, leek, garlic and Chinese chive showed different symptoms by the two organisms. In conclusion, BLSO agent is clearly distinguished from pv. *porri* and considered to be a new pathovar of *P. syringae*.

**P40.002 A new rapid identification method for Japanese *Pectobacterium* strains based on *recA*, *mdh and rpoD* PCR RFLP**

*R. Suharjo, H. Sawada and Y. Takikawa*
*Lab. of Plant Pathology, Graduate School of Science and Technology, Shizuoka University Japan; National Institute of Agrobiological Science, Ibaraki, Tsukuba, Japan*
*Email: abytaki@ipc.shizuoka.ac.jp*

Identification was performed on 189 Japanese *Pectobacterium* strains of MAFF collection isolated from Crucifers and Solanaceous plants using sequence analysis of *recA, mdh, gyrB, rpoD* and PCR RFLP of *recA*, *mdh* and *rpoD*. Using *recA*, *mdh* and *rpoD* PCR RFLP each species and subspecies group of Japanese *Pectobacterium* strains can be easily distinguished. The results of *recA*, *mdh* and *rpoD* PCR RFLP was easy to analyze and corresponded to the result of *recA*, *mdh*, *gyrB* and *rpoD* sequence analysis. Using those methods, the investigated Japanese *Pectobacterium* strains were divided into *P. carotovorum* subsp. *carotovorum*, subsp. *odoriferum*, subsp. *brasiliense*, new subsp. level group of *P. carotovorum*, *P. atrosepticum* and *P. wasabiae*. Here we also found a group that may constitute a new species level group of *Pectobacterium*. This *recA, mdh* and *rpoD* PCR RFLP can be potentially used as rapid method for identification of *Pectobacterium* strains.

**N40.001 Identification of race and biovar of *Ralstonia solanacearum* from tobacco in Jiangxi province**

*Z.K. Zhou, C.Q. Zhang, C.Z. Hu and J.X. Jiang*
*College of Agronomy, JAU, No.1101 Zhimin Street, Changbei District, Nanchang, 330045, P. R. China*
*Email: jxjiang64115@yahoo.com.cn*

In order to provide theoretical basis for breeding and utilizing tobacco bacterial wilt-resistant varieties, race and biovar of *Ralstonia solanacearum* from tobacco in Jiangxi province were identified. Diseased plant samples of tobacco bacterial wilt were collected from six counties of Shicheng, Ruijin, Huichang, Xinfeng, Guangchang and Xiajiang in Jiangxi province, and 24 strains of the pathogen, *Ralstonia solanacearum*, were obtained from these samples by using dilution plate isolation method. Six representative strains of them were chosen for race and biovar determination. Experimental results of differential host assay, infiltration reaction and melanin formation showed that all of the six strains belonged to race 1, and on the basis of their capacity in utilizing three disaccharides and three hexanol, as well as their reduction ability to $KNO_3$, the six strains were also classified as bioval Ⅲ-1.

## Concurrent Session 41-Taxonomy of Plant Pathogenic Fungi

### O41.001 Morphology, molecular phylogeny and host specificity leads to a stable taxonomy for the smut fungi with some examples from Australia

R.G. Shivas [1], A.R. McTaggart[2], Y.M. Li[3], L. Cai[3] and K. Vánky[4]
[1]Department of Agriculture, Fisheries and Forestry, Ecosciences Precinct, Dutton Park, Qld 4102, Australia; [2]School of Agriculture and Food Science, University of Queensland, St. Lucia, Qld. 4067, Australia; [3]Key Laboratory of Systematic Mycology and Lichenology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, P. R. China; [4]Herbarium Ustilaginales Vánky (HUV), Gabriel-Biel-Str. 5, D-72076 Tübingen, Germany
Email: roger.shivas@daff.qld.gov.au

There are about 1,700 species of smut fungi (*Ustilagino mycotina*) in about 100 genera. Almost a third of these genera have been recognised in the last 10 years, which reflects the application of morphological concepts, molecular phylogenetic methods, and host specificity as a basis for delimiting genera. Based mostly on Australian specimens, the *Sporisorium-Ustilago-Macalpinomyces* complex was partly resolved by division into seven genera, namely *Anthracocystis*, *Langdonia*, *Macalpinomyces*, *Sporisorium* s. str., *Stollia*, *Triodiomyces* and *Ustilago*. Three of these genera, *Langdonia*, *Stollia*, and *Triodiomyces* were new and mostly comprised endemic Australian species. Further new genera of smut fungi that have been described from Australia in the last 10 years include *Aizoago*, *Anomalomyces*, *Centrolepidosporium*, *Entylomaster*, *Eriocortex* and *Shivasia*. One polyphyletic genus, *Macalpinomyces*, still needs resolution and we discuss our current concepts for this genus. The creation of a stable taxonomy for the smut fungi at generic level will soon be achieved.

### O41.002 Cryptic species in plant pathogenic fungi, challenge and solution

F. Liu, K. Zhang, Y.H. Gao, Y.Y. Su and L. Cai
State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No.1 West Beichen Road, Chaoyang District, Beijing, 100101, P. R. China
Email: mrcailei@gmail.com

A cryptic species complex is a group of species which satisfy the biological definition of specie but whose morphology is very similar and in some cases virtually identical. In the taxonomy of plant pathogenic fungi, species have long and traditionally been defined based on morphology, but these morph-species have often been shown to contain several to many genetically and biologically distinct species that can only be recognized by their DNA sequences. The first challenge is that most of existing checklists and databases are now outdated, and in most of the literatures, species were referred as species complex. In addition, most of the recently recognized cryptic species have not been well characterized for their physiological features, host range, geographic distribution, and the severity of the diseases they cause, which are important to determine the agricultural, environmental and bio-security significance for these species. Unmasking and understanding cryptic species is one of the major tasks for mycologists and plant pathologists in the next decade. In this paper, we discuss various challenges from the increasingly emerged cryptic species and possible solutions.

### O41.003 Opening the Pandora's box called *Mycosphaerella*

P.W. Crous and J.Z. Groenewald
CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD, Utrecht, the Netherlands
Email: p.crous@cbs.knaw.nl

Phytopathogenic fungi that are mycosphaerella-like in morphology (*Capnodiales*, *Dothideomycetes*) represent close to 50 genera that are distributed over several families, including *Cladosporiaceae*, *Dissoconiaceae*, *Mycosphaerellaceae* and *Teratosphaeriaceae*. These genera represent a range of nutritional modes, being either plant pathogenic, lichenized, saprobic, or parasitic on other fungi or animals. In the strict sense the genus *Mycosphaerella* only applies to species with *Ramularia* anamorphs. Well-known model organisms such as the Sigatoka leaf spot disease complex of bananas involves members of the genus *Pseudocercospora*, while the septoria leaf blotch complex of cereals involve members of *Zymoseptoria*. Not only are most of the genera in the *Mycosphaerella* complex poly- or paraphyletic, but most of the known pathogens actually represent complexes of cryptic taxa that vary in their geographic distributions. A further interesting phenomenon that is common to phytopathogenic species in the *Capnodiales* is the co-occurrence of different species from the same or different genera in a single leaf lesion or stem canker. This finding has important implications for trade in agricultural and forestry produce, as pathogens of different hosts can be introduced into new environments on non-host material, which they colonise as secondary organisms.

### O41.004 Defining boundaries of species and genera in plant pathogenic oomycetes through phylogenetic approaches

C.A. Lévesque, C.F.J. Spies, Q. Eggertson, G. Robideau, T. Rintoul and N. Rodrigue
Agriculture and Agri-Food Canada, Central Experimental Farm, 960 Carling Avenue, Ottawa, ON, Canada,

*K1A 0C6*
*Email: andre.levesque@agr.gc.ca*

The sequencing of complete oomycete genomes and new advances in phylogenetics are providing new data and tools to better define genera within the oomycetes and to resolve species complexes. These tools are being applied by our team in three different ways. 1) A total of 888 single copy orthologous genes from the genomes of several *Pythium* species, other oomycetes and diatoms were identified and used to generate a reference phylogeny. The optimal small subset of phylogenetic markers developed from these genes that can generate a phylogeny that is as robust and with a similar topology to the 888 gene reference phylogeny is being determined. 2) Phylogenies which are determined based on genes that are critical in the life cycle of many oomycete species, namely flagellar genes OCM1 and PF16, can be misleading if based on nucleic or amino acid sequences directly. However an approach using codon modelling can create accurate phylogenies. 3) The first *Pythium* genome sequenced was *P. ultimum*, a species that is highly variable, comprising morphologically defined varieties as well as genetically distinct subgroups that are not yet properly resolved. Genes with high levels of single nucleotide polymorphisms were identified from this genome in order to improve our capacity to genotype single strains. Nuclear multi-gene genealogies determined that the *P. ultimum* species complex is comprised of four genetically distinct species. Altogether, our recent work illustrates how phylogenomics and phylogenetics are providing new insights into the systematics of oomycetes.

**O41.005 A diverse assemblage of Cryphonectriaceae species associated with non-native Myrtales on the Hawaiian Islands**

*G.K. Nkuekam[1], J. Uchida[2], C. Kadooka[2], J.B. Friday[3], L. Loope[4], M.J. Wingfield[1] and J. Roux[1]*
*[1]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Private Bag X20 Hatfield 0028, Republic of South Africa; [2]Department of Plant and Environmental Protection Sciences, Tropical Plant Pathology Program, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu, Hawaii, 96822, USA; [3]Department of Natural Resources and Environmental Management, College of Tropical Agriculture and Human Resources, University of Hawaii, Komohana Research and Extension Centre, Hilo, Hawaii, 96720, USA; [4]US Geological Survey, Pacific Island Ecosystems Research Center, Makawao, Hawaii, USA*
*Email: gilbert.kamgan@fabi.up.ac.za*

The Cryphonectriaceae (Diaporthales) includes at least thirteen genera of ascomycetes, amongst them important fungal pathogens of trees. Well known examples are *Cryphonectria parasitica*, the causal agent of chestnut blight in North America and Europe, and the eucalypt pathogens in the genus *Chrysoporthe*. Species of *Chrysoporthe* are known to infect a broad range of related hosts in the tree order Myrtales, including species of *Syzygium* and *Eucalyptus*. *Chrysoporthe deuterocubensis* has been reported from Hawaii on the island of Kauai, but no information is available regarding the origin of this pathogen on the islands, or the occurrence and identity of Cryphonectriaceae on other Myrtales on the islands. To address this issue, collections of fungi resembling species of Cryphonectriaceae were made from species of *Syzygium* and co-occurring Myrtales on three Hawaiian islands. Bark samples were collected from infected trees and fungi were isolated directly from fruiting bodies on the bark. Pure cultures were identified using both morphological studies and DNA sequencing of the ITS and part of the beta tubulin gene region. Three species of Cryphonectriaceae, spanning three genera were identified from samples originating from four tree genera spanning two orders and two families, yielding more than 150 isolates. Fungi were identified as *Chr. deuterocubensis*, *Microthia havanensis*, *Celoporthe indonesiensis* and two clades within the larger genus *Celoporthe*, most closely related to *Ce. guangdongensis*, *Ce. indonesiensis* and *Ce. syzygii*. Host trees from which the fungi were obtained included *Metrosideros* sp., *Psidium cattleianum*, *Syzygium jambos* and *S. cumini*, all Myrtales, Myrtaceae, as well as one non-Myrtaceae host, *Lycium carolinianum* (Solanales, Solanaceae). This study represents the highest genus and species diversity of the Cryphonectriaceae in a single region. This is most likely due to multiple introductions of these fungi.

**O41.006 *Cercospora*: a weedy patch in the garden?**

*J.Z. Groenewald, C. Nakashima, H.D. Shin, U. Braun and P.W. Crous*
*CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands*
*Email: e.groenewald@cbs.knaw.nl*

The genus *Cercospora* contains numerous important plant pathogenic fungi from a diverse range of plant hosts. Many *Cercospora* species are known only from their morphological characters *in vivo* and not from their morphology *in vitro*. *Cercospora* encompasses more than 5 000 names, but very few of these are available in culture and associated DNA sequence data are rarely provided. In our study, we subjected 360 *Cercospora* strains, obtained from 161 host species, 49 host families and 39 countries, to a molecular phylogeny approach. Partial sequences were derived from five genomic loci, namely the internal transcribed spacer regions and intervening 5.8S nrRNA gene, and partial actin, calmodulin, histone H3 and translation elongation factor 1-alpha genes. The resulting phylogenetic clades were evaluated

for application of existing species names and, in addition, five novel species were introduced. In order to stabilize their species circumscription, 11 species were epi-, lecto- or neotypified. Several clades were found for which existing candidate species names were available; however, it was not always possible to apply North American or European names to African or Asian strains and *vice versa*. We did not identify a single locus to be the ideal DNA barcode gene for the genus, and recommend that species should be identified based on a combination of DNA and morphological characters. In the course of this study, it became evident that several important phytopathogenic *Cercospora* species require taxonomic revision. Fresh collections are required from the original hosts in the countries of origin, however, to facilitate these studies.

### O41.007 Taxonomic and phylogenetic study of a rust genus *Coleosporium* in China

*C.J. You, Y.M. Liang, J. Li and C.M. Tian*
*Key Laboratory for Silviculture & Forest Conservation Affiliated to Ministry of Education, Beijing Forestry University, Beijing 100083, P. R. China*
*Email: chongjuanyou@gmail.com*

Pine needle rusts caused by *Coleosporium* Lév. (Coleosporiaceae, Basidiomycetes) are among the damaging diseases of young pines in plantations and natural forests worldwide, retarding growth by causing premature defoliation and contributing to tree death. Among the approximately one hundred and thirteen *Coleosporium* species described worldwide, fifty nine species have been reported in China. The taxonomy and phylogenetic relationship of *Coleosporium* species in China are challenging. A total of 876 uredinio-telial specimens, from 21 provinces, were used for light microscopy, scanning electron microscopy and molecular phylogenetic investigation. The association between the morphological characters of *Coleosporium* species and the telial host families were summarized, then phylogenetic trees were constructed from the 28S region of nuclear large subunit rDNA, and the internal transcribed spacers regions, respectively. We aimed to define the relations between the phylogenetic group and morphological characteristics, and to evaluate the taxonomic the morphological similar species of *Coleosporium.* Main results are as follows: a new *Coleosporium* species is described, named as *Coleosporium zhuangii* C.M. Tian & C.J. You (Type on *Ligularia fischeri*). The relations between the phylogenetic groups and morphological characteristics were investigated, and the results showed that the urediniospore surface-structure and the teliospore morphological characters reflected the phylogeny, the germ pore arrangement of urediniospores and the shape, size of urediniospores and teliospores did not correspond to the phylogeny. The surface structure of urediniospores, and the morphological characters of teliospores have been proved to be more reliable taxonomic characters for the delimitation and circumscription of *Coleosporium* species.

### O41.008 Identification and biological characterization of *Colletotrichum* species causing strawberry anthracnose in China

*B.C. Jin and G.Z. Zhang*
*Department of Plant Pathology, China Agricultural University, Beijing 100193, P. R. China*
*Email: zhanggzh@cau.edu.cn*

Anthracnose is a major disease of strawberry which attacks all parts of the plant and causes serious crown rot, irregular leaf spot, lesions on petiole and runner, as well as black spot on fruit. Amount of plant death caused by anthracnose crown rot in October and November after transplanting have become a big problem in greenhouse strawberry production in Beijing, China recently. A total of 86 single-conidial isolates were obtained from infected plant tissues collected in eight provinces. Via morphology and comprehensive multilocus analyses all tested isolates were identified as three species, *Colletotrichum fructicola* (23), *C. theobromicola* (42) and *C. nymphaeae* (21) which were considered to be *C. gloeosporioides*, *C. fragariae* and *C. acutatum*, respectively. Among of which, 40 isolates were selected and inoculated to crown, leaf, petiole and runner and fruit, respectively, on cultivar Benihoppe. There were obvious differences in pathogenicity to various parts of strawberry among three species. *C. fructicola* isolates had the highest pathogenicity to crown. *C. nymphaeae* isolates had the highest pathogenicity to fruit and in comparison, the pathogenicity of *C. theobromicola* isolates to all the parts were middle. Sensitivity of the three species to fungicide thiophanate methyl was also obviously different. *C. fructicola* isolates and *C. nymphaeae* isolates were sensitive ($EC_{50}$ values for the mycelia growth were 0.5451 μg/mL ±0.0115 and 5.266 μg/mL ±0.8406, respectively), while *C. theobromicola* isolates were very resistant to the fungicide ($EC_{50}$ value higher than 200 μg/mL).

### P41.001 Confirmation of soybean damping-off by *Pythium myriotylum* Drechsler in Japan

*K. Tomioka, T. Takehara, H. Osaki, H. Sekiguchi and K. Nomiyama*
*Division of Vegetation Resources and Functions, River Basin Research Center, Gifu University, Gifu 501-1193, Japan, Western Region Agricultural Research Center, National Agriculture & Food Research Organization, Fukuyama 721-8514, Japan*
*Email: tomioka@affrc.go.jp*

Severe rotting and blight on seedlings of soybean were found during cultivation trials for stable high-yields of the crop in open fields in Fukuyama City, Hiroshima

Prefecture in the southwest region of Japan, in July 2009. Water-soaked, dark brown, irregular lesions appeared on stem bases. The lesions gradually enlarged towards upper stems, seed leaves and primary leaves. The affected seedlings softened and turned dark brown, resulting in early death. *Pythium myriotylum* Drechsler was isolated from diseased seedlings placed on apricot media. When inoculating soybean, cv. Sachiyutaka, with a representative isolate of the oomycete obtained by single-hyphal isolation, symptoms were reproduced. The isolate was consistently reisolated from the diseased plants, satisfying the Koch's postulates to demonstrate that it was pathogenic to soybean. In Japan, a factor of the soybean monoculture injury, e.g. root browning, growth delaying and yield reduction, is known to be infection of the plant with *P. myriotylum*. But, nor in Japan have any previous reports regarding occurrence of diseases on soybean at a seedling stage caused by the oomycete in natural environments, and the present disease is judged to be new in Japan. Soybean damping-off by *P. spinosum* Sawada, *P. ultimum* Trow var. *ultimum* or *Pythium* sp. has already been noted in Japan. As its symptoms are similar to those of the present disease, we propose to include *P. myriotylum* as one of the causal pathogens of soybean damping-off in Japan. The pathogenic isolate was deposited in the GenBank, National Institute of Agrobiological Sciences, Japan, as accession MAFF243489.

### P41.002 Fruit rot of sweet pepper (*Capsicum annuum* L. var. *grossum* Sendtner) caused by a species of the *Fusarium incarnatum IF. equiseti* complex

*K. Tomioka, T. Aoki, T. Nagai, H. Sawada and T. Sato*
*Genetic Resources Center, National Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan; Western Region Agricultural Research Center, National Agriculture & Food Research Organization, Fukuyama 721-8514, Japan*
*Email: tomioka@affrc.go.jp*

Fruit rot of sweet pepper had been reported in Japan and its causal pathogen was identified at first as *Fusarium lateritium* Nees (Tomioka et al. 2002; Tomioka and Sato 2004; Tomioka 2005). The pathogenic isolate, MAFF238882, was later analyzed phylogenetically based on DNA sequences of the gene-coding regions of histone H3 gene, translation elongation factor 1-α gene, and the rDNA ITS region to be found as a species closely related to *F. incarnatum* (Desmazieres) Saccardo and *F. equiseti* (Corda) Saccardo (Tomioka et al. 2010). Apparently the strains located to the phylogenetic clade of the *F. incarnatum* / *F. equiseti* species complex. Morphological characters of MAFF238882 agreed well with the morphological features of *F. incarnatum* forming blastic multiseptate conidia on branched aerial conidiophores. Contrastedly, *F. equiseti* forms conidia only on sporodochial conidiphores on the agar surface (Leslie and Summerell 2006). Because taxonomic studies on the *F. incarnatum* / *F. equiseti* species complex is not well conducted so far. We, therefore, tentatively identified MAFF238882 as *F. incarnatum*. The disease of sweet pepper by *F. incarnatum* (or a species within the *F. incarnatum* / *F. equiseti* species complex) has not yet been reported in Japan while the fungus has been isolated from the host plant in Malaysia (Liu 1977). It is a new report of the fungus as one of the disease pathogens of sweet pepper in Japan.

### P41.003 Genetic diversity and pathogenicity of *Gibberella fujikuroi* species complex isolated from rice seeds

*Y.A. Jeon, H.S. Lee, Y.Y. Lee, S. Lee, J.S. Sung, Y.G. Kim and S.H. Yu*
*Department of Applied Biology, Chungnam National University, Daejeon 305-764, Republic of Korea; National Agrobiodiversity Center, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-100, Republic of Korea*
*Email: yjeon@korea.kr*

*Gibberella fujikuroi* species complex (GFSC) was isolated from rice (*Oryza sativa* L.) seeds with the geographical origins of Asia, Africa and America, and characterized with respect to genetic diversity. Four species of GFSC, *Fusarium fujikuroi*, *F. proliferatum*, *F. verticillioides* and *F. concentricum*, were detected in the different seed samples with infection rate ranges that varied from 4% to 80% independently to the origin. Phylogenetic analyses with DNA sequences of internal transcribed spacer, translation elongation factor-1α, β-tubulin and histone H3 genes showed a genetic variation among the 50 isolates that were distributed into four different genetic clades. All the isolates showed pathogenicity on rice and slightly reduced seed germination. The seedlings infected with *F. fujikuroi* and *F. concentricum* showed typical Bakanae symptoms. However, *F. proliferatum* and *F. verticillioides* made rice seedlings stunted without Bakanae symptoms. The results provide information on the variation within the GFSC associated with rice seed and Bakanae disease.

### P41.004 Two *Pythium* species associated with root rot of poinsettia caused by *Pythium helicoides* Drechsler and *Pythium myriotylum* Drechsler in Japan

*N. Miyake and K. Kageyama*
*United Graduate School of Agriculture Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
*Email: r6102009@edu.gifu-u.ac.jp*

Poinsettia plants (*Euphorbia pulcherrima* Willd. ex Klotzsch) growing in the ebb-and-flow system showed wilting and root rot during summer growing seasons of 2010 and 2011 in Gifu and Aichi Prefectures, respectively. Two type isolates of *Pythium* species were isolated

from poinsettia plants with a symptom of root rot. In pathogenicity tests, both of the isolates caused severe wilting and root rot. The Gifu isolate caused more severe symptom than the Aichi isolate. Each isolate was re-isolated from lesions of the diseased plants. The Gifu isolate formed ellipsoidal, papillate and proliferating sporangium, one to three smooth and elongate, diclinous antheridia on a smooth oogonium and smooth spherical aplerotic oospore. Hyphal growth occurred at temperatures between 10 ℃ and 40 ℃, and the optimum was 35 ℃. The Aichi isolate formed filamentous and slightly inflated sporangia, three to eight antheridia per a smooth oogonium and aplerotic oospore. Hyphal growth occurred at temperatures between 10 ℃ and 40 ℃, and the optimum was 35 ℃. The Gifu and Aichi isolates were identified as *P. helicoides* and *P. myriotylum*, respectively, on the bases of the morphological characteristics and a sequence homology analysis of the internal transcribed spacer regions of rDNA. To our knowledge, Pythium root rot of poinsettia plants caused by *P. helicoides* and *P. myriotylum* will be the first report in Japan, although *P. aphanidermatum* has been reported as a serious pathogen of poinsettia root rot (Bolton 1978; Watanabe et al. 2008).

**P41.005 Isolation and characterization of *Mycosphaerella graminicola* assocoated with abnormal skin stain symptoms of 'Niitaka' pear in Korea**

*S.Y. Oh*[1], *D.H. Yoon*[1], *E.Y. Lee*[2] *and K.W. Nam*[1,2]
[1]*Research Institute of International Agriculture, Technology and Information, Hankyong National University, 456-749, Republic of Korea;* [2]*Department of Horticulture, Hankyong National University, 456-749, Republic of Korea*
*Email: syoh@hknu.ac.kr*

The abnomal skin stain symptoms in pear (Niitaka) for export rendering them unfit for marketing purposes in recent year in Korea. The AnSS is characterized by dark brown specks and irregular blotches anywhere on the skin of the fruits. The lesions, however, were restricted only on the fruit skin. The exact cause of AnSS is unknown, but it is thought by many to be a mold disease. We investigated the occurrence of the AnSS on pear fruits and major fungi occurred was identified. The highest incidence observed was *Mycosphaerella* (64.2%), followed by *Penicillium* (13.2%) and *Alternaria* (12.0%) genera. The pathogenecity test was conducted; spray spore suspension ($10^4$ spore/ml) onto pear fruit. The isolates caused AbSS symptoms on fruits of pear four weeks after artificial inoculation which were similar to those observed in the storage time. Based on the morphological characteristics and rDNA ITS (internal transcribed spacer) and beta-tubulin genes sequence data, the fungus was identified as a *Mycosphaerella* species showing a separate phylogenetic status as a *Mycosphaerella graminicola*. The colony of the fungus on PDA was velutinous, dark grey and dark pigmentation on reverse side. Sequence analysis by BLAST indicated that the isolates (HKNU1, HKNU3) were closest to *Mycosphaerella* sp. M16 (accession number, HM595520.1) with 99% ITS and *M. graminicola* (accession number, AJ310917) with 99% beta-tubulin sequence similarity. The causal fungus (HKNU1, HKNU3) was determined to be *M. graminicola* based on the morphology and the sequence data analysis. On the basis of mycological characteristics, pathogenicity test, beta-tubulin gene and the ITS sequence analysis, the causal fungus were identified as *M. graminicola*. To our knowledge, the occurrence of the *M. graminicola* on pear fruits has not been reported in Korea.

**P41.006 Diversity of pathogenic and endophytic *Phomopsis* species and *in vitro* biological control of endophytic *Phomopsis* spp. against plant pathogenic fungi**

*A. Danggomen, N. Visarathanonth, O. Piasai and L. Manoch*
*Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand*
*Email: agrlkm@ku.ac.th*

*Phomopsis* species can cause severe fruit rot diseases and can be reported as an endophyte. In the present study, seven fruit rot samples of durian, mango, longkong, tamarind, guava, grape, and jackfruit were collected as well as five samples of various wild plants including *Antirhea lucida, Ficus annulata, Guettarda speciosa, Hunteria zeylanica,* and *Colubrina asiatica*. Tissue transplating method and surface sterilization were used for isolation *Phomopsis* species from diseased and non-diseased fruits respectively. For isolation of endophytic fungi, leaf tissue was cut into small pieces and placed in 10% sodium hypochloride for 3 min and 70% ethanol for 30 sec. Twig and petiole were cut into 2 cm long, put in 75% EtOH for 1 min and 4% sodium hypochloride 3 min then 75% EtOH 30 sec and placed on water agar. They were incubated at 28 C for 7-30 days. Identification of the fungal isolates were based on morphological characteristics as colony growth on agar media, pycnidia, conidiogenous cell and conidia using stereo and light microscopes. Twenty-one isolates of *Phomopsis* spp. were found from diseased fruits including *Phomopsis destructum, P. psidii, P. tamaridii* and *Phomopsis* sp., while 16 isolates were found from non-diseased fruits comprising *Phomopsis amraii, P. archeri, P. durionis, P. elastic, P. lantanae, P. terminaliae* and *Phomopsis* sp. Eighteen isolates of endophytic *Phomopsis* spp. were inoculated on healthy guava fruits and all isolates caused symptomless on guava. *In vitro* antagonistic activity tests were conducted using 12 isolates of endophytic *Phomopsis* spp. against four species of plant pathogenic fungi including *Colletotrichum gloeosporioides, Pestalotiopsis* sp., *Phoma* sp.,

and *Lasiodiplodia theobromae*. Eleven isolates of endophytic *Phomopsis* than 60% of *Pestalotiopsis* sp. and all isolates inhibited more than 50 % of *Phoma* sp. and *C. gloeosporioides*, but failed to control *L. theobromae*.

**P41.007 Root rot diseases on lance asiabell (*Codonopsis lanceolata*) caused by *Fusarium* species**

C.S. Jeon[1], G.H. Kim[1], K.I. Son[1], J.S. Hur[2], K.S. Jeon[3], J.H. Yoon[3] and Y.J. Koh[1]
[1]*Department of Plant Medicine, College of Life Science and Natural Resources, Sunchon National University, Suncheon 540-900, Republic of Korea;* [2]*Department of Environment Education, College of Education, Sunchon National University, Suncheon 540-900, Korea;* [3]*Southern Forest Research Center, Korea Forest Research, Jinju 660-300, Republic of Korea*
*Email: youngjin@sunchon.ac.kr*

Lance asiabell (*Codonopsis lanceolata*) is a kind of mountain herbs whose roots have restorative properties and the cultivating acreage of lance asiabell has been steadily increasing in Korea. Several diseases have been reported to occur on lance asiabell which is cultivating at farmers' high-density fields. Since more frequent rain and high amount of rainfalls as a result of global warming predisposed lance asiabell plants to the outbreaks of root rots in recent years, their severities were surveyed at various cultivation fields of lance asiabell located in Jeonnam, Gyoungnam and Jeju Provinces. The overall disease severities of root rots on lance asiabell were quite variable according to the surveyed fields, which ranged from 0.1% to 50%. The root rot diseases occurred more severely at the paddy or clay soils than the sandy soils and their severities were more severe at lowland than upland in a same field. The diseases increased with aging of the lance asiabell. Root crowns were usually discolored into brown to blackish brown at first and the infected plants showed slight wilting symptom at early infection stage. Severely infected roots were entirely rotted, resulted in eventual death of the whole plants at late infection stage. *Fusarium* spp. were isolated from the diseased root tissues repeatedly and they were identified as *Fusarium solani* and *F. oxysporum* on the basis of their mycological characteristics on potato dextrose agar and carnation leaf agar. The optimum temperature ranges for their mycelial growths were 24℃ and the mycelia growths were most effectively inhibited by tebuconazole EC on potato dextrose agar, respectively. The pathogenic characters of *F. solani* and *F. oxysporum* treated by artificial wound inoculation on healthy roots of lance asiabell revealed that *F. solani* was more virulent than *F. oxysporum*. This is the first report on the root rots of lance asiabell caused by *Fusarium* spp. in Korea.

**P41.008 A new record of *Erysiphe berberidis* var. *asiatica*, in Heilongjiang Province**

*B.N. An, W.T. Jiang, J. Liu, X.Y. Men, Y. Li and S.Y. Liu*
*Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Jilin, Changchun 130118, P. R. China*
*Email: liussyan@163.com*

The plants in genus *Berberis* are widely used as garden shrubs for their ornamental leaves, yellow flowers, and red or blue-black berries. In August 2012, powdery mildew seriously happened on *Berberis poiretii* in Harbin Forest Botanical Garden, Heilongjiang Province, China. White mycelia developed amphigenously on leaves and stems, mostly persistent. Chasmothecia were globose or subglobose, mostly scattered, 76-105 μm in diameter, with irregularly polygonal peridium cells. Appendages were equatorial, fewer than ten, and 72.5-138 μm long, (0.8-) 1-1.5 times of the chasmothecial diameter, brownish at the base and paler towards the tip, uniform thickness, apices 3-5 times dichotomously branched, branches elongated, tips sharp or straight, not recurved. The asci were stalked or not, ovoid or ellipsoid, 4-6 in a chasmothecia, 41.5-53.5×28-34.5 μm. Ascospores were ellipsoid-obovoid, (3-) 4-5 (-6) spores in an ascus, 15-21×8-14 μm, colorless. The fungus was identified as *E. berberidis* var. *asiatica* based on the morphological characteristics. Six species and one variety were reported on *Berberis* spp. in China, i.e. *Erysiphe berberidicola*, *E. berberidis* var. *berberidis*, *E. berberidis* var. *asiatica*, *E. dimorpha*, *E. multappendicis*, *E. sichuanica* and *Phyllactinia berberidis*. *E. berberidis* var. *asiatica* differs from the other species for having straight tip of appendages, and from var. *berberidis* for having relatively short appendages, only 1-2 times, most 1-1.5 times the chasmothecial diameter. *E. berberidis* var. *asiatica* has been reported in Jilin, Gansu, Xinjiang and Sichuan in China. It is the first report in Heilongjiang Province.

**P41.009 Stem anthracnose of red flesh dragon fruit (*Hylocereus polyrhizus*) in Malaysia**

*Z. Latiffah and I.V. Suzianti*
*School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia*
*Email: Lfah@usm.my*

Red flesh dragon fruit (*H. polyrhizus*) has been reported to be infected by fungal diseases that affected the production and reduced the yield. One of the important diseases is stem anthracnose. A survey was conducted in August 2010 to January 2011, with 60 isolates of *Colletotrichum* successfully isolated from the anthracnose lesions from various farms in several states in Peninsular Malaysia. Based on morphological characters and ITS+ 5.8S sequences, two *Colletotrichum* species *C. gloeosporioides* and *C. capsici* were identified. Pathogenicity

test was successfully carried out confirming that *C. gloeosporioides* and *C. capsici* are the causal pathogens of anthracnose on red flesh dragon fruit stem, thus Koch's postulates was fulfilled. This study is the first scientific report of *C. capsici* as one of the causal pathogens of anthracnose of red flesh dragon fruit in Malaysia.

**P41.010 Identification of fungi species in forest ecosystem of internal Alaska**

*M.M. Chen and S. Kim*
*University & Jepson Herbaria, Valley Life Science Building, Berkeley, CA 94720, USA*
*Email: momeichen@berkeley.edu*

Fungi as a natural resource in Alaska have a great potentiality for the ecosystem regard of biodiversity. The Expeditionary Mycology Research Program had a project of forest ecosystem fungi collection in Fairbanks, Alaska (1989-1990). The collections have been preserved in the University of Alaska and University of California, Berkeley, USA. Every specimen was processed in well-equipped laboratories and herbarium. The fungus were identified according to species using the previously citation of taiga forest fungi flora and local fungi collection references. This study was primarily focused on identification with the traditional method which uses the fungus morphological data to identify each specimen. Current identification act upon the nomenclature and with techniques in applied molecular reference level. Under five forest ecosystems, an individual specimen was classified into four categories: edible, medicinal, pathogen, and mycorrhizal. Total 310 specimens are still on process for preservation internal Alaska fungi specimens. The total specimens of fungi arranged in classification position: Ascomycota phylum has one class including two importment forests tree pathogen's two orders of Erysiphales and Rhytismatales (powdery mildew and tar spot diseases). In this project, most of the collections were from Basidiomycota phylum; Agaricomycetes (mushrooms) and Pucciniomycetes (rusts) have Diverse 7 mushroom orders including Agaricales, Boletales, Exobasidiales, Gomphales, Polyporales , Russulales, and Thelephorales, which contain totally 27 families and 28 genera. We will contribute on the future studies to improve the phylogenic and systematic identification of fungi in Alaska.

**P41.011 Morphological and phylogenetic identification of *Botrytis sinoviticola*: a novel cryptic species causing grey mould disease on table grapes (*Vitis vinifera*) in China**

*Y.J. Zhou, J. Zhang and G.Q. Li*
*State Key Laboratory of Agricultural Microbiology and Key Laboratory of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan 430070, P. R. China*
*Email: zhangjing1007@mail.hzau.edu.cn*

Seventy-five isolates of *Botrytis* collected from table grapes (*Vitis vinifera*) showing grey mould symptoms in China were identified based on morpho-cultural characteristics on potato dextrose agar (20℃) and/or phylogenetic analysis using the sequences of three nuclear genes (*G3PDH*, *HSP60*, *RPB2*). Isolates of different species of *Botrytis* were compared using fenhexamid sensitivity, Bc-*hch* gene-RFLP haplotyping and pathogenicity on table grapes. The 75 isolates were found to comprise two species: *B. cinerea* (63 isolates) and *Botrytis* sp. (12 isolates). *Botrytis* sp. is a novel species described here as *Botrytis sinoviticola* Zhang *et al*. sp. nov. Both *B. sinoviticola* (*Bs*) and *B. cinerea* (*Bc*) were found to have optimum temperatures at 20℃ for mycelial growth and at 25℃ for conidial germination. Sensitivity to fenhexamid was significantly greater ($P < 0.05$) for *Bc* ($EC_{50}$ = 0.04 ± 0.01 μg $mL^{-1}$) than for *Bs* ($EC_{50}$ = 0.08 ± 0.02 g $mL^{-1}$). Digestion of the PCR-amplicons of the Bc-*hch* gene with *Hha* I generated two haplotypes, namely Group I haplotype for *Bs* and Group II haplotype for *Bc*. *Bs* infected table grapes (leaves, berries) only through wounds, whereas *Bc* infected both injured and non-injured tissues of table grapes. This study suggests that *Bs* is a cryptic species living in sympatry with *Bc* on table grapes in China.

**P41.012 Identification of pathogenic *Fusarium* spp. causing apple replant disease in Hebei province**

*S.T. Wang, Q.J. Zou, T.L. Hu, Y.N. Wang and K.Q. Cao*
*College of Plant Protection, Hebei Agricultural University, Baoding, Hebei 071001, P. R. China*
*Email: bdstwang@163.com*

Apple replant disease (ARD) distributed in all over the apple producing areas around the world, is the main problem that affects the replacement of the apple orchards. It is also a serious threat to the sustainable development of apple industry in Hebei, China. Among the ARD pathogen isolates, the *Fusarium* spp. which had the highest isolation frequency were tested. In the present study, twenty one strains isolated from different apple production areas of Hebei province were identified by observing of the morphological characteristics and determine with rDNA-ITS (internal transcribed spacer) and TEF-1α (translation elongation factor-1α gene) sequences alignment. *Malus robusta* seedlings were used to test the pathogenicity of the strains. Five different species of pathogenic *Fusarium* include *F. oxysporum*, *F. equiseti*, *F. acuminatum*, *F. proliferatum* and *F. solani* were identified. Among them, *F. proliferatum*, *F. equiseti*, *F. acuminatum* were the first report as the pathogens of the ARD in China. According to the pathogenicity test results, all the tested 21 strains had pathogenicity against *Malus robusta* seedlings. While the virulence of different *Fusarium* strains differed significantly. The corrected

mortality of the seedlings treated by the strongest virulent isolate HS2 was 50% while that of the lowest virulent isolate CZ1 was just 3.3%. The virulence level had no correlation with the species and the geographic origins of these strains.

**P41.013 A new species of the genus *Cytospora* (Diaporthales, Valsaceae) from China on *Salix cupularis***

*X.L. Fan, C.M. Tian and Y.M. Liang*
*Key Laboratory for Silviculture and Conservation of Ministry of Education, Beijing Forestry University Beijing 100083, P. R. China*
*Email: 434535665@qq.com*

The genus *Cytospora* causes canker disease on a wide range of plants worldwide. Over 85 species of plant hosts are susceptible to *Cytospora* canker. *Cytospora* spp. are the anamorphs of the ascomycete genus *Valsa* Fr. Fruiting bodies consist of stromata (conidiomata) that usually contain irregular chambers, having filamentous conidiophores and allantoid hyaline conidia. In moist conditions, the conidia exude from the fruiting bodies in gelatinous matrices, usually as yellow, orange, red or pallid tendrils. The genus *Cytospora* was established by Ehrenberg in 1818. Until now, 110 species of *Cytospora* had been described worldwide based on Dictionary of the Fungi ($10^{th}$). It was indistinct that standard of classification of genus *Cytospora*, but the classification of Cytospora was related to the morphology of the fungi largely. In this study, *C. becuus*, a beaked species of *Cytospora*, was collected on *Salix cupularis* Rehd in Gansu Province, China. Besides, *C. nivea* has been found firstly from *Salix* in China. Identification of fungal species was based on morphological characteristics of fruiting body, spores and cultures. The new species *C. becuus* has a single chamber and a thorn-like beak, which can distinguish from other species. In addition, phylogenetic analysis was performed using the molecular data obtained from sequencing ITS (ITS1-5.8S-ITS2) region with Bayesian posterior probabilities of 0.94. Both morphological and molecular evidence suggest that *C. becuus* is a distinct linage within the genus *Cytospora*.

**P41.014 Symptomological, cultural, morphological and molecular variability of alternaria leaf spot of Broccoli (*Brassica oleracea* var. *italca* L.)**

*G. Chand[1], C.S. Azad[1], S. Kumar[2] and V.K. Yadava[2]*
*[1]Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur-813210, India; [2]Department of Plant Pathology, N. D. University of Agriculture& Technology, Faizabad-224239, India*
*Email: gireesh_76@rediffmail.com*

India is the second most broccoli producer (5mt) after China (8mt) but productivity is low due to various factors. In which alternaria leaf spot disease plays a major role and becomes difficult to diagnose the disease in the field and to indentify the associate species correctly. Maximum number of spots (86) recorded from the sample SHIAT followed by BHU (74) while minimum (17) with the sample CSAUAT. As for as size of spots are concerned maximum size (0.2-1.9 mm) recorded in the samples collected from BHU while minimum size (0.2-0.7mm) with the samples collected from SHIATS with light olivacious brown colour along with concentric rings with black pin point centre and conidia varied in length (13-120 µm), breadth (6-16 µm) and septation (1-8). Maximum growth was recorded on host extract agar followed by Potato Dextrose Agar, Richards Agar Media from CSAUAT isolates. Protein profiling using SDS-PAGE showed variation among the isolates. Highest molecular weight of protein was detected in isolates collected from CSAUAT (22 kDa) followed by isolates sampled from NDUAT (21 kDa) and BHU (21 kDa). SHIATS samples showed minimum molecular weight (20 kDa).

## Concurrent Session 42-Teaching Plant Pathology

### O42.001 Teaching Plant Pathology for undergraduate student in China, opportunity and challenge

*L.Y. Guo*
*Department of Plant Pathology, China Agricultural University, 2 West Yuanmingyuan Rd, Beijing, 100193, P. R. China*
*Email: ppguo@cau.edu.cn*

In the past decade, with the economic growth, the teaching systems in the universities of China have gone through many changes, such as increasing enrolment and providing education to students with varying backgrounds. These changes has also greatly affect the plant pathology teaching for undergraduate student in China, such as the amount of plant pathology courses and the hours of each courses were significantly reduced while the size of classes were greatly increased. However, the advancement of technology has provided opportunity to deliver the knowledge more efficiently. Here, we will share our experiences to reflect the trend of this change and our efforts to meet the challenge.

### O42.002 Outcomes based learning and active learning techniques for plant pathology classes

*D.M. Eastburn*
*University of Illinois, Department of Crop Sciences, 1101 W. Peabody, Urbana, Illinois, 61801, USA*
*Email: eastbur@illinois.edu*

For many years the standard model for instruction in plant pathology courses has included a mix of instructor centered lectures and, in some courses, a lab component based primarily on observation of materials. With this instructor focused format, the students are usually passive recipients of the instruction process. In designing their lectures and labs, instructors often start by outlining the material they want to cover. They then develop activities and assignments, followed by creating assessment tools (papers, quizzes, and exams). Figuring out how to evaluate student achievements and assign grades is often the last step in the process. Current educational research, however, indicates that student focused methods of instruction, which make use of active learning techniques and designed based on desired student outcomes, are more effective in fostering student understanding and retention of concepts and information. With outcomes based learning, design of a course starts with deciding what the students should be able to do at the end of a course or course unit. This is followed by determining how this ability will be evaluated, then by determining the instructional activities that will help the students achieve these competencies, and finally by selecting the specific instructional material to be delivered. With student centered learning, the methods of instruction are redirected from instructors, serving as sources of knowledge, to the students, as seekers of knowledge, with the instructor becoming a facilitator to guide the students. This often involves techniques to make the students more active participants in the learning process.

### O42.003 Virtual labs and other uses of technology in plant pathology courses

*H.D. Shew*
*Department of Plant Pathology, NC State University, Raleigh, NC*
*Email: shew@ncsu.edu*

Demand for effective distance education (DE) offerings is increasing rapidly at all levels of education. However, budget reductions and demands on time can be primary constraints in the development of high-quality DE courses. The selection and use of the most appropriate technology can greatly aid in meeting the demands for web-based learning materials while keeping within your budget and still allowing you to do the rest of your job. One of the greatest challenges for DE courses in our discipline is the effective communication of material for labs. In fact, many face-to-face courses are also dropping labs due to time needed to prep and conduct labs. This presentation will discuss those tools best suited for producing and delivering material in a DE Plant Pathology course, especially material for virtual labs and lab exercises, as well as strategies for preparing material for short courses or extension based materials.

### O42.004 Knowledge transfer outside universities

*A.J. Dik*
*Aleid Dik Consultancy, Wolvenplein 5, 3512 CJ Utrecht, The Netherlands*
*Email: contact@aleiddik.nl*

Universities and colleges provide a vast range of courses for students, which are usually also open for personnel. Universities may also provide courses for growers and other people outside the university. Researchers sometimes have an extension task, but this is not the case for every researcher in every country. In The Netherlands for example, a separate extension service existed, which has later been privatized and subsequently changed. Still, for scientists it can be frustrating when sound results, especially of more applied research projects, are not applied by growers. Apart from technical reasons for this lack of acceptance, it is often due to a gap between the presentation of the results and the details needed by growers. An important source of information for growers is consultants and extension services. It is not always clear how and from where they get their information. A course was developed on recognition and integrated control of plant pathogens, which is now taught regularly in The Netherlands and abroad to growers and

consultants. Much more can be done to optimize knowledge transfer.An international inventory was made on the different groups that growers participate in and the different ways they are informed about new research results. An analysis was made on which methods are most successful. This can be used to improve knowledge transfer which researchers will be able to use to enhance acceptance and practical use of research results.

**O42.005 Negotiating unfamiliar territory: Getting your plant pathology teaching innovations published**

*T.M. Stewart*
*Institute of Agriculture and Environment, Massey University, Tennent Drive, Palmerston North 4474, New Zealand*
*Email:t.stewart@massey.ac.nz*

Along with conducting research and extension work, many plant pathologists also teach. New technologies and educational developments have opened up novel and innovative teaching approaches for engaging students and training them in the plant pathology discipline. Teachers may wish to engage in academic discourse and research on these new teaching approaches, along with their more conventional discipline research. In particular they may wish to expose their work to a wider educational audience beyond specific plant pathology educational outlets such as the ASPnet Plant Health Instructor. Whilst this is a laudable scholarly goal, getting teaching research published in mainstream tertiary teaching education journals can involve major hurdles. These barriers include ethics considerations and clearance, confusing paradigms, educational jargon and unfamiliar research methodologies, both quantitative and qualitative. Funding may be scarce, and faculty and departmental heads unsupportive of this activity, seeing it as a distraction from traditional plant pathology research. This paper discusses these problems and offers possible solutions, the main one being a team approach with an educational researcher or academic developer.

**P42.001 Establishing a new Master degree in sustainable crop protection in Egypt**

*P. Colla[1], D. Spadaro[1], M.E. Abdalla[2], K.A.M. Abo-Elyousr[3], A.Z. Aly[4], M. Badr[5], F. De Luca[6], M. Eizaguirre[7],E.S. Farrag[8], Y. Livieratos[9], A.A. Mosa[10], A.A. Sattar[11]and M.L. Gullino[1,12]*
*[1]Centre for the Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Torino, 10095 Grugliasco, TO, Italy; [2]Plant Pathology Dept., Faculty of Agriculture, Mansoura University, El-Mansoura 35516, Egypt; [3]Plant Pathology Dept., Faculty of Agriculture, AssiutUniversit, Assiut 71526, Egypt; [4]Agr. Bot. & Plant Pathology Dept., Faculty of Agriculture, Zagazig University, Egypt; [5]Agricultural Botany Dept., Faculty of Agriculture, Kafr El Sheikh University, Egypt; [6]Istituto per la Protezione delle Piante, UOS-Bari, Consiglio Nazionale delle Ricerche, Bari, Italy; [7]AGROTECNIO Center, Universitat de Lleida, 25198 Lleida, Spain; [8]Agricultural Botany Dept. Faculty of Agriculture, South Valley University, Qena, Egypt; [9]Mediterranean Agronomic Institute of Chania, AlsylliooAgrokepiou - Chania 73100 Crete, Greece; [10]Plant Pathology Dept., Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt; [11]Plant Pathology Dept., Faculty of Agriculture, Suez Canal University, Ismailia, Egypt; [12]Department of Agricultural, Forest and Food Sciences and Technologies (DISAFA), University of Torino, 10095 Grugliasco, TO, Italy*
*Email: paola.colla@unito.it*

There is a strong pressure on education, training and life-long learning programmes to adapt towards facing the challenges that agriculture is confronting today: securing healthy and safe food and feed supply while ensuring the economic and environmental sustainability. The Project "Establishing a Master degree in Sustainable Crop Protection" supported the modernization of curricula in the field of crop protection in Egyptto respond to societal and job market demands. This initiative, funded by European Tempus Programme, developed a network of Master's degree in Sustainable Crop Protection among seven Egyptian universities (Ain Shams, Assiut, Kafr El Sheick, Mansoura, South Valley, Suez Canal, Zagazig) with the support of four European institutions (University of Torino and National Research Council in Italy, University of Lleida in Spain and Mediterranean Agronomic Institute of Chania in Greece). Tempus MSCP Project was coordinated by Agroinnova, University of Torino, from January 2010 to July 2013 with the goal of produce a new generation of young professionals able to analyze options, define strategies and manage solutions for securing healthy and safe food and feed supply. The first step was to extensively review the curricula taking into account the importance of a good balance between theory and practical activities. Training of universities staff and development of teaching material were two other important tasks. The first generation of MSc students enrolled at Kafr El Sheick University in September 2012. In addition to the main goals, other advantages followed, including the establishment of close collaboration between consortium member institutions and private sector.

## Concurrent Session 43-The Regional Diseases

### O43.001 Cell density-dependent behaviors of *Xylella fastidiosa*: achieving disease control via pathogen confusion

*S.E. Lindow*
*Department of Plant and Microbial Biology, University of California, Berkeley, California 94720, USA*
*Email: icelab@berkeley.edu*

The bacterium *Xylella fastidiosa* causes Pierces disease of grape and other important diseases of citrus, almond and other crops by progressively moving to and multiplying in many in xylem vessels after inoculation by insect vectors. While most xylem vessels harbor only small numbers of the pathogen blockage of some vessels occurs and is associated with disease. *X. fastidiosa* exhibits strong cell density-dependent behaviors that tend to self-limit its population size in plants, presumably to avoid the cell death that is associated with the plugging of vessels due to bacterial over-growth. *X. fastidiosa* employs a quorum sensing system that utilizes fatty acid signal molecules (DSF) including 2-Z-tetradecenoic acid that increase in local concentration in proportion to cell numbers in a vessel to suppress the production of type IV pili enabling twitching motility and extracellular enzymes that enable transit between xylem vessels and also contribute to cell multiplication by liberating consumable carbon sources, while enhancing expression fimbrial and afimbrial adhesins that restrict movement within the plant, but which are required for acquisition by, and hence transmission to, new hosts by insect vectors. The population of cells in a plant is thus partitioned based on their local cell density between those capable of plant colonization and those capable of vector transmission. Expression of *rpfF* from *X. fastidiosa* encoding a DSF synthase in grape results in accumulation of DSF, causing the pathogen to exhibit a restricted movement phenotype in transgenic plants, resulting in high levels of disease resistance.

### O43.002 Insight into the virulence mechanism of the citrus canker pathogen *Xanthomonas citri* subsp. *citri*

*N. Wang*
*Citrus Research and Education Center, Department of Microbiology and Cell Science, University of Florida, Lake Alfred, FL, USA*
*Email: nianwang@ufl.edu*

The genus *Xanthomonas* is an important group of Gram-negative plant pathogenic bacteria, which infects approximately 124 monocotyledonous and 268 dicotyledonous plants. The genus, *Xanthomonas*, has become an important model organism for studying plant-microbe interaction and for understanding bacterial pathogenicity and virulence mechanisms. Among the diseases caused by members of the genus *Xanthomonas*, citrus canker is one of the most serious diseases of most commercial citrus cultivars resulting in significant losses worldwide. This devastating disease is caused by *Xanthomonas citri* subsp. *citri* (XAC). To investigate the virulence mechanism of this pathogen, we screened and characterized virulence deficient mutants of XAC. We will present our recent progress on some important virulence genes and effectors. To further understand the virulence mechanisms of XAC, we designed and conducted genome-wide microarray analyses to characterize important regulatory genes including HrpG and HrpX. The microarray results for HrpX were also confirmed using RNA-seq. Our results indicate that HrpG, together with HrpX, play global roles in coordinating different virulence traits including the novel virulence genes indentified in the mutagenesis analysis. Additionally, some of the virulence genes were also subjected to regulation of the diffusible signal molecules (DSF) as identified in the microarray analysis of the *rpfF*, *rpfC* and *rpfG* mutants compared to wild type.

### O43.003 A brief overview on bacterial panicle blight of rice and its causal agent *Burkholderia glumae*

*J.H. Ham, B.K. Shrestha, H.S. Karki and D.E. Groth*
*Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, Louisiana, USA, LA 70803-0106*
*Email: jham@agcenter.lsu.edu*

Bacterial panicle blight, also known as bacterial grain rot, is an emerging rice disease problem in the southeastern United States and Central and South American countries. *Burkholderia glumae* and *B. gladioli* are the causal agents for this disease but *B. glumae* tends to be prevalent and more virulent than *B. gladioli*. Due to the lack of an effective chemical control measure for this bacterial disease, development of disease-resistant varieties and alternative control measures is imperative. The medium-grain rice variety, Jupiter, and the long-grain line, LM-1 (a mutant line derived from the rice variety, Lemont), show high levels of partial resistance to bacterial panicle blight. Jupiter and LM-1 have been used for both genetic mapping of the partial disease resistance and breeding of new disease-resistant lines in our laboratory. Next-generation sequencing using an Illumina platform shows a great potential in genetic study of rice resistance to bacterial panicle blight. Whole genome sequence data of Jupiter and LM-1 along with those of disease-susceptible parents are being used for designing molecular markers and finding sequence variations associated with disease resistance phenotypes. Several recombinant inbred lines that show significantly higher levels of disease resistance than Jupiter and LM-1 have been obtained from our breeding program. In addition, we have isolated numerous strains of rice endophytic

bacteria and naturally non-pathogenic *B. glumae* that significantly suppress the development of bacterial panicle blight. These bacterial strains are excellent candidates of biological control agents for this disease.

**O43.004 Phytoplasmas effectors modulate plant host development and plant resistance to insects to aid phytoplasma dissemination in crop systems**

*S.A. Hogenhout*
*Department of Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom*
*Email: saskia.hogenhout@jic.ac.uk*

Sap-feeding aphids and leafhoppers cause feeding damage and transmit a number of plant disease agents, including viruses and bacterial pathogens such as phytoplasmas. Whilst phytoplasmas are most devastating for crop productions in tropical and sub-tropical regions of the world, their prevalence have increased in temperate climate regions causing significant yield losses in, for instance, grapevine, citrus, apple, pear, maize, wheat, oilseed rape and vegetable productions worldwide. The phytoplasma insect vectors (leafhoppers, planthoppers and psyllids) often migrate long distances spreading the phytoplasmas efficiently. As well, phytoplasmas produce virulence proteins (effectors) some of which promote insect vector colonization thereby increasing the likelihood of phytoplasma transmission to other plants. Effector protein SAP11 from AY-WB phytoplasma destabilizes TCP plant transcription factors resulting in increased stem production and altered leaf development and reduced jasmonate (JA) synthesis, and AY-WB phytoplasma effector protein SAP54 destabilizes MADS-box transcription factors leading to the conversion of flowers into leaves and delayed plant senescence. AY-WB leafhopper vectors feed and lay eggs on green plant tissues and are sensitive to JA. Both SAP11 and SAP54 promote leafhopper feeding and reproduction contributing to the 60% increase in the number of insect vectors observed on AY-WB-phytoplasma-infected plants. Thus, AY-WB phytoplasmas produce effectors, which control both plant and insect hosts on which phytoplasmas depend for survival and dispersal. Understanding how phytoplasma effectors modulate plant processes will contribute towards the development of sustainable benign methods for reducing outbreaks of phytoplasmas and their insect vectors.

**O43.005 Witches' broom disease of acid lime: a devastating disease in the Middle East**

*A.M. Al-Sadi[1], A.G. Al-Ghaithi[1] and S.A. Hogenhout[2]*
*[1]Department of Crop Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, PO Box 34, Al Khoud 123, Oman; [2]John Innes Centre, Cell and Developmental Biology, Norwich, UK*
*Email: alsadi@squ.edu.om*

Acid lime (*Citrus aurantifolia*) is among the four most important fruit trees in Oman in terms of production and area of cultivation. In the 1970s, disease symptoms were observed on lime trees in the northern part of the country. The symptoms were characterized by clustering of leaves, which become small in size and light green to yellow in color. Affected trees are usually killed within 5 years of appearance of symptoms. The disease, which was called witches' broom disease of lime (WBDL), is caused by *Candidatus* Phytoplasma aurantifolia. It spread to other areas in Oman and was reported in the UAE in 1989 and after that in Iran and Saudi Arabia. Loss of area cultivated with lime trees in Oman is currently 50% of that in 1990 and the disease wiped out over half a million lime trees. Population genetic analysis of acid limes in Oman provided evidence that the low level of genetic diversity of acid lime and frequent movement of acid lime planting material across districts are two main factors which contributed to the spread and high susceptibility of acid limes to WBDL. Empirical data provided evidence for a synergistic effect between phytoplasma and other pathogens and diseases (e.g. CTV, HLB) in the decline of acid limes, a hypothesis which is currently under investigation. Other areas of research on WBDL include genome sequencing of the causal agent, plant-pathogen interactions, effect of the disease on biochemical aspects of acid lime trees, disease resistance and investigating management options.

**O43.006 New regional rice virus disease in China**

*G.H. Zhou, D.L. Xu, D.G. Xu and M.X. Zhang*
*College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, P. R. China*
*Email: ghzhou@scau.edu.cn*

Southern rice black-streaked dwarf virus (SRBSDV) is a novel species in genus *Fijivirus*, family *Reoviridae* recognized in 2008. Rice plants infected with this virus exhibiting symptoms similar to those caused by *Rice black-streaked dwarf virus* (RBSDV) was firstly found in 2001 in southern region of China. Since 2009, the virus spread rapidly throughout Southeast Asia and damaged rice production in southern China and northern Vietnam. The sequence of the viral genome composed of ten dsRNA segments show highly similar to *Maize rough dwarf virus* and RBSDV but the nucleotide identities are less than 80%. The virus can be transmitted by white-backed planthopper (WBPH, *Sogatella furcifera*) in a persistent manner. In addition to rice, the virus can infect a group of Poaceae species including maize, Chinese sorghum (*Coix lacryma-jobi*), *Echinochloa crusgalli* and *Pennisetum flaccidum*. In Southeast Asia, WBPH is a typical large-scale migratory rice pest. The disease cycle might be described as below: the virus and

its vector, WBPH, overwinter in the warm tropical or sub-tropical area; the viruliferous WBPH adults carry the virus from south to north while they migration during early spring, transmit the virus to infect rice seedlings and lay eggs on the infected seedlings in immigration area; the next generation WBPHs developed on the infected seedlings become viruliferous; as a result of the WBPH dispersal, severe disease outbreaks. Control measures based on WBPH protection including seedbed coverage, seed chemical treatment and seedling chemical spray were practiced and successful in China.

**P43.001 Biofertilizer application for Huanglongbing management**

*I.A. Nugraheni, T. Joko and S. Subandiyah*
*Dept. of Plant Protection Faculty of Agriculture, Universitas Gadjah Mada, Bulaksumur Flora St, Yogyakarta 55281, Indonesia*
*Email: ssubandiyah@yahoo.com*

Huanglongbing (HLB) caused by *Candidatus* Liberibacter asiaticus (Las) remain as the most important disease of citrus in Indonesia and other countries. The disease symptom including chlorosis and dying in few years. The roots of infected plants poorely develop and loosing the hairy roots. This reseacrh was conducted in the HLB endemic area in Purworejo, Central Java to study the respons of HLB susceptible citrus plant against the disease by biofertilizer treatments. The trial was using graft inoculated 20 plant replicates of 1 year seedlings treated 4 times with biofertilizer A (manufactured) or biofertilizer B (farmer's formulation). PCR for Las detection was conducted every 2 months and the observation was conducted for 6 months. The results show that uninoculated without biofertilizer application got 9.09% HLB PCR positive through naturally infection; inoculated without biofertlizer treatment got 45.45% HLB positive; uninoculated with Bioferetilizer A application got 9.90% HLB positive; inoculated with Biofertilizer A application got 18.18% HLB positive; uninoculated with Biofertilizer B application got 0% HLB positive; and inoculated with Biofertilizer B application got 9.09% HLB positive. Furthermore, the plants applied with biofertilizers were having better root development. It is suggested that biofertilizer(s) applications used in this experiments promote the plant growth and suppress HLB infection.

**P43.002 Disease mapping of lentil wilt pathogen in Pakistan**

*C.A. Rauf, K. Rafiq, F. Naz and S.S. Naqvi*
*Department of Plant Pathology, PMAS, Arid Agriculture University Rawalpindi, 46300, Pakistan*
*Email: abdul.rauf@uaar.edu.pk*

To investigate the incidence and distribution of *Fusarium* wilt in all major lentil growing districts of Punjab (Pakistan) the survey of Sialkot, Narowal, Gujrat, Jhelum, Attock, Bhakar, Layyah and Chakwal Districts was conducted. Survey was done at seedling and mature stage and disease incidence was calculated with the help of disease incidence key. Disease is prevailing in all major lentil growing areas. On the whole, thirty percent wilt incidence was witnessed. Overall 12 percent mean plant mortality was notice at seedling stage and 18 percent at the crucial reproductive stage. The highest disease incidence was observed in district Layyah (80%), followed by Bhakkar (60%) while the lowest incidence was observed in districts Attock (10%) and Sialkot (10%). The rest of districts showed severe to mild incidence of disease such as Chakwal (35%), Narowal (25%), Gujrat (25%) and Jhelum (20%). Wilted samples were isolated on malt extract medium for associated wilt pathogens in the laboratory and were morphologically characterized employing colony color, growth habit, pigmentation, days to fill 9 cm dish, concentric rings, size of micro-conidia and macro-conidia, shape of micro-conidia and macro-conidia, phialide, shape of apical and basal cells of macro-conidia, septation in macro-conidia, diameter and position of chlamydospores and interseptal distance, on the basis of which 213 *Fusarium* isolate identified and confirmed as *F. oxysporum* showing variability in their characters. The development of disease maps will be helpful in assessing the true relative risk of this disease in Punjab province and in employing necessary management strategies against the disease.

## Concurrent Session 44-Tropical Plant Pathology

### O44.001 Biology and management of *Phytophthora* diseases in tropical horticulture

*D.I. Guest*
*Faculty of Agriculture and Environment, The University of Sydney, NSW 2015, Australia*
*Email: david.guest@sydney.edu.au*

*Phytophthora* is a water-loving soilborne pathogen that causesmost damage to aerial parts of tropical tree crops. Once established in the canopy, epidemics spread rapidly causing massive yield losses and tree deaths under favourable weather conditions. The key to effective management lies in understanding how the pathogen climbs into the canopy, and how it flourishes there if not strictly managed. In this presentation I will outline management options for *Phytophthora* diseases, including the selection and breeding of resistant genotypes, improved nursery hygiene, good drainage and soil management, strict sanitation, early diagnosis andtreatment of infections, and the selective use of pesticides. Extension of good agricultural practices to promote widespread adoption of management practices is essential to control *Phytophthora* epidemics. I will discusscase studies of management options for *Phytophthora* diseases of tropical tree crops that were tested and implemented through Participatory Action Research (PAR), involving farmers and stakeholders. Robust links were established between researchers, extension agents, service providers and farmers, improving the relevance and practicality of research directions.

### O44.002 Diagnosis of *Phytophthora* diseases in the Tropics

*A. Drenth*
*Centre for Plant Science, the University of Queensland, Australia*
*Email: a.drenth@uq.edu.au*

Although many plant pathologists are familiar with a wide range of diseases caused by various species of *Phytophthora* there are quite a number of plant diseases for which we do not know the cause. For example, bud rot has caused major problems in the oil palm industry in the Americas for more than half a century. Until recently bud rot was blamed on a range of biotic and abiotic factors, however none of these culprits was unequivocally demonstrated to be the cause of bud rot. Over the past few years a detailed study by researchers in Colombia demonstrated that *Phytophthora palmivora* was the cause of bud rot. Although a lot of research was directed towards identifying the cause of bud rot the final determination eluded researchers for a rather long time. This is quite unusual as for many novel plant diseases a well focussed research effort involving careful observation of disease symptoms, isolation of the pathogen, infection of healthy plant material and recreation of the disease symptoms can be conducted in a relative short period of time to fulfil Koch Postulates and conclusively determine the cause of disease. Using bud rot in oil palm as case study an analysis of why it took so long to unequivocally establish the causal agent of bud rot is of interest and lessons learnt from this maybe applicable to many other complex and unresolved plant diseases in the tropics as well as elsewhere.

### O44.003 Citrus Huanglongbing in Guangdong, China -- A case analysis

*J. Chen*[1], *X. Deng*[2] *and H. Li*[2]
[1]*Agricultural Research Services, United States Department of Agriculture, Parlier, Ca 93648 U.S.A.;* [2]*Laboratory of Citrus Huanglongbing Research, Department of Plant Pathology, South China Agricultural University, Guangzhou, P. R. China*
*Email: jianchi.chen@ars.usda.gov*

Citrus Huanglongbing (HLB, yellow shoot disease) was observed in the coastal Chaoshan Plain of Guangdong Province, China, in the late 19th century. "*Candidatus*Liberibacterasiaticus" has been considered as a putative pathogen associated with HLB since 1994. Results of a recent survey along with the traditional records showed that HLB was widespread in the province. Interestingly, despite the lack of intentional control of HLB, citrus production in Guangdong remained vigorous in the past 10 years. This could be related to: 1) most citrus groves in the north and west regions are new, suggesting no or low outside sources of initial "*Ca*. L. asiaticus". inoculum; 2) citrus groves are planted primarily on hill slopes with complex geographical topography that may affect insect vector activity; 3) cultivars such as Shatangju were locally propagated and free of HLB, at least initially; and 4) market price for Shatangju has been high, encouraging farmers to optimal orchard management with timely inset controls, flush control and quick removal of unproductive/diseased trees. While all these efforts are suppressive to HLB outbreaks, the high market demands also resulted in a surge of citrus planting. Regulation of production and movement of nursery stocks or propagation materials become difficult. Nursery stocks could be infected with HLB pathogen by citrus psyllids. Even worst, affected trees could be used as mother trees for nursery stocks. In conclusion, HLB control requires both biological knowledge and social efforts. Management of nursery stocks plays a critical role.

### O44.004 Mealybug wilt of pineapple and associated viruses

*J.S. Hu, D.M. Sether, M.J. Melzer, K. Dey, C.V. Subere, K. Cheah, E. Perez and W.B. Borth*
*Department of Plant and Environmental Protection*

*Sciences, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, 3190 Maile Way, Honolulu HI, 96822, USA*
*Email: johnhu@hawaii.edu*

Mealybug wilt of pineapple (MWP) is a devastating disease of pineapple, *Ananas comosus* (L.) Merr., worldwide. The disease is characterized by severe leaf-tip dieback, downward curling of the leaf margins, and loss of leaf turgidity, that can lead to total collapse of the plant. *Pineapple mealybug wilt associated virus-1* (PMWaV-1), PMWaV-2, and PMWaV-3 have been identified in field-grown pineapple throughout Hawaii and are transmitted by the pink and grey pineapple mealybugs, *Dymicoccus brevipes* and *D. neobrevipes*, respectively. Vector transmission characteristics of PMWaV-2, including acquisition access period (AAP), and persistence and retention of the virus in grey pineapple mealybug vectors were evaluated. PMWaV-2 is transmitted by the grey pineapple mealybug in a semi-persistent manner. In Hawaii, PMWaV-2 infection and simultaneous feeding by mealybugs are both involved in the induction and etiology of MWP, whereas infections by PMWaV-1 and -3 do not appear to be necessary for wilt induction. Genomic analyses reveal that PMWaV-1 and PMWaV-3 lack elements that are present in PMVaV-2 including the intergenic region between the RdRp open reading frame (ORF) and the small hydrophobic protein ORF, lack a conserved motif in ORF4, encode a relatively small coat protein, and lack an diverged coat protein (CPd). These characteristics distinguish them from PMWaV-2 and the ampelovirus type member, *Grapevine leafroll associated virus-3* (GLRaV-3). In addition, a badnavirus, designated *Pineapple bacilliform CO virus*-HI1 (PBCOV-HI1), was isolated and sequenced from pineapple in Hawaii. Approaches using non-transgenic and transgenic methods to control *Pineapple mealybug wilt-associated viruses* and badnaviruses were evaluated.

### O44.005 Incidence, severity and symptom development of vascular-streak dieback on local cocoa clones in Sulawesi

A.K. Parawansa[1], A. Purwantara[2], P.J. McMahon[3] and P.J. Keane[3]
[1]*Muslim University of Indonesia (UMI), Jl. UripSumohardjo, Makassar, South Sulawesi, Indonesia;* [2]*Biotechnology Research Institute for Estate Crops, Jl. Taman Kencana 1, Bogor 16151, Indonesia;* [3]*Department of Botany, La Trobe University, Bundoora, VIC 3086, Australia*
*Email: ayuparawansa@yahoo.com*

The incidence and severityof vascular-streak dieback (VSD) of cocoa caused by *Ceratobasidium theobromae* (syn.*Oncobasidium theobromae*) was determined in a range of cocoa clones at two sites in Sulawesi in Pinrang and Polman Districts. In both study sites, all clones were attacked by VSD, with incidence ranging from 39.9% to 94.2%. However, some clones (PBC123, M05, Gene-J) were more resistant to VSD at both sites, sustaining 37-48% infection of branches, while Husbitori was highly susceptible sustaining over 80% infection. Achange in symptoms of VSD has been noted since 2004. The more recent symptoms indicate a greater degree of necrosis of the leaf lamina and vascular tissue compared to the symptoms originally associated with the disease. All clones in the study sustained infections that showed a mix of original and recent symptoms. In most clones the recent symptoms were predominant, but a significantly higher number of original symptoms occurred in BR25. No relation between resistance and the type of symptom was detected in the study. Observations of hyphae in infected twigs and sporocarps on leaf laminae and leaf scars showed that the fungus associated with the new symptoms was identical in all aspects to *C. theobromae*. Isolation of the fungus from infected xylem confirmed that the fungus that first emerges from the xylem is a slow-growing species that cannot be easily subcultured. Further investigations of pathogen populations are underway. This study confirms that VSD is likely to be caused primarily by *C. theobromae*, as originally described for the disease. It is possible that the new symptoms of VSDare caused by changes that affect the host response to the fungus. These could include changes in climate or soil fertility.

### O44.006 Evaluation control of *Meloidogyne* spp. and radopholus similis with local material in Indonesia

Mulawarman
*Plant Pest and Diseases Department, Agriculture Faculty, University of Sriwijaya, Jl. Raya Palembang-unsri KM 32 Inderalaya, Ogan Ilir, Sumatera Selatan, Indonesia*
*Email: Mulaunsri@gmail.com*

Over 80 *Meloidogyne* species have been described so far and to attack over 1000 host plants and survive under a wide range of soil conditions. Root knot nematodes cause economic damage by reducing crop yield and quality. On a worldwide basis, crop loss due to *Meloidogyne* infestation is estimated at 13 %. The Radopholus simlis reported cause big problem especially on banana crops. In Indonesia we evaluate control using bioagents such bacterial and fungus. It showed the biological agent such bacteria and fungus could bring hope to control the diseases. The bio agent could suppress the nematode over 10 percentage. To develop the bio control face with the consistency, formulation and transportation especially the case in Indonesia with lower organic coil contains less than 1 percentage. And the farmer had almost no education with small farm area without good farm practical action.

**O44.007 Competition between individuals induced aggressiveness of *Rigidoporus microporus***

*S. Suwandi*
*Laboratory of Plant Pathology, Faculty of Agriculture, Sriwijaya University, Jl. Palembang-Prabumulih Km.32, Indralaya, Palembang 30662, Indonesia*
*Email: suwandi.saleh@gmail.com*

Local population of *R. microporus*, a white root rot pathogen of *Hevea* rubber tree consists of numerous genetic individual (genet) as revealed by mycelial compatibility. To test the effect of individual competition on pathogenic fitness, coinoculations of rubber seedling with isolates of different mycelial compatibility were conducted in two pot experiments. Single-strain inoculation was used as positive control. Disease severity, taproot colonization and taproot necrosis were significantly increased in coinoculations compared to single-strain inoculations. Coinoculations also caused significant reduction in plant height. These results suggested that within-host competition can favor higher aggressiveness of *R. microporus*.

**O44.008 Histological and transcriptome investigation of the interaction between the Cavendish banana roots and *Fusarium oxysporum* f. sp. *cubense* tropical race 4**

*C.Y. Li, G.M. Deng, J. Yang, R.B. Kuang, Q.S. Yang, C.H. Hu, O. Sheng, Y.R. Wei and G.J. Yi*
*Institution of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, Guangdong Province, P. R. China, Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture, P. R. China*
*Email: yiganjun@vip.163.com*

*Fusarium oxysporum* f. sp. *cubense* (Foc) causes Fusarium wilt, a lethal disease that results in devastating economic losses to banana production worldwide. Among 24 vegetative compatibility groups (VCGs), the most damage is caused by Foc tropical race (TR) 4. 101 Foc isolates from six production areas were collected and VCG identity was determined, and the result showed that the majority of them were Foc TR 4 (VCG 01213/16). Using a high virulent isolate, we developed a green fluorescent protein (GFP)-tagged transformant and investigated its pathogenesis. Our results showed that: (i) Foc TR 4 was capable of invading the epidermal cells of banana roots directly; (ii) potential invasion sites include epidermal cells of root caps and elongation zone, and natural wounds in the lateral root base; (iii) in banana roots, fungal hyphae were able to penetrate cell walls directly to grow inside and outside cells; and (iv) fungal spores were produced in the root system and rhizome. In order to further understand the interaction the Cavendish banana and Foc TR 4, we investigated the transcriptome profiles in the roots of resistant and susceptible Cavendish banana challenged with Foc TR4. Digital gene expression (DGE) analysis revealed large differences in the transcriptome profiles between the Foc TR4 resistant mutant and the wild-type. Our results indicated that the basal defense mechanisms involved the recognition of PAMPs and the high levels of defense-related transcripts may contribute to Foc TR4 resistance in banana.

**P44.001 Molecular analysis on soil fungal community structures and diversity in rhizosphere of fruit tree root rot caused by fungi**

*J.H. Joa, S.C. Kim, C.K. Lim and K.S. Choi*
*National Institute of Horticultural & Herbal Science, RDA, Jeju, 690-150, Republic of Korea*
*Email: choa0313@korea.kr*

Soil-borne disease caused by soil physical and chemical environment changes. Recently, gold kiwi (*Actinidia cinensis*) root rot has increased quickly in summer as effected by precipitation fluctuation according to the climate change in Korea. And also Mangotree died by root rot symptoms in the P.E. house. This study carried out to evaluate the fungal group distribution, species richness estimators, diversity indices, and community structures in rhizosphere soil occurred root rot disease of gold kiwi and mango. Soil samples were taken from rhizosphere kiwi (5 sites) and mango(1 site)shown root rot symptoms in 2011. Pyrosequencing analysis performed using 454 genome sequencer with PCR amplicon after DNA extraction from wet soil. In gold kiwi, rarefaction curves at the 97% sequence similarity showed that distribution range of a clone and the OTUs were at 202-488 and 2,442-8,672. Species richness estimators of Ace, Chao1, and Shannon diversity indices showed higher in volcanic ash closely soil, which were 640.5, 652.6, 5.1, respectively. In mango, rarefaction curves at the 97% sequence similarity showed that distribution of a clone and the OTUs were at 4,680 and 302, respectively. Species richness estimators of Ace, Chao1, and Shannon diversity indices showed 376.2, 379.1, 4.4, respectively. In phylum level, the most abundant fungal group were *Ascomycota* and *Basidiomycota* among fungal community in kiwi and mango rhizosphere soil. And also the most dominant fungal group was *Sordariomycetes and Ascomycota* in genera level. Microbial diversity and community structures showed differently according to cultivating fruit crop.

**P44.002 Distribution of seed borne fungi on rice seeds during seed processing and effect on seed germination**

*S. Sangchote[1,2], S. Laksanaphisut[1,2] and W. Satthai[1]*
*[1]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand; [2]Postharvest Technology Innovation Center (PHTIC) and Office of*

*the Higher Education Commission,Bangkok, Thailand*
*Email: sasivimol_016@hotmail.com*

Investigation of seed-borne pathogens on rice seed after harvest and passed different steps of seed processing to improve the quality including pre-processing, post-processing (cleaning and sizing), and seed treatment from Chiang Mai, Phitsanulok, NakhonSawan, Nakhon-Ratchasima and Chainat Rice Seed Centers were investigated for seed borne pathogens and seedling disease. It showed that in each step of seed processing before seed treatment, *Bipolaris oryzae, Curvularia lunata* and *Alternaria padwickii* was increased and diseased seedling were also increased as compared with pre-processing seeds. However, level of an infection was reduced after seed treatment but not completely control and diseased seedling still occurred. *Bipolaris oryzae* could transmit through seed and caused brown spot to the seedling The relationship of the total and internal seed infection and diseased seedling was shown by linear regression with $r^2$ =85.8% and 86.8% respectively.

**P44.003 Occurrence of gray mold caused by *Botrytis cinerea* on common fig in Korea**

*S.S. Cheong[1], I.Y. Choi[1] and W.H. Lee[2]*
*[1]Jeollabuk-do Agricultural Research and Extension Services, Iksan 570-704, Republic of Korea; [2]Department of Agricultural Biology, Chonbuk National University, Jeonju 561-765, Republic of Korea (Plant Medical Research Center, Industry, Academy, Research, Cooperation Company for Herb, Jeollabuk-do)*
*Email: choiiy21@korea.kr*

In 2010 and 2011, gray mold was found on common fig (*Ficuscarica*) fruit grown at the research field of Jeollabuk-do Agricultural Research and Extension Services, Korea. Gray mold symptoms on common fig fruit mainly occurred after harvest season until December. The typical symptom included brown water-soaked rot and fruit decay. The diseased fruit was covered by gray to brown colored conidiophore and conidia. The conidiophores were tree shape and measured 15-33×2 ㎛. Conidia on conidiophore were ellipsoidal or lemon shape, colorless, single cell, and measured 7.3-14.6× 6.8-11.1 ㎛. The nucleotide sequences of the rDNA ITS region obtained from the pure culture of the gray mold on common fig were 100% similar to the sequences of the GenBank accession number HQ171052, EU519210, HQ171053, FN812726, HM849615, and EU563120 of *B. cinerea* isolates. In phylogenetic tree, the representative isolate was placed within same clade of *B. cinerea*. Based on the morphological characteristics and analysis of rDNA ITS sequence data, the fungus was identified as *B. cinerea*.

**P44.004 Identification and genetic diversity of *Rosellinia* spp. associated with root rot of coffee in Colombia**

*B.L. Castro[1], A.J. Carreño[2], N.F. Galeano[2], J. Rou[1], M.J. Wingfield[1] and A.L. Gaitán[2]*
*[1]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Hatfield, Pretoria 0028, South Africa; [2]National Coffee Research Center (CENICAFE), Manizales, Colombia*
*Email: Berthal.castro@cafedecolombia.com*

The genus *Rosellinia* includes species that cause root rot on a wide range of herbaceous and woody hosts. In Colombia, these fungi cause serious diseases of potato, forest and fruit trees, as well as coffee plants. The aim of this study was to identify isolates of *Rosellinia* collected from coffee and other hosts using DNA sequence comparisons of the internal transcribed spacer (ITS) region. Pathogenicity tests were conducted on coffee seedlings to confirm the role of the collected species in coffee root disease. Twenty six isolates were obtained and these were grouped into two clades representing *R. bunodes* and *R. pepo*. Isolates from *Coffea arabica*, *Hevea brasiliensis*, *Macadamia integrifolia*, *Psidium guajava* and *Theobroma cacao* were identified as *R. pepo*, while *R. bunodes* was obtained only from coffee plants. Low levels of genetic variability were observed among isolates of the two species. Pathogenicity tests on coffee with *R. bunodes* resulted in 98% seedling death in an average of 10 days, while *R. pepo* killed 54% of inoculated seedlings in an average of 16 days confirming the compatibility of both species with this host. Pathogen characterization will be useful for further research in disease diagnosis, soil recovery and breeding for resistance.

## Concurrent Session 45-Vascular Plant Diseases

### O45.001 The global impact of fungal vascular wilt diseases

*R.D. Martyn*
*Department of Botany & Plant Pathology, Purdue University, West Lafayette, IN 47907 USA*
*Email: Rmartyn@purdue.edu*

Fungal vascular wilts are among the most destructive plant diseases known and include such infamous examples as the Fusarium and Verticillium wilts, Dutch elm disease, oak wilt, and many others. Those caused by formae speciales of *F. oxysporum* are among the most destructive plant diseases known. Fusarium wilt of cotton was the first fungal vascular wilt disease described (1892) followed by Fusarium wilt of watermelon (1894). It is difficult to determine the exact origin and means of spread for many vascular wilts, but their rapid emergence in the early 1900s likely is due in part to the narrowing of the genetic base of crops through selective breeding and intensive monoculturing. Many also may be seedborne. For some, such as oak wilt, distribution is limited, while others such as Verticillium wilt have a global distribution. A feature common to all fungal vascular wilts is that they penetrate their host directly and enter the xylem where they remain until the disease is well advanced, a characteristic that has been studied extensively. It is impossible to assess the worldwide economic losses due to vascular wilts, but considering they occur on many of our food, feed, fiber and timber crops, the amount would be staggering. Breeding for genetic resistance in our crop plants has been the most successful means of control, although the appearances of new virulent races possess continual problems. Soil fumigation, cultural methods and fungicide treatments also have some success in controlling fungal vascular wilts.

### O45.002 Tomato immune receptor Ve1 recognizes effector of multiple fungal wilt pathogens

*B.P.H.J. Thomma*
*Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*
*Email: bart.thomma@wur.nl*

Cell surface receptors, generally referred to as pattern recognition receptors (PRR), detect conserved microbial molecules, generally referred to as microbe-associated molecular patterns (MAMPs), to activate MAMP-triggered immunity (MTI). Successful plant pathogens overcome MTI by the use of secreted effectors which perturb host immunity in a pro-active manner. To overcome effector-triggered susceptibility, plants in turn evolved immune receptors that monitor the presence or activity of particular effectors to re-install immunity. The tomato immune receptor Ve1 governs resistance to race 1 strains of the soil-borne vascular wilt fungus *Verticillium dahliae*, while race 2 strains are not recognized. By high-throughput population genome sequencing, the gene that encodes the Ave1 effector (for Avirulence on Ve1 tomato) was identified. Interestingly, Ave1 homologs were also found in the fungal pathogens *Cercospora beticola*, *Colletotrichum higginsianum* and *Fusarium oxysporum* f. sp. *lycopersici*. Some of these homologs are recognized by tomato Ve1. Based on the differential recognition of the Ave1 homologs, the epitope of the Ave1 protein has been identified.

### O45.003 The threat of *Ceratocystis fagacearum*, the oak wilt pathogen, as a global invasive species

*D.N. Appel*
*Dept. of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, USA*
*Email: appel@tamu.edu*

Oak wilt, caused by the fungus *Ceratocystis fagacearum*, is one of the most lethal diseases of oaks (*Quercus* spp.) in the U.S.A. The disease was first described in Wisconsin in 1941 and thirty years later was discovered killing trees in 21 other states, for the most part in the mid-western, north central and mid-Atlantic regions of the country. Since then, the range of the pathogen has remained largely static. There are still oak-dominated forest ecosystems in North America where oak wilt does not occur, and the pathogen has not been found outside of the U.S. One of the reasons given for the relatively limited range of this virulent pathogen is the relatively inefficient insect vector for the pathogen. Lessons have been learned from our experiences with oak wilt regarding the potential damage *C. fagacearum* can cause if introduced into new, susceptible plant communities dominated by oaks. An analysis of the oak wilt epidemic in Texas, for example, illustrates the unique characters of the pathogen that make it such a threat. The unexpected vulnerabilities of the Texas oak savannahs to oak wilt are an example of pathogen resilience by overcoming presumed weaknesses to thrive in a hostile environment and cause enormous losses of trees. Therefore, *C. fagacearum* should be considered a dangerous, potentially invasive species throughout the global range of the oaks.

### O45.004 Fusarium wilt of banana: from landscapes to genes

*M.A. Dita, C. Waalwijk, R. Herai, M. Yamagishi, P. Giachetto, M.T. Souza Jr, R. Ploetz, L.J Ma and G.H.J. Kema*
*Bioversity International, Turrialba, 30501, Cartago, Costa Rica*
*Email: m.dita@cgiar.org*

No other *forma specialis* of the *Fusarium oxysporum* species complex has caused more social and economic impact than *Fusarium oxysporum* f. sp. *cubense* (Foc), the causal agent of Fusarium wilt of banana (also known as Panama disease). The disease wiped out the Gros Michel-based banana industry in the past century causing losses of billions of dollars, unemployment and political instability in several Central America countries. The industry solved this problem by globally introducing resistant varieties of the Cavendish subgroup by 1960. They remain resistant in tropical America and Africa, but began to succumb in the 1990s in South-East Asia to a new Foc genotype called tropical race 4 (TR4). Foc TR4 has already destroyed tens of thousands of hectares of Cavendish bananas, as it spreads, threatens the monoculture export banana industry. Despite the considerable advances over the last years, more research is required to better understand genetic diversity, pathogenicity and virulence in Foc, as well as factors driving host resistance and disease's epidemiology. Here, we provide a detailed overview of the global occurrence of Fusarium wilt in banana with an updated map of incidence and a catalogue of susceptible and resistant cultivars, highlighting specific examples from Asia, Africa and Latin America. Recent genomic and transcriptomic results from the host the pathogen and it interaction will be presented. Future directions towards sustainable disease management will also be discussed.

### O45.005 Genetic mechanisms of resistance to *Fusarium* wilt in watermelon

*Y. Xu, Y. Zhang, J. Zhang, X. Zhou, L. Geng, H. Zhang and S. Guo*
*National Engineering Research Center for Vegetables, Beijing Academy of Agriculture and Forestry Sciences, Key Laboratory of Biology and Genetic Improvement of Horticultural Crops (North China), Beijing 100097, P. R. China*
*Email: xuyong@nercv.org*

Four races of *F. oxysporum* f. sp. *niveurm* have been identified and named as 0, 1, 2, 3. Until now the genetic mechanism of resistance to *Fusarium* wilt in watermelon is still not very clear. In order to elucidate the genetic mechanism of resistance to race 1 of *F. oxysporum* f. sp. *niveurm*, an $F_2$ segregating population derived from a cross between the resistant cultivar 'Calhoun Gray' and the susceptible cultivar 'Black Diamond' was used to investigate. Based on the information of sequenced genome of watermelon, we developed three CAPS/dCAPS markers closely linked to *Fon-1*. The most tightly genetic distance between the marker loci and the *Fon-1* loci was estimated to be 0.8cM, and coincidence rate reached 98.7% was verified by testing 164 different cultivars. To clarify the genetic mechanism of resistance to race 2, a six generation populations of $P_1$, $P_2$, $F_1$, $B_1$, $B_2$, $F_2$ and the RILs($F_2S_8$) population were gotten from PI296341-FR (resistant to race 2) and 97103 (susceptible to race 2). Using the mixed major gene plus polygene inheritance model, the resistance to race 2 was showed to fit one pairs of additive-major genes plus additive-dominant polygene model (D-2 model). In $B_1$, $B_2$ and $F_2$ population, the heritability of major genes with recessive effect were estimated to be 31.8%, 23.4% and 48.6% respectively. Meanwile, three linkage major genes with equal additive effect (F-3) were found to govern the inheritance of race 2 of *Fusarium oxysporum* in the RILs($F_2S_8$) population. This result indicated that the resistance of *Fusarium oxysporum* race2 in PI296341-FR was controlled by major and minor genes, and the additive and dominant effects of minor genes were higher than major genes.

### O45.006 Hog1-MAPK gene *VdHOG1* regulates microsclerotial formation and stress response in smoke-tree vascular wilt fungus *Verticillium dahliae*

*S.X. Xiao, C.M. Tian and Y.L. Wang*
*The Key Laboratory for Silviculture and Conservation of Ministry of Education, College of Forestry, Beijing Forestry University, Beijing 100083, P. R. China*
*Email: ylwang@bjfu.edu.cn*

Smoke-tree wilt, caused by a soilborne fungus *Verticillium dahliae*, is a destructive disease on smoke-tree *Cotinus coggygria* in Beijing region. Mitogen-activated protein kinase (MAPK) signalling pathway plays an essential role in regulating growth, development, pathogenesis and stress response, such as salt, osmotic and hydrogen peroxide in phytopathogenic fungi. HOG1-MAPK pathway in fungi has led to different cellular reactions. However, the function of HOG1-MAPK is less characterized in *V. dahliae*. Thus, we first identified *VdHOG1* and analyzed the transcriptional expression in vegetative growth and microsclerotial formation. Real time qPCR analysis showed that the expression of *VdHOG1* was up-regulated in conidia stage. To elucidate the function, *VdHOG1* was deleted by homologous recombination. We find that the lack of *VdHOG1* inhibits the microslerotial formation apparently, the *ΔVdHOG1* mutant strain forms microslerotia later than the wild type and produces less microslerotia, while it is normal in conidia stage. In detail, the wild-type strain forms microslerotia in 4 to 5 days, however the *ΔVdHOG1* mutant in 9 to 10 days, and it couldn't be seen any microslerotia on PDA in 10 days. Moreover, it is indeed stressed by NaCl and Sorbitol, which is proportional to their concentration, respectively. Interestingly, the *ΔVdHOG1* mutant strain grows faster than the wild type on CM lack of carbon element. In summary, the results demonstrate that *VdHOG1* plays a significant role in microslerotial formation and stress response.

**O45.007 The gene *FoOCH1* encoding a putative α-1, 6-mannosyltransferase in *Fusarium oxysporum* f.sp. *cubense* is required for virulence on banana plants**

*M.H. Li[1], X.L. Xie[1], X.F. Lin[1], J.X. Shi[1], Z.J. Ding[1], J.F. Ling[1,3], P.G. Xi[1],J.N. Zhou[1], S. Zhong[2] and Z.D. Jiang[1]*
*[1]Department of Plant Pathology, South China Agricultural University, Guangzhou, 510642, P. R. China; [2]Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA; [3]Institute of Plant Protection, Guangdong Academy of Agricultural Sciences, Guangzhou, 510640, P. R. China*
*Email: liminhui@scau.edu.cn*

*Fusarium oxysporum* f. sp. *cubense*, the causal agent of banana *Fusarium* wilt, is one of the most destructive pathogens threatening the banana production. However, the molecular mechanisms underlying the virulence and pathogenicity of this fungal pathogen are still poorly understood. In this study, we identified and characterized the disrupted gene in a T-DNA insertional mutant L953 of FOC with significantly reduced virulence on banana plants. The gene disrupted by T-DNA insertion in L953 harbors an open reading frame encoding a protein with homology to α-1,6-mannosyltransferase (OCH1) in fungi. The deletion mutant (Δ*FoOCH1*) of the OCH1 orthologue (*FoOCH1*) and L953 were impaired in fungal growth, exhibited a higher sensitivity to the cell wall perturbing agents, Calcofluor White and Congo Red, produced less cell wall proteins and released more secreted proteins into liquid media than the wild type. Furthermore, the mutation or deletion of *FoOCH1* led to decline in virulence to the banana host. The mutant phenotypes were fully restored by complementation with the wild-type *FoOCH1*. Our data provide a first evidence for the critical role of *FoOCH1* in maintenance of the cell wall integrity and the virulence of *F. oxysporum* f. sp. *cubense.*

**O45.008 Vascular occlusions and resistance/susceptibility mechanism of vascular diseases**

*Q. Sun[1], Y. Sun[2] and J.M. Labavitch[3]*
*[1]Department of Biology, University of Wisconsin-Stevens Point, WI 54481; [2]Department of Biochemistry, University of Wisconsin-Madison, WI 53715; [3]Department of Plant Sciences, University of California-Davis, CA 95616, USA*
*Email: qsun@uwsp.edu*

Vascular occlusions are common structural modifications made by many plant species in response to pathogen infection. However, what roles the occlusions may play in host plants' disease resistance/susceptibility still remains controversial. The report focused on our recent results on grapevine Pierce's disease (PD), a vascular disease caused by the xylem-limited bacterium *Xylella fastidiosa* that can ultimately lead to death of infected vines. With grapevines with different PD resistances, we studied the types, distributions and quantitative characteristics of vascular occlusions in *X. fastidiosa*-infected vines and the occlusions' impact on the water conduction and the pathogen's systemic spread in the vines. Our data have indicated that tyloses were the predominant type of occlusion that forms in the vines of different PD resistances. Tyloses formed throughout PD-susceptible vines with over 60% of the vessels in any examined stem transverse section becoming fully blocked, while tylose development was mainly restricted to a few internodes close to the point of inoculation in PD-resistant vines, impacting only 20% or less of the vessels. The extensive vessel blockage in PD-susceptible vines was correlated to a greater than 90% decrease in stem hydraulic conductivity, compared with an approximately 30% reduction in the stems of PD-resistant vines. The systemic spread of *X. fastidiosa* was detected in PD-susceptible vines, but the pathogen colonized only 15% or less of the vessels in any internode and occurred in relatively small numbers that were far to be enough to directly block the vessels. Therefore, our findings demonstrate that the extensive development of vascular occlusions in PD-susceptible vines does not prevent the pathogen's systemic spread in the vines, but may significantly suppress the vines' water conduction, contributing to PD symptom development and the vines' eventual death.

**P45.001 Pathogenic variation among *Fusarium* isolates associated with wilt of chilli (*Capsicum annumm* L.)**

*M.J. Patil, B.T. Raut, S.M. Ghawade, Ekta Bagde and P.P. Gawande*
*Chilli and Vegetable Research Unit, Dr. Panjabrao Deshmukh Krishi Vidhapeeth, Akola, 444104 Maharashtra, India*
*Email: mohanpatil.1082@rediffmail.com*

Variability among the fifty two isolates collected from different regions of vidarbha of Maharashtra, India was studied in respect of cultural, morphological characteristic and pathogenic ability. Mycelial growth of isolate varied from isolate to isolate. Maximum growth (84.44 mm) was observed in FS-11 followed by FS-9 and FS-13 recording 87.50 and 87.33 mm, respectively. These cultures were obtained from Amravati District. The lowest growth was noticed in FS-1and FS-6 (41.66 mm) followed by FS-41 and FS-8 exhibiting 43.33 and 45.66 mm radial growth, respectively from Akola district and FS-41from Buldhana District. The pigmentation also reflects the variation among the isolates.Three milky white, eight dull white to whitish, four reddish brown, seven yellowish, three purple, eleven light red and ten brownish to dark brown. Out of fifty two isolates 7, 10, 14, 12 and 9 isolates showed non pathogenic, weakly pathogenic, moderately pathogenic, strongly pathogenic and highly pathogenic reactions in pot culture

study against X-235 variety of Chilli.

**P45.002 Xylem features and cell wall compositions affect Pierce's disease resistance of grapevines**

Q. Sun[1] and J.M. Labavitch[2]
[1]Department of Biology, University of Wisconsin-Stevens Point, Stevens Point, WI 54481, USA; [2]Department of Plant Sciences, University of California-Davis, Davis, CA95616, USA
Email: qsun@uwsp.edu

The introduction of *Xylella fastidiosa* to grapevine xylem tissue often results in Pierce's disease (PD) and, ultimately, vine death. The development and progression of PD symptoms depends largely on the ability of the pathogen to spread via the xylem's vessel system in the infected vine. In this report, we will introduce our results on some xylem features which may affect the initial entry of the pathogen to the vessel system. We believe that pit membranes (PMs—two primary cell walls and one middle lamella) separating neighboring vessels should be barriers that *X. fastidiosa* must digest to enhance its systemic spread in a host vine. Production of occlusions in the vessel system in response to the presence of *X. fastidiosa* may also be related to disease symptom development or the host vine's resistance. Laser confocal microscopy and electron microscopy were used to investigate these two host vine factors. Our data revealed that grapevine genotypes with different PD resistances were different in some cell wall polysaccharides of PMs, that the intervessel PMs in PD-susceptible vines were modified for the passage of the pathogen, and that the development of vascular occlusions (e.g., tyloses, gels and crystals) in response to the presence of *X. fastidiosa* should contribute to the PD symptom development of the host vine. These findings are essential to understanding possible roles of these xylem factors in grapevine's resistance/susceptibility to PD.

**P45.003 Strains of *Fusarium oxysporum* f.sp. *cubense* in Asia based on Vegetative Compatibility Groups (VCG) analyses**

A.B. Molina[1], A. Viljoen[2], C. Hermanto[3], W. O'Neil[4], D. Mostert[2], C. Chao[5], E. Fabregar[6], S. Hossain[7], B. Komolong[8], N. Masdek[9], Rajapakse[10], V. Sinohin[1], D.T. Thanh[11], B.R. Thangavelu[12], P. Visarto[13] and Y.Ganjun[14]
[1]Bioversity International- Asia Pacific, Commodities for Livelihoods (CfL) – Musa Programme, IRRI, Los Baños, Laguna, Philippines; [2]University of Stellenbosch, Matieland, Stellenbosch, South Africa; [3]Indonesian Tropical Fruits Research Institute (ITFRI), Solok, Indonesia; [4]Agri Science Queensland, DEEDI, Brisbane, Australia; [5]Taiwan Banana Research Institute, Pintung, Taiwan, China; [6]Lapanday Foods Corporation, Davao City, Philippines; [7]Bangladesh Agricultural Research Institute, Joydebpur, Gazipur-1701, Bangladesh; [8]National Agriculture Research Institute, Papua New Guinea; [9]Malaysia Agricultural Research Institute, Serdang, Malaysia; [10]Horticultural Research and Development Institute, Peradeniya, Sri Lanka; [11]Plant Protection Research Institute, Hanoi, Vietnam; [12]National Research Center for Banana, ICAR, Trichy, India; [13]Cambodia Agricultural Research and Development Institute, Phnom Penh, Cambodia; [14]Guangdong Academy of Agricultural Sciences, Guangzhou, P. R. China
Email: a.molina@cgiar.org

Recent epidemics of Panama disease (Fusarium wilt) in China and the Philippines caused by the virulent Tropical Race 4 (TR4) of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) is a serious threat to the banana industry in Asia, and beyond. This race, belonging to the Vegetative Compatibility Group (VCG) 1213/16, is extremely important because it is the most virulent strain of *Foc* attacking widely grown and traded Cavendish varieties, and many local cultivars grown by smallholder farmers. A study to determine the geographic distribution of the various pathogenic VCGs of *Foc* in Asia was carried out as a key step towards designing policies and measures to prevent further spread of TR4. Samples were collected from diseased banana plants between the period of 2006-2009 from 12 countries in tropical Asia. Foc was isolated from these samples, single-spored, and nit-1 and nit-M mutants generated in laboratories in Australia and South Africa. These mutants were then paired with an international VCG-tester set for *Foc* using the technique described by Puhalla in 1985. Nine VCGs (1213/16, 0120/15, 0121, 0123, 0124/5, 0126, 0128, 01218, 01220) were identified in Asia. VCG1213/16 (TR4), was the dominant VCG from samples collected in China, Indonesia, Malaysia, Philippines, and Taiwan but not found in samples from the other countries. VCG 0124/5, a VCG associated to Foc Race 1, was the dominant VCG in samples from India, Bangladesh Cambodia, Sri Lanka, Vietnam, and Thailand. No *Foc* infection of banana was found in Papua New Guinea. These results are relevant in preventing the movement of TR4 in areas that are not yet affected through effective quarantine measures.

**P45.004 Ecological control on Dwarf mistletoe (*Arceuthobium sichuanense*) based on the research of key factors related to its outbreak**

F. Gao, C. Tian, Y. Hu, B. Xia, Z. Zhou, D. CaiRang and W. Zhou
Key Laboratory for Silviculture and Conservation of Ministry of Education, Beijing Forestry University, Beijing 100083, P. R. China; Forest Pest Control and Quarantine Station of Qinghai Province, Xining 810000, P. R. China
Email: gaofaming.1008@163.com

*Arceuthobium sichuanense* is a hemi-parasite that is said to be the single-most destructive pathogen of commercially valuable spruce forest in Sanjiangyuan area of Qinghai province, China. Infected trees can be identified by large witches brooms, and the hosts can be killed either directly or by rendering them more susceptible to secondary environmental stress eventually. Although much information is available worldwide on various aspects of dwarf mistletoes, there have been very few reports relating to the specie distributed in China. In our study, *A. sichuanense* on *Pinus tabuliformis* is reported firstly; the experiment of seed rain indicated that the ejection of seeds mainly took place during the period from the late August to the middle September, with the maximum rain density of 124 seeds per square meter a day and 70% of the seeds were distributed in the circle area of 3-7 m away from the trees; *A. sichuanense* infection was likely emergence in spruce forest with low canopy density, higher coverage of vegetation and thinner moss layer; the 40% ethephon aqueous solution (diluted 400 and 200 fold in flower and fruit period, respectively) was the best choice for chemical control to spread of *A. sichuanense*. In addition, we reviewed life cycle period, ecological relationships, biotic associates, pathogenic effects and macro-phytopathology study on the threatening of parasite plants. Our aim is to reveal the key inducible factors about large area spread of *A. sichuanense* in local natural spruce forest and establish a scientific and effective technique for ecological control.

### P45.005 A set of special primers for detection of *Fusarium oxysporum* f. sp. *conglutinans*

*J.X. Zhang, J. Ling, Y.H. Yang and B.Y. Xie*
*Institute of Vegetables and Flowers,CAAS, NO.12 Nandajie Zhongguancun, Haidian District, Beijing, 100081, P. R. China*
*Email: qingchaoy@yahoo.com.cn*

*Fusarium oxysporum* Schlechtend:Fr. causes devastating plant diseases worldwide. As a species *F. oxysporum* includes more than 100 forma specialis, which can cause vascular wilt or root rot in important crops species, in which *F. oxysporum* f. sp. *conglutinans* is the main causes of Fusarium wilt on *Brassica oleracea*. Disease monitoring is the basis of integrating plant protection of any disease. However, the lack of rapid, accurate, and reliable devices to detect plant pathogens is one of the main limitations in taking appropriate and timely management measures to control the expansion of diseases. In order to develop a molecular diagnostic tool for identifying *F. oxysporum* f. sp. *conglutinans*, 90 unique sequences of *F. oxysporum* f. sp. *conglutinans* were found by making genomics comparative between *F. oxysporum* f. sp. *conglutinans* and other several forma specialis, and primers were designed according to those specific sequences. One set of those primers had been confirmed with high specificity to *F. oxysporum* f. sp. *conglutinans*, which can identify *F. oxysporum* f. sp. *conglutinans* from twenty-seven isolates of *F. oxysporum* and two other pathogenic fungus.

### P45.006 The T-DNA insertional mutagenesis in *Verticillium dahliae* via *Agrobacterium tumefaciens* mediated transformation

*M. Wang, J. Zhao, S.J. Gu, S. Sang and H.L. Li*
*College of Plant Protection, Henan Agricultural University, No.95 Wenhua Road, ZhengZhou, 450002, P. R. China*
*Email: wangm05@sohu.com*

The fungus *Verticillium dahliae* causes vascular wilt diseases in a wide range of crops. In this study, *Agrobacterium tumefaciens*-mediated transformation (ATMT) was applied for insertional mutagenesis of *V. dahliae* $FGH_2$, a highly virulent and defoliating strain. The collection of 1200 T-DNA random insertion transformants of *V. dahliae* was generated, using the optimized co-cultivation conditions of *V. dahliae* conidia and *Agrobacterium* (1:250 ratio) for 48 h at 25℃, and resulted in 200 to 500 transformants per $10^6$ conidia. A variety of *V. dahliae* mutants were obtained with the phenotypes including reduction in growth rate, sporulation, microsclerotia formation and pathogenicity toward cotton. The T-DNA flanking sequences of several mutants are being isolated through high-efficiency thermal asymmetric interlaced PCR (hiTAIL-PCR).

### P45.007 Differentiation of effectors specific for physiological races in *Phytophthora capsici*

*Y.D. Xi[1,2], G.H, Chen[1] and B.Y. Xie[1]*
*[1]Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China; [2]Institute of Plant Protection, Sichuan Academy of Agricultural Sciences, Chengdu 610066, P. R. China*
*Email: xiyadong2002@126.com*

The Oomycete *Phytophthora capsici* is a worldwide devastating pathogen, which mainly infect solanaceae and cucurbitaceae and other crops. *P. capsici* had 357 predicted RXLR effector genes and also had 29 putative full length and 70 pseudogenes for Crinkling and Necrosis (CRN) effectors. The recognition of the host plants and activation of the host resistance reaction generally belong to RXLR effectors in oomycete effectors, so the RXLR effectors are the focus in this study and the aim is to detect the specific effectors for physiological races. Based on the above reasons, we conducted the following experiments and obtained some ideal results. First, physiological characters test showed that the isolates belonged to the race1, race2 and race3, and the race 3 is probably the superior race in most regions of China. Second, bioinformatics of effectors were analyzed and 25 predicted RXLR effectors were selected.

Third, the primers of these 25 effector genes were designed and synthesized. Fourth, we selected 5 isolates of each race in race1, race2 and race3, and extracted RNA to perform RT-PCR, and then next to detect the effectors specific to physiological races.

**P45.008 Transcriptome analysis of microsclerotial development in smoke tree vascular wilt fungus *Verticillium dahliae***

*D.G. Xiong, Y.L. Wang, J. Ma, S.X. Xiao and C.M. Tian*
*The Key Laboratory for Silviculture and Conservation of Ministry of Education, College of Forestry, Beijing Forestry University, Beijing 100083, P. R. China*
*Email: xiongdianguang@126.com*

Smoke-tree vascular wilt fungus *Verticillium dahliae* is a causal agent of Verticillium wilt disease, which is widespread and devastating to plants. *V.dahliae* can infect more than 200 plant species, causing great economic loss every year. Much worse, the list of hosts is still expanding. Smoke-tree, *Cotinus coggygria*, is one of the most important tree species planted in China and it is also the main component of the red leaf scenery of the Beijing region during autumn. The disease causes stunted growth of stems, early senescence of leaves, and severe mortality of trees, with seriously detrimental effects on the red leaf scenery in Beijing region. The life cycle of *V.dahliae* includes three vegetative phases: parasitic, saprophytic and dormant. In the dormant stage, *V.dahliae* forms a resting structure called microsclerotia, which is vital factor for Verticillium disease, but the mechanism of microsclerotial formation is less understood. In this study, we applied mRNA-Sequencing to gain comprehensive understanding of transcriptional processes during microsclerotia development of *V. dahliae*. Analysis global pattern of gene expression, important genes and processes may contribute to the microsclerotia formation. It revealed that melanin biosynthesis, proteolysis, carbohydrate hydrolysis process were significantly up regulated while the plant cell wall degrading enzymes activities were suppressed during microsclerotial development. Furthermore, we also detected large number of novel transcribed regions and two putative new genes. These results may help us to understand the mechanism of microsclerotial formation and the Verticillium wilt disease, and may provide better guidance for disease prevention and cure.

**N45.001 Phylogenetic analyses and race identification of *Verticillium dahliae* isolates collected from sunflower Verticillium wilt samples**

*G. Zhang[1], J. Ren[1], X. Cao[2] and J. Zhao[1]*
*[1]Department of Agronomy, Inner Mongolia Agricultural University, Huhhot, 010018, P. R. China; [2]Baynnaoer Plant Protection and Quarantine Department, P. R. China*
*Email: zhaojun02@hotmail.com*

Verticillium wilt of sunflower occurred much popular in China, it caused severe yield loss of sunflower. Knowledge on the genetic diversity of sunflower *Verticillium dahliae* is lack. In order to control sunflower Verticillium wilt efficiently, the genetic relationships among *V. dahliae* isolates collected from different sunflower collected from five different provinces in China and identified with ITS1/4 primers. The complete intergenic spacer (IGS) region of the nuclear ribosomal RNA gene were amplified and sequenced. Using the maximum-likelihood distance, the sequences of the complete IGS region were used to analysis the genetic relationships among of all tested sunflower *V. dahliae* isolates. Phylogenetic analyses data set sequences into three distinct groups. Within the three groups, the first two groups only have three strains, and the rest of seventy-seven strains formed the third group indicting less variation among sunflower *V. dahliae* isolates. Using published specific primer of *V. dahliae* Race1, we detected race composition within sunflower *V. dahliae* isolates, all tested isolates did not show clear band while positive control did give right band, suggesting sunflower *V. dahliae* isolates maybe belong to a new race. More experiments need to be done to get the clear answer for that in the future.

**N45.002 The pathogenicity study on the five different colonial morphology of sunflower *Verticillium dahliae* isolates**

*J. Ren, G. Zhang, X. Cao, H.Y. Zhou, L. Jing and J. Zhao*
*Agronomy Department of Inner Mongolia Agricultural University, Saihan District, Hohhot, Inner Mongolia Autonomous region, 010018, P. R. China*
*Email: zhaojun02@hotmail.com*

In this study, we tested growth speed, wilting capability of the crude toxin and pathogenicity among five different colonial morphology of *Verticillium dahliae* isolates. Our results suggested that growth speed of tested isolates exist difference among different colonial morphology. The isolates V33, which produce less aerial hyphae and microsclerotia, grows faster compared with non-production microsclerotia isolate V21. The crude toxin production of different *V. dahliae* isolates showed increased tendency within 15 days after cultured in liquid medium. The toxin level of V33 stands at the top position, the value is 0.36 mg/ml, then followed by isolate V21, V39 and V25, the value is 0.34 mg/ml, 0.31 mg/ml, 0.24 mg/ml, respectively. The V27 stands in the last position and the value is only 0.23 mg/ml. Wilting capability and pathogenicity of tested isolates also showed significant difference. The wilting index of V33 isolate, which produce more microsclerotia and produce high level of crude toxin is 41.67% and 58.33%, respectively, after treated with crude toxin 48 h and 72 h , and

the wilting index of non-microsclerotia production isolate V21 is 39.58% and 27.08%, respectively. Wilting index of V25 and V27 are 25%, 22.91% and 35.41%, 37.50%, respectively. Regarding to the disease index of tested isolate, V33 showed the highest one with the value 53.97%, and V27 showed the lowest disease index with the value 37.44%. The disease index of V21, which is the non-production microsclerotia isolate is 45.90%. In a word, the wilting capability and pathogenicity of different colonial morphology isolates has a positive correlation with crude toxin production ability and have no correlation with the growth speed and the amount of microsclerotia.

# ABSTRACTS OF EVENING SESSIONS

## Evening Session 2-Autophagy in Plant Pathogenic Fungi and Plants

### E02.001 Going Selective: Specific functions of Autophagy during asexual development and pathogenesis in *Magnaporthe oryzae*

*Y.Z. Deng, Y.L. He and N.I. Naqvi*
*Temasek Life Sciences Laboratory, 1 Research Link, NUS, Singapore 117604*
*Email: naweed@tll.org.sg*

Previously, we showed that macroautophagy is necessary for conidiation in the rice-blast fungus *Magnaporthe oryzae*. We analyzed the physiological function(s) of selective autophagy in *Magnaporthe* through functional analysis of the sorting nexin *SNX41*. Loss of *SNX41* abolished conidia formation and pathogenesis in *M. oryzae*. Snx41-GFP localized as dynamic puncta or short tubules that partially associated with autophagosomes and/or autophagic vacuoles. PX domain, but not macroautophagy *per se*, was required for such localization of Snx41-GFP in *Magnaporthe*. We identified Oxp1, an ATP-dependent oxoprolinase in the gamma-glutamyl cycle, as a binding partner and potential retrieval target of Snx41-dependent protein sorting. Exogenous Glutathione, a product of the gamma-glutamyl cycle, significantly restored pathogenicity in the *snx41*-deletioṇmu- tant, likely through counteracting the oxidative stress imposed by the host. We propose that the gamma-glutamyl cycle is subject to regulation by Snx41- dependent vesicular trafficking, and mediates anti-oxidant defense crucial for *in planta* growth and pathogenic differentiation of *Magnaporthe* at the onset of blast disease in rice. Furthermore, Atg24-mediated Mitophagy, selective degradation of mitochondria via autophagy, plays a crucial role in regulating the redox homeostasis during asexual development and pathogenesis. The transition from biotrophic to necrotrophic growth *in planta* requires the remodeling and mitophagic regulation of mitochondria in the rice blast fungus. Lastly, pexophagy per se did not contribute to any specific developmental process in *M. oryzae*.

### E02.002 The pathways of autophagy in tobacco BY-2 cells

*Y. Inoue and Y. Moriyasu*
*Graduate School of Science and Engineering, Saitama University, Saitama 338-8570, Japan*
*Email: moriyasu@mail.saitama-u.ac.jp*

Tobacco BY-2 cells execute a large-scale degradation of intracellular proteins under nutrient-starvation conditions. We have shown that the degradation of cellular proteins is performed by macroautophagy, where newly-formed autolysosomes act as a lytic compartment. The autophagy pathway merges with the endocytosis pathway. The plasma membranes of tobacco cells expressing a fusion protein of Arabidopsis Atg8 and GFP were stained with FM4-64, and the cells were transferred to starvation medium. After 16 h of incubation, numerous autophagosomes with GFP fluorescence were observed and 15% of them had FM4-64 fluorescence, suggesting that the endocytosis pathway merges with the autophagy pathway immediately after autophagosome formation. In contrast, when the cells with their vacuolar membranes stained with FM4-64 were transferred to starvation medium, only 5% of autophagosomes had FM4-64 fluorescence. These results suggest that transport of vacuolar membranes to autophagosomes occurs after the endocytosis pathway merges with autophagosomes. Macroautophay ceases within 24 h after nutrient deprivation, and another type of autophagy, in which the central vacuole functions as a lytic compartment, subsequently occurs. Studies by electron microscopy showed that numerous small vacuoles appeared in cells cultured for 24 h under nutrient starvation conditions. We propose that these vacuoles fuse with the central vacuole and as a result, the parts of the cytoplasm among them and the central vacuole are delivered into the central vacuole.

### E02.003 Biogenesis and functions of peroxisome in the rice blast fungus *Magnaporthe oryzae*

*L. Li, J.Y. Wang, Y.L. Wang, H. Jiang, Z. Zhang and G.H. Sun*
*State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and Disease Control, Institute of Plant Protection Microbiology, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, P. R.China*
*Email: jiaoyuwang1@gmail.com*

Peroxisomes present ubiquitously in eukaryotes and participate in various metabolisms. Their matrix proteins and membrane proteins are post-translationally imported into the organelles. The import machinery consists of a specific group of proteins encoded by *PEX* genes. To date, dozens of the *PEX* genes have been identified in different eukaryotes and some were demonstrated to be involved in pathogenicity of fungal plant pathogens. Disruption of *PEX5*, *PEX6* or *PEX7* resulted in severally reduction in pathogenicity of the rice blast fungus *Magnaporthe oryzae*. To learn more about peroxisomal biogenesis and functions in the pathogenicity of fungal pathogens, we investigated the rest *PEX* genes in *M. oryzae* in present work. Our data showed that most of *PEX* deleted mutants exhibited greatly reduced virulence, mainly presented as reduction in conidiation, appressoria formation and appressorial turgor, and decrease in lipid utilization, cell viability and cell wall integrity in different degrees. The peroxisomal matrix proteins or mem-

branes proteins were partially or completely mislocated in these mutants, and interestingly in some cases, the numbers and sizes of peroxisomes were altered and even completely absent. The data indicated that the *PEX* genes participated in peroxisomal biogenesis and regulated peroxisome metabolism in *M. oryzae* and disruption of these genes led to multiple disorders in fungal development and pathogenicity.

### E02.004 Regulation of autophagy by the TOR signaling pathway in *Arabidopsis thaliana*

*D.C. Bassham and Y. Pu*
*Department of Genetics, Development and Cell Biology, Iowa State University, Ames, IA 50011, USA*
*Email: bassham@iastate.edu*

Autophagy is a major pathway for delivery of proteins and organelles to the plant vacuole for degradation and recycling. Autophagy functions in nutrient recycling during macronutrient deficiency and in the degradation of aggregated and damaged proteins during stress conditions such as oxidative, salt and drought stress. There is also evidence that autophagy is involved in programmed cell death and control of disease progression during pathogen immune responses. In animal and yeast cells, Target of Rapamycin (TOR) kinase was identified as a central regulator of cellular responses to nutrient concentrations, activating biosynthetic and growth pathways and inhibiting catabolic pathways such as autophagy under nutrient replete conditions. We have shown that an Arabidopsis TOR homolog can negatively regulate autophagy, as decreased levels of components of the TOR complex lead to constitutive autophagy, even in the absence of stress. We have also shown that TOR overexpression lines have reduced autophagy in response to various stresses, suggesting that the TOR signaling pathway is involved activating autophagy in response to stress conditions in plants.

### E02.005 The functions of *MoATG15* in *Magnaporthe oryzae*

*H.B. Shi, X.H. Liu and F.C. Lin*
*Biotechnology Institute, Zhejiang University, Hangzhou, 310058, P. R. China*
*Email: fuchenglin@zju.edu.cn*

Rice blast, caused by the pathogen *Magnaporthe oryzae*, threatens the rice production and human sustenance worldwide. Autophagy is a conserved physiological process in eukaryotes playing significant roles in the pathogenic mechanism of filamentous fungi. *MoATG15*, the homologue gene of *ATG15* in *Saccharomyces cerevisiae*, is a lipase required for the intravacuolar disintegration of membrane vesicle including autophagic bodies in the late process of autophagy. In our research, the *MoATG15* null mutant was acquired using gene target replacement. Compared with the wild type strain Guy11, the Δ*moatg15* mutant exhibited decreased conidiation and alleviated pathogenicity on rice CO39. To visualize the cellular localization of *MoAtg15*, the fusion protein was generated by GFP conjugating to the carboxyl terminus of MoAtg15 and expressed in the Δ*moatg15* mutant. The results showed that MoAtg15 localized to the cell membrane and vacuolar membrane. Meanwhile, the fusion protein complemented the defects of the Δ*moatg15* mutant.

### E02.006 Compositional identification of PI3K complex in tobacco

*H.N. Zhou, Y.L. Xu, N. Zhai, P.J. Cao, Z.P. Luo, D.W. Jin and F.C. Lin*
*China tobacco gene research center, Zhengzhou Tobacco Research Institute, CNTC*
*Email: fuchenglin@zju.edu.cn*

The class III phosphatidylinositol 3-kinase (PI3K) complex, formed around ATG6/Beclin1, play an essential role during autophagosome formation. Besides Beclin1 and PI3K(Vps34), several other proteins including Bcl-2, UVRAG, Bif-1, Ambra1, Vps15, and newly described Rubicon and Atg14L had been identified as the components of PI3K complex, which controlled the autophagosome formation by functioning as an activator or inhibitor. Autophagic process is conserved among fungi, plant, animal and human, while the detail composition of PI3K complex in plant, and relationship among them still remains much unclear. Previous attempts by blast search of sequenced plant species genomes like arabidopsis did find some homologous genes, while some key regulators like UVRAG and Ambra1 were not detected. Tobacco plays a more similar autophagic procedure to animal and human by fusion of autophagosome with the lysosome prior to degradation, comparing with the autophagosome directed to vacuole to be degradated in arabidopsis. Here, a deep blast search of partners in PI3K complex was done based on tobacco genome sequences. Together with the experimentally detection by yeast two-hybrid, the first profile of PI3K complex in plant would be identified.

## Evening Session 3-Blackleg A Global Threat to Canola, What Can We Do about It

### E03.001 Monitoring frequency of avirulence genes of *Leptosphaeria maculans*

*A.P. Van de Wouw, S.J. Marcroft and B.J. Howlett*
*School of Botany, the University of Melbourne, VIC 3010, Australia*

The interaction between the blackleg fungus, *Leptosphaeria maculans* and *Brassica napus* is a ‘gene for gene’ relationship. Blackleg can be particularly devas-

tating as fungal populations have a high evolutionary potential and rapidly adapt to selection pressure from sowing of varieties with major gene resistance. Thus within a few years of release of a variety resistance can be 'overcome'. With French colleagues, we have sequenced the genome of the blackleg fungus and discovered the basis of the high evolutionary potential of this fungus. Key disease-related genes, including avirulence effector genes, are embedded in gene-poor regions of the genome that have transposable elements degenerated by Repeat Induced Point (RIP) mutations. Thus these key avirulence genes are easily gained, lost or inactivated during sexual reproduction, which occurs prolifically on stubble. Molecular markers for three avirulence genes have been developed and are now being used in high throughput methods to monitor virulence of blackleg populations. Ascospores released from canola stubble collected across canola-growing regions of Australia are trapped on tape in a Burkard liberation tunnel and then fungal DNA on the tape is PCR-analysed for changes in frequency of avirulence genes complementary to particular resistance genes. This information, along with field and glasshouse data, is then used to predict whether disease outbreaks will occur on canola varieties with a particular resistance source. If an epidemic is predicted then farmers are advised to plant a different canola variety that will not readily succumb to blackleg disease.

### E03.002 Epidemiology and spread of *Leptosphaeria* species (phoma stem canker) on brassicas

*B.D.L. Fitt[1], D.J. Hughes[1], H.U. Stotz[1], B.A. McDonald[2], T. Rouxel[3]*
*[1]University of Hertfordshire, Hatfield, Hertfordshire AL10 9AB, UK; [2]ETH Zurich, LFW 1316, Universitatstrasse 2, CH-8092, Zurich, Switzerland; [3]INRA, UR1290 BIOGER, Avenue Lucien Bretignieres, BP 01, F-78850 Thiverval-Grignon, France*
*Email: b.fitt@herts.ac.uk*

*Leptosphaeria maculans* and *L. biglobosa*, cause of phoma stem canker disease of brassicas, are related pathogens of brassicas that were originally considered as one species but occupy slightly different ecological niches and are now reproductively isolated. Both pathogens are naturally dispersed by air-borne ascospores, produced on infected crop debris, which may be dispersed over longer distances as a contaminant in internationally traded seed cargoes. Globally, the invasion by the more damaging *L. maculans* into areas where only *L. biglobosa* was present occurred in North America in the 1980s and Eastern Europe in the 1990s, whereas there are still areas of the world, such as China, where only *L. biglobosa* is present. In addition, the worldwide population of *L. maculans* is much less variable than that of *L. biglobosa*, which has populations related to particular geographic areas (Australia, Canada and the Indo-European continent) that should be considered as different species that diverged > 4MYA. Further evidence is provided by the massive invasion of the genome of *L. maculans* by numerous repeated elements; recent evidence suggests that divergence between *L. biglobosa* and *L. maculans* occurred 72MYA. As the environment and brassica cropping patterns alter in response to climate change, further evolution of these two *Leptosphaeria* species will occur, with possible increases in the range and severity of phoma stem canker epidemics.

### E03.003 The Canadian response to the rapid face-changing (Bian Lian) of the blackleg pathogen populations in the Prairies

*W.G. Dilantha Fernando[1], X. Zhang[1], S.H. Liban[1], D.J. Cross, G. Peng, and H.R. Kutcher*
*[1]Department of Plant Science, University of Manitoba, 66 Dafoe Road, Winnipeg, Manitoba, R3T 2N2, Canada*
*Email: Dilantha.Fernando@ad.umanitoba.ca*

*Leptosphaeria maculans* is the causal agent of blackleg (aka phoma stem canker), an economically important disease of oilseed brassicas that has led to epidemics in both Australia and France. This pathogen is now a growing concern affecting the Canadian Canola (*Brassica napus*) industry and has appeared on cultivars previously rated as resistant. Tighter rotations driven by economic prospects, low cultivar diversity, and an excess of 20 million acres has led to emergence of new virulent races of the pathogen. Resistance to blackleg is analogous to the gene for gene interaction model in which Rlm genes defend against isolates with Avrlm genes. However no single resistance gene can remain effective against dispensable avirulence genes and a changing pathogen population. Collaborative efforts between industry and researchers have led to the screening of both the avirulence genes in the pathogen population and major resistance genes in commercial varieties. The results have demonstrated that pathogen adaptation has rendered the most common resistance gene in the Canadian germplasm, Rlm3 (65% of lines), ineffective by selecting against isolates carrying Avrlm3 (7% of isolates) in *L. maculans*. In response the Canadian industry will establish a cultivar rotation program that switches between varieties in different resistance groups to mitigate the effects of pathogen adaptation. Five different resistance groups will initially be deployed. More resistance groups will be incorporated as more resistance genes are identified. Ongoing monitoring of the pathogen population will be maintained to observe the durability of different resistance genes and pre-emptively switch cultivars before significant yield losses can occur.

### E03.004 Getting worse–constant increase of pathogen aggressivness and yield loss in Poland: Can

**aerobiology help to manage blackleg in oilseed rape/canola?**

*M. Jedryczka, J. Kaczmarek, A. Dawidziuk*
*Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland*
*Email: mjed@igr.poznan.pl*

Blackleg or stem canker of crucifers is caused by two closely related fungal ascomycete pathogens *Leptosphaeria maculans* and *L. biglobosa*. Although the latter was described as a separate taxon 12 year ago, it is now regarded as an older species than *L. maculans*. Disease symptoms on stems, caused by *L. biglobosa* are more profound due to its fast growth rate, but it is *L. maculans* that causes higher yield losses. Large scale field experiments performed in Poland and France revealed great differences in the evolutionary speed of avirulence genes. Under no selection pressure from resistance genes, virulence alleles *avrLm6* and *avrLm7* were almost nonexistent in Poland, whereas in France a rapid buildup of virulent populations was observed soon after the introduction of the corresponding resistance genes. In Poland a gradual shift towards *L. maculans* has been observed since 1984. Large scale studies of fungi inhabiting the stubble of winter oilseed rape show considerable changes in the ratio between *Leptosphaeria* species over one season. Inspite of the high infection rate of oilseed rape by *L. biglobosa*, it is *L. maculans* that produces more pseudothecia and spores. When stressed, mutation rate of *L. maculans* can be extremely high. Moreover, sexual reproduction allows for increased genetic diversity. Asexual reproduction with its abundance of genetically identical spores leads to immediate propagation of new fungal variants. Monitoring spores is a very useful tool in disease forecasting. For the last 10 years aerobiological methods have been used extensively in Poland and to a lesser extent also by the other countries of central Europe to reduce the disease.

**E03.005 Effect of fungicides and application timing on control of blackleg of canola**

*G. Peng[1], W.G. Dilantha Fernando[2], C.L. Kirkham[1], C. Liu[2], R.M. Lange[3], H.R. Kutcher[4], D.L. McLaren[5], E.N. Johnson[1], T.K. Turkington[6]*
*[1]Agriculture and Agri-Food Canada (AAFC)Research Centre, 107 Science Place, Saskatoon, Saskatchewan S7N 0X2; [2]Dept. Plant Sciences, Univ. Manitoba, 222 Agriculture Building, Winnipeg, Manitoba R3T 2N2; [3]Alberta Innovates, P.O Bag 4000, Vegreville, Alberta T9C 1T4; [4]Dept. Plant Sciences, Univ. Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan S7N 5A8; [5]AAFC Research Centre, 2701 Grand Valley Road, Brandon, Manitoba R7A 5Y3; [6]AAFC Research Centre, 6000 C & E Trail, Lacombe, Alberta, T4L 1W1, Canada*
*Email: Gary.Peng@agr.gc.ca*

Blackleg, caused by the fungus *Leptosphaeria maculans* (Desmaz.) Ces. & de Not, is the most widespread disease of canola on the Canadian prairies. The disease has been managed mainly through use of resistant cultivars in conjunction with a 3 to 4 year crop rotation. Over the past few years, crop rotation has been shortened in favor of canola production and the pathogen population has been evolving. As a result, the blackleg disease is on the rise in many areas. To mitigate the risk of potential loss of cultivar resistance, a study was conducted to assess the effect of registered fungicides and their application timing for blackleg control using replicated (n=4) research plots (4×8 m). The fungicides pyraclostrobin, azoxystrobin, propiconazole, and propiconazole + azoxystrobin were applied at the 2-4 leaf stage of a susceptible canola cultivar at three trial locations in 2011 and five locations in 2012. Pyraclostrobin was also applied at bolting or in combination with propiconazole at both 2-4 leaf stage and bolting, and to a resistant and a moderately resistant cultivar at the 2-4 leaf stage as checks. The early application (2-4 leaf) of pyraclostrobin or azoxystrobin reduced blackleg damage substantially in 3 of the 5 site-years that recorded >1.0 disease severity, while propiconazole alone was ineffective. Pyraclostrobin applied at bolting was also less effective, reducing blackleg in only one site-year. An additional treatment prior to bolting provided no further efficacy relative to one application at the 2-4 leaf stage. None of the fungicide treatments, however, increased canola seed yield substantially, regardless of cultivar resistance.

## Evening Session 5-Overview of Edible and Medicinal Mushroom in the USA and China

**E05.001 Overview of edible and medicinal mushroom in the U.S and China**

*M.M. Chen*
*University of California, Berkeley and Beijing Normal University; University & Jepson Herbaria, Valley Life Science Building, Berkeley, CA 94720*
*Email:momeichen@berkeley.edu*

Edible and Medicinal mushroom is the healthy fine agricultural crop produced from the world's rich and diverse resources and advanced technology. Approximately 700 species of mushrooms are edible worldwide and fewer than 200 species are considered to have medicinal value including many species of mushrooms have shown preventive and therapeutic effects when included in the daily diet. Edible and medicinal mushrooms can not only convert lignocelluloses biomass waste into digestible human food but also produce many biologically active compounds that promote five reasons to eat mushrooms: the mushroom is 1) non-polluting 2) nutritious 3) serves as various cooking ingredients, 4) promotes weight loss and 5) boosts health and beauty. The aim of this session is to provide scientific infor-

mation of edible mushrooms and their cultivation. For a long time people have been looking for information on Chinese edible and medicinal mushroom in an English book which can now be found in the first English edition encyclopedic book Fungi Treasure. While achieving my research in the United States for 30 years and have Chinese-American expertise, the new encyclopedia will provide new information on the identity of each edible mushroom including morphology, ecology, nutrition and medicinal value, as well as requirements for cultivation techniques, and culinary uses. It can also clarify all kinds of mystified questions about mushrooms with information supported by scientific results by co-research with Chinese and American mycologists and amateur.

### E05.002 The main cultivation species of edible mushroom in China

*Q. Tan*[1, 2, 3] *and M.M. Chen*[4]
[1]*National Engineering Research Center of Edible Fungi, Ministry of Science and Technology (MOST), P. R. China;* [2]*Key Laboratory of Edible Fungi Resources and Utilization (South), Ministry of Agriculture, P. R. China; Institute of Edible Fungi;* [3]*Shanghai Academy of Agricultural Sciences, Shanghai, P. R. China;* [4]*University of California, Berkeley, USA*
*Email: syj0@saas.sh.cn*

A historical monograph of China recorded thousand years ago, during the Yang Shao civilization, that people collected and consumed wild mushrooms. Since this time, mushrooms have become a traditional food and delicacy in the Chinese culture. This long history has created a wealth of knowledge on the cultivation and culinary qualities of mushrooms. According to newest international information records shown, presently the Chinese produce 25.71 million tons of edible mushrooms to per year which is 70% cultivating of the world in total production. Main cultivation species of edible mushroom in China are: Largel mount high productivities *Pleurotus* spp., *Lentinula edodes, Auricularia auricular, Agaricus bisporus, Flammulina velutipes, Pleurotus erygi,* and *P. nebrodensis.*, *Hypsizygus* marmoreus, As the demand for mushrooms continues to increase with the rapidly growing global population, the natural resources for mushroom cultivation will become limiting. Coming into the new millennium, humans can hope to use fungi even more widely for food and medicine. To achieve our lofty goal of increasing mushroom cultivation for production of food, we must work hard on such aspects as acceptance of a greater variety of mushrooms by public, standards of production (quality control), biodiversity of species in nature, sustainability of natural resources, and optimization of quality of spawn. Mushroom production can be non-commercial as well as industrialized. A free exchange between science and technology as well as improved marketing practices are essential to modernization of the production of edible mushrooms.

### E05.003 Research and cultivation of Morels (Morchella) thirty years

*T.F. He*[1] *and M.M. Chen*[2]
[1]*Forestry Academy of Sichuan Province, P.R. China;* [2]*University of California, Berkeley*
*Email:Tanfanghe668@yahoo.com.cn*

According to the Dictionary of the Fungi 10th edition (2009), *Morchalla* comprises 292 records and is classified as Ascomycota, Pezizales, Morchellaceae. Traditionally, morels have always been a favored group of wild edible mushrooms for North Americans, Europeans, and Asians. Mycologists and amateurs often collect them in mountains during spring time. In 1964, Liu Bo study world literature record: "until today still cannot use mycelium to produce morel fruiting indoors, later mycologist they use fruiting body tissue or spores mycelia only (Gilbert, 1960). Until Yang Xinmei (1986), Write in *Chinese mushroom cultivation Monograph* noted: "Janardhanan (1971), R. Ower (1982) scientific research results improved morel physiologic many fundamental questions but even though since, to date, mass morel fruiting of commodity production has made little progress. The morel has defied all attempts at consistent artificial cultivation that Chinese Mycologist Tan Fang He recently 30 years was one accomplishment mycologist on morel cultivation. 1980's, We starts on morel fruiting indoor experimental in Chengdu, China. Then, We research focused on solution of the cultivation of the complexity and instability of forming morel fruiting. The U. S and China cooperation between Chengdu and University of California, Berkeley we has been cooperated research on *Mochella* systematic, spores collection, spores bank establishing and delicious strain. Especially, we consider the sclerotia function as a key structure for production, The Sclerotia promote to mass production research for 20 years. Finally, 2013, we has been harvest morel from 500 Mu land and dried morel 5000 Chinese weights, It's gain 4 million RNB.

## Evening Session 9-Prospects and Limitations of Novel Action Fungicides

### E09.001 Impact of future trends on the introduction of new fungicides

*A.J. Leadbeater*
*Syngenta Crop Protection AG., Schwarzwaldallee 215, CH-4058 Basel, Switzerland*
*Email: andy.leadbeater@syngenta.com*

Fungicides have been used to good effect in agriculture since the 1940s resulting in crop security and helping to optimise yields. Since around the 1960s however the

impact of pathogen resistance to the specific, single site fungicides has become more significant and important, driving the need for novel fungicides with new modes of action. Thus the search in industry for new classes of chemistry with quite different characteristics to the older, more established fungicides. At the same time there is a clear requirement for new fungicides to be highly effective at low use rates. This search has proved rather challenging since increasing hurdles in the regulatory area drive industry to develop products focusing on human and environmental safety rather than biological effectiveness. These trends can be expected to have an impact on the ability of industry to come up with new solutions. To avoid the potential consequence of a narrower range of less effective products innovation is required across the industry.

**E09.002 Update on the evolution and selection for azole and Succinate DeHydrogenase Inhibitor (SDHI) fungicide insensitivity in UK *Mycosphaerella graminicola* populations**

*B.A. Fraaije*
*Department of Biological Chemistry and Crop Protection, Rothamsted Research, Harpenden, AL5 2JQ, UK*
*Email: bart.fraaije@rothamsted.ac.uk*

Septoria leaf blotch caused by the fungus *Mycosphaerella graminicola* is mainly controlled by programmed foliar application of fungicides. Due to the development of resistance to methyl benzimidazole carbamates (MBCs) and Quinone outside Inhibitors (QoI), the use of fungicide mixtures containing azoles, SDHIs and/or multisite inhibitors is common practice in the UK to control Septoria epidemics whilst minimising the risk of resistance. Due to the recent erosion of azole efficacy and the perceived high risk of resistance to SDHIs, continuous monitoring of fungicide sensitivities in field populations is required. Here we report the latest findings of these studies and discuss implications for future disease control.

**E09.003 Temporal changes in fungicide resistance in Czech populations of *Pseudoperonospora cubensis***

*A. Lebeda, B. Sedláková and J. Pavelková*
*Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 11, 783 71, Olomouc-Holice, Czech Republic*
*Email: ales.lebeda@upol.cz*

Cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, is a major disease of cucurbit crops worldwide, as well as in the Czech Republic. The disease is mainly managed by fungicide applications. A total of 142 Czech *P. cubensis* isolates (from 2005-2010) were used for fungicide resistance/tolerance screening. Six commonly used and registered fungicides (except Ridomil Plus 48 WP that served as control fungicide) were screened for efficiency against *P. cubensis*. The majority of isolates (134) originated from *Cucumis sativus* and 8 isolates from new hosts (*Cucurbita* spp. and *Citrullus lanatus*). Fosetyl-Al (Aliette 80 W) and propamocarb (Previcur 607 SL) were the most effective fungicides. All tested isolates were sensitive on all tested concentrations of fosetyl-Al. However, some isolates expressed resistance or tolerance to lower and/or even to recommended concentrations of propamocarb in the years 2006 and 2008-2010. Metalaxyl (Ridomil PLUS 48 WP) and metalaxyl-M (Ridomil Gold MZ 68 WP) were ineffective. Isolates collected in 2008 and 2009 showed greater variation in tolerance or resistance at higher dosages of fungicide. While cymoxanil (Curzate K) was ineffective in the years 2005-2008 and in 2010, there was 68% of isolates controlled at the recommended concentration in 2009.

**E09.004 *CYP51* and evolution of azole resistance**

*N.J. Hawkins, H.J. Cools and B.A. Fraaije*
*Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom*
*Email: nichola.hawkins@rothamsted.ac.uk*

The azoles are an important class of fungicides for a wide range of arable and horticultural crops as well as clinically. They have remained important in agriculture for three decades, in part because qualitative, cross-azole resistance has not emerged. However, quantitative sensitivity shifts have taken place, compromising control of some pathogens by some compounds, caused by a range of different resistance mechanisms. The azole target site is sterol 14-α demethylase, encoded by *CYP51*. Some species possess a single paralogue, *CYP51B*. Multiple *CYP51B* mutations have been reported in some species, including *Mycosphaerella graminicola*, with the exact combinations reflecting functional constraints on enzyme structure and the history of azole use. In other species, a second paralogue, *CYP51A*, confers lower intrinsic sensitivity to some azoles, and sensitivity shifts may result from mutations, over-expression or, in *Rhynchosporium commune*, from re-emergence of *CYP51A* in pathogen populations. A third paralogue, *CYP51C*, has only been found in *Fusarium* species and appears to be functionally diverged. Non-target-site mechanisms have also been implicated in some cases.

**E09.005 An overview of fungicides and resistance development in vegetable crops in the USA**

*M.T. McGrath*
*Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Long Island Horticultural Research & Extension Center, 3059 Sound Avenue, Riverhead, NY, 11901, USA*

*Email: mtm3@cornell.edu*

Managing resistance before it develops is an important aspect of effectively managing diseases in vegetable crops today. This is because most fungicides have targeted mode of action, which enables them to be mobile in plants and imparts high efficacy, low potential impact on the environment and non-target organisms, but also risk of resistance developing in the pathogen. There are targeted fungicides registered for use in the USA to manage diseases of vegetable crops in FRAC (Fungicide Resistance Action Committee) Groups 1, 2, 3, 4, 7, 9, 11, 12, 13, 14, 21, 22, 25, 27, 28, 30, 33, 40, 43, 45, and U6. Pathogens and the fungicide chemistry to which they have developed resistance include *Alternaria solani* and *A. tomatophila* (fungicides in FRAC Group 11), *Botrytis cinerea* (2 and 11), *Bremia lactucae* (4 and 33), *Corynespora cassiicola* (11), *Didymella bryoniae* (1, 7, and 11), *Fusarium* species associated with dry rot of potato (1), *Helminthosporium solani* (1), *Phytophthora capsici* (4 and 21), *Phytophthora erythroseptica* (4), *Phytophthora infestans* (4), *Podosphaera xanthii* (1, 3, 7, and 11), *Pseudoperonospora cubensis* (4, 11, and 43 suspected), *Pythium ultimum* causing potato leak (4), and *Sclerotinia minor* (2). Typically resistance is detected after the fungicide exhibited lower efficacy than expected in a research or commercial planting, rather than through pathogen monitoring. Challenges include predicting risk (for both pathogen and fungicide), identifying best anti-resistance strategies (in particular fungicide mixtures versus alternations), lack of tools (other fungicides, resistant varieties), detecting resistance, and increased management costs.

### E09.006 Multifungicide resistance in strains of *Monilinia fructicola* and evidence of fungicide-induced mutagenesis

*G. Schnabel and F. Chen*
*School of Agricultural, Forest, and Environmental Sciences, Clemson University, Clemson SC 29634*
*Email: schnabe@clemson.edu*

Fungicide resistance in *Monilinia fructicola* is becoming a problem for producers, resulting in outbreaks of brown rot disease in stone fruits. We collected strains from problem orchards in Maryland (MD), Pennsylvania (PA), and South Carolina (SC) and found a variety of fungicide resistance phenotypes. In addition to strains resistant to either propiconazole (Paz), thiophanate methyl (TM), or boscalid, we identified strains resistant to more than one fungicide: Paz and TM in all regions and Paz plus boscalid in SC. The molecular basis of fungicide resistance differed between regions. Paz resistance in SC was associated with the insertion of genetic element Mona upstream of Mf*cyp51* and increased gene expression. In contrast, the *cyp51* in Paz-resistant strains from PA and MD lacked the insertion was not overexpressed, suggesting a different resistance mechanism. TM resistance was associated with mutations E200Y and E198Q in β-tubulin in the north and E198A in the south. Boscalid resistance was not associated with point mutations in *Sdh* subunits. Examination of the impact of sublethal exposure of fungicides to *M. fructicola* isolates revealed unexpected results. After exposing *M. fructicola* isolates to sublethal doses of azoxystrobin plus salicylhydroxamic acid, we observed transposon movement and microsatellite alteration suggesting that azoxystrobin might aid the pathogen's struggle to adapt to fungicide stress.

### E09.007 Challenges and opportunities in developing and positioning novel SDHI fungicides: Disease control and beyond

*M. Scheller, C. Schlatter and D. Hermann*
*Syngenta Crop Protection AG, Research Center, WST 540.1.71, 4332 Stein, Switzerland*
*Email: dietrich.hermann@syngenta.com*

Carboxamide fungicides inhibiting the complex II in fungal respiration are known since more than 45 years, with carboxin being introduced as a first representative in 1966. This mode of action, now described as succinate dehydrogenase (SDH) inhibition was for a long time assumed to provide only a narrow biological spectrum. However recently, highly active, broad spectrum SDH inhibitors have entered the market or are in late stage development, providing novel solutions to the farmer. Syngenta has developed a range of products based on the active ingredients isopyrazam, sedaxane and benzovindiflupyr for key crop segments globally. The combination of broad spectrum activity with unique physical-chemical properties is a key feature of this new SDHI class. With Vibrance®, based on sedaxane, Syngenta has developed a product specifically designed for seed treatment and has created a new market segment: Root Health. Sedaxane leads to stronger and healthier roots under a broad range of conditions. Discovery and development of new compounds requires intense studies in chemistry, biochemistry and numerous experiments under laboratory-, greenhouse- and field conditions to select suitable candidates and to understand their full potential. For sedaxane, these tests also included features like seed safety, mobility in the rhizosphere and systemicity in the root system. In addition to excellent broad spectrum disease control, studies in collaboration with research institutes have demonstrated root biostimulant effects and improved photosynthetic efficiency of plants under drought stress conditions. The growers and the entire industry will benefit from these new tools to secure and improve crop productivity.

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