15th ISPVE Madrid 2022

International Symposium of Plant Virus Epidemiology

ISPP INTERNATIONAL SOCIETY FOR PLANT PATHOLOGY

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

"Epidemiology and Management of Plant Viruses under a Changing Climate"

Madrid, Spain. 5-8th June, 2022

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BOOK OF ABSTRACTS

15TH ISPVE-MADRID 2022
International Symposium of
Plant Virus Epidemiology
CSIC

15th International Symposium of Plant Virus Epidemiology

"Epidemiology and Management of Plant Viruses under a Changing Climate"

These Symposia are organized under the auspices of the Plant Virus Epidemiology Committee of the International Society of Plant Pathology

List of IPVE Symposia Series:

1. UK, Oxford, 28 - 31 July 1981
2. Australia, Corowa, 25 - 27 August 1983
3. USA, Orlando, 6 - 8 August 1986
4. France, Montpellier, 1 - 5 September 1989
5. Italy, Valenzano (Bari), 27-31 July 1992
6. Israel, Jerusalem, 23 - 28 April 1995
7. Spain, Aguadulce (Almeria), 11 - 16 April 1999
8. Germany, Aschersleben, 12 - 17 May 2002
9. Peru, Lima (CIP), 4 - 7 April 2005
10. India, Hyderabad (ICRISAT), 15 - 19 October 2007
11. USA, Ithaca (Cornell University), 20 - 24 June 2010
12. Tanzania, Arusha (IITA), 28 January – 1 February 2013
13. France, Avignon (INRA), 6-10 June 2016
14. Korea, Seoul 13-17 May 2019

The photograph in the front page illustrates typical symptoms of a zucchini plant infected with zucchini yellow mosaic virus. It was taken by Dr. Gemma Clemente.

Welcome Message

We are pleased to invite you to participate in the 15th International Symposium of Plant Virus Epidemiology (ISPVE 2022) from June 5th to June 8th, 2022, CSIC, Madrid, Spain.

ISPVE 2022 will bring together a collection of research scientists who are at the front of plant virus epidemiology and related scientific fields and will provide opportunities for **in-person conversation to encourage collaboration** among meeting participants. After more than 2 years without in person meetings, we are delighted to welcome all of you in Madrid. Actually, it has been 23 years since we hosted the memorable VIIth IPVE Symposium in Spain. It was back in 1999, in Almeria, where several of you were part of the 280 participants from 28 different countries that attended the meeting.

The focus of the present meeting is to **broaden the scope** of plant virus epidemiology to other vector-borne diseases (e.g. bacterial diseases) and to bring together people working in Diagnostics, Surveillance and Modeling, Ecology and Evolution, Virus-Vector Interactions and Disease Management under the context of a Changing Climate.

This program will include oral and poster sessions as well as social events (welcome reception, a tour around old-town Madrid and a closing banquet) that will give an opportunity to exchange information, engage in stimulating discussions and collaborate with your fellow members from around the world. We are sure that our expert keynote speakers will provide you with **the most critically relevant and up-to-date information**.

We hope all of you will participate in the 15th ISPVE and enjoy this opportunity to meet again face to face and to have the chance to taste some Spanish food and know more about Madrid its culture and its surroundings.

ISPVE 2022 SPONSORS

ISPVE 2022 has been partially supported by the sponsorship of Koppert, CORTEVA, BASF, Syngenta and Sistemas DR and has been convened by Prof. Alberto Fereres, past Chairman of ISPVE Organizing Committee.

Organizing and Scientific Committees

Organized by

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
INSTITUTO DE CIENCIAS AGRARIAS
MADRID, SPAIN

Chair of ICPVE: William M. Wintermantel, USDA, Salinas, California, USA

Organizing Committee

Alberto Fereres (Chair)	ICA-CSIC, Madrid, Spain	a.fereres@csic.es
Aránzazu Moreno	ICA-CSIC, Madrid, Spain	amoreno@ica.csic.es
Elisa Garzo	ICA-CSIC, Madrid, Spain	elisa.garzo@ica.csic.es
Inmaculada Ferriol	ICA-CSIC, Madrid, Spain	iferriol@ica.csic.es
María Plaza	ICA-CSIC, Madrid, Spain	mariap@ica.csic.es
Gemma María Clemente Orta	ICA-CSIC, Madrid, Spain	gemma.clemente@udl.cat
Rocío Galán	ICA-CSIC, Madrid, Spain	rociogalancu@gmail.com
Clara Lago	ICA-CSIC, Madrid, Spain	clara.lago@ica.csic.es
Carolina Junca Morales	ICA-CSIC, Madrid, Spain	carolinajuncam@yahoo.com
Konstantina Lyra	AUA, Athens, Greece	konstantina1999@hotmail.com
Myrto Mela	AUA, Athens, Greece	myrtomelaa@gmail.com
Eirini Biniari	AUA, Athens, Greece	irenebiniari@gmail.com
Zoe Pentheroudaki	U. of Crete, Athens, Greece	zpentheroudaki@gmail.com

Scientific Committee

William M. Wintermantel	USDA, Salinas, California. USA
Michael J. Jeger	Imperial College, Silwood Park. Askot, UK
Peter Palukaitis	Seoul Women's University, Seoul, Korea
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P. Lava Kumar	IITA-CGIAR. Ibadan, Nigeria
Saskia Hogenhout	John Innes Centre, Norwich, UK

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ISPVE 2022 Program

- The ISPVE 2022 venue will be at the CSIC main campus located in Calle Serrano 117, 28006, Madrid, Spain.
- The Registration and Welcome Reception will be on Sunday at 19:00 at the "Residencia de Estudiantes". Calle del Pinar 21, Madrid.
- Registration will continue on Monday at 7:45 am at the Rocasolano Building. The Oral Sessions will be held from Monday to Wednesday at the Rocasolano Building. Poster Sessions and Coffee Breaks will be held at CSIC Atrium located at the Instituto de Física Fundamental. All locations are within the CSIC main campus.

Sunday 5th, June

19:00-22:00 **REGISTRATION AND WELCOME RECEPTION**

"Residencia de Estudiantes". Calle del Pinar 21. Madrid.
2 min-walk from Rocasolano Building

Monday 6th, June

7:45-8:30 **REGISTRATION (Rocasolano Building)**

8:30-9:00 **OPENING CEREMONY (Juan A.Hermoso, Nilsa.Bosque-Perez, Alberto Fereres)**

Session 1. General Epidemiology

Chairs: Michael J. Jeger and Alberto Fereres

9:00-9:45		***Keynote Lecture 1*** Emerging themes and approaches in plant virus epidemiology. *Michael J. Jeger.*
9:45-10:00	S1-O1	Whitefly-transmitted virus dominance in mixed infections varies among three cucurbit production regions in the United States. *William M. Wintermantel.*
10:00-10:15	S1-O2	Cucurbit cytorhabdovirus 1: a novel whitefly transmitted cytorhabdovirus infecting zucchini crops in Greece. *Chrysoula Orfanidou.*
10:15-10:45		*Coffee Break and Poster Exhibit*
10:45-11:00	S1-O3	Epidemiology and genetic diversity of cucurbit aphid-borne yellows virus and watermelon mosaic virus in cucurbit crops. *Pedro Gómez.*
11:00-11:15	S1-O4	Molecular insights on potato yellow vein crinivirus infections in single and mixed infections with a potyvirus, in Colombia. *Liliana Franco-Lara.*
11:15-11:30	S1-O5	Epidemiology of tomato brown rugose fruit virus in active greenhouses. *Zafeiro Zisi.*
11:30-11:45	S1-O6	Tomato brown rugose fruit virus in aqueous environments – survival and significance of water-mediated transmission. *Nataša Mehle.*
11:45-12:00	S1-O7	Cotton leafroll dwarf disease: an enigmatic virus disease on cotton in Georgia, USA. *Sudeep Bag.*

Monday 6th, June (cont.)

12:00-12:15	S1-O8	Prevalence and vector transmission of new maize viruses in São Paulo State, Brazil. *João Lopes.*
12:15-12:30	S1-O9	High-throughput sequencing survey on cereal and barley yellow dwarf viruses indicates their underestimated diversity and spread. *Merike Sõmera.*
12:30-14:15		*Lunch and Poster Exhibit*

Session 2. Diagnostics, Surveillance and Modeling

Chairs: Jan Kreuze and Marleen Botermans

14:15-14:45		***Keynote Lecture 2*** Developing elements for global plant virus management: diagnostics, surveillance, and modelling. *Jan Kreuze.*
14:45-15:00	S2-O1	Applying high throughput sequencing in a generic surveillance workflow: a case study using UK peas. *Aimee R. Fowkes.*
15:00-15:15	S2-O2	Detection of global soybean viruses in metagenomic sequence data using Microbe Finder (MiFi®). *Marcos R. Ribeiro-Junior.*
15:15-15:30	S2-O3	HTS as a powerful tool to assist the phytosanitary risks, associated with newly introduced tuber crops in Belgium. *Kris De Jonghe.*
15:30-15.45	S2-O4	Tomato brown rugose fruit virus in the Netherlands: The rise of a novel clade. *Marleen Botermans.*
15:45-16:00	S2-O5	Evaluating the threat of introducing non-European virus isolates of tomato leaf curl New Delhi virus into Europe. *Stephan Winter.*
16:00-16:30		*Coffee Break and Poster Exhibit*
16:30-16:45	S2-O6	The spread of cassava brown streak viruses, CBSV and UCBSV, from coastal Africa to the continent. *Samar Sheat.*
16:45-17:00	S2-O7	Dispersion and evolutionary history of rice yellow mottle virus in West and Central Africa: tales of rice and men. *Nils Poulicard.*
17:00-17:15	S2-O8	Deciphering the influence of soil structure and nutrients on furovirus infection rates in wheat. *Annette Niehl.*
17:15-17:30	S2-O9	Global risk predictions for Pierce's disease of grapevines. *Alex Giménez-Romero.*
17:30-18:30		Poster Session 1 (odd numbers, w/presenters)

Tuesday 7th, June

Session 3. Virus Ecology and Evolution

Chairs: Fernando García-Arenal and Peter Palukaitis

8:30-9:00		***Keynote Lecture 3*** Virus host ranges and transmission dynamics in heterogeneous environments. *Fernando García-Arenal.*
9:00-9:15	S3-O1	Plant virus mixed infections modulate vertical transmission. *Alberto Cobos.*
9:15-9:30	S3-O2	Global diversity of solanum nigrum ilarvirus 1 among diverse plant hosts and associated metagenomes and its biological characterization. *Mark P. S. Rivarez.*
9:30-9:45	S3-O3	Genome formula and coordinated gene expression in within-host populations of a multipartite virus. *Stéphane Blanc.*
9:45-10:00	S3-O4	A cross-environment viromics study of tomatoes, weeds and water reveals many new plant virus species and links between sample types. *Denis Kutnjak.*
10:00-10:15	S3-O5	Factors that determine the epidemiology of a crop pathogen, tobacco mild green mosaic virus, in its wild reservoir *Nicotiana glauca. Rafael De Andrés Torán.*
10:15-10:45		*Coffee Break and Poster Exhibit*
10:45-11:00	S3-O6	Phylogenetics and evolution wheat streak mosaic virus: its origin and the source of the Australian epidemic. *Adrian Fox.*
11:00-11:15	S3-O7	Decoding the wheat virome using metagenomics. *Shahideh Nouri.*
11:15-11:30	S3-O8	Viral diversity of cassava mosaic begomoviruses in coastal and western Kenya. *Anna E. Dye.*
11:30-11:45	S3-O9	Rice yellow mottle disease in western Burkina Faso: incidence, diversity and dynamics at local scale. *Charlotte Tollenaere.*
11:45-12:00	S3-O10	Phylogenetic and population analyses of cotton leafroll dwarf virus reveals extensive genomic variability and global sub-populations. *Judith K. Brown.*
12:00-12:15	S3-O11	The rare case of the first whitefly-transmitted polerovirus. *Murad Ghanim.*
12:15-12:30	S3-O12	Potato virus Y adaptation to various resistance QTL combinations in pepper and impact on host tolerance. *Thibaud Jayet.*
12:30-14:15		*Lunch and Poster Exhibit*

Tuesday 7th, June (cont.)

Session 4. Virus-Vector Interactions

Chairs: Veronique Brault and Murad Ghanim

14:15-14:45		***Keynote Lecture 4*** When plants and aphids are under the control of viruses *Veronique Brault.*
14:45-15:00	S4-O1	Yellowing viruses promoting their own spread by reducing Mature Plant Resistance to aphids in sugar beet. *Sharella Schop.*
15:00-15:15	S4-O2	Aphid symbionts influence the transmission of a plant virus. *Patricia Sanches.*
15:15-15:30	S4-O3	Complex interactions in co-occurring vector-borne pathosystems: a case study of potato virus Y and zebra chip disease on a solanaceous crop. *Arash Rashed.*
15:30-15.45	S4-O4	Virus-induced changes in tomato plants infected with tomato yellow leaf curl virus and tomato chlorosis virus influence host selection by their common vector *Bemisia tabaci. Irene Ontiveros.*
15:45-16:00	S4-O5	How do you make a mixed infection: effect of acquisition sequence on propagation of TYLCV and ToMoV by *Bemisia tabaci. Alana L. Jacobson.*
16:00-16:30		*Coffee Break and Poster Exhibit*
16:30-16:45	S4-O6	Relating acquisition of cassava mosaic begomovirus components A and B to transmission of single and co-infections by *Bemisia tabaci* SSA1-SG1. *George G. Kennedy.*
16:45-17:00	S4-O7	Specificity in transmission of old- and new-world begomoviruses by *Bemisia tabaci* MEAM1 and MED cryptic species. *Rajagopalbabu Srinivasan.*
17:00-17:15	S4-O8	Investigation of the replacement between two tomato-infecting begomoviruses from the perspective of vector transmission. *Wei-Hua Li.*
17:15-17:30	S4-O9	*In planta* production of filamentous virus-like particles: insights into 3-D models of different sweet potato infecting viruses vectored by aphids or whiteflies. *Ornela Chase.*
17:30-18:30		Poster Session 2 (even numbers, w/presenters)
18:30-21:00		**FREE CITY TOUR "Madrid de los Austrias"**

Wednesday 8th, June

Session 5. Other Vector-Borne Diseases

Chairs: Cecilia Tamborindeguy and Saskia Hogenhout

8:30-9:00		***Keynote Lecture 5*** How does '*Candidatus* Liberibacter solanacearum' manipulate plant and insect immunity?. *Cecilia Tamborindeguy.*
9:00-9:15	S5-O1	Florida citrus growers' potential 'toolbox' for Huanglongbing (HLB) management: an alphabet soup (ISVs, PDIs, IPCs, NATI etc.). *Ozgur Batuman.*
9:15-9:30	S5-O2	Vector biology, abundance, dispersal and temporal transmission dynamics shape *Xylella fastidiosa* epidemiology in Apulia. *Domenico Bosco.*
9:30-9:45	S5-O3	Elucidating the inoculation mechanism of *Xylella fastidiosa. Daniele Cornara.*
9:45-10:00	S5-O4	The immunodominant membrane protein (Imp) of Flavescence dorée phytoplasma interacts with gut proteins of insect vectors. *Luciana Galetto.*
10:00-10:15	S5-O5	Investigation of the diversity of the destructive 16SrV phytoplasma group in grapevine, hazelnut and leafhoppers. *Zala Kogej.*
10:15-10:45		*Coffee Break and Poster Exhibit*

Session 6. Disease Management

Chairs: Hanu R. Pappu and P. Lava Kumar

10:45-11:15		***Keynote Lecture 6*** Disease management in the omics era: Status and future prospects. *Hanu R. Pappu.*
11:15-11:30	S6-O1	The interplay between Autophagy and defense hormone Salicylic acid shape disease during viral infection and contribute to host resistance. *Aayushi Shukla.*
11:30-11:45	S6-O2	Habitat manipulation for sustainable management of *Philaenus spumarius,* the European vector of *Xylella fastidiosa. Alberto Fereres.*
11:45-12:00	S6-O3	Specificity of resistance and tolerance to cucumber vein yellowing virus in melon accessions and evidence for resistance-breaking associated with a single mutation in VPg. *Cécile Desbiez.*
12:00-12:15	S6-O4	Advances in epidemiology and control of banana bunchy top virus disease pandemic in sub-Saharan Africa. *P. Lava Kumar.*
12:15-12:30	S6-O5	Integrated control of a polerovirus. *John A. Walsh.*
12:30-14:15		*Lunch and Poster Exhibit*
14:15-14:30	S6-O6	Efficacy of the insecticide dimpropyridaz (AxaliON™) against the transmission of barley yellow dwarf virus (BYDV). *Jorge Sanz-Gomez.*
14:30-14:45	S6-O7	Use of glandular trichomes to control whitefly-transmitted viruses in tomato: modulation by natural enemies. *Enrique Moriones.*

Wednesday 8th, June (cont.)

Session 7. Climate Change

Chair: Tomás Canto

14:45-15:15		***Keynote lecture 7*** Anthropogenic climate change and its impact on interactions between viruses and plants. *Tomás Canto.*
15:15-15:30	S7-O1	Milder autumns may increase risk for infection of crops with turnip yellows virus. *Anders Kvarnheden.*
15:30-15:45	S7-O2	Modelling the effects of climate change on plant virus vertical transmission and prevalence. *Álvaro Gutiérrez-Sánchez.*
15:45-16:00	S7-O3	Stability of the resistance conferred by *Sbm1* and *Sbm2* against soil-borne furoviruses in wheat in the context of climate change. *Kevin Gauthier.*
16:00-16:30		*Coffee Break and Poster Exhibit*
16:30-17:30		**BUSINESS MEETING**
20:30-22:30		**CLOSING BANQUET AT RESTAURANTE JAI ALAI**

List of Posters

SESSION 1: GENERAL EPIDEMIOLOGY

S1-P1- Spread and genetic diversity of two badnaviruses infecting grapevine in Greece

C.L. Sassalou, L. Lotos, E. Palla, P. Panailidou, N. I. Katis, and V. I. Maliogka
Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Plant Pathology Laboratory, 54124 Thessaloniki, Greece.

S1-P2- The application of high-throughput sequencing reveals new viral pathogens implicated in the etiology of pepper yellows disease in Greece

V. Gavrili, L. Lotos, N.I. Katis, and V.I. Maliogka
Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, 54124, Thessaloniki, Greece.

S1-P3 - Occurrence of tomato yellow leaf curl virus on resistant tomato cultivars in Korea

M. Bae[1], M. Kwak[1], M. C. Son[1], H. S. Choi[2], H. R. Kwak[2], H. S. Byun[2], and E. J. Kil[1]
[1]Department of Plant Medicals, Andong National University, Korea; [2]Crop Protection Division, National Institute of Agricultural Sciences, RDA, Korea.

S1-P4- Adventitious plants act as a reservoir of cucumber mosaic virus in chili-pepper crops in northern Spain

M. Ojinaga[1], S. Mendarte[2], B. Juaristi[1], U. Apodaka[1], A. Revillas[1], A. Ortiz-Barredo[1], and S. Larregla[1]
[1]Plant Production and Protection Department, NEIKER - Basque Institute for Agricultural Research and Development, Derio (Bizkaia), Spain; [2]Conservation of Natural Resources Department, NEIKER - Basque Institute for Agricultural Research and Development, Derio (Bizkaia), Spain.

S1-P5- Infectious clones of tomato black ring virus fused with green fluorescence protein (GFP) as a tool for pathogenesis monitoring in plant tissues

A. Zarzyńska-Nowak[1], J. Minicka[1], P. Wieczorek[2], and B. Hasiów-Jaroszewska[1]
[1]Department of Virology and Bacteriology, Institute of Plant Protection-National Research Institute, Poznań, Poland; [2]Department of Molecular Biology and Biotechnology, Institute of Plant Protection-National Research Institute, Poznań, Poland.

S1-P6- Molecular characterization of sugarcane streak mosaic virus in Côte d'Ivoire

M. M. Ouattara[1], C. Desbiez[2], G. Girardot[2], B. Ble[3], K. A. Yao[4], K. D. Kouame[1] , and A. Schoeny[2]
[1]UFR Biosciences, Laboratoire de Biotechnologie, Agriculture et Valorisation des Ressources biologiques, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire; [2]Pathologie Végétale, INRAE, Montfavet, France; [3]Sucrivoire, UAI, Borotou-Koro, Côte d'Ivoire ; [4]Sucrivoire, UAI, Zuénoula, Côte d'Ivoire.

S1-P7- **The emergence of Tomato Leaf Curl New Delhi Virus in France**

A. Patthamapornsirikul , E. Verdin, and C. Desbiez
Pathologie Végétale, INRAE, Avignon, France.

S1-P8- **Effects of the serial passages of tomato severe rugose virus (ToSRV) by different hosts on the viral infection rate, genetic diversity and population structure**

C. G. Ferro, G. M. Favara, H. D. Kraide, and J. A. M. Rezende
Plant Pathology and Nematology Department, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, SP, Brazil.

S1-P9- **Effect of temperature and between-host transmission on the induction of tomato black ring virus defective RNA particles**

D. Budzyńska, J. Minicka, A. Taberska, and B. Hasiów-Jaroszewska
Department of Virology and Bacteriology, Institute of Plant Protection-National Research Institute, Poznań, Poland.

S1-P10- **Virome analyses of *Cnidium officinale* infecting viruses based on next-generation sequencing in Korea**

J. H. Kang[1], M. Kwak[1], C. R. Jung[2], J.-B. Yoon[3], and E. J. Kil[1]
[1]Department of Plant Medicals, Andong National University, Korea; [2]Forest Medicinal Resources Research Center, NiFoS, Korea; [3]Horticulture and Herbal Crop Environment Division, National Institute of Horticultural and Herbal Science, RDA, Korea.

S1-P11- **The effect of satellite RNAs on the accumulation of tomato black ring virus in different hosts**

J. Minicka[1], A. Taberska[1], A. Zarzyńska-Nowak[1], S.F. Elena[2,3], and B. Hasiów-Jaroszewska[1]
[1]Department of Virology and Bacteriology, Institute of Plant Protection – National Research Institute, Poznań, Poland; [2]Instituto de Biología Integrativa de Sistemas, CSIC-Universitat de València, València, Spain; [3]The Santa Fe Institute, Santa Fe, NM, USA.

S1-P12- **Epidemiology and factors influencing the spatial spread of yam mosaic virus in yam fields in Nigeria**

B.O. Osundahunsi[1], B. Odu[2], B. Aighewi[3], N. Maroya[1] and P. Lava Kumar[1]*
[1]International Institute of Tropical Agriculture (IITA), Oyo Road, PMB 5320, Ibadan, Nigeria; [2]Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria; [3]IITA, Kubwa, PMB 82, Abuja, Nigeria.

S1-P13- **Evaluation of the RNA silencing suppression ability of three cherry virus F encoded proteins**

L. Lotos*, A. Katsiani*, N.I. Katis, and V.I. Maliogka
*Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Plant Pathology Laboratory, 54124, Thessaloniki, Greece. *Both authors have contributed equally.*

S1-P14- **Differences in the 3' intergenic region and the V2 protein of two variants of Tomato curly stunt virus play an important role in disease pathology in *Nicotiana benthamiana***

M. E. C. Rey, A. M. Zwolinski, and A. Brigden

University of the Witwatersrand, School of Molecular and Cell Biology, South Africa.

S1-P15- Survey for identification and epidemiology of citrus viroids in various *Citrus spp.* in Greece

N. Tektonidis, A. Karagianni, L. Mikalef, and M. M. Mathioudakis
Plant Pathology Laboratory, Institute of Olive tree, Subtropical Crops & Viticulture / ELGO-DIMITRA, Chania,Crete, Greece.

S1-P16- High throughput sequencing of the tomato viromes in Korea

M. Kwak[1], M. Bae[1], M. C. Son[1], H. S. Choi[2], H. R. Kwak[2], H. S. Byun[2], and E. J. Kil[1]
[1]Department of Plant Medicals, Andong National University, Korea; [2]Crop Protection Division, National Institute of Agricultural Sciences, RDA, Korea.

S1-P17- Virome release of an invasive exotic plant species in Southern France

O. Moubset[1], D. Filloux[1], H. Fontes[2], C. Julian[1], E. Fernandez[1], L. Claude[1], F. Chiroleu[3], F. Mesleard[2,4], S. Kraberger[8], J. Custer[8], A. Salywon[5], E. Makings[6], D. P. Martin[7], A. Varsani[8,9], and P. Roumagnac[1]
[1]CIRAD, BGPI, Montpellier, France; [2]Tour du Valat, Institut de recherche pour la conservation des zones humides méditerranéennes, Le Sambuc-Arles, France; [3]CIRAD, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France; [4]Institut Méditerranéen de Biodiversité et Ecologie, UMR CNRS-IRD, Avignon Université Aix-Marseille Université, IUT d'Avignon, 84911 Avignon, France; [5]Desert Botanical Garden, Phoenix, AZ, United States; [6]Arizona State University, Tempe, AZ, USA; [7]Department of Integrative Biomedical Sciences, Institute of infectious Diseases and molecular Medicine, University of Cape Town, Cape Town, South Africa; [8]The Biodesign Center for Fundamental and Applied Microbiomics, Center for Evolution and Medicine, School of Life Sciences, Arizona State University, Tempe, AZ, USA; [9] Department of Integrative Biomedical Sciences, Structural Biology Research Unit, University of Cape Town, Observatory, Cape Town, South Africa.

S1-P18- Identification of a new 'old' tobamovirus originating from pepper

R.A.A. van der Vlugt[1], P. van Bekkum[1], and C.C.M.M. Stijger[2]
[1]Wageningen Plant Research, Wageningen University and Research, Wageningen The Netherlands; [2]Wageningen Greenhouse Horticulture and Flowerbulbs, Wageningen University and Research, Wageningen, The Netherlands.

SESSION 2: DIAGNOSTICS, SURVEILLANCE AND MODELING

S2-P1- High throughput sequencing identifies a divergent strain of cherry latent virus 1 in sweet cherry in Greece

C.G. Orfanidou, A. Katsiani, N.I. Katis, and V.I. Maliogka

Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Plant Pathology Laboratory, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.

S2-P2- Improving strategies for the detection of tomato brown rugose fruit virus

A. Skelton[1], A. Fowkes[1], L. Frew[1], J. van Gemert[2], Y. L. Loh[1], O. Maksimovic[3], R. Macarthur[1], M. Botermans[2], and A. Fox[1]

[1]Fera Science Ltd, York Biotech Campus, Sand Hutton, York, United Kingdom; [2]National Plant Protection Organization, Geertjesweg 15, 6706 EA Wageningen, The Netherlands; [3]National Institute of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia.

S2-P3- Development of quick and accurate diagnostic methods for tomato infecting viruses based on RPA and RT-RPA techniques

M. Kwak[1], W. K.Cho[2], and E. J. Kil[1]

[1]Department of Plant Medicals, Andong National University, Korea; [2]College of Biotechnology and Bioengineering, Sungkyunkwan University, Korea.

S2-P4- Unveiling the virome of stone fruit trees in Greece using high throughput sequencing approaches

A. Katsiani[1], C.L. Sassalou[1], C. Orfanidou[1], C. Beta[1], N. I. Katis[1], P. Drogoudi[2], and V.I. Maliogka[1]

[1]Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Plant Pathology Laboratory, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; [2]Department of Deciduous Fruit Trees, Hellenic Agricultural Organization-'DIMITRA', Institute of Plant Breeding and Genetic Resources, 38 R.R. Station, 59035 Naoussa, Greece.

S2-P5- A decade of foreign pest interceptions and introductions in Spain

B. Dáder[1], E. Viñuela[1], and P. del Estal[1,2]

[1]Department of Crop Production, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain; [2]National Reference Laboratory for Arthropods, Spain.

S2-P6- Characterization of the viral community associated with pineapple mealybug wilt diseased plants in Reunion Island through a metagenomic approach

D. Massé[1,2], T. Candresse[3], D. Filloux[4], S. Massart[5], N. Cassam[1], B. Hostachy[1], A. Marais-Colombel[3], E. Fernandez[4], P. Roumagnac[4], E. Verdin[6], P. Y. Teycheney[7], P. Lefeuvre[7], and J. M. Lett[7]

[1]ANSES - LSV RAPT, Saint Pierre, La Réunion, France ; [2]Université de La Réunion, UMR PVBMT, Pôle de Protection des Plantes, Saint-Pierre, Ile de La Réunion, France ; [3]INRAe, UMR 1332 Biologie du Fruit et Pathologie, INRAe-Univ. Bordeaux, Villenave d'Ornon, France ; [4]CIRAD, UMR PHIM, Campus International de Montferrier-Baillarguet, Montpellier, France ; [5]Plant Pathology Laboratory, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgique ;

[6]INRAe, UR407 Unité de Pathologie Végétale, Montfavet, France ; [7]CIRAD, UMR PVBMT, Pôle de Protection des Plantes, Saint-Pierre, Ile de La Réunion, France.

S2-P7- **Early warning system and mitigation strategies for Tomato brown rugose fruit virus**

E. Vogel[1], Z. Zisi[2], N. Ortega-Parra[3], C. Vos[2], and I. Hanssen[1]

[1]De Ceuster Meststoffen NV (DCM), Belgium; [2]Scientia Terrae Research Institute VZW, Belgium; [3]De Ceuster Meststoffen BV (DCM), the Netherlands.

S2-P8- **Schlumbergera virus X in Dragon Fruit (*Hylocereus spp.*) in Spain**

D. Janssen, C. García, and L. Ruiz

Centro La Mojonera, IFAPA, Almeria, Spain.

S2-P9- **Development of a reverse transcription loop-mediated isothermal amplification assay for detection of pepper viruses**

E. Baek[1], J. Y. Yoon[2], and P. Palukaitis[1]

[1]Department of Horticultural Science, Seoul Women's University, Seoul, Republic of Korea; [2]Graduate School on Plant Protection and Quarantine, Jeonbuk National University, Jeonju, Republic of Korea.

S2-P10- **Detection of Augusta disease in tulip and other flowerbulbs**

B. Mulder[1], E. T.M. Meekes[2], I. van Duivenbode[3], C. de Krom[4], I. C.C.M.M. Stijger[5], M. Verbeek[6], and I. J.E. Stulemeijer[1]

[1]Flower Bulb Inspection Service (BKD), Lisse, The Netherlands; [2]Naktuinbouw, Roelofarendsveen, The Netherlands; [3]Dutch General Inspection Service for agricultural seeds and seed potatoes (NAK), Emmeloord, The Netherlands; [4]National Plant Protection Organization (NPPO-NL), Wageningen, The Netherlands, [5]Greenhouse horticulture & Flower Bulbs, Wageningen University & Research (WUR), Bleiswijk, The Netherlands; [6]Biointeractions and Plant Health, Wageningen University & Research (WUR), Wageningen, The Netherlands.

S2-P11- **Comparative sensitivity of nucleic acid-based diagnostic assays for detecting the banana bunchy top virus in banana plants and aphid vector**

A. O. Adediji[1,2], G.I. Atiri[2], and P. L. Kumar[1]

[1]International Institute of Tropical Agriculture, Oyo Road, PMB 5320, Ibadan, Nigeria; [2]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria.

S2-P12- **Rapid and sensitive detection of rice stripe virus by RT-RPA and real-time RT-RPA methods**

J. Jeon, and E. J. Kil

Department of plant Medicals, Andong National University, Andong, Korea.

S2-P13- **Metagenomic probes for the rapid identification of quarantine viruses in cereal grains**

R. Gomes Ruschel[1,2], A.S. Espindola[2], F. Ochoa-Corona[1,2], M.R. Ribeiro-Junior[1,2,3], M. Malapi-Wight[4], X. Hu[5], and O. Hurtado-Gonzales[5]

[1]Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma, United States; [2]Institute of Biosecurity and Microbial Forensic, Stillwater,

Oklahoma, United States; [3]Department of Plant Protection, School of Agriculture, Sao Paulo State University, Botucatu, Sao Paulo, Brazil; [4]USDA-APHIS-BRS Biotechnology Risk Analysis Programs, Riverdale, Maryland, United States; [5]USDA-APHIS-PPQ Plant Germplasm Quarantine Program – Beltsville, Maryland, United States.

S2-P14- Mix infection of garlic virus D (GarVD), onion yellow dwarf virus (OYDV), and leek yellow stripe virus (LYSV) in garlic in Oklahoma, USA

M. R. Ribeiro-Junior[1,2,3], D. M. do Nascimento[2] , R. Gomes Ruschel[1,2], J. Olson[1], S. Wallace[1], and F.M. Ochoa-Corona[1,2]

[1]Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma, United States; [2]Institute of Biosecurity and Microbial Forensic, Stillwater, Oklahoma, United States; [3]Department of Plant Protection, School of Agriculture, Sao Paulo State University, Botucatu, Sao Paulo, Brazil.

S2-P15- Diversity of yellow dwarf viruses in south-eastern Australia

N. Nancarrow[1,2], S. Maina[3], S.K. Lam[1], B. Rodoni[4], M. Afab[2], and P. Trębicki[1,2]

[1]The University of Melbourne, Melbourne, Australia [2]Agriculture Victoria, Grains Innovation Park, Horsham, Australia [3]NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, Australia [4]Agriculture Victoria, AgriBio, Bundoora, Australia.

S2-P16- Toward the characterization of a novel Bymovirus infecting rice in Burkina Faso by combining metagenomics and targeted sequencing

M. Bangratz[1], M. Barro[2], D. Sérémé[2], I. Wonni[2], A. I. Kassankogno[2], P. Roumagnac[1], D. Filloux[1], E. Fernandez[1], J. Orjuela[1], A. Comte[1], C. Tollenaere[1]*, and N. Poulicard[1]*

*[1]PHIM Plant Health Institute, Univ Montpellier, IRD, CIRAD, INRAE, Institut Agro, Montpellier, France; [2]Laboratoire de Phytopathologie, INERA, Institut de l'Environnement et de Recherches Agricoles du Burkina Faso, Bobo-Dioulasso, Burkina Faso. * Equal contribution.*

S2-P17- Cucurbit chlorotic yellows virus is widespread in mixed infections on cucurbits and also infects wild radish (*Raphanus raphanistrum*), a common weed in Georgia, USA

S.R. Kavalappara[1], S. Bag[1], A. Sparks[2], C. Mcgregor[3], D. G. Riley[2], and W.M. Wintermantel[4]

[1]Department of Plant Pathology, University of Georgia, Tifton, GA, USA; [2]Department of Entomology, University of Georgia Tifton, GA, USA; [3]Department of Horticulture, University of Georgia, Athens, GA, USA; [4]United States Department of Agriculture-Agricultural Research Service, Salinas, CA, USA.

S2-P18- Vector-borne diseases with non-stationary vector populations: the case of growing and decaying populations

A. Giménez-Romero[1], R. Flaquer-Galmés[2], and M. A. Matías[1]

[1]Instituto de Física Interdisciplinar y Sistemas Complejos, IFISC (UIB-CSIC), 07122 Palma de Mallorca, Spain; *[2]Grup de Física Estadística, Departament de Física. Facultat de Ciències, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain.*

S2-P19- **Modelling plant resistance deployment: the R package landsepi**

L. Rimbaud[1], J. Papaïx[2], J. F. Rey[2], J. L. Gaussen[2], M. Zaffaroni[3], and F. Fabre[3]

[1]INRAE – Pathologie Végétale, 84140, Montfavet, France; [2]INRAE – BioSP, 84914, Avignon, France; [3]INRAE – SAVE, 33882 Villenave d'Ornon, France.

SESSION 3: VIRUS ECOLOGY & EVOLUTION

S3-P1- Plant-virus interactions in a heterogeneous landscape: analysis of four tobamovirus species

A.D. Zamfir, B.M. Babalola, A. Fraile, M. J. McLeish, and F. García-Arenal
Centro de Biotecnología y Genómica de Plantas (CBGP), Universidad Politécnica de Madrid (UPM) and Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and E.T.S.I. Agronómica, Alimentaria y de Biosistemas, Campus de Montegancedo, UPM, 28223 Pozuelo de Alarcón, Madrid, Spain.

S3-P2- Genetic variability and evidence of a new subgroup in Watermelon mosaic virus isolates infecting cucurbits in the United States

O. A. Abdalla[1, 2], and A. Ali[1]
[1]Department of Biological Science, The University of Tulsa, Tulsa Oklahoma, 74104, US; [2]Present address: Department of Plant Pathology, Faculty of Agriculture, Assiut University, Assiut, Egypt.

S3-P3- Influence of habitat on incidence, host use & genetic structure of cucumber mosaic virus (CMV)

B. M. Babalola, A. Fraile, M. J. McLeish, and F. García-Arenal
Centro de Biotecnología y Genómica de Plantas (CBGP), Universidad Politécnica de Madrid (UPM) and Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and E.T.S.I. Agronómica, Alimentaria y de Biosistemas, Campus de Montegancedo, UPM, 28223 Pozuelo de Alarcón, Madrid, Spain.

S3-P4- Targeting of genomic and minus strands of viral RNA contributes to amiRNA-mediated antiviral resistance and promotes the emergence of complex viral populations

F. Mesel[1], M. Zhao[1,2], B. García[1], J. A. García[1], and C. Simón-Mateo[1]
[1]Department of Plant Molecular Genetics, Centro Nacional de Biotecnología (CNB-CSIC), Campus Universidad Autónoma de Madrid, 28049 Madrid, Spain; [2]College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot 010018, China.

S3-P5- Virus diversity in natural Dutch plant ecosystems along a chronosequence

D.E. Boezen[1], C.M. Malmstrom[2], R.A.A. van der Vlugt[3], and M.P. Zwart[1]
[1]Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands; [2]Department of Plant Biology, Michigan State University, East Lansing (MI), USA; [3]Laboratory of Virology, Wageningen University and Research, Wageningen, The Netherlands.

S3-P6- Wild plant species: enemies or allies of carrot crops?

F. Salavert[1], H. McGrath[2], A. Fox[3], I. Adams[3], and N. Boonham[1]
[1]Newcastle University, Newcastle Upon Tyne, UK; [2]Rothamsted Research, Harpenden, UK; [3]FERA Science Ltd, York, UK.

S3-P7- **Characterization of the biological properties of a chimeric potyvirus, and of its adaptation to a compatible experimental host**

H. Sun[1], F. del Toro[1], M. Makki[2], F. Tenllado[1], and T. Canto[1]

[1]Department of Microbial and Plant Biotechnology. Margarita Salas Center for Biological Research. Ramiro de Maeztu 9, 28040 Madrid, Spain; [2]Laboratory of Molecular Genetics, Immunology and Biotechnology. Faculty of Sciences. University of Tunis El Manar. Manar II, Tunis 2092, Tunisia.

S3-P8- **Molecular characterization of watermelon mosaic virus isolates infecting zucchini and pumpkin plants in the Czech Republic**

K. Ben Mansour[1], M. Komínková[2], P. Komínek[2], J. Brožová[2], J. Kazda[1], M. Zouhar[1], and P. Ryšánek[1]

[1]Department of Plant Protection, Czech University of Life Sciences, Prague, Czech Republic; [2]Crop Research Institute, Prague, Czech Republic.

S3-P9- **Multipartite virus genome formula variability in local lesions of *Chenopodium quinoa***

M. Johnson[1, 2] and M. Zwart[1]

[1]Microbial Ecology, Netherlands Institute of Ecology, NIOO-KNAW, Wageningen, The Netherlands; [2]Department of Virology, Wageningen University and Research, Wageningen, The Netherlands.

S3-P10- **TuYV isolates found in Sugar beet**

S. Schop[1], E. Raaijmakers[2], and R.A.A. van der Vlugt[1]

[1]Laboratory of Virology, Wageningen University and Research, The Netherlands; [2]IRS (Dutch Sugar Beet Research Institute), Dinteloord, The Netherlands.

S3-P11- **High genetic diversity of *Plum pox virus* in subspontaneous trees in North Macedonia sheds new light into its evolutionary history**

S. Dallot[1], M. Brevet[1], D. Filloux[2], E. Fernandez[2], R. Rusevski[3], B. Kuzmanovska[3], and G. Thébaud[1]

[1]PHIM Plant Health Institute, INRAE, Univ Montpellier, CIRAD, Institut Agro, IRD, Montpellier, France; [2]PHIM, CIRAD, Montpellier, France; [3]Department of Plant Pathology, Ss. Cyril and Methodius University, Skopje, North Macedonia.

SESSION 4: VIRUS-VECTOR INTERACTIONS

S4-P1- **Two populations of *Bemisia tabaci* Mediterranean in Brazil are unable to transmit three native begomoviruses**

A.M. Nogueira[1], V. H. Bello[1], E. Vicentin[1], C. S. de Oliveira[1], C. C. Martines[1], T.M.C. Barbosa[2], E. S. Gorayeb[1], L. F. M. Watanabe[1], J. M. Marubayashi[1], F. M. Zerbini[2], M. Ghanim[3], J. A. M. Rezende[4], M. A. Pavan[1], and R. Krause-Sakate[1]

[1]Departamento de Proteção de Plantas, Faculdade de Ciências Agronômicas, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, Brazil; [2]Departmento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Brazil; [3]The Volcani Center, Institute of Plant Protection, Bet Dagan, Israel; [4]Departmento de Patologia de Plantas e Nematologia, E.S.A. Luiz de Queiroz, Universidade de São Paulo, Piracicaba, Brazil.

S4-P2- **Towards understanding the importance of mixed infections for vector-mediated spread of sweet potato virus diseases**

C. Ontañón, O. Chase, and J.J. López-Moya

Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Cerdanyola del Vallès, Barcelona, Spain.

S4-P3- **Aphid response to volatiles emitted by melon plants after single and mixed virus-infection**

E. Garzo, A. Moreno, and A. Fereres

Instituto de Ciencias Agrarias, CSIC, Madrid Spain

S4-P4- **Detecting virus-carrying *Xiphinema* spp. as an alternative to species identification of *Xiphinema* in trade**

E. Everaert, N. Viaene, and K. De Jonghe

Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Merelbeke, Belgium.

S4-P5- Bemisia tabaci **Mediterranean cryptic species survey on soybean in Sao Paulo State (Brazil) and its interaction with cowpea mild mottle virus**

F. Barreto da Silva[1], J. Uzan[1], J. M. Marubayashi[1], R. S. Raposo[1], C. C. Martines[1], M. R. Ribeiro-Junior[1,2], A. M. Nogueira-Portilho[1], M. A. Pavan[1], and R. Krause-Sakate[1]

[1]Departamento de Proteção de Plantas, Faculdade de Ciências Agronômicas, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, Brazil; [2]Institute of Biosecurity and Microbial Forensic – Oklahoma State University - Stillwater, Oklahoma, United States.

S4-P6- **Pre-infection of tomato plants carrying Sw-5 gene with tomato chlorosis virus does not seem to alter the infection with groundnut ringspot virus**

H. D. Kraide[1], V. M. Camelo-García[2], G. M. Favara[1], F. F. de Oliveira[1], C. G. Ferro[1], E. Y. N. Carmo[1], E. F. B. Lima[3], A. B. Filho[1], and J. A. M. Rezende[1]

[1] Plant Pathology and Nematology Department, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, SP, Brazil; [2] United States Department of Agriculture, Agricultural Research Service, Salina, CA, USA; [3] Biological Sciences Departmente, Campus Amilcar Ferreira Sobral, Federal University of Piauí, Floriano, PI, Brazil.

S4-P7- **New insights about the biology and structure of insect-transmitted plant viruses of the family *Secoviridae***

I. Ferriol[1,2], M. Byrne[3], A. Javed[3], N. Ranson[3], G. P. Lomonossoff[4], and J. J. López-Moya[1]

[1]Centre for Research in Agricultural Genomics (CRAG-UAB-UB-CSIC-IRTA), Spain; [2]Instituto de Ciencias Agrarias (ICA-CSIC), Spain; [3]University of Leeds, Leeds, England; [4]John Innes Centre (JIC), Norwich, England.

S4-P8- **Endosymbiont community structure of rice stripe virus-viruliferous *Laodelphax striatellus* (Hemiptera: Delphacidae) in Korea**

M. Kwon, and E.-J. Kil

Department of Plant Medicals, Andong National University, Korea.

S4-P9- **A database on the transmission of plant viruses**

D. Peters[1], P. van Vredendaal[2], and R.A.A. van der Vlugt[1]

[1]Laboratory of Virology, Wageningen University and Research, The Netherlands; [2]Wageningen Library, Wageningen University and Research, The Netherlands.

S4-P10- **The role of HCPro in the transmission properties of nine PVY field isolates from Tunisia**

M. Makki[2], H. Sun[1], F. del Toro[1], K. Necira[2], F. Tenllado[1], F. Khouaja[2], and T. Canto[1]

[1]Department of Microbial and Plant Biotechnology. Margarita Salas Center for Biological Research. Ramiro de Maeztu 9, 28040 Madrid, Spain; [2]Laboratory of Molecular Genetics, Immunology and Biotechnology (LR99ES12). Faculty of Sciences. University of Tunis El Manar. Manar II, Tunis 2092, Tunisia.

SESSION 5: OTHER VECTOR-BORNE DISEASES

S5-P1- Impact of insecticides in the feeding behaviour of *Philaenus spumarius* associated to the transmission of *Xylella fastidiosa*

C. Lago[1,2], D. Cornara[1,3,4], S. A. Minutillo[4], A. Moreno[1,5], and A. Fereres[1,5]

[1]Instituto de Ciencias Agrarias (ICA), Consejo Superior de Investigaciones Científicas (CSIC), Calle Serrano 115dpdo, 28006 Madrid, Spain; [2]Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas (ETSIAAB), Universidad Politécnica de Madrid (UPM), Campus, Av. Puerta de Hierro, nº 2, 4, 28040 Madrid, Spain; [3]Department of Soil, Plant and Food Sciences, Entomological and Zoological Section, University of Bari Aldo Moro, Bari, Italy; [4]International Centre for Advanced Mediterranean Agronomic Studies - Institute of Bari (CIHEAM-Bari), Via Ceglie 9, 70010 Valenzano (BA), Italy; [5]Associate Unit IVAS (CSIC-UPM), Control of Insect Vectors of Viruses in Horticultural Sustainable Systems, Madrid, Spain.

S5- P2- Landscape complexity promotes the abundance of potential vectors of *Xylella fastidiosa* in Portuguese vineyards

I. Rodrigues[1,2], M. Villa[1], P. Baptista[1], and J. A. Pereira[1]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; [2]Departamento de Ingeniería Agrária, Universidad de Léon, Av. Portugal, n° 41, 24071 Léon, Spain.

S5- P3- Olfactory behavior of *Philaenus spumarius* and *Cicadella viridis* to cis-3-hexen-1-ol and cis-3-hexenyl acetate

I. Rodrigues[1,2], J. Benhadi-Marín[1], N. Rodrigues[1], P. Baptista[1], and J. A. Pereira[1]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; [2]Departamento de Ingeniería Agrária, Universidad de Léon, Av. Portugal, n° 41, 24071 Léon, Spain.

S5- P4- Association of '*Candidatus* Phytoplasma asteris' (group 16SrI) and '*Candidatus* Phytoplasma fraxini' with a new syndrome in potato crops in Colombia

L. Franco-Lara [1], C. A. Varela-Correa [1], G. P. Guerrero Carranza [1], and J. C. Quintero Vargas[2]

[1] Faculty of Basic and Applied Sciences, Universidad Militar Nueva Granada, Km2 via Cajicá - Zipaquirá, Cajicá, Colombia; [2] Instituto Colombiano Agropecuario (ICA), Carrera 68A N° 24B – 10 - Edificio Plaza Claro- Torre 3, Bogotá D.C., Colombia.

S5-P5- In planta distribution of '*Candidatus* Phytoplasma asteris' (16SrI) and '*Candidatus* Phytoplasma fraxini' (16SrVII) infecting *Quercus humboldtii* trees in mixed infections

J. Lamilla[1], L. Franco-Lara [1], and Y. Arocha Rosete[2]

[1]Faculty of Basic and Applied Sciences, Universidad Militar Nueva Granada, Cundinamarca, Colombia; [2]Sporometrics Inc., Toronto, Ontario M6K 3J1, Canada.

S5-P6- **Preparedness for *Xylella fastidiosa* in Australia; understand biology, physiology and ecology or potential vectors**

P. Trębicki[1,2]

[1]The University of Melbourne, Melbourne, Australia; [2]Agriculture Victoria, Grains Innovation Park, Horsham, Australia.

S5-P7- **Finding a suitable host plant for transmission trials and infectivity screening with *Xylella fastidiosa* and its vector *Philaenus spumarius***

S. Avosani[1], G. Cavallo[1], M.L. Vitale[1], M. Ripamonti[3a], N. Bodino[3], D. Bosco[4], V. Verrastro[1], and D. Cornara[1,2]

[1]CIHEAM-Bari, Valenzano, Italy; [2]Department of Environmental Science, Policy, & Management, UC Berkeley, USA; [3]Institute for Sustainable Plant Protection - National Research Council of Italy, IPSP-CNR, Torino, Italy; [4]Department of Agriculture, Forest, and Food Sciences (DiSAFA), University of Turin, Grugliasco (TO), [a]Present address: Luxembourg Institute of Science and Technology (LIST), Luxembourg.

S5-P8- **Population genomics of the meadow spittlebug *Philaenus spumarius*, the main insect vector of *Xylella fastidiosa* in Europe**

R. Biello[1], Q. Liu[1], S. T. Mugford[1], A. Stewart[2], C. Harkin[2], K. Lester[3], R. Cairns[3], M. Wilson[4], S. Conyers[5], D. Allen[5], D. De Marzo[5], G. Clover[1], T. C. Mathers[1], and S. A. Hogenhout[1]

[1]Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich, United Kingdom; [2]University of Sussex, Brighton, United Kingdom; [3]SASA, Edinburgh, United Kingdom; [4]National Museum Wales, Cardiff, United Kingdom; [5]FERA, York, United Kingdom.

S5-P9- **New sustainable approaches for interfering with vector-borne plant pathogens transmission**

V. Zaffaroni-Caorsi[1], D. Cornara[2,3], D. Bosco[4], C. Marzachi[5], and V. Mazzoni[6]

[1]C3A Centro Agricoltura, Alimenti e Ambiente, University of Trento, Trento, Italy; [2]DISSPA, University of Bari Aldo Moro, Bari, Italy; [3]CIHEAM—IAMB International Centre for Advanced Mediterranean Agronomic Studies, Bari, Italy; [4]DISAFA, University of Torino, Grugliasco (TO), Italy; [5] Institute for Sustainable Plant Protection, CNR, Torino, Italy; [6]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy.

SESSION 6: DISEASE MANAGEMENT

S6-P1- **Survival and disinfection of tomato brown rugose fruit virus on common glasshouse surfaces**

A. Skelton, L. Frew, A. Fowkes, and A. Fox
Fera Science Ltd, York Biotech Campus, Sand Hutton, York, United Kingdom.

S6-P2- **Impact of dimpropyridaz (AxaliON™) on the feeding behaviour of aphid and whitefly vectors of plant viruses**

A. Moreno [1], A. Herraiz[1], J. Sanz-Gomez[2], and A. Fereres[1]
[1]Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, Madrid, Spain; [2]BASF SE, Agricultural Solutions, Limburgerhof, Germany.

S6-P3- **Integrated pest management controls the spread of tomato leaf curl New Delhi virus in zucchini crops.**

D. Janssen, M.M. Tellez, E. Rodriguez, A. Simón, M. Boulares, and L. Ruiz
Centro La Mojonera, IFAPA, Almeria, Spain.

S6-P4- **Secondary dissemination of tomato severe rugose virus (ToSRV) and tomato chlorosis virus (ToCV) in tomato fields under the effect of insecticides**

F. F. de Oliveira, G. M. Favara, V. H. Bello, H. D. Kraide, C. G. Ferro, E. Y. N. Carmo, A. B. Filho, and J. A. M. Rezende
[1] Plant Pathology and Nematology Department, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, SP, Brazil.

S6-P5- **A high-throughput image analysis method for assessing pepper quantitative resistance to Cucumber mosaic virus**

J. Hirsch[1], M. Szadkowski[1], S. Piry[1,2], C. Lacroix[1], L. McLeod[3], B. Moury[1], V. Lefebvre[3]
[1]Pathologie Végétale, INRAE, 84140 Montfavet, France; [2]CBGP, INRAE, CIRAD, IRD, Institut Agro, Univ. Montpellier, Montpellier, France; [3]INRAE, GAFL, F-84140 Montfavet, France.

S6-P6- **Degeneration of Clean Virus-tested Sweetpotato Seed in High and Low Virus Pressure Areas at the Lake Zone, Tanzania**

K.O. Ogero[1, 2*], J.F. Kreuze[3], M.A. McEwan[4], C.J.M. Almekinders[5], P.C. Struik[6], and R.A.A. van der Vlugt[2]
[1]International Potato Center (CIP), C/o Tanzania Agricultural Research Institute (TARI) Tanzania; [2]Laboratory of Virology, Wageningen University and Research (WUR), The Netherlands; [3]International Potato Center, (CIP) Peru; [4]International Potato Center, Regional Office for Sub-Saharan Africa (CIP – SSA), Kenya; [5]Knowledge Technology and Innovation Group, Social Sciences, Wageningen University and Research (WUR), The Netherlands; [6]Centre for Crop Systems Analysis, Wageningen University and Research (WUR), The Netherlands.

S6-P7- **Field biotest development with virus inoculation in sugar beet – efficacy of dimpropyridaz (AxaliON™) against *Beet mild yellowing virus* (BMYV) transmitted by *Myzus persicae***

M. Varrelmann[1], R. Hossain[1], C. Lachmann[1], and J. Sanz-Gomez[2]

[1]*Institute of Sugar Beet Research at the University of Göttingen, Department of Phytopathology, Germany;* [2]*BASF SE, Agricultural Solutions, Limburgerhof, Germany.*

S6-P8- **Application of a reverse genetic system for Beet necrotic yellow vein virus to study Rz1 resistance breaking in sugar beet**

S. Liebe[1], E. Maiss[2], and M. Varrelmann[1]

[1]*Institute for Sugar Beet Research, Department Phytopathology, Holtenser Landstr. 77, D-37077 Göttingen;* [2]*Leibniz University Hannover, Institute of Horticultural Production Systems, Dept. Phytomedicine, Herrenhäuser Str.2, 30419 Hannover.*

S6-P9- **Development of *Agrobacterium tumefaciens* infiltration of infectious clones of grapevine geminivirus A directly into greenhouse-grown grapevine and *Nicotiana benthamiana* plants**

Y.-W. Kuo[1], A. Bednarska[1], M. Al Rwahnih[1,2], and B. W. Falk[1]

[1]*Department of Plant Pathology, University of California, Davis, CA 95616, U.S.A;* [2]*Foundation Plant Services, University of California, Davis, CA 95616, U.S.A.*

S6-P10- **Epidemiology and management of tomato spotted wilt virus in *Chrysanthemum morifolium* in South Korea**

J. Y. Yoon[1], J. B. Yoon[2], M. H. Seo[2], and S. K. Choi[2]

[1]*Graduate School on Plant Protection and Quarantine, Jeonbuk National University, South Korea;* [2]*Division of Horticultural and Herbal Crop Environment, NIHHS, RDA, South Korea.*

SESSION 7: CLIMATE CHANGE

NO POSTERS

Location of Events

WELCOME RECEPTION: RESIDENCIA DE ESTUDIANTES

Welcome Reception and Registration (Sunday June 5th, 19:00 –22:00) at the "Residencia de Estudiantes" located in Calle del Pinar 21, Madrid, just behind the CSIC Main Building.

Residencia de Estudiantes (Calle del Pinar 21, Madrid)

ORAL SESSIONS: ROCASOLANO BUILDING

Registration and Oral Sessions. Registration **(June 6th, 7:45 – 8:30)** and Oral Sessions (June 6th-7th, 8:30-18:30 and June 8th, 8:30–16:30) will be held at the **Instituto de Química Física Rocasolano** Calle Serrano 119, Madrid.

Instituto de Química Física Rocasolano (Rocasolano Building, Calle Serrano 119, Madrid)

POSTER SESSIONS: INSTITUTO DE FÍSICA FUNDAMENTAL

Poster Sessions and Coffee Breaks (June 6th-8th, 2022) will be held at CSIC Atrium located at the **Instituto de Física Fundamental** Calle Serrano 113b (2 min walking from the auditorium of the Rocasolano Building).

Atrium located at the Instituto de Física Fundamental (Calle Serrano 113b, Madrid)

LUNCH: INSTITUTO DE QUÍMICA ORGÁNICA

Lunch (June 6th-8th, 12:30 - 14:15) will be served each day at the **Química Orgánica Building** (Calle Serrano 148; located at 5-min walk from the Rocasolano Building).

Instituto de Química Orgánica (Calle Serrano 148, Madrid)

CLOSING BANQUET: RESTAUTANTE JAI ALAI

Closing banquet (June 8th, 20:30 - 22:30) will be held at the **Restaurante Jai Alai**, located at walking distance of the CSIC Main Campus (Calle de Balbina Valverde 2, Madrid).

Jai Alai Restaurant (Calle de Balbina Valverde 2, Madrid)

Street View Location of Events

Information for Attendees

Oral Presentations

Keynote speaker presentations will be 30-45 minutes long. The rest of the oral presentations will be 15 minutes long including time for questions. Presentations should be prepared in PowerPoint for Windows and delivered to local organizers at the Registration desk at the Instituto de Física Química (Rocasolano Building) but not later than the day before the talk. Presentations for Monday 6th of June can be delivered at the Welcome Reception on Sunday night at the Residencia de Estudiantes. There will be a limited number of computers available to preview Powerpoint presentations at the **Instituto de Ciencias Agrarias (ICA-CSIC, Calle Serrano 115b, Madrid)** building. We strongly recommend to bring your laptop to edit or preview your presentation.

Poster Presentations

Posters should be put up during the morning of the first day of the Symposium (June 6th, 2022) on the atrium located at the **Instituto de Física Fundamental** Calle Serrano 113b (2 min walking from the auditorium of the Rocasolano Building). Posters will be displayed for the entire Conference and can be viewed over the coffee break and poster exhibit hours, lunch break and during the Poster Session from 17:30 to 18:30 each day. Authors with odd numbers are requested to be present at their poster on Monday evening's Poster Session (June 6th, 2022). Authors with even numbers are requested to be present at their poster on Tuesday evening's poster session (June 7th, 2022). Posters should be taken down by 17:00 Wednesday afternoon (June 8th, 2022). Poster boards are 100 cm x 160 cm and will accommodate 90 cm wide x 120 cm high (portrait orientation) standard posters. Pins for securing posters will be provided.

Email access on the CSIC Campus and Eduroam (Configure this prior to arrival at CSIC)

There will be access to Eduroam on campus. Those participants that have no access to Eduroam will have internet access at the **Rocasolano Building** (meeting venue) using the following SSDI connection:

1. Activate your Wi-Fi
2. Connect to the Wireless SSDI Network: **ISPVE-2022**
3. Introduce the password: **1Spv3-Ev3nt15$**

Certificate of attendance and invoices

Certificates and invoices will be available upon request. We will need an ID number, Tax Number and Address to prepare invoices of registration fees.

Access to the ISPVE-2022 website

The following QR code will provide access to the online ISPVE-2022 website. Scan this QR code with your cell phone if you want to get access to the website:

SCAN ME

QR code for the ISPVE-2022 website

Covid-19 health measures

The health measures to be taken for the Covid-19 pandemic will be updated at every moment and explained during the Open Ceremony at the Instituto de Física Química Rocasolano (Rocasolano Building) on Monday 6th. Masks will be provided with the welcome package. Masks are mandatory in public transportation and some public services. We encourage to bring extra masks for your travel safety and check the health measures before your

Session

01

General Epidemiology

Emerging themes and approaches in plant virus epidemiology

M. J. Jeger[1], F. Hamelin[2], and N. Cunniffe[3]

[1]Department of Life Sciences, Imperial College London, Silwood Park, United Kingdom; [2]IGEPP INRAE, University of Rennes, Rennes, France; [3]Department of Plant Sciences, University of Cambridge, Cambridge, United Kingdom.

(*m.jeger@imperial.ac.uk)*

Keynote Lecture 1

Epidemiology is the study of the rates of temporal and spatial change of disease in populations and the determining factors underlying change. For plant viruses, transmission is a key determining factor of disease dynamics and, in most cases, depends on the interaction of viruses, host plants, and vectors, subject to the biotic and abiotic environment. The complexity of these interactions makes field-based studies difficult to interpret without supporting studies, either laboratory or microcosm-based, that provide information on the parameters involved in transmission. Problems then arise in scaling up information to the field. The hope has been that mathematical models based on the known or assumed biology can make a bridge between the specific information provided by laboratory or microcosm studies and field observations on disease dynamics; whether to provide a greater understanding, to test hypotheses, to make predictions on future change, or to improve disease management. In practice, there are few examples of such bridges being made and models used, largely because of the broader biotic and abiotic environment of field populations that add further layers of ecological complexity. How to address these challenges is an emerging theme in plant virus epidemiology. The different approaches that can be taken will be illustrated by the widespread occurrence of co-infections in plants (1), the extent to which the consequences of virus manipulation of hosts and vectors can be assessed in the field (2), and whether the ambiguities in usage of tolerance as a host defence mechanism can be modelled (3). A final emerging theme is the need to consider wild plants as a major biotic factor in the epidemiology of viruses in crops. In many cases, much epidemiological activity occurs in wild plant populations but has been rarely modelled, with the crop only an incidental host even if severe crop damage results.

References

(1) Hamelin, F.M., et al., (2019). PLoS Biol 17: e3000551.
(2) Cunniffe, N.J., et al., (2021). PLoS Comp. Biol. 17: e1009759.
(3) Jeger, M.J., et al., (2018). Plant Dis. 102: 837-854.

Whitefly-transmitted virus dominance in mixed infections varies among three cucurbit production regions in the United States

W. M. Wintermantel[1], S. Mondal[1], E. Sikora[2], T. Turini[3], R. A. Melanson[4], B. Poudel[5], J. Palumbo[5], R. Singh[6], J. D. McCreight[1]

[1]USDA-ARS, Salinas, CA, USA; [2]Auburn University, Auburn, AL, USA; [3]University of California Extension, Fresno, CA, USA; [4]Mississippi State University Extension, Raymond, MS, USA; [5]University of Arizona Extension, Yuma, AZ, USA; [6]Louisiana State University Agricultural Center, Baton Rouge, LA, USA.

(bill.wintermantel@usda.gov) *S1-O1*

Several whitefly-transmitted viruses affecting cucurbit crops have been introduced into the United States (US) over the past twenty years and have spread among production regions. Two introduced viruses in the genus *Crinivirus*, cucurbit yellow stunting disorder virus (CSYDV) and cucurbit chlorotic yellows virus (CCYV), are now present in several production areas. Multiplex RT-PCR and qRT-PCR systems were developed to examine virus prevalence and dominance during mixed infections and were used for virus detection in surveys in three US production regions, which differ in crop predominance: the Sonoran Desert production region in the Southwest, the Central Valley of California, and the Central Gulf Coast. In the Sonoran Desert where these viruses are well-established, CCYV was consistently more prevalent than CYSDV in spring, but CYSDV was the predominant virus in fall. In the Central Gulf Coast, where these viruses are just becoming established, early studies indicate a prevalence of CYSDV in early summer, compared to CCYV. CYSDV and CCYV were initially identified in the Central Valley in 2020 and in low numbers compared to CABYV, which has been prevalent in the area for decades; however, all melons with yellowing symptoms in 2021 were infected with CYSDV, suggesting its establishment in the region. Controlled melon (*C. melo* cv. Topmark) inoculation studies comparing accumulation of CYSDV and CCYV during mixed infections indicate dominance of CYSDV when it is introduced first or at the same time as CCYV and dominance of CCYV only when it infects plants prior to CYSDV. This suggests CYSDV and CCYV prevalence is partially dependent upon sequence of introduction and alternate hosts may greatly influence which virus is most prevalent during a production season. Understanding prevalence is critical to determining virus management.

Cucurbit cytorhabdovirus 1: a novel whitefly transmitted cytorhabdovirus infecting zucchini crops in Greece

C.G. Orfanidou[1], C. Beta[1], J.-S. Reynard[2], N.I. Katis[1], V.I. Maliogka[1]

[1]Plant Pathology Laboratory, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece; [2]Laboratory of Virology, Agroscope, Route de Duillier 50, 1260, Nyon, Switzerland.

(chorfani@agro.auth.gr) *S1-O2*

A new cytorhabdovirus was identified in zucchini (*Cucurbita pepo*) in Greece with the aid of high-throughput sequencing (HTS) technology. The negative-sense, single-stranded genomic RNA of the virus was determined and includes 7 open reading frames in the order 3'-N-P-P3-P4-M-G-L-5' in the antigenomic orientation. Transmission electron microscopy (TEM) revealed rhabdovirus-like particles in infected leaves. Moreover, a small-scale survey was conducted to unravel the distribution of the virus in cucurbit crops (melon, watermelon, zucchini, cucumber) and results indicated its occurrence in 30.1% (50/136) in zucchini samples and in only one melon plant (10.0%, 1/10). Based on the genome organization, TEM observations, sequence and phylogenetic analyses, we suggest its classification as a new species within the genus *Cytorhabdovirus* (family Rhabdoviridae) under the tentative name cucurbit cytorhabdovirus 1 (CuCV1) (1). In transmission studies, using the whitefly species *Bemisia tabaci* MED, CuCV1 was transmitted to 1 out of 6 zucchini test plants, and to our knowledge, this is the third whitefly transmitted cytorhabdovirus. So far, two cytorhabdoviruses, namely bean-associated cytorhabdovirus (2) and papaya virus E (3), have been reported to be transmitted by whiteflies, an unknown feature for a plant rhabdovirus so far. Therefore, future studies should focus on elucidating the transmission mechanism of CuCV1 by whiteflies, the identification of the whitefly species vectors and whether CuCV1 replicates in the vector.

References

(1) Orfanidou, C.G., et al. (2020). Virus Res., 287: 198095.
(2) Pinheiro-Lima, B., et al. (2020). Viruses, 12: 1028.
(3) Cornejo-Franco, J.F., et al. (2022). Plant Dis.: https://doi.org/10.1094/PDIS-08-21-1785-RE

Epidemiology and genetic diversity of cucurbit aphid-borne yellows virus and watermelon mosaic virus in cucurbit crops

M.P. Rabadán[1], M. Juárez[2], C. De Moya-Ruiz[1] and P. Gómez[1]

[1]Centro de Edafología y Biología Aplicada del Segura (CEBAS)- CSIC, Departamento de Biología del Estrés y Patología Vegetal, PO Box 164, 30100, Murcia, Spain; [2]Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández de Elche, Orihuela 03312, Alicante, Spain.

(pglopez@cebas.csic.es) *S1-O3*

Systematic monitoring of plant viral diseases is fundamental to increase our knowledge in the epidemiology of their populations and understand their evolutionary dynamics in order to facilitate the design of efficient and sustainable control measures. However, there is scant long-term epidemiological and genetic monitoring of plant viral populations elucidating their evolutionary dynamics. Here, we monitored the occurrence of six aphid-borne viruses in cucurbit crops by surveying apical-leaf samples of cultivated plant species that displayed yellowing and mosaic symptoms for eleven consecutive (2011-2021) cropping seasons in Spain. We show that cucurbit aphid-borne yellows virus (CABYV) and watermelon mosaic virus (WMV) are the most common viruses, with the relatively low occurrence of cucumber mosaic virus (CMV), papaya ring spot virus (PRSV), and zucchini yellow mosaic virus (ZYMV). Remarkably, CABYV and WMV were often co-detected in the same sample, supporting the relatively high proportion of mixed infections in cucurbit crops. We then hypothesized that the co-circulation of both viruses could be epidemiologically relevant in shaping the population structure and genetic diversity of their viral populations. To this end, a collection of CABYV and WMV isolates were sequenced by using the Pacific Biosciences single-molecule real-time (SMRT, PacBio) high-throughput technology. Our genetic analysis, showed that the contemporary genetic variability of either CABYV or WMV isolates is higher than for preceding isolates, and probe links between hosts, symptoms, and type of infection at complete viral entity-level. In particular, the analysis of nucleotide substitutions reveals a complex population structure within CABYV Mediterranean group, that could in part be explained by the significant level of the variance in the mixed infected samples. These results are also consistent with the appreciation that the agricultural practices may maintain virus populations diversity and, despite severe seasonal bottlenecks, also be very dynamic in determining the prevalence and emergence of the viral diseases.

Molecular insights on potato yellow vein crinivirus infections in single and mixed infections with a potyvirus, in Colombia

F. J. del Toro[1], F. Tenllado[1], T. Canto[1], and L. Franco-Lara[2]

[1]Department of Environmental Biology, Center for Biological Research, CIB-CSIC, Ramiro de Maeztu 9, Madrid 28040, Spain; [2]Faculty of Basic and Applied Sciences, Universidad Militar Nueva Granada, Cajicá, Colombia.

(Liliana.franco@unimilitar.edu.co) *S1-O4*

Potato yellow vein virus (PYVV) (family *Closteroviridae,* genus *Crinivirus*) causes a disease in potato crops in Colombia, Ecuador, Perú and Venezuela, producing yield losses between 30 and 50%. PYVV has a tripartite, (+) ss RNA genome (1). *Solanum tuberosum* or *Solanum phureja* plants, with typical PYVV-yellowing symptoms, atypical yellowing and non-symptomatic were sampled at three separate geographical locations in the Colombian highlands. PYVV presence was assessed by RT-PCR in 12 plants, and five of them, including one non-infected healthy plant, were subject to HTS (High-Throughput Sequencing) of the sRNA populations. Almost complete sequences of four PYVV isolates were obtained from the four symptomatic plants. Three plants, showing PYVV typical yellow leaf lamina symptoms were infected only with PYVV. A fourth plant displaying yellow leaf laminas with green patches, was co-infected with a potyvirus. In this plant, the relative proportion of the three PYVV genomic RNAs was only 1% of the total viral sRNA reads, whereas the potyvirus sRNAs accounted for the remaining 99%. However, the relative proportions of the sRNAs of the three viral PYVV genomic RNAs remained similar in the four infected plants, where RNA1 generated less sRNAs per Kb than RNA2 and RNA3. The relative proportion of the three genomic RNAs was also estimated by semi-quantitative RT-PCR producing similar results. Genomic regions were identified as hotspots to sRNA formation, while other regions induced poor sRNAs production. Since the proportion of the genomic RNAs in the mixed vs. the single infections remained comparable, we concluded the absence of a synergistic/antagonistic effect of the potyvirus on the accumulation of PYVV. Progeny plants raised in the greenhouse from tubers of these infected, field-sampled plants, displayed mild PYVV-infection symptoms, In some cases the symptoms disappeared with time demonstrating the occurrence of recovery and asymptomatic infection phenotypes in this patho-system. Proyects COOPB20310, CIAS-2742.

Reference

(1) Livieratos, I.C., et al. (2004). J. Gen. Virol., 85:2065-2075.

Epidemiology of tomato brown rugose fruit virus in active greenhouses

Z. Zisi[1,2], E. Vogel[3], N. Ortega-Parra[4], A. Justé[3], C. Vos[1], J. Matthijnssens[4], I. Hanssen[3]

[1]Scientia Terrae Research Institute VZW, Belgium; [2]Laboratory of Viral Metagenomics, University of Leuven, Belgium; [3]De Ceuster Meststoffen NV (DCM), Belgium; [4]De Ceuster Meststoffen BV (DCM), the Netherlands.

(zzi@scientia.be)

S1-O5

Since its first report in 2016, Tomato brown rugose fruit virus (ToBRFV) has become a major threat in commercial tomato cultivation (1). The virus quickly spread over several continents and has also quickly gained terrain in Europe (2). ToBRFV can cause a significant reduction in yield and the fruit symptoms dramatically reduce the quality of the fruit, leading to vast amounts of non-marketable fruit (3). Additionally, strict hygienic measures should be applied, further increasing the economic impact on growers (3). For the past two years, we followed cases of ToBRFV outbreaks in greenhouses in Belgium and the Netherlands. The molecular analysis of a wide range of sample types gave insight into the time between detection and symptom development, viral accumulation in different plant parts and typical infection patterns within the greenhouse. Furthermore, it was observed that environmental conditions, such as sudden temperature fluctuations, could increase the viral load and symptom severity. Illumina sequencing technology was used to determine (near) complete ToBRFV genomes of these samples. A phylogenetic analysis of the isolates from subsequent cropping cycles in the same greenhouse revealed cases of new introduction but also reinfection. To identify possible sources of reinfection in the crop clean-out, an analysis of critical points was performed using viral swab testing of different greenhouse surfaces before and after various disinfection treatments. An improved understanding of ToBRFV epidemiology through close collaboration with growers will lead to tailored advice and future solutions.

Acknowledgements

This project has received funding from VLAIO Baekeland Mandate HBC.2020.2306 and the Belgian Logistical Auction Association (LAVA).

References

(1) Oladokun, J. O., et al. (2019). Plant Pathol., 68 (9): 1579-1586.
(2) https://gd.eppo.int/taxon/TOBRFV/distribution.
(3) Tomato brown rugose fruit virus. Bull OEPP. 2020;50(3): 529–34.

Tomato brown rugose fruit virus in aqueous environments – survival and significance of water-mediated transmission

A. Vučurović[1], D. Kutnjak[1], I. Gutiérrez-Aguirre[1], M. Ravnikar[1], K. Bačnik[1,2], O. Maksimović Carvalho Ferreira[1,2], A. Fox[3], J. Brodarič[1], I. Bajde[1], M. Kitek[4] and N. Mehle[1,5]

[1]Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia; [2]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia; [3]Fera Science Ltd, Sand Hutton, York, United Kingdom; [4]Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [5]School for Viticulture and Enology, University of Nova Gorica, Vipava, Slovenia.

(natasa.mehle@nib.si) *S1-O6*

Tomato brown rugose fruit virus (ToBRFV), which emerged in Israel around 2014 and subsequently caused outbreaks worldwide, seriously threatens tomato and pepper production. ToBRFV is thought to be introduced into new countries and regions mainly via infested seeds and infected planting material. Due to its physical and biological properties, ToBRFV is particularly stable and can be easily transferred to other plants by mechanical transmission after introduction to a new site. It has already been confirmed that ToBRFV can survive on various surfaces for long period of time and is environmentally stable. Therefore, once ToBRFV has entered the greenhouse, it is difficult to eradicate it using existing disinfection techniques. We detected ToBRFV RNA in some wastewater samples (1), in samples from rivers, and in samples from irrigation systems. We confirmed that the source of infectious ToBRFV particles in irrigation water may be the release of virus particles from the roots of infected plants and that the virus can survive in water stored at room temperature for at least four weeks. The objective of our current studies is to investigate the efficiency of transmission of ToBRFV to plants through irrigation water when grown in soil and hydroponics, and whether infection can occur through the roots. In addition, our preliminary results have shown that viral RNA can be detected on the surface of greenhouses and on the surface of plants in the presence of heavily infected plants without direct contact, so further studies will be conducted to investigate other possible sources of plant infection. The results of our studies will fill the knowledge gaps in the epidemiology of ToBRFV and provide a reliable risk assessment to identify critical points for monitoring and control.

Reference

(1) Bačnik, K., et al. (2020). Water Res.: 115628.

Cotton leafroll dwarf disease: an enigmatic virus disease on cotton in Georgia, USA

S. Bag[1], P. M. Roberts[2], R. C. Kemerait[1], and P. W. Chee[3]

[1]Department of Plant Pathology, University of Georgia, Tifton, GA, USA; [2]Department of Entomology, University of Georgia, Tifton, GA, USA; [3]Institute of Plant Breeding, Genetics and Genomics, University of Georgia, Tifton, GA, USA.

(sudeepbag@uga.edu) *S1-O7*

Cotton leafroll dwarf virus is an emerging plant virus in cotton in Georgia and other cotton-producing states of the USA. Since 2018 an extensive survey has been conducted in the cotton-producing region in GA. The foliage symptoms that appear early in the season diminish as the plant develops. The virus may remain latent in the asymptomatic plants and express in the late season. The symptomatic samples were tested for the presence of the virus using RT-PCR protocols developed in-house. Samples exhibiting diverse symptoms were subjected to genomic analysis to elucidate the virus diversity. The genome of CLRDV from six isolates from Georgia and one isolate from Alabama. CLRDV isolate from GA, and AL was 5,866 nucleotides long and encoded seven functional proteins, and >94% identical with other isolates from the USA and South America. In the silencing suppressor protein (P0), at amino acid position 72, the isolates from Georgia and Alabama had a valine (V), similar to resistant-breaking 'atypical' genotypes in South America. In contrast, the Texas isolate had isoleucine (I), similar to the more aggressive 'typical' genotypes of CLRDV. CLRDV was detected from 23 non-host weed species. Overwintering cotton stalks (48%) and regrowth leaves (75%) were both found to harbor CLRDV. Despite the detection of CLRDV in most of the growing areas of the USA, the economic importance and epidemiology are poorly understood. An increased understanding of CLRDV population structure and genetic diversity will help develop management strategies and breeding approaches for CLRDV. The persistence of CLRDV on weeds, overwintering cotton stalks, basal regrowth, and asymptomatic plants complicate the viral pathosystem by acting as a reservoir of either vectors or viruses, or both.

Prevalence and vector transmission of new maize viruses in São Paulo State, Brazil

J. R. S. Lopes[1], E. S. Vilanova[1], A. Ramos[1], M. C. S. de Oliveira[2], M. B. Esteves[1], A. P. Duarte[3], and M. C. Gonçalves[2]

[1]Dept. Entomology and Acarology, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, SP, Brazil; [2]Crop Protection Research Center, Instituto Biológico, São Paulo, SP, Brazil; [3]Grains and Fibers Center, Instituto Agronômico, Campinas, Brazil.

(jrslopes@usp.br) *S1-O8*

The substantial increase in maize cultivation in the second crop season (fall and winter) and the frequency of volunteer maize transgenic plants in soybean fields (first season – spring and summer) over the last decade in Brazil was followed by a dramatic increase in insect vector populations, notably the corn leafhopper, *Dalbulus maidis*, and by epidemics of vector-borne maize pathogens e.g. viruses and mollicutes. In addition, two new maize viruses have been detected in frequent association with previously known pathogens. One of them is maize yellow mosaic virus (MaYMV), genus *Polerovirus,* family *Luteoviridae*, originally reported in China and then found in other regions of Asia, Africa and South America (1). The other is a novel member of the genus *Mastrevirus*, family *Geminiviridae* originally detected in Central Brazil, named maize striate mosaic virus (MSMV) (2). Here we report a survey of maize viruses conducted in different regions of São Paulo State during 2019-20 (first crop season) and 2020 (second season), with detection of MaYMV and MSMV in 59 and 79% of samples (n=48), respectively, showing mosaic symptoms, most often in mixed infections with other viruses. Laboratory assays showed that these two viruses are not mechanically transmitted. MaYMV is transmitted by the corn leaf aphid, *Rhopalosiphum maidis*, and retained for at least 24 h after acquisition, while MSMV is transmitted by *D. maidis* for up to 12-16 days following acquisition, with gradual loss in efficiency over time, suggesting a persistent, but non-propagative mode of transmission. Additional studies on transmission biology, host plant range and interactions with other maize viruses are needed for a better understanding of the epidemiology and management of MaYMV and MSMV.

References

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(2) Fontenele, R. S., et al. (2018). Arch. of Virol., 163: 263–267.

High-throughput sequencing survey on cereal and barley yellow dwarf viruses indicates their underestimated diversity and spread

M. Sõmera[1], S. Massart[2], L. Tamsier[2], P. Sooväli[3] and A. Kvarnheden[4,5]

[1]Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia; [2]Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium; [3]Department of Plant Protection, Estonian Crop Research Institute, Jõgeva, Estonia; [4]Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [5]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia.

(merike.somera@taltech.ee) *S1-O9*

Worldwide, barley/cereal yellow dwarf viruses (YDVs) are the most widespread and damaging group of cereal viruses. High-throughput sequencing based virus identification survey that involved 47 fields in Estonia allowed us to assemble the complete genome sequences for one isolate of cereal yellow dwarf virus RPS, one isolate of barley yellow dwarf virus PAV, two isolates of barley yellow dwarf virus GAV, eleven isolates of barley yellow dwarf virus PAS, and seven isolates of the putative novel species barley yellow dwarf virus OYV (1). Combining the genome sequences generated during this study and the sequences retrieved from the NCBI database, we propose new diagnostic primers for specific detection of these BYDV species in single or multiplex-RT-PCR. Through systematic *in silico* analysis of published primers for YDVs´ detection revealed that the widely used RT-PCR assays do not detect BYDV-OYV. Moreover, the potential to detect a subset of different BYDV species and isolates, as well as the specificity and sensitivity of commonly used primer pairs varied greatly. As known earlier, also the existing antisera fail to detect or do not allow proper discrimination between several YDV species which may lead to biased epidemiological analysis. Taking account the unexpected diversity of YDVs in small area explored in this study, accompanied by the fact that the presence of several of these species have been reported very seldom so far, we suggest that there is a need for the comprehensive surveys about the geographical and host distribution of the different species of the YDV complex and their prevalence in cereal/barley yellow dwarf disease epidemics worldwide.

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Spread and genetic diversity of two badnaviruses infecting grapevine in Greece

C.L. Sassalou, L. Lotos, E. Palla, P. Panailidou, N. I. Katis, and V. I. Maliogka

Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Plant Pathology Laboratory, 54124 Thessaloniki, Greece.

(sassalou@agro.auth.gr) *S1-P1*

Viruses belonging to the genus *Badnavirus* have been recently identified in grapevine with the help of high throughput sequencing (HTS). Among them, grapevine Roditis leaf discoloration associated virus (GRLDaV) was first identified in Greece and then reported in other countries (1,2,3,4,5) and more recently, grapevine Badnavirus-1 (GBV-1) was identified in Croatia (3). In this study, HTS analysis revealed for the first time the presence of GBV-1 in a vine of the Greek variety Assyrtiko in Thessaloniki (Greece). To study further the spread of the two badnaviruses, PCR was applied by using specific primers that amplify the RNase-H and RT regions of GBV-1 and GRLDaV, respectively. Grapevine samples were collected from different viticultural areas of Greece. GBV-1 was present at 8% (10/130) of the vines tested, whereas GRLDaV was more abundant (27%, 212/796). To study the genetic diversity of both viruses seven grapevine samples from indigenous varieties were subjected to HTS analysis. One was dually infected with GRLDaV and GBV-1 whereas six of them were singly infected with GRLDaV. The analysis of the complete genomes of GBV-1 revealed sequence identities between 85%-95% at nt level with the sequence of the GBV-1 isolate recently identified in Croatia. Phylogenetic analysis also showed that the isolates from Greece are classified in a different clade. The GRLDaV genomes were more divergent and showed 80%-90% sequence identity at the nt level with the sequences deposited in GenBank. Phylogenetic analysis of the GRLDaV genomes showed that some of them are more divergent whereas others are grouped together with the already deposited sequences. Further sampling and HTS analysis will provide more information about the distribution and genetic variability of the two badnaviruses populations in Greece.

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The application of high-throughput sequencing reveals new viral pathogens implicated in the etiology of pepper yellows disease in Greece

V. Gavrili, L. Lotos, N.I. Katis, and V.I. Maliogka

Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, 54124, Thessaloniki, Greece.

(vmaliogk@agro.auth.gr) *S1-P2*

Pepper yellows disease (PYD) is endemic in pepper crops in Tympaki and Ierapetra areas in Crete for more than 15 years and pepper vein yellows virus-6 (PeVYV-6) (formerly pepper yellows virus) has been associated with it (1). In order to further investigate the etiology of the disease, pepper samples were collected in February 2021 from 312 symptomatic and 140 asymptomatic plants from both regions. Initially, all samples were subjected to a generic RT-PCR assay as described by Lotos et al. (1) to confirm the presence of pepper-infecting poleroviruses. The majority of the symptomatic plants (275 out of 312) tested positive for polerovirus infection, whereas all the asymptomatic plants tested negative. Subsequently, four composite samples were assembled from fields that tested polerovirus-positive and two from those found negative and were submitted to high-throughput sequencing (HTS) analysis. HTS results revealed the presence of tomato chlorosis virus (ToCV) and of different variants of PeVYV-6 which exhibited genetic variability in the ORF5/P5 region. Tobacco mild green mosaic virus (TMGMV) was also identified, even though it doesn't seem to be associated with the PYD. Furthermore, pepper plants exhibiting PYD-like symptoms but with variable intensity were reported in the region of Mandriko in Rhodes. To investigate the viruses present, 51 symptomatic and 20 asymptomatic plants were collected and tested for poleroviruses as described above. All samples were found negative to polerovirus infection. To identify the causal agent(s) of the disease, a composite sample containing 4 plants from a single field was submitted to HTS. Two ophioviruses were found, namely ranunculus white mottle virus (RWMV) and lettuce necrotic ring virus (LNRV). All the HTS results were verified either by RT-qPCR or RT-PCR assays. Taken together, our results indicate that although PeVYV-6 is the key-pathogen in PYD etiology in Greece other viral species from different genera with distinct transmission modes and epidemiology might also contribute to its development.

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Occurrence of tomato yellow leaf curl virus on resistant tomato cultivars in Korea

M. Bae[1], M. Kwak[1], M. C. Son[1], H. S. Choi[2], H. R. Kwak[2], H. S. Byun[2], and E. J. Kil[1]

[1]*Department of Plant Medicals, Andong National University, Korea;* [2]*Crop Protection Division, National Institute of Agricultural Sciences, RDA, Korea*.

(dwc04151@naver.com) *S1-P3*

Tomato is one of the important economic crop grown all over the world. Tomato yellow leaf curl virus (TYLCV), a representative virus that infects tomato plants, a belongs to the family *Geminiviridae*, and TYLCV causes severe economic damage to tomato production by inducing leaf curling, yellowing, and stunting. In order to reduce the damage caused by TYLCV, farmers sometimes grow tomato resistant varieties. However, recently, a lot of damages caused by TYLCV have been reported in farms growing resistant varieties, so we confirmed the possibility of resistance breaking through TYLCV sequence mutation in Korea. Through a nationwide survey, the leaves were collected from tomato plants showing symptoms in tomato greenhouses in Korea, Using TYLCV resistance gene markers, it was confirmed which resistance *Ty* gene(s) was present. Co-infection with other viruses including TYLCV, ToMV, ToCV, STV, and CMV were also identified. Sequence analysis was carried out by securing the TYLCV genome through next generation sequencing using illumina Novaseq system, and mutation and recombination were confirmed through comparison with the TYLCV sequences previously reported in Korea.

Adventitious plants act as a reservoir of cucumber mosaic virus in chili-pepper crops in northern Spain

M. Ojinaga[1], S. Mendarte[2], B. Juaristi[1], U. Apodaka[1], A. Revillas[1], A. Ortiz-Barredo[1], and S. Larregla[1]

[1]Plant Production and Protection Department, NEIKER - Basque Institute for Agricultural Research and Development, Derio (Bizkaia), Spain; [2]Conservation of Natural Resources Department, NEIKER - Basque Institute for Agricultural Research and Development, Derio (Bizkaia), Spain.

(mojinaga@neiker.eus) *S1-P4*

In the last seasons there has been a high incidence of the cucumber mosaic virus (CMV) in Ibarra yellow chili-pepper crops. The Ibarra yellow chili-pepper is a local variety whose immature fruits are consumed as a processed packaged product (pickled) and as a fresh product (fried). This variety is grown open-field conditions in Gipuzkoa (northern Spain) and does not present genetic resistance to CMV virus. This virus, transmitted by aphids in a non-persistent way, is seriously affecting the chili production and packaging sector. The virus produces very serious alterations in fruits morphology that depreciate their commercial quality (thickening, twisting, early and excessive seed formation). The economic losses are important for the farmer and are compromising the future viability of the crop. For all these reasons, epidemiological aspects of this virus have begun to be studied in order to establish better control strategies. In this study, results obtained in the analysis of adventitious flora of several plots dedicated to the commercial cultivation of chili are shown. Plots that had suffered high incidences of CMV virus in previous years were selected. The analysis of plants from adventitious flora to CMV was carried out by the nonisotopic dot-blot hybridization molecular technique. In the adventitious flora of these plots, CMV-positive plants were detected in different phases of the crop cycle. Before transplanting the chili pepper crop, 1140 adventitious flora plants were analyzed and 2.3% of these plants were positive for CMV which belonged to 11 different species. At the end of the chili pepper cultivation cycle, 1,183 adventitial flora plants were analyzed and 3.2% of these plants were positive for CMV which belonged to 13 different species.

Infectious clones of tomato black ring virus fused with green fluorescence protein (GFP) as a tool for pathogenesis monitoring in plant tissues

A. Zarzyńska-Nowak[1], J. Minicka[1], P. Wieczorek[2], and B. Hasiów-Jaroszewska[1]

[1]Department of Virology and Bacteriology, Institute of Plant Protection-National Research Institute, Poznań, Poland; [2]Department of Molecular Biology and Biotechnology, Institute of Plant Protection-National Research Institute, Poznań, Poland.

(a.zarzynska@iorpib.poznan.pl) *S1-P5*

Tomato black ring virus (TBRV), a member of the *Nepovirus* genus is a serious plant pathogen distributed worldwide. It infects a wide range of economically important, ornamental, weeds, herbaceous and woody plants. The TBRV bipartite genome consists of two polyadenylated single-stranded positive-sense RNA. Moreover, the genomic RNAs of some TBRV isolates might be accompanied by subviral RNA particles such as defective RNAs (D RNAs) and satellite RNAs (satRNAs). The knowledge regarding molecular mechanisms underlying the interaction between TBRV and its hosts remain largely unknown. Therefore, infectious TBRV cDNA clones fused with green fluorescence protein (GFP) were constructed. The open reading frame encoding GFP were inserted within movement protein and capsid protein on RNA2 of the recombinant TBRV using In-Fusion®HD Cloning system (Takara). The obtained TBRV-GFP clones were introduced into *Agrobacterium tumefaciens*, and then agroinfiltrated into leaves of test plants. The symptoms on the agroinfiltrated plants, TBRV-GFP clones infectivity, the presence of GFP insertions and the fluorescence in the plants were monitored within one month. Because of low level of gene expression and GFP concentration in plant tissues, the obtained constructs were further modified by (1) changing the length of the flanking sequences of the GFP insert, (2) introducing additional synonymous mutations within them, (3) changing the location of the GFP insert within the RNA2 polyprotein, (4) inserting homing protien gene, or (5) adding a self-cutting 2A protein sequence. Unfortunately, the level of gfp expression in all tested constructs was insufficient. Among 13 constructs tested, only 3 had the ability to systematically infect plants. In order to obtain an adequate GFP fluorescence signal in TBRV-GFP agroinfiltrated plants, further optimization of the above-mentioned constructs is required.

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Molecular characterization of sugarcane streak mosaic virus in Côte d'Ivoire

M. M. Ouattara[1], C. Desbiez[2], G. Girardot[2], B. Ble[3], K. A. Yao[4], K. D. Kouame[1] , and A. Schoeny[2]

[1]UFR Biosciences, Laboratoire de Biotechnologie, Agriculture et Valorisation des Ressources biologiques, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire; [2]Pathologie Végétale, INRAE, Montfavet, France; [3]Sucrivoire, UAI, Borotou-Koro, Côte d'Ivoire ; [4]Sucrivoire, UAI, Zuénoula, Côte d'Ivoire.

(alexandra.schoeny@inrae.fr) *S1-P6*

Since its development in the 1970s, the cultivation of sugarcane has played an important role in Côte d'Ivoire's economy, but the domestic demand is only 80% satisfied. Production stagnates mainly due to biological constraints (pests and diseases). Sugarcane mosaic disease affects the photosynthesis and growth of sugarcane, leading to a significant decrease in cane yield and sucrose content, and thus serious economic losses. It can be caused by sugarcane mosaic virus (SCMV), sorghum mosaic virus (SrMV), and/or sugarcane streak mosaic virus (SCSMV). SCMV and SrMV are distributed worldwide and SCSMV mainly exists in Asia (1). More recently, the virus has also been reported in Côte d'Ivoire in West Africa (2,3) where it appears responsible of increasing yield losses. In order to characterize the impact and genetic diversity of SCSMV in two of the three production areas, Borotou-Koro (North-West) and Zuénoula (Center), an intensive sampling campaign took place from September to October 2020. For each site, 22 plots (from 1 to 35 ha) were assessed. For each plot, five 100m^2 micro-plots were defined in which four randomly selected plants were sampled. A partial sequence encompassing the N-terminal variable part of the coat protein was obtained for 470 isolates. Mixed infections were observed for 157 isolates, 49 from Borotou-Koro (10% of sequenced samples) and 108 from Zuénoula (23% of sequenced samples). The remaining sequences presented a high molecular diversity, including isolates of the two major molecular groups described at the global level (4) as well as a specific group of unknown origin, observed so far only in Côte d'Ivoire.

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The emergence of Tomato Leaf Curl New Delhi Virus in France

A. Patthamapornsirikul , E. Verdin, and C. Desbiez

Pathologie Végétale, INRAE, Avignon, France.

(atiwich.patthamapornsirikul@inrae.fr) *S1-P7*

The Mediterranean basin and Mainland France are facing the risk of *Tomato leaf curl New Delhi virus* or ToLCNDV (genus *Begomovirus*, family *Geminiviridae*) transmitted by the whitefly *Bemisia tabaci*. In Mediterranean countries, ToLCNDV was detected in 2012 in Spain (1) then throughout Southern Europe and North Africa (2). In September 2020, symptoms reminiscent to ToLCNDV were observed on zucchini in the Bouches-du-Rhône and Gard regions of France. The presence of ToLCNDV was confirmed using RCA (Rolling Circle Amplification) and PCR (Polymerase chain reaction) (3). Complete genome sequencing confirmed that the French isolates belonged to the "Mediterranean" ToLCNDV clade. However, molecular diversity studies suggested that at least two different variants, differing by 1-2%, have been introduced, possibly from different origins. Isolates from the two variants were cloned and characterized biologically, displaying contrasted severities in cucurbits with a recovery phenotype associated with one variant on susceptible melon and zucchini. Experiments are underway to characterize the molecular determinants of the phenotypic differences, the potential trade-off with other properties (host range in cultivated and wild hosts, transmission specificity and efficiency) and the risks of durable establishment of the virus in the environment.

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Effects of the serial passages of tomato severe rugose virus (ToSRV) by different hosts on the viral infection rate, genetic diversity and population structure

C. G. Ferro, G. M. Favara, H. D. Kraide, and J. A. M. Rezende

Plant Pathology and Nematology Department, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, SP, Brazil.

(camilaferro@usp.br) *S1-P8*

Tomato severe rugose virus (ToSRV) is the most important begomovirus in Brazilian tomato crops (1). Many weeds and other solanaceous are associated with tomatoes, and some are hosts of begomoviruses, but it remains unknown what is the primary source of ToSRV inoculum for tomatoes in the field. Here we tried to identify differences in the nucleotide sequences of ToSRV isolates after serial passages throughout different hosts to obtain molecular markers. The ToSRV-TF isolate, originally maintained on tomato, was transmitted by *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1) for six successive generations to tomato (control), soybean and *Nicandra physalodes* plants. At the end of the fourth and sixth passages, viral isolates present in the different hosts were recovered for tomato plants. The ToSRV-TF isolate was able to infect tomato, soybean, and *N. physalodes*. However, not with the same efficiency. There was a higher infection rate in tomato plants compared to other hosts. Furthermore, the viral population's “disappearance” scenario was observed in soybean and *N. physalodes* plants during serial passages, which was not seen in tomato plants. It was found that there was an increase in the infection rate by the viral population resulting from serial passages in *N. physalodes* when this population was recovered in tomatoes. Phylogenetic analysis based on the complete nucleotide sequence of DNA-A showed low genetic variability among isolates and no evidence of genetic structuring by the host. The distribution of mutations was not informative to effectively correlate the decrease in the viral infection rate in soybean and *N. physalodes* plants with changes in the level of diversity of the viral population. None of the mutations were fixed during the serial passages. Therefore, it has not been possible to identify differences in nucleotide sequences that could represent molecular markers.

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Effect of temperature and between-host transmission on the induction of tomato black ring virus defective RNA particles

D. Budzyńska, J. Minicka, A. Taberska, and B. Hasiów-Jaroszewska

Department of Virology and Bacteriology, Institute of Plant Protection-National Research Institute, Poznań, Poland.

(D.Budzynska@iorpib.poznan.pl) *S1-P9*

The genomic RNAs of tomato black ring virus (TBRV) can be associated with subviral defective RNAs (D RNAs). D RNAs are derived from the viral genome by deletions or rearrangements and show a high similarity to the genomic sequence. Our previous research showed that D RNAs TBRV have 335-570 nt in length, arise both from RNA1 and RNA2, and are generated *de novo* during prolonged passages of the virus in one host (1). D RNAs interfere with TBRV replication. Here, we investigated the influence of temperature and between-host transmission on the D RNA TBRV induction. Firstly, TBRV isolates from black locust, lettuce and tomato were passaged 15 times through four hosts: quinoa, tobacco, cucumber and tomato. Plants were grown in greenhouse conditions at two temperatures,18°C and 26°C, with a photoperiod of 16 hr. light:8 hr dark. Secondly, between-host transmission of TBRV isolate from tomato was performed. In the first part of experiment TBRV isolate evolved in tobacco (5 passages) /quinoa (5 passages) /tobacco (5 passages) /quinoa (5 passages). In the second experiment, TBRV was passaged 20 times through randomly chosen hosts. In each lineage, the setup of plants was different. After both experiments the purified virus preparations were obtained in sucrose gradient and the RNA profile of TBRV was analyzed. The preliminary results indicated that: i) the presence of potential D RNA particles about ~500 nt was observed in some lineages ii) the temperature has an effect of D RNA formation eg. D RNAs arose in cucumber plants grown in 26°C whereas no additive RNAs were observed in 18°C iii) no additional small RNAs were observed in TBRV passaging in tomato iv) arising of D RNA seems to be dependent on host in which virus evolve and virus isolate.

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Virome analyses of *Cnidium officinale* infecting viruses based on next-generation sequencing in Korea

J. H. Kang[1], M. Kwak[1], C. R. Jung[2], J.-B. Yoon[3], and E. J. Kil[1]

[1]*Department of Plant Medicals, Andong National University, Korea;* [2]*Forest Medicinal Resources Research Center, NiFoS, Korea;* [3]*Horticulture and Herbal Crop Environment Division, National Institute of Horticultural and Herbal Science, RDA, Korea*.

(wjdgns3771@naver.com) *S1-P10*

In Korea, *Cnidium officinale* Makino is cultivated as a medicinal plant, mainly cultivated in the highland regions of Gangwon-do and northern Gyeongsangbuk-do. Viruses such as apple stem grooving virus (ASGV), cucumber mosaic virus (CMV), cnidium vein yellowing virus 1 (CnVYV-1), and cnidium vein yellowing virus 2 (CnVYV-2) have been identified and reported in cultivated *C. officinale* (1,2). During May and June 2021, *C. officinale* plants grown in four cities (Hoengseong, Samcheok, Bonghwa, and Yeongyang) located in the eastern part of South Korea and *Thrips nigropilosus* living therein were collected. Mainly samples in which symptoms of vein clearing and chlorotic spots observed in leaves, and thrips occurring in nearby areas were also used for analysis. Total RNA was extracted and the complementary DNA libraries were generated. Next-generation sequencing (NGS) was performed using an Illumina Novaseq 6000 (100 bp paired-end read), and then analyzed raw read data with CLC Genomic Workbench. CnVYV-1, CnVYV-2, CMV, and ASGV previously reported in Korea were commonly identified in *C. officinale* leaf samples, and cnidium virus X was also identified in all but one region. In addition, sequences of viruses suspected to be novel viruses belonging to the *Solemoviridae* and *Betaflexiviridae* families have been additionally identified. In thrips, reads for CnVYV1, CnVYV2 and CMV were confirmed. Nucleic acid sequence similarity among trimmed and rearranged contig sequences identified in each region was analyzed. Thereafter, to recheck, virus infection was verified through RT-PCR using primer sets specific to each virus.

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The effect of satellite RNAs on the accumulation of tomato black ring virus in different hosts

J. Minicka[1], A. Taberska[1], A. Zarzyńska-Nowak[1], S.F. Elena[2,3], and B. Hasiów-Jaroszewska[1]

[1]Department of Virology and Bacteriology, Institute of Plant Protection – National Research Institute, Poznań, Poland; [2]Instituto de Biología Integrativa de Sistemas, CSIC-Universitat de València, València, Spain; [3]The Santa Fe Institute, Santa Fe, NM, USA.

(j.minicka@iorpib.poznan.pl) *S1-P11*

Viral satellite RNAs (satRNAs) are small subviral RNAs that are associated with helper virus (HV) and depends on it for replication, encapsidation and movement (Simon et al., 2004). SatRNAs are rather short RNA molecules ranging in size from 220 to 1400 nucleotides (nt) and most share little or no sequence homology to the helper viruses (Hu et al., 2009). The presence of these subviral particles can have neutral, exacerbating or attenuating effects on the virulence, pathogenicity and accumulation of its HV, depending on satRNA variants, host and HV genotype (Simon et al., 2004). In this study, the impact of satRNAs on the accumulation of tomato black ring virus (TBRV) was investigated. Three different TBRV isolates designated as TBRV-K8, TBRV-MJ and TBRV-Pi and three different host plants (*Chenopodium quinoa, Nicotiana tabacum* and *Spinacia oleracea)* were used. Five plants per each host were infected using TBRV with and without satRNAs, respectively, and maintained under greenhouse conditions (24 °C, 16/8 photoperiod) for 28 dpi. The accumulation of TBRV genomic RNAs was measured 7, 14, 21, and 28 dpi using RT-qPCR (LightCycler96, Roche). *C. quinoa* and *S. oleracea* plants infected with TBRV+satRNAs displayed more intense symptoms in comparison to those infected only with TBRV. Furthermore, the RT-qPCR results indicate that the presence of satellite RNA has an effect on TBRV accumulation, that depends on the combination of viral isolate and the host species. While in *C. quinoa* and *S. oleracea* the presence of satellite RNA increases the accumulation of HV, in tobacco the effect was the opposite. Moreover, the magnitude of this effect varies between isolate and host plant. Our data provide evidence that presence of satRNA can have significant effect on TBRV replication and pathogenicity.

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Epidemiology and factors influencing the spatial spread of yam mosaic virus in yam fields in Nigeria

B.O. Osundahunsi[1], B. Odu[2], B. Aighewi[3], N. Maroya[1] and P. Lava Kumar[1]*

[1]International Institute of Tropical Agriculture (IITA), Oyo Road, PMB 5320, Ibadan, Nigeria; [2]Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria; [3]IITA, Kubwa, PMB 82, Abuja, Nigeria.

(L.kumar@cgiar.org) *S1-P12*

Yam mosaic disease caused by the yam mosaic virus (YMV, genus Potyvirus) is a major threat to yam production (*Dioscorea* spp.) in the Western African sub-region, contributing to nearly 90% of the global food yam production. Very little information is available on epidemiological factors contributing to the spread of YMV in the yam fields. In this study, we determined factors contributing to the spread of YMV in yam fields established 2016–2020. Trials were conducted at IITA, Ibadan, Nigeria, in fields planted with virus-free vines of *Dioscorea rotundata*. Yellow water traps were set in fields to monitor aphid diversity and movement. YMV infection was assessed visually and by RT-PCR diagnosis at monthly intervals in 10 m x 10 m marked plots. The YMV infected seedlings were tagged and mapped their positions in the field. The identity of the aphid species was determined by morphological features and mitochondrial COI DNA barcoding representative specimens. Results from the study revealed no significant difference ($P>0.05$) in total aphids trapped at different positions within each trial, while the number of aphids trapped reduced with time ($P< 0.05$) for each year. The overall distribution of aphids demonstrated a biphasic pattern marked by an initial increase in the aphid population and then a rapid decline. DNA barcoding revealed that dominant aphid species were *Aphis spiraecola*. YMV incidence was highest in 2019 trials (46.4±3.3%) and lowest in 2016/17 (7.9±4.5%). Distance to surrounding yam fields was negatively correlated to YMV incidence. Results indicated that YMV spread in the yam field as dependent on the movement of non-yam colonizing aphids, planting date, and proximity to the inoculum sources. The findings of this study are useful to refine YMV control measures and develop a model for disease risk assessment under different epidemiological scenarios.

Evaluation of the RNA silencing suppression ability of three cherry virus F encoded proteins

L. Lotos*, A. Katsiani*, N.I. Katis, and V.I. Maliogka

*Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Plant Pathology Laboratory, 54124, Thessaloniki, Greece. *Both authors have contributed equally.*

(vmaliogk@agro.auth.gr) *S1-P13*

Cherry virus F (CVF) (family *Secoviridae*, genus *Fabavirus*) is a recently identified sweet cherry (*Prunus avium*) and Japanese plum (*Prunus mume*) infecting virus (1,2). Until recently, CVF has been identified in a small number of countries and it hasn't been associated with a discrete symptomatology. CVF has a bipartite genome with each RNA coding for a polyprotein which, after translation, gets proteolytically processed into functional proteins. Studies on other fabaviruses and comoviruses have indicated that the three proteins (movement protein - MP, large coat protein - CPL, small coat protein - CPS) produced from RNA2 could act as possible RNA silencing suppressors (RSSs). To assess the putative RSS role of these three proteins encoded by the CVF genome, they were separately cloned into the binary vector pART27 and *Agrobacterium tumefaciens* C58C1 was transformed with each of the three recombinant plasmids. The clones were used for transient expression experiments in *Nicotiana benthamiana* leaves, using co-agroinfiltration with a GFP-expressing vector. A cymbidium ringspot virus P19 expressing vector was used as the positive control and an empty pART27 vector as the negative. The plants were observed under UV light and the levels of fluorescence were monitored for 10 days after the infiltration. The two coat proteins exhibited a rapid decrease in fluorescence, comparable to the negative control, whereas the MP retained the GFP's fluorescence even though this was observed in a small number of infiltrated leaves. To evaluate the levels of GFP expression in the presence of the MP, leaf disks of the infiltrated area were collected 4, 5, 6 and 8 dpi and total RNA was subjected to RT-qPCR for GFP using L23 as the reference gene. Preliminary results showed that the MP of CVF is possibly implicated in the suppression of the RNA silencing pathway. Further experiments are currently underway to characterize the mode of action of this protein.

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Differences in the 3' intergenic region and the V2 protein of two variants of Tomato curly stunt virus play an important role in disease pathology in *Nicotiana benthamiana*

M. E. C. Rey, A. M. Zwolinski, and A. Brigden

University of the Witwatersrand, School of Molecular and Cell Biology, South Africa.

(Chrissie.Rey@wits.ac.za)

S1-P14

Tomato production in the subtropical growing regions of South Africa is threatened by the emergence of Tomato curly stunt virus (ToCSV), a monopartite ssDNA virus in the genus *Begomovirus*. Two variant groups have been identified: V30 causing severe disease, and V22 causing mild disease in tomato. We investigated the role of several sequence differences present in the 3' intergenic region (IR) and the V2 protein on infectivity in *Nicotiana benthamiana*. Partial 3' IR sequence swap, full V2 ORF swap and V22 V2 point mutants were generated and the effect of mutations on symptom phenotype and viral load was recorded. Swapping of a 78 nt segment of the IR in V30 with the corresponding sequence in V22 resulted in the development of upward leaf roll (ULR), a symptom characteristic of V22 infection. A significant increase in viral load at 28 dpi was also observed. The reverse swap of this mutant (V22 containing V30 IR segment) resulted in a complete knockout of ULR phenotype but with no significant difference in viral load. Altered symptom phenotypes induced by shorter IR swap mutants suggested that the unique arrangement of a novel *cis*-acting TATA-associated composite element (1) may contribute to the observed phenotype differences. A full V2 ORF swap mutant of V30 resulted in a significant reduction in symptom severity, whilst the reverse swap of this mutant in V22 resulted in a loss of symptom recovery phenotype with a significant increase in symptom severity and viral load at both peak and late infection timepoints. The observed changes in the V22 V2 swap mutant were also observed with a V27S V2 mutant to a lesser degree whilst a V2 T57S mutant resulted in a delay in symptom expression. Our findings indicate that the 3' IR region is a symptom phenotype determinant in ToCSV whilst the V2 coding region plays a critical role in symptom severity and disease recovery in *N. benthamiana*.

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Survey for identification and epidemiology of citrus viroids in various *Citrus spp.* in Greece

N. Tektonidis, A. Karagianni, L. Mikalef, and M. M. Mathioudakis

Plant Pathology Laboratory, Institute of Olive tree, Subtropical Crops & Viticulture / ELGO-DIMITRA, Chania,Crete, Greece.

(mathioudakis@elgo.iosv.gr) *S1-P15*

Citrus plants are infected by several viroid species belonging to the *Pospiviroidae* family, causing in some cases significant yield losses worldwide (1). The information about the presence and the epidemiology of citrus viroids in Greece is limited to a study conducted at a national repository collection and the identification of viroids in lime in Crete (2). In spring and autumn of 2021, a survey was conducted from the two most important citrus-growing areas (Chania at Crete, Nafplio at Peloponnese). A total of 1738 samples were randomly collected from six different citrus species and various varieties: sweet orange (518 samples; 6 varieties), lemon (411; 5 varieties), mandarin (429; 5 varieties), lime (95), grapefruit (215; 2 varieties) and blood orange (70; 2 varieties). Most of the sampled trees were asymptomatic, whereas in some cases they showed symptoms such as yellowing in leaves, stunting and shoot bark cracking. From Chania area the samples were collected from 11 villages (45 different orchards) and from Naflpio area the samples were collected from 6 villages (16 different orchards). All the samples were tested for the presence of citrus exocortis viroid (CEVd), hop stunt viroid (HSVd), citrus dwarfing viroid (CDVd), citrus bark cracking viroid (CBCVd) and citrus bent leaf viroid (CBLVd) by reverse transcription-polymerase chain reaction (RT-PCR) amplifying the entire viroid genome. So far, the results have shown the presence of all five viroids in the six different *Citrus* species. The HSVd and the CDVd have shown high infection rates (>70%) and a spread epidemiology in all surveyed areas and citrus hosts, followed by CEVd and CBCVd in lower infection rates (40-50%), whereas the CBLVd was occasionally detected mostly in sweet orange and lemons. Taken together this study illustrates the wide spread of viroids in citrus plants and the need to use certified propagative material.

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High throughput sequencing of the tomato viromes in Korea

M. Kwak[1], M. Bae[1], M. C. Son[1], H. S. Choi[2], H. R. Kwak[2], H. S. Byun[2], and E. J. Kil[1]

[1]*Department of Plant Medicals, Andong National University, Korea;* [2]*Crop Protection Division, National Institute of Agricultural Sciences, RDA, Korea.*

(kwak2604@gmail.com) *S1-P16*

Tomato (*Solanum lycopersicum*) is one of the most popular and extensively consumed economic crops. However, viral disease is still a major factor limiting tomato production. There are at least 312 virus, satellite virus, or viroid species (in 22 families and 39 genera) associated with tomato, which is likely the highest number recorded for any plant (1). The advent of high throughput sequencing (HTS) has accelerated virus detection and characterization techniques, allowing researchers, for the first time, to characterize all or nearly all viruses in a sample without prior information about which viruses might be present (2). In this study, we carry out virome analysis using Illumina NGS(Next-generation sequencing) platforms and bioinformatic to understand the virosphere in different geographical locations in Korea where tomatoes are cultivated. We identified tomato-infecting virus species reported continuously in Korea, namely, Tomato Yellow leaf curl virus (TYLCV), Tomato mosaic virus (ToMV), Cucumber mosaic virus (CMV), Tomato chlorosis virus (ToCV) and Tomato spotted wilt virus (TSWV). Collected tomatoes were generally co-infected. Our tomato virome study provides the viral population and a comprehensive overview of viral communities in tomato.

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Virome release of an invasive exotic plant species in Southern France

O. Moubset[1], D. Filloux[1], H. Fontes[2], C. Julian[1], E. Fernandez[1], L. Claude[1], F. Chiroleu[3], F. Mesleard[2,4], S. Kraberger[8], J. Custer[8], A. Salywon[5], E. Makings[6], D. P. Martin[7], A. Varsani[8,9], and P. Roumagnac[1]

[1]CIRAD, BGPI, Montpellier, France; [2]Tour du Valat, Institut de recherche pour la conservation des zones humides méditerranéennes, Le Sambuc-Arles, France; [3]CIRAD, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France; [4]Institut Méditerranéen de Biodiversité et Ecologie, UMR CNRS-IRD, Avignon Université Aix-Marseille Université, IUT d'Avignon, 84911 Avignon, France; [5]Desert Botanical Garden, Phoenix, AZ, United States; [6]Arizona State University, Tempe, AZ, USA; [7]Department of Integrative Biomedical Sciences, Institute of infectious Diseases and molecular Medicine, University of Cape Town, Cape Town, South Africa; [8]The Biodesign Center for Fundamental and Applied Microbiomics, Center for Evolution and Medicine, School of Life Sciences, Arizona State University, Tempe, AZ, USA; [9] Department of Integrative Biomedical Sciences, Structural Biology Research Unit, University of Cape Town, Observatory, Cape Town, South Africa.

(oumaima.moubset@cirad.fr) *S1-P17*

Globalisation and increase world travel in the last century has resulted in a dramatic increase in human-mediated introduction of plant species to regions outside their natural geographical ranges. Invasive exotic plants (IEPs) can significantly impact biodiversity, ecosystem processes and food production. In many cases the introduction of IEPs to a new region is through seed, and given that most pathogens are not vertically transmitted it is likely the plants are therefore pathogen free or at the least contain a limited number of viruses. Also, most pathogens are not evenly distributed across the Earth, such that IEPs colonizing a new territory are likely to rarely, if ever, encounter pathogens from their native regions: a situation that may result in escape or release of IEPs from pathogen-mediated ecological processes that naturally control plant population sizes, distributions and densities. This so-called enemy-release hypothesis, posits that decreases in pathogen-mediated selective pressures on IEPs in colonized territories will likely promote increases in IEP population sizes, densities and geographical distributions (1). Although few studies have tested the enemy release hypothesis, compiled data on plant species in the US Department of Agriculture (USDA) Plants Database has revealed that 84% fewer fungi and 24% fewer virus species infected each plant species in its naturalized range than in its native range (1). To further explore the pathogen release hypothesis, we compared the virome of cane bluestem (*Bothriochloa barbinodis*) - a C4-grass that is an IEP species in southern France – with the viromes of four grass species that are indigenous to southern France (*Brachypodium phoenicoides*, *Cynodon dactylon*, *Dactylis glomerata* and *Elytrigia campestris*). We show that the IEP *Bothriochloa barbinodis* has significantly lower viral infection loads than do the indigenous grasses: an observation that is consistent with the enemy-release hypothesis.

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Identification of a new 'old' tobamovirus originating from pepper

R.A.A. van der Vlugt[1], P. van Bekkum[1], and C.C.M.M. Stijger[2]

[1]Wageningen Plant Research, Wageningen University and Research, Wageningen The Netherlands; [2]Wageningen Greenhouse Horticulture and Flowerbulbs, Wageningen University and Research, Wageningen, The Netherlands.

(rene.vandervlugt@wur.nl) *S1-P18*

Wageningen Plant Research (WPR) maintains an extensive virus collection as reference material. Various methods are used for checking identity and possible contamination of virus isolates including regular use of High Throughput Sequencing (HTS). Following analyses of an isolate of Calibrachoa mottle virus (CbMV) suspicious tobamovirus particles were observed in transmission electron microscopy (TEM) studies and inoculation on *Nicotiana glutinosa* plants showed distinct local lesions suggesting the presence of a tobamovirus. Illumina HTS analyses indeed revealed the presence of a tobamovirus sequence (representing >95% of the reads) showing the closest overall nucleotide identity of 88% to bell pepper mottle virus (BePMoV) indicating this virus might classify as a new tobamovirus. The sequence was compared to those of other BePMoV isolates present in our virus collection and it showed near identity to a BePMoV isolate obtained in the 1970's through A.Th.B. Rast (1). Following the trail back its most likely first description dates back to 1968 where it was described an aberrant TMV strain from pepper in Argentina (2, 3). Purified virus material of this isolate and closely related isolates collected in the 1970's by A.Th.B. Rast and still present at Wageningen Greenhouse Horticulture and Flowerbulbs, was investigated for their relationships to the newly identified 'pepper' tobamovirus, which will be reported.

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Session

02

Diagnostics, Surveillance and Modeling

Developing elements for global plant virus management: diagnostics, surveillance, and modelling

J. Kreuze

Centro de Investigacion de la Papa. Avenida la Molina 1895. Lima, Peru.

(j.kreuze@cgiar.org)

Keynote Lecture 2

Plant viruses pose a continuous and serious threat to crop production worldwide. At the same time globalization and climate change are providing opportunities for new viruses to emerge and spread rapidly. At the same time, developments in sequencing technology, nucleic acid amplification techniques and risk modeling approaches are providing plant health specialist with unprecedented opportunities to confront these increasing risks. This keynote will describe, using examples from potato and sweetpotato, how high throughput sequencing based surveillance approaches can provide information to better understand the impact of plant viruses on crops, support development of more targeted field and lab based diagnostic tools for use in virus management and will look into how modeling approaches can be used to support surveillance and preparedness of emerging viruses in the face of climate change.

Applying high throughput sequencing in a generic surveillance workflow: a case study using UK peas

A. R. Fowkes[1], S. McGreig[1], H. Pufal[3], S. Duffy[2], B. Howard[2], I. P. Adams[1], R. Macarthur[1], R. Weekes[1], and A. Fox[1]

[1]Fera Science Limited, Sand Hutton, York, UK; [2]Processors & Growers Research Organisation (PGRO), Peterborough, PE8 6HJ, UK; [3]Institute for Agri-Food Research and Innovation, University of Newcastle, Newcastle, NE1 7RU.

(aimee.fowkes@fera.co.uk) *S2-O1*

High throughput sequencing (HTS) is increasingly being used in plant health, including within surveys. Traditional conventional surveys consist of testing for a selected suite of viruses known to be present as well as viruses of concern, which only gives information on the presence of those specific viruses. By using HTS, viruses not previously known to be in the region or in the host can also be identified. Pea (*Pisum sativum*) is an important crop worldwide, yet within the United Kingdom very little research has gone into the presence of viruses since the 1970s (1). Recently, emerging viruses in Europe and reports of unusual symptoms in peas in the UK have led to a renewed interest in the virus health of this crop. Over two years, forty fields across the UK have been sampled according to the approach presented by Fowkes *et al* (1). Briefly, from each site, a 'bulked field sample' was tested by HTS to identify candidate targets. Mixed sized bulks were then tested by real-time RT-PCR which gave both confirmation and incidence of the virus. Over the two years, 8 viruses, a satellite RNA and associated RNA have been detected by HTS. Turnip yellows virus, was the most prevalent virus, present in over half the fields surveyed and at incidences up to 100%. Unexpectedly, pea enation mosaic virus-2 was found at sites without pea enation mosaic virus-1 (1,2) or at a greater incidence. The approach also revealed new country records for the UK. Soybean dwarf virus was present in a limited number of fields, but with broad geographic distribution. Pea necrotic yellow dwarf virus was recorded in a single field in the second year of the survey. The advantages and limitations of this method compared with a conventional survey method will be discussed.

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Detection of global soybean viruses in metagenomic sequence data using Microbe Finder (MiFi®)

M. R. Ribeiro-Junior[1,2,3], F. M. Ochoa-Corona[1,2], A. S. Espindola[2], F. Barreto da Silva[3], D. M. do Nascimento[2], and R. Krause-Sakate[3]

[1]Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma, United States; [2]Institute of Biosecurity and Microbial Forensic, Stillwater, Oklahoma, United States; [3]Department of Plant Protection, School of Agriculture, Sao Paulo State University, Botucatu, Sao Paulo, Brazil.

(marcos.ribeiro@unesp.br) *S2-O2*

Soybean (*Glycine max*) is an important crop worldwide as a source of oilseed and protein. Brazil and the USA are the two largest producers in the world. Nevertheless, each year, soybean growers lose significant yields to diseases caused by viruses and precise diagnosis is crucial for management. To date, 47 virus species have been described infecting soybean worldwide. Plant virus identification based on serological and traditional molecular techniques is time-consuming and does not allow simultaneous identification of all viruses present in a single sample. Microbe Finder (MiFi®) is a web application for quick detection and identification of known pathogen species where highly curated, target-specific electronic probes (E-probes) are used to query in raw, unassembled High-throughput sequencing (HTS) metagenomic data (1). E-probes ranging from 30-60 nucleotides in length were designed for the 47 viruses that infect soybean using MiProbe™ within MiFi® platform. Further curation and validation were performed using mock metagenomic databases assembled with each virus reference positive controls and host genome sequences. *In silico* results validate the usage of e-probes to detect up to 47 viruses in metagenomic soybean data. *In vitro* analyzes were performed using Illumina HTS data from soybean plants collected in Brazil. Nine viruses were identified in the Brazilian soybean samples. The results were confirmed by PCR, RT-PCR, and mapping reads to virus reference genomes using Geneious software. Here we propose a sequence-based method for rapid (less than an hour) detection and identification of all viruses described in soybean. Curated soybean virus e-probes were made available to the public for diagnostics through MiDetect™ within MiFi® platform.

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HTS as a powerful tool to assist the phytosanitary risks, associated with newly introduced tuber crops in Belgium

K. De Jonghe [1], Y. Foucart[1], A. Tack[2], S. Buysens[2], and N. Viaene[1]

[1]Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Merelbeke, Belgium; [2]PCG - Vegetable Research Centre (PCG), Kruisem, Belgium.

(kris.dejonghe@ilvo.vlaanderen.be) *S2-O3*

The increasing introduction of new exotic crops in Europe are an opportunity for growers to produce for niche markets. These new crops, as well as “forgotten” crops, are mainly grown and marketed outside the general, large-scale commercial agriculture. This local production, and its associated short food supply chain, obtain planting materials (seed, tubers, cuttings) and products from different sources. The phytosanitary status of these materials is very rarely checked. Entry and spread of possible plant pathogens and pests could pose a threat to traditional crops, besides hampering the cultivation of the new crops. Especially when planting material is obtained from non-European countries, phytosanitary risks can be high. We present the results of a project focusing on viruses and nematodes – both holding a high potential of being introduced without being noticed - in yacon (*Smallanthus sonchifolius*), ulluco (*Ullucus tuberosus*), sweet potato (*Ipomoea batatas*), crosne (*Stachys affinis*), mashua (*Tropaeolum tuberosum*), and oca (*Oxalis tuberosa*) as new crops, and Jerusalem artichoke (*Helianthus tuberosus*) as a forgotten crop in Belgium. Together with classic detection techniques, HTS is used to conduct an unprejudiced mapping of the presence of high risk viruses, particularly in starting material. A multitude of viruses were detected in small scale production units, and in tubers being traded through the internet, and this in any of the included tuber crops, in particular sweet potato. Currently, the phytosanitary impact of these viruses is being assessed, eg. for sweet potato chlorotic stunt virus (SPCSV), a regulated pathogen that is definitely present in some of the sweet potato starting material. In addition, the fact that viruses are so numerous in some of these tubers indicates that not much attention is paid to their presence when trading and propagating these crops. At least one virus, *Physostegia* chlorotic mottle virus (PhCMoV) is drawing our attention, since it is a relatively new virus for our region and has been reported to induce serious disease symptoms in tomato and pepper. Even if several *Pratylenchus* sp. were detected in some samples, no nematodes of major concern were found in tubers from the field nor in planting material. Although none of the *Pratylenchus* sp. have a quarantine status, and all are commonly found in Belgium, attention should be paid, as it can lead to damage on the tuber crops that were assessed.

Tomato brown rugose fruit virus in the Netherlands: The rise of a novel clade

M. Botermans[1], P.P.M de Koning[1], A.G. Oplaat[1], A.R. Fowkes[2], S. McGreig[2], I.P. Adams[2], A. Fox[2], K. De Jonghe[3], J.E. Demers[4], J.W. Roenhorst[1], M. Westenberg[1], and B.T.L.H. van de Vossenberg[1]

[1]National Reference Centre of plant health, National Plant Protection Organization, the Netherlands; [2]Fera Science Ltd, York Biotech Campus, Sand Hutton, York, United Kingdom; [3]Plant Sciences Unit, Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium; [4]Bayer Crop Science, Bayer Crop Science, St. Louis, Missouri, United States.

(m.botermans@nvwa.nl) *S2-O4*

In the Netherlands, tomato brown rugose fruit virus (ToBRFV) was first identified in tomato crops in 2019. Since then, the National Plant Protection Organization (NPPO-NL) is performing surveys to track and trace this regulated virus aiming for its eradication. According to the EU implementing regulation, samples are tested with real-time RT-PCR for virus detection and confirmation. Additionally, to gain more insight in the epidemiology of this virus, for samples with sufficiently high virus concentration, whole genomes are assembled based on Illumina RNA sequencing data. We integrated whole-genome phylogenetics with meta-data such as host, variety, seed batch, geographic location, but found no associations. This indicates that multiple introductions may have accounted to the outbreaks in the Netherlands. Furthermore, our analyses suggested that that the virus was probably already present in the Netherlands in 2018 or 2017 and had been introduced at least three times (1). Thanks to strict hygiene measures, the majority of infested growers managed to eradicate the virus during crop rotation. Those who did not succeed, remained infested with the same virus sequence type, suggesting elimination was unsuccessful. Intriguingly, in 2021 this pattern changed, when many re-infestations concerned a common novel virus sequence type, indicating a shared source. Therefore, investigations on the epidemiology of ToBRFV will be continued, including the analysis of sequence data available from other countries.

NPPO-NL maintains a publicly available interactive ToBRFV Nextstrain webpage (2) displaying data from our tracing research supplemented with genome sequences that are either retrieved from NCBI GenBank or kindly shared by international partners. This dataset facilitates a better understanding of the global diversity and spread of ToBRFV.

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Evaluating the threat of introducing non-European virus isolates of tomato leaf curl New Delhi virus into Europe

S. Winter and P. Margaria

Plant Virus Department, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany.

(stephan.winter@dsmz.de) *S2-O5*

Tomato leaf curl New Delhi virus (ToLCNDV) causing a severe disease of zucchini and melon, was first reported from Spain in 2012 and has since spread to other Mediterranean countries, Italy, Greece and France. The European strain, ToLCNDV-ES, forms a monophyletic sequence clade and all viruses so far analyzed in Europe likely originate from a single introduction. ToLCNDV is associated with cucurbit diseases but can also infect tomato and eggplant. We have conducted a comparative study between a ToLCNDV isolate from India (ToLCNDV) and the strain ToLCNDV-ES, to evaluate spread parameters and the risk associated with introduction of new, *non-European*" isolates of this virus. Both viruses share 90% identical DNA-A sequences, within the diverse species cluster of ToLCNDV and in addition, form viable pseudo-recombination. In contrast to ToLCNDV-ES, the Indian virus is readily and efficiently transmitted by *B. tabaci* to tomato to cause a severe yellow mosaic disease, while it cannot be transferred to zucchini or other cucurbits. Whitefly transmissions of ToLCNDV-ES onto tomato were less efficient and erratic. Whitefly transmission studies with TYLCV and TYLCNDV showed that the Indian virus, similar to TYLCV, rapidly infects tomato. Both viruses cause stable mixed infections with mixed yellow mosaic/leaf curl symptom types that eventually pass over to severe leaf curl symptoms of new growth when plants shed old leaves. More critically, impact is expected from infections of Ty-1 tomatoes with ToLCNDV because this resistance is not effective. While the onset of ToLCNDV infections in Ty-1 resistant plants is quite delayed compared to susceptible genotypes, plants eventually succumb to the disease and show similar symptoms. The effects of a TYLCNDV infection on TYLCV in resistant tomato genotypes will be presented.

The spread of cassava brown streak viruses, CBSV and UCBSV, from coastal Africa to the continent

S. Sheat, and S. Winter

Plant Virus Department, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Culture, Braunschweig, Germany.

(samar.sheat@dsmz.de)

S2-O6

Cassava brown streak disease (CBSD) is caused by two virus species, CBSV and UCBSV (U/CBSV), that differ in virulence and plant invasion however causing an almost indistinguishable disease and impact in cassava. We are studying spread parameters of the two viruses to elucidate and predict the direction of further outbreaks and to assess the composition of viral populations. We used nextstrain (www.nextstrain.org) to reconstruct the geographic structure of 140 complete virus genomes and to explore the spread of the viruses. From the Kenyan and Tanzanian coast, the likely origin of the viruses, severe outbreaks of CBSD started only from 2004/2005 onwards and were triggered by an increased regionwide movement of new cassava materials. Thus, focal points of the outbreaks were located to plant breeding institutions and active propagation sites. Our nextstrain analysis attributed geography and time to virus isolates and thus pathways of virus movement and introduction could be identified. We could also show that the geographic expansion of U/ CBSV is not consistent with an increase in genetic diversity. The westward expansion of the viruses causing CBSD into Eastern Congo (Kivu North, Kivu South) can be traced to recurrent introductions of diverse virus isolates from Uganda and from Rwanda. We also can conclude from 3 years field trials in the epidemic zone, that human assisted spread is key to virus dissemination even on a field scale and vector transmission of the viruses does not play a critical role in spreading the viruses opening avenues for virus control.

Dispersion and evolutionary history of rice yellow mottle virus in West and Central Africa: tales of rice and men

N. Poulicard[1], O. Traoré[2], S. Issaka[3], A. Pinel-Galzi[1], J. Aribi[1], D. Fargette[1], E. Traoré[2], Y. Séré[4], D. Silué[4], E. Hébrard[1]

[1]PHIM, Université Montpellier, IRD, INRAE, Cirad, Institut Agro, Montpellier, France; [2]INERA, LNB, Ouagadougou, Burkina Faso; [3]Université Boubakar Bâ, FSA, Tillabéri, Niger; [4]AfricaRice, Côte d'Ivoire.

(nils.poulicard@ird.fr) *S2-O7*

Rice has become a pillar of food security in Africa. During the 20th century, rice cultivation intensified to cope with the rising demand due to demographic changes in West and Central Africa (WCA). Rice yellow mottle virus (RYMV) is a major biotic constraint to rice cultivation in Africa. RYMV is a (+)ssRNA virus transmitted at short distance by beetles and by contact during cultural practices. Six major strains were identified with a marked spatial-based diversity (1). Several sources of resistance against RYMV were identified in rice, but none of them are currently widely deployed in field. Resistance-breaking risk maps were proposed based on the spatial distribution of the strains and their pathogeny in controlled conditions (2). However, the validity and the sustainability of these risk maps are strictly dependent on the dispersal and adaptive characteristics of the RYMV in field conditions. The main objectives of this study are i) to reconstruct the dispersion dynamics of RYMV in WCA, ii) to identify the main drivers of RYMV evolution and dispersion, and iii) to estimate the impact of RYMV evolutionary history on the sustainability of the resistance genes in the fields. Thus, based on the RYMV genetic data collected in WCA since the 1970's, the phylogeography of RYMV was reconstructed using Bayesian evolutionary inference. These spatio-temporal reconstructions revealed links between RYMV expansion dynamics and the rice cultivation history in Africa. In addition, we demonstrated that the geographic dispersion of the RYMV in WCA shaped genetic evolution, with the emergence of adaptive mutations to new host species and of inter-strain recombination events. In addition, experiments in controlled conditions demonstrated that virulence factors were exchanged in fields, even without selection pressure from resistance genes. Altogether, we partially deciphered the balance and the interplay between genetic determinants and stochasticity in evolution and epidemiology of a plant virus.

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Deciphering the Influence of soil structure and nutrients on furovirus infection rates in wheat

K. Gauthier[1], D. Pankovic[2], M. Nikolić[3], C. Paris[4], C. U. Germeier[5], F. Ordon[2], D. Perovic[2] and A. Niehl[1]

[1]Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn Institute, Brunswick, Germany; [2]Institute for Resistance Research and Stress Tolerance, Julius Kühn Institute, Quedlinburg, Germany; [3]Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia; [4]State Institute for Agriculture and Horticulture Saxony-Anhalt, Centre for Agronomy and Crop Production, Bernburg, Germany; [5]Institute for Breeding Research on Agricultural Crops, Julius Kühn Institute, Quedlinburg, Germany.

(annette.niehl@julius-kuehn.de) *S2-O8*

The influence of nutrients and chemical elements on plant-pathogen interactions has been extensively studied during the last two decades (e.g. (1, 2)). However, the effect of the elements on infection and symptom severity depends on the plant-pathogen system under study. Here, we studied the influence of soil-structure parameters and mineral nutrients on furovirus infection rates in wheat. Furoviruses are transmitted by the obligate biotrophic plasmodiophorid *Polymyxa graminis.* As the vector infects and replicates in root cells as well as transmits virus during its replication cycle, and *P. graminis* zoospores are mobile in the soil to infect new roots, understanding interactions between soil, root, vector and virus and identifying factors influencing infection would allow to develop new strategies for virus control. Susceptible wheat lines were sown in infected soil gathered in France, Germany, Italy and UK. Soil samples were taken in different years from the same locations. After confirming that infection rates differed among fields using ELISA, we identified soil parameters and element composition in soil, leaves and roots of plants grown in different soils. Using stepwise backward modelling along with model averaging approaches, our study identified crucial parameters linked to furovirus infection. Interestingly, common parameters were identified to influence infection by two different furoviruses, soil-borne cereal mosaic virus (SBCMV) and soil-borne wheat mosaic virus (SBWMV), suggesting a possible influence of these parameters on the vector. By contrast, both analyses performed separately in the leaves and in the roots mostly identified the same parameters to explain SBCMV infection rates. None of these parameters was linked to SBWMV infection rates. The identified parameters were used to further predict SBWMV and SBCMV infection rates under specific conditions. The identification of crucial parameters influencing infection may eventually aid to the development of a microenvironment-adapted agriculture.

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Global risk predictions for Pierce's disease of grapevines

A. Giménez-Romero[1], J. Galván[1], M. Montesinos[2], J. Bauzà[3], M. Godefroid[4], A. Fereres[4], J. J. Ramasco[1], M. A. Matías[1], and E. Moralejo[2]

[1]Instituto de Física Interdisciplinar y Sistemas Complejos, IFISC (UIB-CSIC), 07122 Palma de Mallorca, Spain; [2]Tragsa, 07009 Palma de Mallorca, Spain; [3]Departamento de Geografía, Universidad de las Islas Baleares, 07122 Palma de Mallorca, Spain; [4]Instituto de Ciencias Agrarias, ICA-CSIC, 28006 Madrid, Spain.

(alex@ifisc.uib-csic.es) *S2-O9*

The clonal lineage of the bacterium *Xylella fastidiosa* (Xf) responsible for Pierce's disease (PD) poses a threat to viticulture worldwide. Although this vector-transmitted disease has remained mainly restricted to the United States, recent introductions on the islands of Majorca (Spain) and Taiwan have raised concerns about the risk of spreading worldwide. To assess this risk, here we build a climate-driven epidemiological model that simulates PD progression. The model considers the temperature-dependent infection process based on a 3-year inoculation assay and assume local disease propagation when climatic conditions are favourable. The model was successfully validated with spatiotemporal data of the PD distribution in the United States yielding a remarkable ~90% accuracy. Thereafter the model was applied to the main winegrowing regions worldwide, specially focusing in Europe as a case study based on the distribution of the main vector, *Philaenus spumarius*. Our model simulation reveals that most wine-quality producing areas in China, Europe, Argentina, Chile, South Africa, and Australia currently thrive in non-risk or transient-risk zones. To a lesser extent, epidemic-risk zones with low to moderate risk indices appear in coastal zones such as Mallorca and Apulia, where Xf outbreaks have been already detected. The European case shows how models assuming a vector heterogeneous distribution yield lesser extended epidemic-risk zones than previous risk maps. Overall, a global expansion of PD epidemic-risk zones is projected for 2050, although with low increase in risk indices. Our study highlights the importance of considering climate variability and an invasive criterion to obtain precise risk maps for plant health decision-making.

High throughput sequencing identifies a divergent strain of cherry latent virus 1 in sweet cherry in Greece

C.G. Orfanidou, A. Katsiani, N.I. Katis, and V.I. Maliogka

Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Plant Pathology Laboratory, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.

(chorfani@agro.auth.gr) *S2-P1*

Sweet cherry (*Prunus avium* L.) is an economically important fruit crop worldwide. Nevertheless, it is jeopardized by a large number of viral infections (1), with the majority of them being latent. In this study, leaf tissue from 8 sweet cherry trees collected from 7 orchards (2 counties) at different time periods (2009, 2014, 2019, 2020) was subjected to high throughput sequencing (HTS) analysis, in order to evaluate their phytosanitary status. Total RNAs were extracted and used as templates for cDNA library construction followed by HTS on an Illumina HiSeq 4000 platform. Reads were assembled into contigs using the *de novo* assembler 'Trinity' (v.2.2.0) and iterative mapping was then applied with 'Bowtie2'. Finally, BLASTn/BLASTx analyses were performed against local and online databases. Seven out of the 8 sweet cherry samples contained several contigs, 3,032-5,310 nucleotides (nts) in length showing high nt identity (82.3-84.2%) with a recently identified trichovirus from Georgia, i.e. cherry latent virus 1 (CLV-1) (2). *In silico* analysis succeeded in obtaining the full-length sequences of CLV-1, which revealed that the 7 isolates from Greece exhibit >95% nt identity among them and 82.1-84.3% nt (88-90.1% amino acid identity in translated proteins) identity with the Georgian CLV-1 isolate "Mskhvil Nakota" (MK770441). RT-PCR and Sanger sequencing confirmed the presence of the divergent CLV-1 in 7 out of the 8 analyzed samples. Further phylogenetic analysis indicated that the Greek CLV-1 isolates are separately grouped from the other characterized Georgian isolate. Moreover, 151 sweet cherry samples (126 from 20 commercial orchards and 25 from a nursery) were also tested for the presence of CLV-1 by RT-PCR. Interestingly, the virus was found in 31.1% (47/151) of the tested samples. Finally, Sanger sequencing of the partial capsid protein gene of 19 CLV-1 Greek isolates revealed high nucleotide similarity (94.9%) among them. Overall, the divergent CLV-1 strain seems to be widespread in Greek sweet cherry orchards. Further studies are required to evaluate its pathogenicity in sweet cherry and, potentially, in other *Prunus* species.

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Improving strategies for the detection of tomato brown rugose fruit virus

A. Skelton[1], A. Fowkes[1], L. Frew[1], J. van Gemert[2], Y. L. Loh[1], O. Maksimovic[3], R. Macarthur[1], M. Botermans[2], and A. Fox[1]

[1]Fera Science Ltd, York Biotech Campus, Sand Hutton, York, United Kingdom; [2]National Plant Protection Organization, Geertjesweg 15, 6706 EA Wageningen, The Netherlands; [3]National Institute of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia.

(adrian.fox@fera.co.uk) *S2-P2*

The distribution of the target pathogen within the individual sampled plant is a dynamic situation influenced by timing of infection, host life cycle, season and variety. Multiple molecular assays have been designed to detect tomato brown rugose fruit virus (ToBRFV), however, reliable plant sampling strategies are needed to support pathogen surveillance. The optimum sampling regime for reliable detection of ToBRFV throughout the growing season was determined with reference to timing of infection. These studies, based on the work of Samuel (1), compared detection from different plant parts with respect to variety, cropping season and timing of inoculation. Tomato plants of two varieties were inoculated at 8- or 17-weeks post emergence, then sampled at regular intervals from different parts of each plant for up to 20 weeks post-inoculation and tested by qPCR. In plants infected early in the growing cycle the virus was detected in the growing tips after 13 days. In older plants, irrespective of season or variety, the virus was detected earliest in sepals and fruit (14 – 35 days post inoculation). Where sepals are present, these present the best sample matrix for reliable early detection of the ToBRFV. Studies in the Netherlands to investigate optimum sampling strategies were conducted during active outbreaks in tomato crops. Samples were taken from plants with and without symptoms comprising old and young leaves, sepals and fruit. These were tested by qPCR for the presence of ToBRFV. Detection Cq values were compared to identify the most reliable plant parts for sampling. Sepals and young leaves were the most reliable sample type for virus detection. The implications for sampling strategies will be discussed. Alternative diagnostic surveillance strategies, including environmental monitoring in glasshouses are being investigated. A comparison of LAMP and qRT-PCR approaches for testing surface swabs and irrigation water samples will also be discussed.

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Development of quick and accurate diagnostic methods for tomato infecting viruses based on RPA and RT-RPA techniques

M. Kwak[1], W. K.Cho[2], and E. J. Kil[1]

[1]Department of Plant Medicals, Andong National University, Korea; [2]College of Biotechnology and Bioengineering, Sungkyunkwan University, Korea.

(kwak2604@gmail.com)

S2-P3

Unlike PCR, isothermal amplification reaction methods have been used for various diagnostics because they can produce amplification products that can be used for diagnosis under one temperature condition without a complicated process. Recombinase polymerase amplification (RPA) is also a type of isothermal amplification reaction, which usually produces sufficient amplification products with a reaction time of less than 30 minutes at a temperature of 37 degrees. We developed an RPA-based diagnostic method for seven major viruses that occur mainly in tomatoes grown in Korea [tomato yellow leaf curl virus (TYLCV), tomato chlorosis virus (ToCV), cucumber mosaic virus (CMV), tomato mosaic virus (ToMV), Southern tomato virus (STV), tomato bushy stunt virus (TBSV), and tomato spotted wilt virus (TSWV)]. TwistAmp® Basic kit was directly used only in the case of TYLCV, which has DNA as its genome, and RevertAid Reverse Transcriptase was added to the reagent to diagnose the rest of the viruses through RT-RPA directly from RNA. After designing at least 5 sets of primers for each virus, the primer showing the clearest amplification product was selected (1). For viruses with multiple segments, primer sets targeting different genes were prepared and selected. Afterwards, each diagnostic condition (temperature, time, magnesium ion concentration, etc.) was optimized. After that, in order to perform real-time diagnosis, Miami Green, a DNA-Binding Fluorescent, was added to the reaction, and then a method was also developed to confirm the amplification product through the amplification curve using real-time PCR equipment.

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Unveiling the virome of stone fruit trees in Greece using high throughput sequencing approaches

A. Katsiani[1], C.L. Sassalou[1], C. Orfanidou[1], C. Beta[1], N. I. Katis[1], P. Drogoudi[2], and V.I. Maliogka[1]

[1]Faculty of Agriculture, Forestry and Natural Environment, School of Agriculture, Plant Pathology Laboratory, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; [2]Department of Deciduous Fruit Trees, Hellenic Agricultural Organization-'DIMITRA', Institute of Plant Breeding and Genetic Resources, 38 R.R. Station, 59035 Naoussa, Greece.

(sassalou@agro.auth.gr) *S2-P4*

During the last decade the application of high throughput sequencing (HTS) has facilitated the identification of several new fruit tree viruses. Since 2018, screening tests are being performed in several prunus species in Greece for the detection of viruses included in the European Commission Directive concerning official inspections (2014/98/EU). To identify putative new viral pathogens and/or divergent virus isolates infecting Prunus, five sweet cherry samples and one pooled (sweet cherry and plum) sample were subjected to HTS. Total RNA extraction was performed with Total RNA purification Kit (Norgen Biotek, Canada) and the samples were sequenced in NovaSeq6000 Illumina platform. For the *de novo* assembly of the genome the Geneious Prime and the 'Trinity' (v.2.2.0) algorithm were employed. All contigs were compared with available in NCBI database (Blastn, BlastX, TBlastX) nucleotide and aminoacid sequences. Several already known viruses were determined along with some newly emerged viruses belonging to different genera such as cherry latent virus 1 (CLV-1), prunus virus F (PrVF), cherry virus F (CVF), prunus virus T (PrVT) and prunus virus I (PrVI). Plum bark necrosis and stem pitting-associated virus (PBNSPaV) and nectarine stem pitting-associated virus (NSPaV) were detected in the pooled sample, and this is the first time these viruses are reported in stone fruits in Greece. All newly recorded viral pathogens are currently under characterization. This study has enriched the list of the viruses infecting Prunus species in Greece.

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A decade of foreign pest interceptions and introductions in Spain

B. Dáder[1], E. Viñuela[1], and P. del Estal[1,2]

[1]Department of Crop Production, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, Spain; [2]National Reference Laboratory for Arthropods, Spain.

(beatriz.dader@upm.es) *S2-P5*

The Spanish Reference Laboratory for the identification and diagnosis of arthropods was created by the Ministry of Agriculture in 1983 and established at the Polytechnic University of Madrid from 1997 to 2020. Samples from vegetal material intercepted at the Spanish border inspection points or by the Plant Protection Services of the different provinces in the last ten years were mounted in cardboards or microscope slides according to standardized procedures and identified with existing literature. The species name, family, order, host plant, host category, point of interception and country of origin were annotated. Samples collected in the country were processed similarly but distinguished as introductions. The number of interceptions have decreased since 2016, due to the lower amount of samples received for diagnosis. However, the percentage of interceptions referred to analyzed samples shows an upward trend. Intercepted pests mostly belong to order Hemiptera (34%, being the most prevalent families Diaspididae and Aleyrodidae), followed by Lepidoptera (23%, Tortricidae) and Diptera (18%, Tephritidae). America is the continent of origin with more samples intercepted (41%, Brazil), followed by Africa (33%, South Africa). Fruits (46%, orange and mango) and vegetables (19%) are the predominant hosts. Barcelona, Valencia and Madrid are the three border inspection points with more interceptions (42, 22 and 17%, respectively). Potential vectors are highlighted (17% of interceptions). With regard to introductions in Spain, a mean rate of 3.5 new species per year was recorded in the last 6 years. Hemiptera (Psylloidea and Aphididae), Coleoptera (Chrysomelidae) and Thysanoptera (Thripidae) are the most prevalent orders introduced. Ornamentals and fruits (citrus) are the most attacked hosts. Introductions were mostly reported in Tenerife and Barcelona. A timeline of the most damaging pests introduced in the country is included, with a rate of 33% of potential vectors.

Characterization of the viral community associated with pineapple mealybug wilt diseased plants in Reunion Island through a metagenomic approach

D. Massé[1,2], T. Candresse[3], D. Filloux[4], S. Massart[5], N. Cassam[1], B. Hostachy[1], A. Marais-Colombel[3], E. Fernandez[4], P. Roumagnac[4], E. Verdin[6], P. Y. Teycheney[7], P. Lefeuvre[7], and J. M. Lett[7]

[1]ANSES - LSV RAPT, Saint Pierre, La Réunion, France ; [2]Université de La Réunion, UMR PVBMT, Pôle de Protection des Plantes, Saint-Pierre, Ile de La Réunion, France ; [3]INRAe, UMR 1332 Biologie du Fruit et Pathologie, INRAe-Univ. Bordeaux, Villenave d'Ornon, France ; [4]CIRAD, UMR PHIM, Campus International de Montferrier-Baillarguet, Montpellier, France ; [5]Plant Pathology Laboratory, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgique ; [6]INRAe, UR407 Unité de Pathologie Végétale, Montfavet, France ; [7]CIRAD, UMR PVBMT, Pôle de Protection des Plantes, Saint-Pierre, Ile de La Réunion, France.

(delphine.masse@anses.fr) *S2-P6*

Pineapple is the third most cultivated tropical fruit crop in the world and the leading fruit production sector in Reunion Island, where Queen Victoria pineapple (*Ananas comosus*) is the flagship local fruit for export. However, pineapple plantations throughout Reunion have been affected by an upsurge of pineapple mealybug wilt disease (PMWD) for several years. Data available from the literature suggest that the etiology of PMWD involves a complex of viruses (1). We characterized the virome of symptomatic pineapple leaf samples collected from plantations throughout Reunion Island, using second (Illumina, HiSeq) and third generation (Oxford Nanopore Technologies, MinION) sequencing tools. Our results show the presence of up to nine distinct viruses in the analyzed samples. Seven viruses were characterized previously and belong to families *Closteroviridae* (PMWaV1, -2, -3, -5 and -6), *Caulimoviridae*, (PBCoV) and *Secoviridae* (PSVA). Two new viruses were characterized: pineapple mealybug wilt-associated virus 7 (PMWaV7) belong to genus *Ampelovirus* (family *Closteroviridae*) and pineapple vitivirus A (PinVA) to genus *Vitivirus* (family *Betaflexiviridae*). Our results underline the existence of an important viral community potentially associated with PMWD in Reunion. They stress out the importance of developing accurate diagnostic tools to assess the sanitary status of pineapple in Reunion and the need to implement the use of certified pineapple planting material to control the spread of PMWD.

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Early warning system and mitigation strategies for Tomato brown rugose fruit virus

E. Vogel[1], Z. Zisi[2], N. Ortega-Parra[3], C. Vos[2], and I. Hanssen[1]

[1]De Ceuster Meststoffen NV (DCM), Belgium; [2]Scientia Terrae Research Institute VZW, Belgium; [3]De Ceuster Meststoffen BV (DCM), the Netherlands.

(evo@dcm-info.com) *S2-P7*

Tomato brown rugose fruit virus (ToBRFV) is a recently emerged tobamovirus that poses a huge threat to commercial tomato cultivation worldwide. While closely related to Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV), research has shown that this devastating virus is able to overcome the robust Tm-2^2 resistance in tomato (1,2). Due to its high persistence and easy mechanical transmission, ToBRFV is extremely hard to get rid of once it enters the greenhouse. Current management strategies therefore centre around preventive measures in an attempt to keep the virus out of the crop. Consequently, early detection of the virus plays an important role. Our research shows that ToBRFV circulates in drain water and can be detected in drain water samples before plants start showing symptoms. We illustrate that drain water can be used to monitor ToBRFV outbreaks and thus can serve as an early warning system. Furthermore, new screening methods were developed to determine viral (in)activity, indicating that ToBRFV found in drain water can still be infectious. Disinfection of water in recirculation systems is thus a vital point of defence for the grower. This will be illustrated with several practical examples. Our research identifies drain water as a cornerstone in the current containment efforts for ToBRFV.

We will also present the EU-funded VIRTIGATION project, in which ToBRFV is a major focus. This project will develop solutions and mitigation strategies to emerging viral diseases caused by tobamoviruses and begomoviruses in cucurbits and tomatoes in Northern Europe and the Mediterranean Basin.

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Schlumbergera virus X in Dragon Fruit (*Hylocereus spp.*) in Spain

D. Janssen, C. García, and L. Ruiz

Centro La Mojonera, IFAPA, Almeria, Spain.

(dirk.janssen@juntadeandalucia.es) *S2-P8*

The Dragon fruit (*Hylocereus undatus*) is a high-value fruit crop, introduced about a decade ago in the mainland of Spain. In 2021, chlorotic spots were observed on young cladodes in a commercial dragon fruit orchard in the province of Seville (southern Spain). Total RNA was extracted from the symptomatic cladodes. Reverse transcription (RT)-PCR, which was carried out using general tobamovirus, and specific Cactus virus X primers, failed to produce any amplicons. Instead, potexvirus group primers (Potex F5/Potex R2), amplified an expected 584-bp amplicon from RNA extracts of field-collected samples. The RT-PCR products from four samples were Sanger-sequenced. All showed identical sequence results (GenBank Accession MZ614940) with a predicted amino acid identity of 99% with the corresponding RNA-dependent RNA polymerase amino acid sequence of Schlumbergera virus X (SchVX). SchVX-specific primers that were designed based on the new sequence, amplified the expected amplicon of around 430 nucleotides from the total RNA extracts of the four samples. This pair of primers were used in RT-PCR tests on subsequent surveys in 2 commercial dragon fruit greenhouses from the province of Seville, and in 1 experimental greenhouse in the province of Almeria. All samples from 25 symptomatic plants of *H. undatus, H. hybridum, H. costaricensis,* and *H. purpusii* in Seville and from 1 symptomatic *H. undatus* plant from Almeria tested positive for SchVX, while 15 asymptomatic plants tested negative. The results obtained in this investigation support that SchVX is present in the cladodes of dragon fruit plants expressing the symptoms. It is concluded that SchVX has been introduced in dragon fruit farms from Spain and propagation of this emerging crop through planting of cuttings should include testing for this virus in order to prevent further spread.

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Development of a reverse transcription loop-mediated isothermal amplification assay for detection of pepper viruses

E. Baek[1], J. Y. Yoon[2], and P. Palukaitis[1]

[1]Department of Horticultural Science, Seoul Women's University, Seoul, Republic of Korea; [2]Graduate School on Plant Protection and Quarantine, Jeonbuk National University, Jeonju, Republic of Korea.

(eseul@swul.ac.kr)

S2-P9

Cucumber mosaic virus (CMV), tomato spotted wilt virus (TSWV), pepper mild mottle virus (PMMoV), broad bean wilt virus 2 (BBWV2) and pepper mottle virus (PepMoV) are the major viruses in pepper and other solanaceous crop-growing areas in Korea. Using the Reverse Transcription-Loop Mediated Isothermal Amplification (RT-LAMP) assay, a rapid method that does not require a thermal cycler, diagnosis systems for these five of viruses were constructed in this study. The primer sets consisted of six primers [F3, B3, FIP, BIP, LF (Loop-F), and LB (Loop-B)], designed for RT-LAMP assays, and optimal RT-LAMP conditions were established. These primer sets were tested for primer specificity and sensitivity using viral cDNA as LAMP templates. Then, total RNAs were extracted from the virus-infected plants and healthy plants using a commercial total RNA extraction kit. RT-LAMP reactions were performed at 65°C for 45 min, and then heated to 80°C for 10 min for termination. The results of the RT-LAMP assay were observed visually, based on color changes due to lowering of the pH during the polymerase reaction. The detection limit of the RT-LAMP assay was shown to be more sensitive than that of conventional RT-PCR. The RT-LAMP assay system developed in this study is expected to be widely applied for detection of viruses in the major vegetable crops with a rapid nucleic acid extraction technology for field diagnosis.

Detection of Augusta disease in tulip and other flowerbulbs

B. Mulder[1], E. T.M. Meekes[2], I. van Duivenbode[3], C. de Krom[4], I. C.C.M.M. Stijger[5], M. Verbeek[6], and I. J.E. Stulemeijer[1]

[1]Flower Bulb Inspection Service (BKD), Lisse, The Netherlands; [2]Naktuinbouw, Roelofarendsveen, The Netherlands; [3]Dutch General Inspection Service for agricultural seeds and seed potatoes (NAK), Emmeloord, The Netherlands; [4]National Plant Protection Organization (NPPO-NL), Wageningen, The Netherlands, [5]Greenhouse horticulture & Flower Bulbs, Wageningen University & Research (WUR), Bleiswijk, The Netherlands; [6]Biointeractions and Plant Health, Wageningen University & Research (WUR), Wageningen, The Netherlands.

(Iris.Stulemeijer@bkd.eu) *S2-P10*

Augusta disease was observed for the first time in 1928 in The Netherlands, in the tulip cultivar 'Augusta' and causes stunted growth and chlorotic and necrotic stripes on leaves and flowers. Originally, Augusta disease was reported to be caused by *Tobacco necrosis virus* (TNV), a necrovirus transmitted by the vector *Olpidium brassicae* (1). Additional serological research revealed that two types of TNV could be distinguished, type A and D (2). Initially, isolates from tulip were characterized as TNV-D. However, in 2009 these isolates were recharacterized as *Olive mild mosaic virus* (OMMV), as the isolates had a remarkably high homology to this new necrovirus (3). Interestingly, it has been reported in Japan that another necrovirus *Olive latent virus 1* (OLV-1) can also infect tulips (4). To detect and confirm Augusta disease infection in tulips, an ELISA test based on an antiserum raised against Augusta disease in tulips (most likely now classified as OMMV) is used in our laboratory. As OMMV and TNV-D have homologous coat proteins, it is likely that the ELISA test detects both viruses as well as other related necroviruses. This however complicates correlation analysis between symptoms and virus. Another challenge of the ELISA test is that it is only applicable for testing of symptomatic material, which hampers screening of larger lots. To investigate the occurrence of the Alphanecroviruses OMMV, TNV-A and OLV-1, and the Betanecrovirus TNV-D in tulip, hyacinths and liliums, specific TaqMan PCRs were developed. Using these PCRs, we will further enhance our understanding of Augusta disease in various flower bulb crops.

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Comparative sensitivity of nucleic acid-based diagnostic assays for detecting the banana bunchy top virus in banana plants and aphid vector

A. O. Adediji[1,2], G.I. Atiri[2], and P. L. Kumar[1]

[1]International Institute of Tropical Agriculture, Oyo Road, PMB 5320, Ibadan, Nigeria; [2]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria.

(L.kumar@cgiar.org)

S2-P11

Banana bunchy top virus (BBTV, genus *Babuvirus*) is a major threat to banana (and plantain, *Musa* spp.) production worldwide. The virus gained prominence in sub-Saharan Africa as an emerging virus of high quarantine importance. The virus spreads through vegetative propagation of planting material and by the banana aphid, *Pentalonia nigronervosa*. In the absence of durable host resistance, virus control strategies focused on surveillance for infections in plantations and planting material for early detection and eradicating infected plants. This study evaluated five diagnostic methods, polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and TaqMan® real-time PCR, to determine the relative sensitivities of each assay for the detection of BBTV in plants and aphids during surveillance programs. New oligonucleotide primers were designed targeting the BBTV DNA-N segment and evaluated their detection sensitivity and specificity in PCR and LAMP assays. Compared with the existing BBTV DNA-R-based primers, the assays targeting the DNA-N segment were ten-fold more sensitive in both PCR and LAMP, with detection limits at 10 pg/µl and 100 fg/µl of banana host DNA, respectively. Direct DNA binding PCR using leaf and pseudostem sap extracts was sensitive at 1 ng/µl total DNA. Direct-binding LAMP and RPA were as sensitive as conventional PCR, thus potentially eliminating the need for DNA extraction in routine diagnostics. All these assays were useful for virus detection in single aphids. The TaqMan® qPCR detection sensitivity was similar to that of conventional PCR. Improved and rapid detection targeting DNA-N provided the most sensitive detection. Assessed the relative merits of these various diagnostics options for BBTV detection and provided recommendations to select the best fit tool according to the testing objective and available facilities by plant health workers for decision-making in the management of BBTV.

Rapid and sensitive detection of rice stripe virus by RT-RPA and real-time RT-RPA methods

J. Jeon, and E. J. Kil

Department of plant Medicals, Andong National University, Andong, Korea.

(wjswlgh15@naver.com)

S2-P12

Rice (*Oryza sativa*) is the main crop in East Asia, and infection with various pathogens has been reported. Rice stripe virus is a virus that mainly occurs in China, Japan, and Korea, and is transmitted by the small brown planthopper (*Laodelphax striatellus*)(1). In Korea, intermittent outbreaks have been reported mainly by migrated insects from China. We tried to develop a diagnostic technology that can quickly and reliably diagnose RSV in the field based on the Recombine polymerase amplification (RPA) method. TwistAmp® Basic kit was used after adding RevertAid Reverse Transcriptase to diagnose RSV through one-step RT-RPA without additional cDNA synthesis step from RNA. After designing at least 5 sets of primers from RNA dependent RNA polymerase coding sequence, the primer set showing the clearest amplicon was selected, and then optimized conditions for temperature, time, magnesium ion concentration, etc. was confirmed. After that, to identify real-time amplification, Miami Green, a DNA-Binding Fluorescent, was added to the reaction compounds, and amplification curves were described using real-time PCR equipment. Through qRT-RPA, it was observed that the amplification product was confirmed in 6-7 cycles (about 120-140 seconds). Through this, it was confirmed that this method can be usefully used for quantitative analysis of RSV within a short time.

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Metagenomic probes for the rapid identification of quarantine viruses in cereal grains

R. Gomes Ruschel[1,2], A.S. Espindola[2], F. Ochoa-Corona[1,2], M.R. Ribeiro-Junior[1,2,3], M. Malapi-Wight[4], X. Hu[5], and O. Hurtado-Gonzales[5]

[1]Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma, United States; [2]Institute of Biosecurity and Microbial Forensic, Stillwater, Oklahoma, United States; [3]Department of Plant Protection, School of Agriculture, Sao Paulo State University, Botucatu, Sao Paulo, Brazil; [4]USDA-APHIS-BRS Biotechnology Risk Analysis Programs, Riverdale, Maryland, United States; [5]USDA-APHIS-PPQ Plant Germplasm Quarantine Program – Beltsville, Maryland, United States.

(marcos.ribeiro@unesp.br) *S2-P13*

Infections of viruses in Poaceae crops can occur as single or mixed infections, often leading to a significant decline of yields. At least 33 viruses are known to infect crops grown for cereal grains, biofuel, and lawns. Several are of quarantine interest for the U.S. Current methods for detection and diagnosis are laborious and time-consuming since they target one virus at a time. This research aims to adapt High-Throughput Sequencing (HTS) into routine quarantine diagnostics using E-probe Diagnostic Nucleic Acid Analysis (EDNA). This method is capable of simultaneously detecting all 33 queried viruses infecting cereal grains. The MiFi® server was developed to host metagenomic databases, e-probe design programs, and detection software. E-probes are unique genomic sequences designed to target the virus of interest with high specificity and representative genome coverage. E-probes were designed and curated by BLAST screening to confirm specific detection for 33 virus species and seven host crops (barley, corn, miscanthus, sugarcane, sorghum, switchgrass, and rice). *In-silico* simulations of HTS-metagenomics-derived data were performed during the development and design process. A total of 16,876 e-probes were confirmed to detect only the 33 targeted viruses. Nine sugarcane samples previously known to contain 11 different viruses, out of the 33-virus target, were tested by RT-PCR and HTS sequenced (MinION®). The evidence presented here demonstrates that MiFi® detection via HTS sequencing outputs matches with RT-PCR detection in 90 percent of the samples tested. Thus, we showed that EDNA is a flexible bioinformatics and diagnostic pipeline that can assist during quarantine pathogen detection.

Mix infection of garlic virus D (GarVD), onion yellow dwarf virus (OYDV), and leek yellow stripe virus (LYSV) in garlic in Oklahoma, USA

M. R. Ribeiro-Junior[1,2,3], D. M. do Nascimento[2] , R. Gomes Ruschel[1,2], J. Olson[1], S. Wallace[1], and F.M. Ochoa-Corona[1,2]

[1]Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, Oklahoma, United States; [2]Institute of Biosecurity and Microbial Forensic, Stillwater, Oklahoma, United States; [3]Department of Plant Protection, School of Agriculture, Sao Paulo State University, Botucatu, Sao Paulo, Brazil.

(marcos.ribeiro@unesp.br) *S2-P14*

Garlic (*Allium sativum* L.) is an important crop grown worldwide and used as a seasoning and medicinal vegetable. In 2020, the production in the United States of America showed significant growth, reaching 9,996 ha harvested and a total production of 175.675 tons. In June 2021, virus-like-symptoms (mosaic, chlorotic streaking, and stunting) were observed in garlic cv. Lorz Italian Red from a local market producer in Payne county, Oklahoma, USA. Three garlic plants were submitted to the Oklahoma State University Plant Disease & Insect Diagnostic Laboratory (PDIDL). Symptomatic leaves were confirmed as potyvirus positive by using a ImmunoStrip® Lateral Flow Device (Agdia, Inc), which detects most members of the potyvirus group. A total of five leaves of the three plants were used for RNA extraction. A pooled RNA sample was subjected to viral metagenomics analysis after using MinIon Sequencing Technology (Oxford Nanopore Technologies). The total number of reads (72,806) were trimmed and aligned using BLASTn against the GenBank virus Database using Geneious Software. The number matching reads for garlic virus D - GarVD (NC022961), onion yellow dwarf virus - OYDV (NC005029), and leek yellow stripe virus - LYSV (JX429967) were 11,207, 3,212, and 2,907, respectively. The MinIon obtained matches were confirmed testing the five individual RNA used for high throughput sequencing by RT-PCR using primers Cpallexi-anti1/Cpallexi-senso2 (1), O3-F/O3-R (2), and L-F/L-R (2). The mixed infection was confirmed for each of the 5 leaves sourced from the 3 plants samples. The obtained amplicons were purified and sequenced, confirming the presence of GarVD, OYDV, and LYSV in the symptomatic garlic plants. After reviewing state records and literature, this study concludes that this is the first report of the occurrence of GarVD, OYDV, and LYSV naturally infecting garlic in Oklahoma. Allexiviruses and potyviruses significantly impact garlic production worldwide and are a concern for garlic production in the USA.

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Diversity of yellow dwarf viruses in south-eastern Australia

N. Nancarrow[1,2], S. Maina[3], S.K. Lam[1], B. Rodoni[4], M. Afab[2], and P. Trębicki[1,2]

[1]The University of Melbourne, Melbourne, Australia [2]Agriculture Victoria, Grains Innovation Park, Horsham, Australia [3]NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, Australia [4]Agriculture Victoria, AgriBio, Bundoora, Australia.

(piotr.trebicki@agriculture.vic.gov.au) *S2-P15*

Yellow dwarf viruses (YDV) are transmitted by aphids and infect cereals and other members of the *Poaceae* family. YDVs are prevalent in south-eastern Australia and cause significant yield losses in cereals in Australia and worldwide. While four YDV species (BYDV PAV, BYDV MAV, CYDV RPV and MYDV RMV) are commonly detected in Australia, the majority of Australian YDV studies have been based on serological tests which cannot differentiate between closely related species. To explore the diversity of YDVs in Australia, plant samples were collected from symptomatic wheat, barley, oat, wild oat and brome grass plants within and around 28 cereal paddocks across the Wimmera region of south-eastern Australia over two years (2020-2021). Each sample was individually tested for YDVs using tissue blot immunoassay (TBIA) with selected samples further tested by reverse-transcription PCR (RT-PCR) and high-throughput sequencing (HTS). While YDVs were detected at each site in each year of the study, virus infection was more prevalent in 2020 than 2021. Some differences were obtained between the results of TBIA and RT-PCR tests, and between different RT-PCR tests for the same YDV species, were obtained, indicating possible diversity which will be clarified by HTS. Not all symptomatic samples tested positive for YDVs, and these samples are being examined more closely. This information is critical for the development of more effective and targeted diagnostic methods, resistance-screening and control strategies.

Toward the characterization of a novel Bymovirus infecting rice in Burkina Faso by combining metagenomics and targeted sequencing

M. Bangratz[1], M. Barro[2], D. Sérémé[2], I. Wonni[2], A. I. Kassankogno[2], P. Roumagnac[1], D. Filloux[1], E. Fernandez[1], J. Orjuela[1], A. Comte[1], C. Tollenaere[1]*, and N. Poulicard[1]*

*[1]PHIM Plant Health Institute, Univ Montpellier, IRD, CIRAD, INRAE, Institut Agro, Montpellier, France; [2]Laboratoire de Phytopathologie, INERA, Institut de l'Environnement et de Recherches Agricoles du Burkina Faso, Bobo-Dioulasso, Burkina Faso. * Equal contribution.*

(martine.bangratz@ird.fr) *S2-P16*

Only two viruses have been described to infect rice in Africa, the rice yellow mottle virus (RYMV) and the rice stripe necrosis virus (RSNV). Using viral metagenomics, we aimed at exploring the diversity of viruses infecting rice in southwestern Burkina Faso. To this purpose, we collected rice samples in three irrigated perimeters and three rainfed lowland sites within two consecutive years (2016 and 2017). Within each site, 5-10 rice fields were surveyed and 16 plants were sampled following a grid-based regular approach. The 16 samples collected within each rice field were pooled to constitute a 'field-scale' sample. Eighty-four of these 'field scale' samples were used for Virion-Associated Nucleic Acid (VANA) metagenomics-based approach and sequenced by Illumina HiSeq. Results revealed a number of reads related to unexpected viruses, among which one belongs to the genus Bymovirus (family *Potyviridae*), which shared ca. 80% nucleotidic identity with the rice necrosis mosaic virus (RNMV). RNMV is a bipartite (+)ssRNA virus (1) transmitted by the soil protist *Polymyxa graminis* and causes stunting and yellow lesions (2). Only a partial genome was retrieved from the novel bymovirus-like virus, as Illumina reads sharing identity with RNMV were scattered around the viral genome. Thus, we further combined Sanger and long-read sequencing (ONT Nanopore) to characterize the full viral genome of this novel rice virus. Preliminary results showed that the assembled complete genome shares 73% nucleotide homology with the published RNMV sequence, so we are certainly in presence of a new virus species of the genus Bymovirus infecting rice in Burkina Faso. In addition, we demonstrated by specific RT-PCR detection assays that this virus is present whatever the rice cultivation mode and widely distributed among the different sites. In perspective, we plan to better assess and understand the epidemiology of this new Bymovirus "RNMV like"-species in African rice fields.

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Cucurbit chlorotic yellows virus is widespread in mixed infections on cucurbits and also infects wild radish (*Raphanus raphanistrum*), a common weed in Georgia, USA

S.R. Kavalappara[1], S. Bag[1], A. Sparks[2], C. Mcgregor[3], D. G. Riley[2], and W.M. Wintermantel[4]

[1]Department of Plant Pathology, University of Georgia, Tifton, GA, USA; [2]Department of Entomology, University of Georgia Tifton, GA, USA; [3]Department of Horticulture, University of Georgia, Athens, GA, USA; [4]United States Department of Agriculture-Agricultural Research Service, Salinas, CA, USA.

(sarirk@uga.edu) *S2-P17*

Over the past several years, cucurbit production in the southeastern USA has incurred severe losses due to increased whitefly (*Bemisia tabaci*) populations and the incidence of viruses transmitted by them. In Georgia, a major cucurbit-producing state, cucurbit leaf crumple virus (CuLCrV) and cucurbit yellow stunting disorder virus (CYSDV) were consistently detected from diseased samples. Another crinivirus, cucurbit chlorotic yellows virus (CCYV) was recently identified in the region by unbiased small RNA sequencing and de-novo assembly. Surveys conducted in the fall of 2019 and 2020 in the state showed that these viruses are more widely distributed than previously assumed. In a total of 820 samples of cantaloupes, cucumbers, and yellow squash tested, CuLCrV was detected in 76%, CCYV in 60%, and CYSDV in 43% of the total samples. The level of mixed infections was high in all the cucurbits, with most samples being infected with at least two of these viruses. Natural infection of CCYV was also detected on wild radish (*Raphanus raphanistrum* L.), a common weed in the family *Brassicaceae* that grows throughout the southeastern USA. Transmission assays using whiteflies demonstrated that wild radish is an excellent host for CCYV from which whiteflies can acquire and transmit the virus to cucurbit hosts. The role of crops and weeds, particularly those in the family *Brassicaceae* to serve as overwintering hosts of CCYV and other criniviruses in the southeastern USA and other parts of the world needs to be further studied.

Vector-borne diseases with non-stationary vector populations: the case of growing and decaying populations

A. Giménez-Romero[1], R. Flaquer-Galmés[2], and M. A. Matías[1]

[1]Instituto de Física Interdisciplinar y Sistemas Complejos, IFISC (UIB-CSIC), 07122 Palma de Mallorca, Spain; *[2]Grup de Física Estadística, Departament de Física. Facultat de Ciències, Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain.*

(alex@ifisc.uib-csic.es) *S2-P18*

Since the last century, deterministic compartmental models have emerged as powerful tools to predict and control epidemic outbreaks, helping to mitigate its impacts. A key quantity for these models is the so-called *Basic Reproduction Number*, that measures the number of secondary infections produced by an initial infected individual in a fully susceptible population. Standard methods have been developed to allow the direct computation of this quantity, provided that some conditions are fulfilled, such that the model has an initial disease-free equilibrium state. However, in vector-borne diseases, this is only accomplished when the vector population is *stationary*, this is, when the number of vector deaths are balanced with the same amount of vector births. On the other hand, many situations could lead to *non-stationary* vector populations. Here we study a vector-borne epidemic model with growing and decaying vector populations. We show that traditional methods to determine the basic reproduction number are valid only under some conditions and propose an alternative when standard methods fail. Furthermore, we show that growing vector populations produce a delay in the epidemic dynamics when compared to the case of the stationary vector population. Finally, we show that the model can be reduced to a traditional SIR model if some conditions are fulfilled, which helps in solving the problem of parameter unidentifiability of many vector-borne epidemic models.

Modelling plant resistance deployment: the R package landsepi

L. Rimbaud[1], J. Papaïx[2], J. F. Rey[2], J. L. Gaussen[2], M. Zaffaroni[3], and F. Fabre[3]

[1]INRAE – Pathologie Végétale, 84140, Montfavet, France; [2]INRAE – BioSP, 84914, Avignon, France; [3]INRAE – SAVE, 33882 Villenave d'Ornon, France.

(loup.rimbaud@inrae.fr) *S2-P19*

Owing to their evolutionary potential, plant pathogens are able to rapidly adapt to genetically-controlled plant resistance, often resulting in resistance breakdown and major epidemics in agricultural crops, as well as a continuing need to breed new resistant cultivars. Several strategies (e.g. gene pyramiding, crop rotations and mixtures, landscape mosaics) have been proposed to improve resistance deployment. They rely on the careful selection of resistance sources and their sensible combination at various spatio-temporal scales. However, experimental assessment of their efficiency (i.e. ability to reduce disease impact), durability (i.e. ability to limit pathogen evolution and delay resistance breakdown) and cost-efficiency (i.e. ability to maximise profit) at large spatio-temporal scales presents a major challenge (1). The R package landsepi provides a general modelling framework to help compare deployment strategies and understand the impact of epidemiological, evolutionary and genetic factors for a wide range of pathosystems. The model is based on (i) a spatial geometry for describing heterogeneous landscape and allocating different cultivars, (ii) a dispersal kernel for the dissemination of the pathogen, and (iii) a stochastic SEIR ('Susceptible-Exposed-Infectious-Removed') structure with a discrete time step for the description of the host-pathogen interaction, which involves both with qualitative and quantitative resistance genes. It accounts for pathogen evolution (e.g. via mutation, selection, drift, sexual reproduction) and provides epidemiological, evolutionary and economic outputs to assess the performance of the simulated deployment options. The model has initially been calibrated to represent spore-borne pathogens as typified by rust fungi of cereal crops (genus *Puccinia*); parameterization of other pathosystems including downy mildew on grapevine (caused by the oomycete *Plasmopara viticola*) and two viral diseases of pepper (caused by *Potato virus Y* and *Cucumber mosaic virus*) is ongoing.

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Session

03

Virus Ecology and Evolution

Virus host ranges and transmission dynamics in heterogeneous environments

F. García-Arenal

Centro de Biotecnología y Genómica de Plantas (CBGP), Universidad Politécnica de Madrid (UPM) and Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), and E.T.S.I. Agronómica, Alimentaria y de Biosistemas, Campus de Montegancedo, UPM, 28223 Pozuelo de Alarcón, Madrid, Spain.

(fernando.garciaarenal@upm.es)

Keynote Lecture 3

Understanding disease systems requires the analysis of multi-host – multi-parasite interactions that occur within heterogeneous communities. This approach is necessary for understanding the emergence of viral diseases in plants, which results from novel transmission dynamics between wild and crop plant communities. High throughput techniques allow approaching this goal without *a priori* biases towards "focal" hosts and viruses within communities, including potential non-antagonistic symbioses in wild plants. Following a metagenomic approach we analyse plant-virus interactions in an agricultural landscape in central Spain. We focus on four major habitats, each under different levels of human management: Crop fields (Crop), the edges between crop fields (Edge), successional scrublands with no particular human intervention (Wasteland), and the native Mediterranean evergreen oak forests (Oak). Four sites of each habitat were sampled following a strategy used to optimise species representation in each community. Pools of total RNA preparations from plant species sampled from each habitat were deep-sequenced, Field sampling and virus OTUs detection by high throughput sequencing libraries has generated a large data set on the interaction of about 160 virus OTUs with 120 plant species. On this data set which we test hypotheses relative to infection dynamics and host range evolution. I will present results showing that environmental heterogeneity: i) leads to non-random plant-virus associations organised into ecological compartments, ii) allows identification of reservoir communities, iii) conditions realised host ranges and intensity of host usage that iv) are largely independent of virus adaptation to host.

Plant virus mixed infections modulate vertical transmission

A. Cobos, M. Gil-Valle, and I. Pagán

Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Campus de Montegancedo, Universidad Politécnica de Madrid, Pozuelo de Alarcón, Madrid, Spain.

(alberto.cobos@upm.es) *S3-O1*

Mixed infections are widespread both in wild and agroecosystems. These virus-virus interactions may have great impact on the pathogen's fitness, which can be positive or negative depending on their interaction being synergistic or antagonistic, respectively. Most analyses on this subject used within-host multiplication and/or efficiency of vector transmission as virus fitness proxies. However, more than 25% of plant viruses are seed transmitted, this trait being a key fitness component of these viruses. Although exploring the consequences of mixed infections for virus seed transmission rate is central to fully understand the determinants of virus fitness, and consequently virus epidemiology and evolution, to date it received little attention. To address this question, we analyzed the efficiency of *Turnip mosaic virus* (TuMV) and *Cucumber mosaic virus* (CMV) seed transmission in six *Arabidopsis thaliana* genotypes, in which both viruses differ in their efficiency of vertical transmission in single infections, under co- and super-infection scenarios (i.e., co-inoculation and sequential inoculation, respectively). In the infected plants, we also quantified virus multiplication and virulence. Results indicated synergistic effects for TuMV and antagonistic effects for CMV seed transmission rate as compared with single infection. In addition, mixed infections did not affect the number of TuMV-infected seeds, but generally reduced CMV-infected seeds. Interestingly, in host genotypes where one of the viruses was not seed transmitted in single infections, co- and super-infection always resulted in transmission of both viruses. These changes in the efficiency of seed transmission were related to modifications in virus multiplication but not in virulence. Thus, this work provides novel information on the effect of mixed infections in the efficiency of plant virus seed transmission.

Global diversity of solanum nigrum ilarvirus 1 among diverse plant hosts and associated metagenomes and its biological characterization

M.P.S. Rivarez[1,2], C. Faure[3], L. Svanella-Dumas[3], A. Marais[3], M. Ravnikar[1], D. Kutnjak[1], and T. Candressse[3]

[1]National Institute of Biology, Večna pot 111, Ljubljana 1000, Slovenia; [2]Jožef Stefan IPS, Jamova cesta 39, Ljubljana, 1000 Slovenia; [3]Universite de Bordeaux, INRAE, UMR 1332 Biologie du Fruit et Pathologie, 33140 Villenave d'Ornon, France.

(mpsrivarez@gmail.com) *S3-O2*

Solanum nigrum ilarvirus 1 (SnIV1) is a new ilarvirus associated with tomato and wild *Solanum nigrum* from France (1). Interestingly, SnIV1 was concurrently detected in various metagenomic studies involving *Pisum sativum* and other *Fabaceae* plants from Germany (2), *Plasmopara viticola*-infected grapevines (*Vitis vinifera*) from Italy (3), and *Erysiphe necator*-infected grapevines from Spain (4), thus prompting the current study. First, we verified by RT-PCR the detection of SnIV1 in *Physalis* sp. from Slovenia, and grapevines, *Solanum villosum* and wild carrots (*Daucus carrota* subsp. *carota*) from France. We further investigated the global distribution of SnIV1 through palmID search on the Sequencing Read Archive (SRA) database (as of January 2021) in Serratus (5). We detected SnIV1 in 80 SRAs (100% identity, E-value=8.4×10^{-74}), which originated from the USA, Venezuela, Europe, and China, from sources dominated by plants, but also non-plant sources. We assembled SnIV1 genomes from high coverage hits in CZ-ID (6), from libraries of *Daphnia magna*, *Lithospermum erythrorhizon*, and *Jaltomata repandidentata*. We likewise obtained full SnIV1 genome from a BLAST search of the Transcriptome Shotgun Assembly of *Humulus lupulus* from Japan (7). When compared to existing genomes in our diversity and phylogenetic analyses, SnIV1 global isolates showed low sequence divergence. Moreover, we detected SnIV1 in greenhouse-introduced grapevine and *S. villosum* plants. Surface disinfected *S. villosum* leaves, stems, roots, fruits, seeds, flowers, and grapevine bark and phloem tissues were all positive in RT-PCR assays. Additional mechanical, graft and seed transmission assays are underway to verify its infectivity, and the possible presence of a culturable endophytic fungal host. Overall, we showed the global presence of a new ilarvirus, and initiated its biological characterization. Whether SnIV1 will be an emerging pathogen in tomato, grapevine and other crops cannot be determined, but our study may give us a head start in understanding its epidemiology and biology.

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Genome formula and coordinated gene expression in within-host populations of a multipartite virus

M. Bonnamy[1], Y. Michalakis[2], and S. Blanc[1]

[1]PHIM, Univ Montpellier, CIRAD, INRAE, IRD, Institut Agro, Montpellier, France; [2]MIVEGEC, Univ. Montpellier, CNRS, IRD, Montpellier, France.

(stephane.blanc@inrae.fr)

S3-O3

Multipartite viruses have a segmented genome, with each segment encapsidated independently. In these viral systems, complementary parts of the genetic information are physically separated, yet they must interact at least at some key stages of the life cycle. Because of this physical separation of distinct parts of their genetic information, multipartite viruses have evolved some "behaviors" or "capacities" that may be unique to this genome architecture and/or that have thus far been overlooked in virology. Here we address one such capacity that is the regulation of gene expression through gene copy number variation at the within-host population level. In all multipartite virus species for which the question has been addressed, the distinct segments reproducibly accumulate at a specific and host-dependent relative frequency, defined as the 'genome formula'. Using Faba bean necrotic stunt virus (FBNSV) as a model, we provide evidence that changes of the frequency of specific segments correlate with changes of the corresponding mRNAs production, indicating that genome formula variations modify the viral gene expression patterns. We further show that the genome formula modification upon host-switching is a "plastic" property of the FBNSV viral genome, as it does not depend on any sequence mutation. The mechanism governing the establishment and stabilization of the genome formula remains elusive. Two alternatives are imaginable (and not mutually exclusive): i) the genome formula results from processes acting at the level of individual segments, and/or ii) the genome formula is shaped by a process acting at a supra-segment level, possibly that of a group of interacting segments where not only the nature of the segments but also their relative copy number may be instrumental. Preliminary results strongly suggest that the second alternative is at least partly acting and that processes at a supra segment level are involved.

A cross-environment viromics study of tomatoes, weeds and water reveals many new plant virus species and links between sample types

D. Kutnjak[1], M. P. Selda Rivarez[1,2], O. Maksimović Carvalho Ferreira[1,2], K. Bačnik[1,2], Z. Kogej[1,2], A. Pecman[1,2], Ž. Lengar[1], N. Mehle[1,3], A. Vučurović[1], I. Gutiérrez-Aguirre[1], and M. Ravnikar[1]

[1]Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia; [2]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia; [3]School for Viticulture and Enology, University of Nova Gorica, Vipava, Slovenia.

(denis.kutnjak@nib.si)

S3-O4

High-throughput sequencing based virome studies enable an untargeted insight into the diversity of viruses in different plant species and in environmental samples, which allows discovery of unknown or unexpected viruses in plants and environment and identification of links by studying the co-occurrence of viruses in different sample types. We have investigated the virome of tomato, associated weed species, and irrigation and surface water samples from several locations in Slovenia. During two years, we collected 293 tomato samples, 143 wild plants samples from different botanical families and 24 samples of tap, underground and surface water. RNA was extracted from individual plant samples, and extracts were further pooled and sequenced using Illumina approach. RNA was also extracted from concentrated water samples, preamplified, and sequenced using Illumina approach. Bioinformatic analyses of sequencing data from plant samples, coupled with further targeted confirmation studies, revealed 37 known, and at least 56 new viruses. Most of the new viruses were associated with different species of wild plants growing close to tomato production sites (around 70% of RNA viruses detected in wild plants). A great diversity of plant viruses was also discovered in investigated water samples, where viruses with stable virions (tobamoviruses, tombusviruses) predominated. A diversity of viruses and their abundance was dependent on the water source type (e.g., surface vs. underground water). Most of the viruses were detected in one sample type (tomatoes/weeds/water), however, few of them were also detected across different sample types, implying their persistence in the ecosystem, e.g., a new tobamovirus, detected in *Plantago* sp. at one location, was further detected in water samples from different locations. This comprehensive virome study represents a baseline for understanding the epidemiological links between plants and environmental waters and will help us detecting and better understanding possible future emergences of viral diseases in tomato and other crops.

Factors that determine the epidemiology of a crop pathogen, tobacco mild green mosaic virus, in its wild reservoir *Nicotiana glauca*

R. De Andrés Torán, A. Fraile, F. García-Arenal

Centro de Biotecnología y Genómica de Plantas (CBGP), Universidad Politécnica de Madrid (UPM) and Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and E.T.S.I. Agronómica, Alimentaria y de Biosistemas, Campus de Montegancedo, UPM, 28223 Pozuelo de Alarcón, Madrid, Spain.

(rafaelde.andres@upm.es) *S3-O5*

Understanding the emergence of virus diseases in crops is hampered by limited knowledge of virus epidemiology in wild reservoirs (1). The tobamovirus tobacco mild green mosaic virus (TMGMV) is an important pathogen of pepper crops in SE Spain, where it emerged in the 1980s from its wild reservoir host *Nicotiana glauca* Grah. A duplication in the 3' end untranslated region of the virus genome is associated with differential multiplication in both hosts: genotypes with a long 3'UTR (Long-3'UTR) multiply to higher levels in *N. glauca*, while a Short-3'UTR determines higher multiplication in pepper (2). TMGMV incidence in *N. glauca* populations in SE Spain is very high (>60%) (2, 3), and the frequency of Short-3'UTR genotypes is maintained over years at about 20% despite its reduced multiplication in this host. Here we analyse determinants of the high incidence of TMGMV in *N. glauca* populations and the maintenance of Short-3'UTR genotypes. To this aim we analysed if Short-3'UTR and Long-3'UTR genotypes differ in traits that determine virus transmission in nature: i) incidence along seasons and years, ii) multiplication at different temperatures, iii) seed transmission rate and, iv) virulence under various conditions of temperature and water availability to the host plant. For these analyses virus multiplication was quantified in leaves of field infected plants and in experiments under controlled conditions. Results allow developing a model to explain the maintenance of the pepper-adapted Short-3'UTR genotypes of TMGMV in the populations of its wild reservoir at a constant frequency.

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Phylogenetics and evolution wheat streak mosaic virus: its origin and the source of the Australian epidemic

R. A.C. Jones[1,2], I. Vasques[3], S. McGreig[3], A. Fox[3], A. J. Gibbs[4]

[1]UWA Institute of Agriculture, University of Western Australia, Crawley, WA, Australia; [2]Department of Primary Industries and Regional Development, South Perth, WA, Australia; [3]Fera Science Ltd, Sand Hutton, York, UK; [4]Emeritus Faculty, Australian National University, Canberra, ACT, Australia.

(roger.jones@uwa.edu.au) *S3-O6*

Wheat streak mosaic virus (WSMV) seriously damages wheat worldwide, including in the Great Plains Region (GPR) of North America and in southern Australia's grainbelts. Its carry over between annual wheat crops sown either in quick succession or after a dry summer break is attributed to bridging infected volunteer wheat plants (GPR) or infected wheat seeds, both sown and volunteer (Australia), respectively. We report here the phylogeny of WSMV based on the complete genomic (CG) sequences from seven Australian wheat isolates, together with the published sequences of 56 CG and 134 coat protein (CP) genes. Eleven CG and three CP sequences were recombinant so were discarded. ML phylogenies of the remaining 52 CG and their CP sequences were closely correlated (R=0.994, *P*=<.00001). The phylogeny of all 183 CP genes had four well-supported phylogroups (I - IV): I, a single sequence from Mexico; II, six from Iran; and the crown groups, III and IV, each with basal Iranian sequences and either mostly Eurasian (III) or American (IV) terminal sequences. The Australian sequences formed a single sub-cluster of phylogroup IV linked by an Argentinian isolate to a cluster from Pacific north-west USA. No CG sequences from phylogroup II have been reported. The known sample collection dates of 40 CG sequences allowed WSMV dating by the RTDT method in MEGA-X using a ML phylogeny. The most recent ancestor was dated at 1415 CE, and the ancestor of the Australian cluster 1999.7 CE which is two years before WSMV was first reported in Australia. The simplest biogeographical explanation of the phylogeny is that WSMV first entered wheat in its domestication centre in the Middle East, and a basal lineage was introduced to Mexico from Spain after the 1519 Spanish conquest (NB no WSMV sequences from the Iberian peninsula or north-west Africa have been reported yet).

Decoding the wheat virome using metagenomics

C. Redila, V. Prakash, and S. Nouri

Department of Plant Pathology, Kansas State University, USA.

(shahidehnr@ksu.edu) *S3-O7*

Wheat viruses including wheat streak mosaic virus, Triticum mosaic virus, and barley yellow dwarf virus cost substantial losses in crop yields every year. Although there have been extensive studies conducted on these known wheat viruses, currently, there is limited knowledge about all components of the wheat (*Triticum aestivum* L.) virome. Here, we determined the composition of the wheat virome through total RNA deep sequencing of field-collected leaf samples. BLASTx searches using *de novo* assembled contigs not only identified documented wheat viruses but also novel plant and fungal-associated viral sequences. We obtained the full genome sequence of the first umbra-like associated RNA virus tentatively named wheat umbra-like virus in cereals. Moreover, a novel bi-segmented putative virus tentatively named wheat-associated vipovirus sharing low but significant similarity with both plant and fungal-associated viruses was identified. This new putative virus is probably the result of cross-kingdom infections and genetic exchanges. Additionally, new putative fungal-associated viral sequences were discovered in wheat samples. The discovery and characterization of novel viral sequences associated with wheat is important to determine if these putative viruses may pose a threat to the wheat industry or have the potential to be used as new biological control agents for wheat pathogens either as wild-type or recombinant viruses.

Viral diversity of cassava mosaic begomoviruses in coastal and western Kenya

A. E. Dye[1], Y. Rosado[2], V. Ly[2], B. Muga[3], J. Mwai[3], P. Labadie[4], E. Ateka[3], G. Kennedy[4], L. Hanley-Bowdoin[1], and J. T. Ascencio-Ibáñez[2]

[1]Plant and Microbial Biology Department, North Carolina State University, Raleigh, NC, USA; [2]Molecular and Structural Biochemistry Department, North Carolina State University, Raleigh, NC, USA; [3]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya; [4]Entomology and Plant Pathology Department, North Carolina State University, Raleigh, NC, USA.

(aedye@ncsu.edu) *S3-O8*

Cassava is a staple crop for over 800 million people globally, and its production is threatened by cassava mosaic begomoviruses (CMBs). These viruses led to a CMB pandemic in East Africa in the early 2000s, aided by viral recombination and a sharp increase in whitefly populations (1). CMBs are transmitted by vegetative propagation and whitefly transmission. Further study is needed to understand how these viruses evolve in the field. This study examined viral diversity in the field and after successive vegetative regrowth to measure the effect of vegetative propagation on viral evolution. Cassava plants with symptoms of CMD were sampled and collected from the Lake Victoria region and Coastal regions of Kenya in 2015 and were maintained by cuttings in a greenhouse for three years. Plants were cut back approximately every 6 months and leaf samples were taken in 2018. Samples were analyzed for the presence of geminiviruses by PCR and then sequenced using a next-generation sequencing pipeline developed for ssDNA viruses (2). Viral variants were analyzed using Galaxy pipelines, and variants in paired samples from the same plants were compared between 2015 and 2018. Viral diversity was also compared by region. The results of this study provide further insight into initial viral diversity in the field and the effect of vegetative propagation in the greenhouse on viral variants.

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Rice yellow mottle disease in western Burkina Faso: incidence, diversity and dynamics at local scale

D. Sérémé[1], N. Poulicard[2], E. Billard[2], M. Barro[1], M. Bangratz[2], I. Wonni[1], M. Koala[1], A. I. Kassankogno[1], E. Hébrard[2], G. Thébaud[2], C. Brugidou[2], and C. Tollenaere[1]

[1]Département Productions Végétales, INERA, Institut de l'Environnement et de Recherches Agricoles du Burkina Faso, Burkina Faso; [2] PHIM Plant Health Institute, Univ. Montpellier, IRD, CIRAD, INRAE, Institut Agro, Montpellier, France.

(charlotte.tollenaere@ird.fr) *S3-O9*

The rice yellow mottle virus (RYMV), genus *Sobemovirus*, family *Solemoviridae* constitutes a threat to the actual development of rice cultivation in Africa, inducing severe yield losses in all types of rice growing systems (1). While RYMV is a model in plant virus molecular epidemiology (2,3), the patterns of viral incidence and viral diversity at local geographical scale were much less described to date. This study includes a five-years monitoring of RYMV incidence in six sites, characterized by two rice growing systems (irrigated vs. rainfed lowland), in western Burkina Faso. This allows confirming the highly local patterns of prevalence, and to identify a disease hotspot. A screening of RYMV genetic diversity was then performed in this site though Coat Protein sequencing (132 samples). We found a very high genetic diversity, with the co-occurrence of four distinct lineages at small geographical scale (within one irrigated perimeter, 1100ha). One isolate from each group was fully sequenced (four complete genomes), revealing that one of these lineages results from a recombination event between two others. Temporal dynamics suggest an evolution of RYMV population (modifications in the relative frequencies of each genetic lineage) within the studied period (2015-2019). Then, in controlled conditions, we evaluated the virulence of viral isolates from each genetic group on a set of rice cultivars, including the three most frequently grown in western Burkina Faso. Induced symptoms, as well as viral load (estimated though quantitative PCR) were affected by the rice cultivar, the viral isolate and their interactions. The implications of these results, and potential consequences for the evolution of viral population, are discussed. The perspectives include taking into account multiple isolate infections and potential within-plant competition between the genetic lineages in this system.

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Phylogenetic and population analyses of cotton leafroll dwarf virus reveals extensive genomic variability and global sub-populations

J. K. Brown and R. O. Adegbola

School of Plant Sciences, The University of Arizona, Tucson, AZ 85721, USA.

(jbrown@ag.arizona.edu) *S3-O10*

Cotton blue disease (CBD) symptoms were first reported in cotton plantings heavily infested by the cotton aphid in Central Africa Republic during 1949. Subsequently, CBD-like symptoms were observed in aphid-infested, commercial cotton fields in Brazil during 1962 and twenty years later in 1982-1983 in the Misiones Province of Argentina. Molecular characterization of partial and complete genome sequencing revealed the presence of Cotton leafroll dwarf virus (CLRDV), belonging to the genus, *Polerovirus* (family, *Solemoviridae*). The virus has a linear, positive-sense, single-stranded, monopartite RNA genome of ~5.8 kb in size, encapsidated in a spherical virion of ~23 nm in diameter. Subsequently, CLRDV etiology has been confirmed in cotton plants exhibiting CBD-like symptoms in localities in Argentina, Brazil, East Timor, India, and Thailand based on complete or partial genome sequences. A disease reminiscent of CBD was reported for the first time in the United States (US), in the state of Alabama (AL) during 2016-2017 (1). Additional CLRDV field isolates have recently been sequenced from symptomatic cotton plants in seven US states in the 'US cotton belt'. To investigate the genomic variability among US CLRDV isolates and 'typical' and 'atypical' CLRDV isolates from South America for which complete genome sequences are available in GenBank, high-throughput sequencing (HTS) was carried out using total RNA isolated from cotton plants exhibiting 'mild' and more 'severe' symptoms collected in AL and Texas. The eight newly determined CLRDV genomes (5,865 to 5,867 bp) shared the highest nucleotide identity with previously sequenced US isolates, at 95.9-98.7%. Among complete genome sequences, predicted recombination was evident based on breakpoints identified in ORFs 2 and 3, and ORF5. The ORF 0 encoding the P0 host plant silencing suppressor was the most divergent gene, at 88.5-99.6% nucleotide and 81.2-89.3% amino acid sequence similarity, among known field isolates of CLRDV (2). Based on partial sequencing of ORFs 0 and 3 (n= >100) isolates exhibiting the greatest sequence divergence from the original AL isolates have been identified in Georgia, North Carolina, and Texas (3). The results are provisionally suggestive of multiple independent introductions, perhaps over time, and or of selective spread of certain isolates over others that originated from one or a few infection foci in the US.

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The rare case of the first whitefly-transmitted polerovirus

S. Ghosh, S. Kanakala, S. Kontsedalov, G. Lebedev, and M. Ghanim

Department of Entomology, Volcani Center, Rishon LeZion, Israel

(ghanim@volcani.agri.gov.il)

S3-O11

Virus-vector interactions are highly specific and only one vector or a group of vectors from the same family is able to transmit a given virus. Poleroviruses (Luteoviridae) are phloem-restricted RNA plant viruses, which are exclusively transmitted by aphids. Multiple aphid-transmitted polerovirus species commonly infect pepper, causing vein yellowing, leaf rolling and fruit discoloration. Despite low aphid populations, recent outbreaks with such severe symptoms in many bell pepper farms in Israel led to reinvestigation of the disease and its insect vector transmission. The outbreak was caused by a new whitefly (*Bemisia tabaci*)-transmitted polerovirus, which we coined Pepper whitefly borne vein yellows virus (PeWBVYV). PeWBVYV is highly homologous (>95%) to Pepper vein yellows virus (PeVYV) from Israel and Greece on its 5' end half, while it is homologous to African eggplant yellows virus (AeYV) on its 3' half. By constructing a PeWBVYV infectious clone, we were able to show that the generated virus following agro-infection was transmitted by *B. tabaci* but not by aphids. PeWBVYV is specifically transmitted by MEAM1 species of *B. tabaci* and not by MED. Both PeWBVYV and PeVYV-2 follow a persistent, circulative mode of transmission and were detected in the hemolymph of both whitefly species, however, with significantly different viral loads. PeVYV-2 and PeWBVYV compete inside the host plant and also inside insect vectors. PeWBVYV was the weaker competitor inside the host plant while PeVYV-2 was the weaker competitor inside the insect vector. The virus was found to mainly concentrate in the fruit calyx, while it was barely detected in the pericarp of the fruit and the leaves. Field surveys revealed the presence of the new virus in the Jordan Valley for the last 5 years, while new infections in northern and southern Israel were detected recently.

Potato virus Y adaptation to various resistance QTL combinations in pepper and impact on host tolerance

T. Jayet[1,2], W. Billaud[1,2,3], L. Tamisier[1,2], M. Szadkowski[1], C. Wipf-Scheibel[1], L. Rimbaud[1], J. Hirsch[1], G. Girardot[1], J. Gaudin[1], A. Schoeny[1], K. Berthier[1], K. Nozeran[1], E. Lepage[1], S. de-Colle-Guiheneux[1,2,3], T. Pressecq[1], E. Verdin[1], V. Lefebvre[2], and B. Moury[1]

[1]Pathologie Végétale, INRAE, 84140 Montfavet, France; [2]GAFL, INRAE, 84140 Montfavet, France; [3]Avignon Université, UMR Qualisud, 84916 Avignon, France.

(thibaud.jayet@inrae.fr) *S3-O12*

Plant resistance is one of the best strategies to control pathogens for an environmentally friendly agriculture. Resistances are of two types: monogenic or polygenic, the latter frequently controlled by resistance quantitative trait loci (rQTL). However, both resistance types face pathogen adaptation, leading regularly to a complete breakdown. Pathogen adaptation may also have an impact on the plant tolerance, which is the ability of the plant to reduce the damages caused by pathogen infection (i.e. the ability to reduce the pathogen virulence). Studies of viral molecular mechanisms linked with resistance breakdown are frequent for monogenic resistance but scarce for adaptation to rQTL. The main objectives of the current study are to (i) evaluate the adaptation of experimentally-evolved PVY strains on pepper lines containing various combinations of rQTL and tolerance QTL, (ii) determine tolerance changes induced by this adaptation, (iii) analyse mutations appeared during experimental evolution. Two PVY strains were experimentally evolved for nine months corresponding to nine successive passages on five doubled haploid lines, each containing one specific combination of rQTL and tolerance QTL. Following the experimental evolution, quantitative ELISA and fresh weight measurements were performed to compare the fitness and virulence of ancestral and evolved strains. Significant adaptation was only recorded on the most resistant doubled-haploid line. Two evolved strains from another treatment (strain-pepper line combination) showed a fitness decrease compared to the ancestral strain. Virulence increase was recorded for 2 treatments involving a non-tolerant doubled-haploid line, but they were not associated with a fitness increase. Actually, any evolved population showed both a change in fitness and in virulence. Sequencing of the viral population revealed at least one mutation related to either fitness or virulence changes. However, mutation validation is yet to be performed to confirm the impact of these mutations on the fitness and the virulence of viral populations.

Plant-virus interactions in a heterogeneous landscape: analysis of four tobamovirus species

A.D. Zamfir, B.M. Babalola, A. Fraile, M. J. McLeish, and F. García-Arenal

Centro de Biotecnología y Genómica de Plantas (CBGP), Universidad Politécnica de Madrid (UPM) and Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and E.T.S.I. Agronómica, Alimentaria y de Biosistemas, Campus de Montegancedo, UPM, 28223 Pozuelo de Alarcón, Madrid, Spain.

(ad.zamfir@upm.es) *S3-P1*

Plant virus emergence is a complex process, yet poorly understood, that relies on a number of ecological and evolutionary factors. In nature, viral infections occur in diverse host plant communities at different spatial scales. Thus, transmission dynamics, host range, and disease risk will be determined by a set of ecological factors including community structure, its spatio-temporal variation, and the connectivity between host and virus populations. Although data on the incidence of plant viruses in wild hosts is still scarce, it is widely accepted that perturbations of natural ecosystems which includes loss of biodiversity favors disease emergence (1). We have analysed virus infection at the landscape scale in an agricultural ecosystem in Central Spain. We analysed plant communities at four habitats with different levels of human management: Crops, Edges between crop fields, Wastelands with no particular human usage and the native Mediterranean evergreen Oak Forests. Plant communities were sampled at four sites, taken as replicates, for each habitat, and virus infection was detected by high throughput sequencing of total RNA extracts. We analysed host range, incidence and diversity of four contact transmitted viruses in the genus Tobamovirus: Pepper mild mottle virus (PMMoV), Tobacco mild green mosaic virus (TMGMV), Tobacco mosaic virus (TMV) and Youcai mosaic virus (YoMV). Tobamoviruses are considered to have narrow host ranges in nature (2, 3), and contact transmitted viruses were described to have low incidence in wild ecosystems (4). Contrary to these expectations, we observed these viruses to be generalists that specialize in host usage and to show high incidence in wild plant communities. We also compare their host range, incidence and genetic diversity, and we analyze the data to test effects of spatial scale, community structure and host range on infection risk and genetic diversity.

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Genetic variability and evidence of a new subgroup in Watermelon mosaic virus isolates infecting cucurbits in the United States

O. A. Abdalla[1, 2], and A. Ali[1]

[1]Department of Biological Science, The University of Tulsa, Tulsa Oklahoma, 74104, US; [2]Present address: Department of Plant Pathology, Faculty of Agriculture, Assiut University, Assiut, Egypt.

(Akhtar-ali@utulsa.edu)

S3-P2

Watermelon mosaic virus (WMV) is one of the important potyviruses that infect cucurbits worldwide. To better understand the population structure of WMV in the United States (U.S.), 57 isolates were collected cucurbit fields located in nine southern states. The complete coat protein gene of all WMV isolates was cloned, sequenced and compared with 89 reported WMV isolates. The nucleotide and amino acid sequence identities among the U.S. WMV isolates ranged from 88.9-99.7% and 91.5-100%, respectively. Phylogenetic analysis revealed that all the U.S. WMV isolates irrespective of their geographic origin or hosts belonged to Group 3. However, the 57 isolates made three clusters in G3, where two clusters were similar to previously reported subgroups EM-I and EM-II, and the third cluster, containing nine WMV isolates, formed a distinct subgroup named EM-V. The ratio of non-synonymous to synonymous nucleotide substitution was low indicating the occurrence of negative purifying selection in the CP gene of WMV. Phylogenetic analysis of selected 37 complete genome sequences of WMV isolates also supported the above major grouping. Recombination analysis in the CP genes confirmed various recombinant events, indicating that purifying selection and recombination are the two dominant forces for the evolution of WMV isolates in the U.S.

Influence of habitat on incidence, host use & genetic structure of cucumber mosaic virus (CMV)

B. M. Babalola, A. Fraile, M. J. McLeish, and F. García-Arenal

Centro de Biotecnología y Genómica de Plantas (CBGP), Universidad Politécnica de Madrid (UPM) and Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and E.T.S.I. Agronómica, Alimentaria y de Biosistemas, Campus de Montegancedo, UPM, 28223 Pozuelo de Alarcón, Madrid, Spain.

(bisolamercy.babalola@upm.es) *S3-P3*

In ecological studies, spatial scale, host range, and the biotic communities associated with different habitats are known to affect the evolution, distribution, and interactions of viruses with their hosts (1). The recent hypothesis that virus–plant interactions differ according to the communities in which they occur is of widespread interest, but few studies have supported it. Thus, we investigate whether changes among communities of distinct habitats have an influence on the incidence, host use, and genetic diversity of the generalist virus CMV. We focus on four habitats of an ecosystem of central Spain, each varying in the degree of human management: Crop fields (Crop), edges between crop fields (Edge), scrublands with no particular human intervention (Wasteland), and native Mediterranean oak forests (Oak). Four sites of each habitat were sampled following a strategy to optimise plant species representation. Pools of total RNA preparations from plant species sampled from each habitat were deep-sequenced. Analyses detected CMV infection in all habitats, with a host range of 84 plant species and RT-PCR of individual plants was used to estimate incidence of CMV. Incidence was highest in Crop and Edge (83% & 91% respectively) than in Oak and Wasteland (53% & 54%), showing a positive association with the degree of human management (2). Although CMV hosts were often shared among habitats, host use specificity depended on habitat. For example, in species like *Bromus* sp. and *Convolvulus arvensis* present in crop or edge and at least one other habitat, incidence was higher in edge. Genetic diversity showed a major role of habitat on CMV diversification and different evolutionary dynamics for the three genomic RNAs of CMV. Thus, our data supports the hypothesis to be tested.

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Targeting of genomic and minus strands of viral RNA contributes to amiRNA-mediated antiviral resistance and promotes the emergence of complex viral populations

F. Mesel[1], M. Zhao[1,2], B. García[1], J. A. García[1], and C. Simón-Mateo[1]

[1]Department of Plant Molecular Genetics, Centro Nacional de Biotecnología (CNB-CSIC), Campus Universidad Autónoma de Madrid, 28049 Madrid, Spain; [2]College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot 010018, China.

(csimon@csic.es) *S3-P4*

Technology based on artificial microRNAs (amiRNAs) has been successfully employed to generate resistance against different animal and plant viruses. However, not all amiRNAs directed to viral targets are able to efficiently block virus multiplication, and the properties that confer efficacy to antiviral amiRNAs are largely unknown. Moreover, evolutionary outcomes of the selective pressure imposed by antiviral amiRNAs have been barely explored. In this work, we have transformed *N. benthamiana* plants with amiRNA constructs designed against the potyvirus *Plum pox virus* (PPV), and obtained transgenic lines immune to PPV infection in our experimental conditions. But our work has focused more deeply on other lines that only showed partial resistance, as they can shed light on the molecular basis of this interesting biotechnological approach. Usually, approaches to generate resistance mediated by artificial small RNAs against RNA viruses have directed them to the genomic RNA. In contrast with data reported for animal viruses, our results demonstrate that the minus strand RNA of PPV can be cleaved by the amiRNA-guided RNA silencing machinery, although targeting this strand provides weaker antiviral resistance than directing the mature amiRNA strand to the genomic RNA. Moreover, the genome analysis of escaping mutants strongly suggested that the activity of both mature and star amiRNA strands contribute to the resistance. The selection pressure posed by this dual activity caused an evolutionary explosion resulting in the emergence of a wide range of virus species. Interestingly, this range further expanded after propagation under amiRNA antiviral challenge or even in its absence rather than reverting to the original sequence, broadening the viral genetic repertoire to better adapt to different hosts and environments.

Virus diversity in natural Dutch plant ecosystems along a chronosequence

D.E. Boezen[1], C.M. Malmstrom[2], R.A.A. van der Vlugt[3], and M.P. Zwart[1]

[1]Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands; [2]Department of Plant Biology, Michigan State University, East Lansing (MI), USA; [3]Laboratory of Virology, Wageningen University and Research, Wageningen, The Netherlands.

(d.boezen@nioo.knaw.nl) *S3-P5*

Historically, plant virology research has focused on viruses which are pathogenic to crops. In agricultural systems, plant diversity is generally low but disease risk is high. In uncultivated plants, virus prevalence is high, but viruses often do not cause overt symptoms. The role and diversity of plant viruses in nature is understudied. However, the rise of metagenomics and high throughput sequencing (HTS) has enabled a less biased characterization of the viruses of plants in natural ecosystems. To estimate virus diversity as a function of land use change, we are using a chronosequence approach: ecological sites that are similar, but are at different successional stages. Our chronosequence consists of five former agricultural fields, which were taken out of production at different points in time and rewilded through natural succession. These sites are part of a long-term ecological study, with data available on land use change, soil, and plant biodiversity. We characterized the virome for eight host plants (*Plantago lanceolata, Jacobea vulgaris, Achillea millefolium, Rumex acetosella Geranium molle, Viola arvensis, Hypericum perforatum,* and *Bromus hordeaceus*) over two seasons (spring and summer) and across five chronosequence sites varying in age since abandonment. For each site, ten specimens were sampled for each plant species irrespective of the presence of viral symptoms (n=551). Samples were pooled per plant species (n=66), RNA was extracted and sequenced, and virus prevalence and diversity were estimated by comparing the HTS data to the virus reference database. These results will provide us with a first survey of plant-virus diversity in a natural grasslands ecosystem in The Netherlands. Based on prior work on virus diversity along the agricultural-wild interface, we expect results to show higher virus prevalence and diversity at the younger sites, potentially due to the 'dilution effect' resulting from higher plant biodiversity limiting transmission opportunities at the older sites.

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Wild plant species: enemies or allies of carrot crops?

F. Salavert[1], H. McGrath[2], A. Fox[3], I. Adams[3], and N. Boonham[1]

[1]Newcastle University, Newcastle Upon Tyne, UK; [2]Rothamsted Research, Harpenden, UK; [3]FERA Science Ltd, York, UK.

(f.salavert2@newcastle.ac.uk) *S3-P6*

The agroecosystem associated with the carrot crop have proven to be much more complex than initially thought. Intensive cropping strategies are used in the UK where carrots can be found in the field year around, from seedlings to carrots stored under straw. In addition the adoption of regenerative agricultural practices where flowering borders are used as part of an IPM approach are increasingly common, increasing the number and diversity of plant species in association with the crop. In the UK, the biggest carrot producer of Europe, many viruses have been reported infecting carrot crops and causing major economic losses (e.g., Carrot red leaf virus, Carrot closterovirus, Carrot mottle virus, etc.). These viruses have not only been found in the crops but also in the neighbouring wild species. The close contact in which these different elements of the agroecosystem are found raises questions about their role in virus communities and virus species evolution. In the framework of INEXTVIR, a Marie Sklodowska-Curie Innovative Training Network, and with the collaboration with other research institutions (Fera Science Ltd and Rothamsted Research), our aim is to achieve a better understanding of the virus flow within and between the plants that form the carrot agroecosystem. We took samples of carrot and wild plant species within two different agricultural management approaches (conventional and IPM). The samples were processed and sequenced in pools of total RNA using Miseq (Illumina) to reveal the diversity of the virome present. The development of qPCR detection assays for all the viruses that were found will enable the back-testing of the samples, enabling the incidence and distribution of individual viruses to be more finely mapped. This will allow us to ascertain the relationship between different viruses and vectors to be established between the carrot crops and the wild species.

Characterization of the biological properties of a chimeric potyvirus, and of its adaptation to a compatible experimental host

H. Sun[1], F. del Toro[1], M. Makki[2], F. Tenllado[1], and T. Canto[1]

[1]*Department of Microbial and Plant Biotechnology. Margarita Salas Center for Biological Research. Ramiro de Maeztu 9, 28040 Madrid, Spain;* [2]*Laboratory of Molecular Genetics, Immunology and Biotechnology. Faculty of Sciences. University of Tunis El Manar. Manar II, Tunis 2092, Tunisia.*

(hao.sun01@estudiante.uam.es) *S3-P7*

For functional studies on potyviral HCPro, we created a virus chimera in which the *P1-HCPro* bi-cistron in a plum pox virus construct (PPV-GFP) was replaced by that of a potato virus Y (PVY) isolate (1). We characterized the virulence of the chimera in *Nicotiana benthamiana* plants, and found that it accumulated systemically to levels that were 5-10 % those of the parental PPV-GFP at 20 days post inoculation. Symptoms were very mild. We tested whether tobacco, which is not infected systemically by PPV but allows this virus to replicate in it, and arabidopsis plants, which are not infected by PVY, were compatible hosts of this chimera. We also determined the chimera transmissibility by aphids. We finally tested whether the chimera would increase its virulence through mechanical passages, and how this could relate to alterations in its genome. We found that after four mechanical passages chimera titers rose to 20-30% those of PPV-GFP, and symptoms increased. We sequenced over half of the genome of the chimeras present in two plants after those passages. The regions sequenced (*P1-HCPro-P3*; *Vpg/NIa*; *GFP-CP*) were selected for being potential sites of mutations/deletions leading to adaptation. We only found a very low number of single amino acid substitutions in the two adapted chimeras, two in one case (one in HCPro, one in Vpg/NIa) and 3 in the other (one in HCPro, one in Vpg/NIa, and one in the CP). No deletions were found. The roles in adaptation of the two substitutions found in HCPro are discussed.

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Molecular characterization of watermelon mosaic virus isolates infecting zucchini and pumpkin plants in the Czech Republic

K. Ben Mansour[1], M. Komínková[2], P. Komínek[2], J. Brožová[2], J. Kazda[1], M. Zouhar[1], and P. Ryšánek[1]

[1]Department of Plant Protection, Czech University of Life Sciences, Prague, Czech Republic; [2]Crop Research Institute, Prague, Czech Republic.

(Karina79@hotmail.fr) *S3-P8*

Watermelon mosaic virus (WMV) is among the most important potyviruses infecting cucurbit vegetables in the Czech Republic. The occurrence of WMV in the eastern part of the Czech Republic was first reported by Svoboda (2011) based on ELISA testing. In 2019, symptoms of mosaic, leaf deformations, and fruit discoloration occurred on pumpkin and zucchini plants in a private garden in the western part of the Czech Republic, where WMV had not been reported before. High throughput sequencing was used for further characterization of the virus. The full WMV genome (acc. n MW188031) consists of 10,027 nt. One statistically significant recombination event was detected using the RDP4 program; the detected event was positive for six out of seven algorithms implemented in the RDP4 program. It covers NIb and CP coding regions. The putative parents are KP164988 (major parent, Argentina) and AB369278 (minor parent, South Korea). Both putative parents belong to the G3 molecular group. The phylogenetic analysis of the complete genome revealed that this isolate belongs to sub-group EM1 and clustered together with KP164988 sharing 98% and 94% nucleotide and amino acid identity, respectively. Therefore, leaf samples were collected from six different locations in the western part of the Czech Republic to check the occurrence of this emerging group revealed that the tested samples belonged to the same group, G3, based on the NIb-CP region (273 bp), and to sub-group EM1 based on the CP gene. This result provides evidence for the higher incidence of WMV in cucurbits compared to previous years in the Czech Republic (unpublished data).

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Multipartite virus genome formula variability in local lesions of *Chenopodium quinoa*

M. Johnson[1, 2] and M. Zwart[1]

[1]Microbial Ecology, Netherlands Institute of Ecology, NIOO-KNAW, Wageningen, The Netherlands; [2]Department of Virology, Wageningen University and Research, Wageningen, The Netherlands.

(M.Johnson@nioo.knaw.nl) *S3-P9*

Multipartite viruses have segmented genomes in which individual segments are independently packaged and transmitted. For successful infection, all genome segments must be present in the same cell. Studies have shown that the segments accumulation and stoichiometry is host specific, termed the genome formula (GF) (1). The individual transmission of segments and the requirement for appropriate dose to initiate infection present a challenge, as the GF signal would be lost between transmission events. We investigated the variability of inoculum GF and the cost of infectivity combining experimental and modelling approaches. Dose response - local lesion assays were conducted with the tripartite +ssRNA Cucumber mosaic virus (CMV) infections in *Chenopodium quinoa*. The number of lesions on CMV infected plants was recorded at 10 dpi and individual lesions were excised for analysis of relative and total accumulation by RT-qPCR and digital PCR. We use the ΔGF metric to measure the variability of the GF between individual local lesions and the relationship between the GF and accumulation. We show that there is high variability in the GF of local lesions, to the extent that ΔGF approaches model predictions for random noise. The relationship between the GF and viral accumulation shows that the mean GF is associated with increased viral accumulation and that deviations from this position may result in markedly lower accumulation. This model system therefore lets us study GF variability under extreme circumstances of small virus populations replicating under harsh conditions, and the results suggest GF variation may be associated with different infection outcomes.

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TuYV isolates found in Sugar beet

S. Schop[1], E. Raaijmakers[2], and R.A.A. van der Vlugt[1]

[1]Laboratory of Virology, Wageningen University and Research, The Netherlands; [2]IRS (Dutch Sugar Beet Research Institute), Dinteloord, The Netherlands.

(Sharella.Schop@wur.nl) *S3-P10*

In a sugar beet field trial in 2020 plants showing clear yellowing symptoms were observed. Specific primers for the three well-known beet infecting yellowing viruses (beet yellows virus, beet mild yellowing virus and beet chlorosis virus) were used in a RT-PCR reaction but did not generate any amplicons in 15 plant samples while generic polerovirus primers did generate amplicons of the expected size. Sequencing of these amplicons suggested that the plants were infected with turnip yellows virus (TuYV) as the sequence showed over 95% identity to the type isolate (NC_003743). TuYV has a wide host range infecting plant species such as turnip, oil seed rape, lettuce, faba bean, chickpea and many common weed species. However, based on previous host range studies TuYV is classified by ICTV as a non-beet infecting Polerovirus (1). Therefore, it is surprising that we have found multiple TuYV infections in sugar beet. Illumina High Throughput Sequencing on one of the plant samples confirmed the presence of TuYV. In addition, based on the sequence information obtained specific primers were designed to generate amplicons of TuYV which were subsequently sequenced by Sanger sequencing. These results confirmed the presence of TuYV however also revealed a high sequence diversity, whereby the obtained sequences of three isolates differed up to 10% for a 800 bp amplicon located at the 5' end of the viral genome (bp 204 to 1012). This strongly suggests that the virus infections observed in the field originated from different sources. This is supported by the fact that the infected plants were not located in one yellow patch but spread across the field (approx. 1000m^2). With TuYV having a wide host range in multiple field-grown crops, it is important to investigate the possible spread and risk of these beet-infecting TuYV isolates. Especially now that the availability of insecticides in many crops, including sugar beet, decreases.

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High genetic diversity of *Plum pox virus* in subspontaneous trees in North Macedonia sheds new light into its evolutionary history

S. Dallot[1], M. Brevet[1], D. Filloux[2], E. Fernandez[2], R. Rusevski[3], B. Kuzmanovska[3], and G. Thébaud[1]

[1]PHIM Plant Health Institute, INRAE, Univ Montpellier, CIRAD, Institut Agro, IRD, Montpellier, France; [2]PHIM, CIRAD, Montpellier, France; [3]Department of Plant Pathology, Ss. Cyril and Methodius University, Skopje, North Macedonia.

(sylvie.dallot@inrae.fr)

S3-P11

Understanding plant virus spread and evolution at the agro-ecological interface is crucial to design appropriate disease management strategies and prevent variant emergence. Perennial plants are prone to harbor a high viral genetic diversity due to repeated and long-lasting infections and may thus provide a conducive environment to the emergence of new virus genotypes. Sharka, a serious disease of stone fruits (*Prunus*) worldwide, is caused by the plum pox virus (PPV, genus *Potyvirus*). Ten strains have been described so far, among which only three are widely distributed across Europe. In this study, we assessed the role of the wild compartment as a potential reservoir of PPV genetic diversity. We carried out a countrywide survey in North Macedonia, targeting cultivated trees, but also subspontaneous myrobalan trees (*P. cerasifera*) and wild blackthorn (*P. spinosa*) bushes. For PPV diagnosis and strain assignment, we designed new or used previously published polyvalent and strain-specific RT-PCR tests followed by partial sequencing. In *P. cerasifera*, we identified the three strains that are widespread in Europe (i.e., PPV-M, -D, -Rec), the geographically restricted PPV-T recombinant as well as PPV-An, one of the putative parents of the epidemic PPV-M strain and previously detected only once (in Albania). Furthermore, a PPV isolate distant from all known strains was detected and fully sequenced using Nanopore and Sanger technologies. Based on genetic distance, this isolate belongs to a new PPV strain, that we called PPV-P. Its aphid transmissibility and its ability to infect cultivated *Prunus* species were assessed experimentally, evidencing that this new strain has the potential to be epidemic in stone fruits. In contrast to *P. cerasifera*, cultivated species harbored only one (peach), two (apricot) or four (plum) PPV strains. These results suggest that *P. cerasifera* in the Balkans may be involved in the emergence of the PPV strains spreading in Europe.

Session

04

Virus-Vector Interactions

When plants and aphids are under the control of viruses

Q. Chesnais[1], F. Bogaert[1], C. Krieger[2], D. Halter[1], V. Ziegler-Graff[2], A. Marmonier[1], P. Claudel[1], M. Drucker[1], and V. Brault[1]

[1]INRAE-Université de Strasbourg, Colmar, France; [2]IBMP-CNRS-Université de Strasbourg, Strasbourg, France.

(veronique.brault@inrae.fr)

Keynote Lecture 4

There are increasing evidences that viruses may affect plant traits in ways that facilitate their transmission by vectors. For example, virus infection can induce leaf yellowing, promote emission of volatile compounds and modify phloem sap composition that can have an indirect effect on vector behavior. These modifications can affect vector preference for infected plants and change their feeding behavior that is directly linked to virus acquisition. In addition, plant-virus infection can alter vector performances, like fecundity influencing viruliferous vector population, which also can affect virus dispersion. More intriguingly, plant viruses can have a post-acquisition effect on vectors by altering their locomotor behavior and survival following virus uptake. When these vector behavioral modifications are conducive to transmission, there are referred to as “Manipulation”. Many examples of plant and vector manipulations have been described in the last few years but the molecular mechanisms underlying these effects are largely unknown. We have conducted projects on poleroviruses/aphids/plants pathosystems to try to unravel these mechanisms. Poleroviruses are phloem-limited and transmitted by aphids in a circulative and persistent manner. For efficient aphid transmission of poleroviruses, infected plants must be attractive to aphids and aphids need to settle on the plant long enough to reach the phloem and ingest sap; a feeding phase during which the viral particles are acquired. We developed transcriptomic, metabolomic, genetic and behavioral approaches on both aphids and plants, to unravel these amazing mechanisms and increase our understanding on host and vector manipulations by plant viruses.

Yellowing viruses promoting their own spread by reducing Mature Plant Resistance to aphids in sugar beet

S. Schop[1], E. Raaijmakers[2], and R.A.A. van der Vlugt[1]

[1]Laboratory of Virology, Wageningen University and Research, Netherlands; [2]IRS (Dutch Sugar beet Research Institute), Dinteloord, The Netherlands.

(Sharella.schop@wur.nl) *S4-O1*

Yellowing viruses, mainly persistently transmitted by the aphid species *Myzus persicae,* have a major negative impact on sugar beet yield. These yellowing viruses belong to different virus families; *Closteroviridae* (beet yellows virus)*, Luteoviridae* (beet mild yellowing virus, beet chlorosis virus and beet western yellows virus) and *Potyviridae* (beet mosaic virus). In sugar beet, a resistance mechanism is naturally present and is called "Mature Plant Resistance" (MPR). When sugar beet plants reach their 10 to 12 leaf stage aphid mortality is increased (1). Prior to the aphid's death, a black stomach deposit is produced, which is apparently directly related to their death. Interestingly, reduced aphid mortality is observed for plants that are infected by yellowing viruses compared to healthy plants (2). This is beneficial to the virus, as aphids are required for yellowing virus spread. We will report on experiments in which we quantify the clear negative effects of MPR and plant yellowing on aphid behavior, survival and fecundity. We will also report on our investigations into the underlying biochemical pathway of MPR which suggest that polyphenol oxidases (localized in the chloroplast) may play a key role in the formation of the black deposit in the aphids stomachs and MPR. Yellowing virus infection results in the breakdown of chloroplasts, which leads to the typical discoloration of the leaves. This results in lower MPR, higher aphid survival rates and thus increased viral spread. Better knowledge of the effects and underlying mechanisms of MPR is crucial for implementing more sustainable measures to control yellowing viruses and their aphid vectors. The effects of biotic stresses, as well as the effects of yellowing virus infection on MPR and its underlying pathway will be presented. In addition, the impact of this knowledge on virus control will be discussed.

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Aphid symbionts influence the transmission of a plant virus

P. Sanches, C. De Moraes, and M. Mescher

Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland.

(patricia.sanches@usys.ethz.ch) *S4-O2*

Nearly all aphids harbor obligate and facultative symbiotic bacteria that influence a wide range of insect and host-plant traits (1). Endosymbionts can also influence aphid-plant interactions in ways that may impact pathogen transmission, for example via effects on the probing behavior of aphids on virus-infected plants (2). To date, however, the influence of aphid endosymbionts on plant virus transmission remains largely unexplored. We investigated the effects of five different aphid endosymbionts on the transmission of pea enation mosaic virus (PEMV) to fava beans by pea aphids (*Acyrthosiphon pisum*), as well as on aphid behavioral preferences and performance. In dual-choice feeding and emigration assays, only aphids harboring the facultative bacteria *Regiella insecticola* or *Hamiltonella defensa* exhibited plant preferences, with viruliferous individuals settling more frequently on healthy plants. In addition, aphid biomass and population growth were enhanced in the colonies harboring one of these two symbionts, but only on virus-infected plants. Most other endosymbionts had limited or no effects on aphid behavior or performance. Aphid vectors carrying facultative bacteria led to high virus transmission rates, and the colonies carrying *R. insecticola* and *H. defensa* exhibited some of the intermediate and the highest transmission rates, respectively. Finally, metabolic analyses revealed distinct profiles for aphids harboring different endosymbionts and also showed that aphids harboring facultative bacteria had higher levels of critical amino acids when feeding on virus-infected plants. Taken together, these findings demonstrate that endosymbionts can influence the aphid behavior and performance in ways that effect virus transmission. Furthermore, the specificity of these interactions, including the finding that virus-infection of host plants can enhance the performance of aphids harboring particular symbionts, has intriguing implications for the evolution of viral pathosystems, as well as potentially important implications for epidemiology.

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Complex interactions in co-occurring vector-borne pathosystems: a case study of potato virus Y and zebra chip disease on a solanaceous crop

R. K. Cruzado[1], R. Sadeghi[1], S. M. Prager[2], C. L. Casteel[3], E. J. Wenninger[4], N. A. Bosque-Pérez[1], A. V. Karasev[1], and A. Rashed[1]

[1]Department of Entomology, Plant Pathology and Nematology, University of Idaho, Moscow, ID, USA; [2]Department of Plant Science, University of Saskatchewan, Saskatoon, SK Canada; [3]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY, USA; [4]University of Idaho, Department of Entomology, Plant Pathology and Nematology, Kimberly Research & Extension Center, Kimberly, ID 83341, USA.

(arashed@uidaho.edu) *S4-O3*

Plants are hosts for herbivorous arthropods and microbial pathogens that harm plants, sometimes causing economic losses. In vector-borne pathosystems, relationships between individual plant enemies and host plants are complex. Yet studies of vector-borne pathosystems are usually isolated, with plants infected by only one pathogen or infested by only one arthropod vector. However, multiple pests often attack crop fields in nature, resulting in spatiotemporal mosaics of infection, infestation, and damage. We highlight this perspective and illustrate its broad implications by introducing a case study focused on potato virus Y (PVY) and zebra chip disease (ZC), which are major threats to solanaceous crop production in North America. PVY can be spread by aphid vectors and through vegetative propagation in potatoes. ZC is associated with "*Candidatus* Liberibacter solanacearum" (Lso), which is transmitted by the potato psyllid, *Bactericera cockerelli* Šulc (Hemiptera: Triozidae). Greenhouse choice and no-choice bioassays were conducted to evaluate the psyllid response to PVY-infected and uninfected tomatoes. Lso-positive psyllids preferentially settled on PVY-negative hosts, whereas Lso-negative psyllids preferred PVY-positive tomatoes. Oviposition of Lso-positive psyllids was lower on PVY-infected than uninfected tomatoes, but Lso transmission, titer, and psyllid egg fertility were not significantly affected by PVY infection. The induction of salicylic acid and its related responses, not nutritional losses, may explain the reduced attractiveness of the PVY-positive host to the Lso-positive psyllids. This case study suggests that pre-existing PVY infection may influence patterns of Lso spread in a field. Oversimplifying studies of vector-borne pathosystems could generate results that ignore outcomes of complex interactions in nature, impacting the effectiveness and sustainability of management approaches.

Virus-induced changes in tomato plants infected with tomato yellow leaf curl virus and tomato chlorosis virus influence host selection by their common vector *Bemisia tabaci*

I. Ontiveros, [1,2], J.J. López-Moya [1], and J.A. Díaz Pendón [2]

[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Cerdanyola del Vallès, Barcelona, Spain; [2]Institute for Mediterranean and Subtropical Horticulture "La Mayora" (IHSM-UMA-CSIC), Algarrobo-Costa, Málaga, Spain.

(irene.ontiveros@csic.es) *S4-O4*

Mixed viral infections in susceptible host plants can differentially affect the expression of symptoms compared with single infections, and also cause other sensory-relevant alterations for the interactions with insect vectors, influencing recruitment and behaviour parameters governing virus dissemination with epidemiological consequences (1). We have focused on single and mixed infections frequently occurring in tomato crops in Spain, caused by *Tomato yellow leaf curl virus* (TYLCV, genus *Begomovirus,* family *Geminiviridae*) and *Tomato chlorosis virus (*ToCV, genus *Crinivirus,* family *Closteroviridae*), both viruses being transmitted by the whitefly *Bemisia tabaci.* After assessing symptoms and measuring the relative abundances of viruses, we observed that co-infections resulted in more severe symptoms at late stages when compared to single infections, resulting in an asymmetrical synergism that correlated with the dynamics of ToCV accumulation and the expression of the salicylic acid responsive gene PR-P6. When vector choices were assayed, we observed preference for symptomatic leaflets infected with either TYLCV or TYLCV+ToCV, compared with those infected with ToCV alone or with non-infected controls, suggesting that TYLCV drives host selection by *B. tabaci*. Results were similar for both viruliferous and non-viruliferous whiteflies. To distinguish visual and olfactory cues, experiments in a Y-tube olfactometer assays suggested that olfactory cues have neutral effects on whiteflies preferences, while choice assays with colored sticky traps confirmed that the preference was driven mainly by visual cues that mimicked the color of TYLCV symptomatic leaves, irrespective of whether the vector is viruliferous or non-viruliferous. These results might help to better understand how the TYLCV and ToCV mixed infections in tomato plants can contribute to the spread of both viruses, showing that viruses could have effects on the host with the potential to alter the behavior of insect vectors.

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How do you make a mixed infection: effect of acquisition sequence on propagation of TYLCV and ToMoV by *Bemisia tabaci*

A. L. Jacobson[1], A. A. McLaughlin[1], L. Hanley-Bowdoin[2], and G. G. Kennedy[3]

[1]Department of Entomology and Plant Pathology, Auburn University, USA; [2]Department of Plant and Microbial Biology, North Carolina State University, USA; [3]Department of Entomology and Plant Pathology, North Carolina State University, USA.

(alj0043@auburn.edu) *S4-O5*

Co-infections of plant viruses can be important factors influencing disease severity, genetic diversity of the co-infecting viruses, transmission rates, and virus evolution. Although the insect vector is crucial to the spread and survival of most plant viruses, vector-mediated effects on virus acquisition, transmission, and infection are poorly understood. We report on the role of vector acquisition and transmission on the propagation of single and co-infections of tomato yellow leaf curl virus (TYLCV) and tomato mottle virus (ToMoV) (*Geminiviridae, Begomovirus*) by the whitefly vector, *Bemisia tabaci* MEAM1 (Gennadius), in a tomato pathosystem. We investigated how the acquisition of both viruses by *B. tabaci* from singly infected or co-infected plants influences 1) probability of virus acquisition, 2) virus titers accumulated in vectors after acquisition, 2) probability of virus transmission, 3) titers of transmitted virus, 4) probability of infection in inoculated host plants, and 5) accumulation of viruses in infected host plants. The order of virus acquisition did not change the probability of acquisition by vector cohorts, but did influence the transmission efficiency and probability of host plant infection. Co-infections were propagated in all co-acquisition treatments, but their occurrence was low due to antagonistic virus-virus interactions in the vector and/or the host plant. These interactions also changed the accumulation of viruses that persisted in single and co-infections. Our results reveal that there is a complex interplay among virus-virus-vector-plant interactions as viruses are acquired, circulate through their vectors, and are inoculated into host plant tissue. These interactions determine virus persistence and accumulation in new hosts.

Relating acquisition of cassava mosaic begomovirus components A and B to transmission of single and co-infections by *Bemisia tabaci* SSA1-SG1

G. G. Kennedy[1], W. Sharpee[1], M. Wambugu[2], B. Mware[2], L. Hanley-Bowdoin[3], and A. L. Jacobson[4]

[1]Entomology and Plant Pathology, North Carolina State University, Raleigh, USA; [2]BecA-ILRI Hub, Nairobi, Kenya; [3]Department of Plant and Microbial Biology, North Carolina State University, Raleigh, USA; [4]Department of Entomology and Plant Pathology, Auburn University, USA.

(gkennedy@ncsu.edu) *S4-O6*

Cassava mosaic disease (CMD) represents a serious threat to cassava, a major food crop in Africa and Asia. CMD is caused by a complex of cassava mosaic begomoviruses (CMBs) that are transmitted by *Bemisia tabaci.* A pandemic of CMD in Sub-Saharan Africa in the 1990s and early 2000s was associated of co-infections of two CMBs, which together caused severe disease. CMB genomes consist of two DNA components (DNA-A and DNA-B) both of which are required for infection and are packaged separately into virions. Hence, both must be acquired and transmitted by the vector for plant-to-plant spread to occur. Interactions between these component virions within whiteflies may influence acquisition and/or transmission. These potential interactions are not well understood, especially in co-infections. Our study examines transmission of African cassava mosaic virus (ACMV) and East African cassava mosaic Cameroon virus (EACMCV) by individual *Bemisia tabaci* SSA-1 SG1 that acquired virions of one or both viruses. We present results describing the relationships between titers of the cognate DNA components of each virus that were acquired and transmitted by whiteflies and the ways that those relationships were affected by the presence of one or both components of the other virus.

Specificity in transmission of old- and new-world begomoviruses by *Bemisia tabaci* MEAM1 and MED cryptic species

R. Srinivasan[1], S. Gautam[1], H. Mugerwa[1], S. Ghosh[1], B. Dutta[2], and S. Adkins[3]

[1] Entomology, University of Georgia, Griffin, Georgia 30223, United States; [2]Plant pathology, University of Georgia, Tifton, Georgia 31793, United States; [3]USDA-ARS, Fort Pierce, Florida 34945.

(babusri@uga.edu) *S4-O7*

Vegetable production in the southeastern United States is severely impacted by the whitefly, *Bemisia tabaci* (Gennadius), and begomoviruses it transmits. Old-world monopartite tomato yellow leaf curl virus (TYLCV) and new-world bipartite cucurbit leaf crumple virus (CuLCrV) and sida golden mosaic virus (SiGMV) are prevalent. Bipartite viruses are relatively recent introductions than TYLCV. *Bemisia tabaci* MEAM1 is the predominant cryptic species, and MED has been restricted to greenhouses. Recently, there is evidence for MED colonizing outdoor crops. This study assessed how virus transmission dynamics would vary if MED populations also colonized outdoor crops. Transmission of CuLCrV, SiGMV, and TYLCV by MEAM1 and MED whiteflies was compared. MEAM1 efficiently transmitted all three viruses, whereas MED transmitted the monopartite TYLCV but not the bipartite viruses. TYLCV accumulation levels in MEAM and MED was similar, but CuLCrV and SiGMV accumulation levels were substantially lower in MED than in MEAM1. TYLCV accumulation levels in the midgut, hemolymph, and salivary glands were not different between MEAM1 and MED. In contrast, CuLCrV and SiGMV accumulation levels in the midgut, hemolymph, and salivary glands were much lower in MED than in MEAM1. Fluorescent *in situ* hybridization further substantiated the findings, suggesting that CuLCrV and SiGMV did not effectively traverse the midgut barrier and/or endocytose into salivary glands in MED whiteflies in comparison with MEAM1. Fitness parameters on respective hosts of CuLCrV, SiGMV, and TYLCV *viz.*, squash, snap bean, and tomato did not differ between MEAM1 and MED. Endosymbiont profiles also were not different between MEAM1 and MED. Therefore, inherent differences between MEAM1 and MED likely influenced the observed variation in transmission. To further understand this phenomenon, transcriptomes and proteomes of viruliferous and non-viruliferous MEAM1 and MED were synthesized and annotated. Several dissimilarities explaining the differential transmission abilities between MEAM1 and MED were identified and will be discussed.

Investigation of the replacement between two tomato-infecting begomoviruses from the perspective of vector transmission

W.-H. Li, and C.-W. Tsai

Department of Entomology, National Taiwan University, Taipei, Taiwan.

(f06632007@ntu.edu.tw)

S4-O8

According to a field survey, tomato yellow leaf curl Thailand virus (TYLCTHV) quickly replaced tomato leaf curl Taiwan virus (ToLCTV) after the former invaded Taiwan (1). Both viruses are exclusively transmitted by *Bemisia tabaci*, so the virus replacement in tomato fields is possibly related to the virus-vector interaction. In this study, *B. tabaci* was given two virus acquisition periods on single infected or co-infected plants, and the amount of virus in the organs and hemolymph of *B. tabaci* were determined by quantitative PCR to study the interaction of these two viruses in whitefly. Further, transmission assays were conducted to examine the effect of virus-virus interaction on whitefly transmission of the viruses. When the whiteflies acquired TYLCTHV first and ToLCTV later, the titers of ToLCTV in the midgut and salivary glands of whiteflies were significantly lower than those of whiteflies that acquired ToLCTV only. However, the titer of ToLCTV in the organs and hemolymph of whiteflies were not different between the whiteflies acquired ToLCTV first and TYLCTHV later and the whiteflies acquired ToLCTV only. In contrast, the titers of TYLCTHV in the organs and hemolymph of whiteflies were not changed no matter ToLCTV was acquired earlier or later than TYLCTHV. Whitefly transmission assays found that the infection rate of TYLCTHV were higher than that of ToLCTV no matter that the whiteflies acquired these viruses first or later. Taken together, TYLCTHV had an antagonistic effect on the infection of ToLCTV in *B. tabaci*, and TYLCTHV was more competitive than ToLCTV in host plant. This study provides a new insight to understand the effect of virus-vector interaction on the epidemiology of plant viruses.

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In planta production of filamentous virus-like particles: insights into 3-D models of different sweet potato infecting viruses vectored by aphids or whiteflies

O. Chase[1], M. Byrne[2], E. Thuenemann[3], N. Ranson[2], G. P. Lomonossoff[3], and J.J. López-Moya[1]

[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Cerdanyola del Vallès, Barcelona, Spain; [2]Astbury Centre for Structural Molecular Biology, School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK; [3]Department of Biochemistry and Metabolism, John Innes Centre, Norwich Research Park, Norwich, UK.

(ornela.chase@cragenomica.es) *S4-O9*

The *Potyviridae* constitutes the largest family of plant-infecting single-stranded RNA viruses, including many members of high agronomical importance. All potyvirids, regardless of their vector, share similar virion structures consisting of flexuous elongated particles, composed by hundreds of copies of a single type of coat protein (CP) surrounding the RNA genome in helical arrangement. Some members of the family can infect sweet potato (*Ipomoea batatas*), an important staple crop for food security. These belong to either the aphid-transmitted genus *Potyvirus* or the whitefly-transmitted genus *Ipomovirus*. To gain insights on the molecular mechanisms required for vector specificity and vector-mediated dissemination of these viruses, we have conducted structural studies based on the production of flexuous virus-like particles (VLPs) in plants, a system with a great potential for nanobiotechnological uses. VLPs of two potyviruses, *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato virus 2* (SPV2), and one ipomovirus, *Sweet potato mild mottle virus* (SPMMV) were produced through transient expression of their respective CPs in *N. benthamiana* plants, using a self-replicating expression vector (1). Western blotting with specific antibodies and electron microscopy (EM) imaging of crude extracts of infiltrated leaves confirmed overexpression of CPs and their subsequent assembly into VLPs that resemble the flexuous filaments of the parent virus. The VLPs were purified and used for Cryo-EM studies, allowing us to solve their structure to near-atomic resolution. Our results allowed comparison of the structures of particles corresponding to a potyvirus and an ipomovirus that are able to infect the same host plant but are disseminated by different insect vectors. The observed structural differences, confirmed also by divergences in thermal stability, might contribute to better understand their biological properties, and hopefully to design future control measures.

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Two populations of *Bemisia tabaci* Mediterranean in Brazil are unable to transmit three native begomoviruses

A.M. Nogueira[1], V. H. Bello[1], E. Vicentin[1], C. S. de Oliveira[1], C. C. Martines[1], T.M.C. Barbosa[2], E. S. Gorayeb[1], L. F. M. Watanabe[1], J. M. Marubayashi[1], F. M. Zerbini[2], M. Ghanim[3], J. A. M. Rezende[4], M. A. Pavan[1], and R. Krause-Sakate[1]

[1]Departamento de Proteção de Plantas, Faculdade de Ciências Agronômicas, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, Brazil; [2]Departmento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, Brazil; [3]The Volcani Center, Institute of Plant Protection, Bet Dagan, Israel; [4]Departmento de Patologia de Plantas e Nematologia, E.S.A. Luiz de Queiroz, Universidade de São Paulo, Piracicaba, Brazil.

(angelica.nogueira@unesp.br) *S4-P1*

The whitefly *Bemisia tabaci* Middle East-Asia Minor 1 (MEAM1, formerly B biotype) has been the predominant species in Brazil since the mid-1990's. *B. tabaci* Mediterranean (MED, formerly Q biotype) was detected in 2014 in Rio Grande do Sul State, and since then, high infestations of the insect in greenhouses and open field production of *Solanaceae* in the South and Southeast regions of the country have occurred (1,2). Most viruses transmitted by whiteflies belong to *Begomovirus* genus. Previous studies demonstrated the ability of a MED population (MED-SP) to transmit tomato severe rugose virus (ToSRV) and bean golden mosaic virus (BGMV), the main begomoviruses that infect tomatoes and beans, respectively, in Brazil (3). Studies to evaluate MED potential as a vector of Brazilian begomoviruses are scarce. We evaluated the transmission efficiency by *B. tabaci* MEAM1 and MED of ToSRV and tomato rugose mosaic virus (ToRMV) for tomatoes and BGMV for beans. Results of transmission experiments by MED-SP, when compared with MEAM1, showed high efficiency of MEAM1 in the transmission of ToRMV, BGMV and three different ToSRV isolates, while MED-SP did not transmit any of the begomoviruses. Transmission tests of ToSRV by an additional MED population, denominated MED-Oleo, were also negative. Furthermore, transmission experiments by MEAM1, MED-SP and MED-Oleo using a higher number of insects and 72h of viral acquisition and inoculation access periods were also performed. MEAM1 transmitted the viruses with 100% efficiency, however, MED populations were unable to transmit any of the viruses. Our results conclude that the two populations of *B. tabaci* MED are unable to transmit Brazilian begomoviruses, for unknown reasons, and suggest that the interaction between the begomoviruses and whiteflies in Brazil is a highly dynamic process and should be investigated over time.

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Towards understanding the importance of mixed infections for vector-mediated spread of sweet potato virus diseases

C. Ontañón, O. Chase, and J.J. López-Moya

Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Cerdanyola del Vallès, Barcelona, Spain.

(clara.ontanon@cragenomica.es) *S4-P2*

Sweet potato (*Ipomoea batatas*) is threatened by many pests and pathogens, with viral diseases being among the most important constraints for yield and quality of this worldwide major food crop. The most prevalent RNA viruses in sweet potato belong to the family *Potyviridae,* such as the aphid-transmitted potyvirus *Sweet potato feathery mottle virus* (SPFMV). Frequently the presence of the whitefly-transmitted crinivirus *Sweet potato chlorotic stunt virus* (SPCSV), family *Closteroviridae*, contributes to cause severe diseases when combined in mixed infections with potyvirids (1). Single infection of SPFMV in susceptible plants usually causes mild symptoms only, and our attempts to transmit one isolate of SPFMV by the aphid vector *Myzus persicae* failed when tested in absence of SPCSV. On the other hand, in plants superinfected with SPCSV using the natural vector *Bemisia tabaci*, a much higher accumulation of the potyvirus occurred (2), resulting in successful SPFMV transmission by aphids. To further explore risks of SPFMV spread associated to coinfection with the crinivirus, experiments are being conducted with SPCSV viruliferous whiteflies. Different plant species, belonging to up to five botanical families, were tested in a first investigation, finding several previously uncharacterised susceptible hosts that could support replication and movement to distal parts of the plants, where SPCSV was detected by RT-PCR. Despite this unexpected expansion of the host range, their capacity to act as reservoirs for subsequent transmission was uncertain, and at least in our laboratory conditions SPCSV infected *Nicotiana tabacum* plants failed to serve as inoculum source for reinfection of sweet potatoes, suggesting that many of the new susceptible species might behave as dead-end hosts. Our results highlight the importance of mixed infections to understand the epidemiology of sweet potato viruses.

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Aphid response to volatiles emitted by melon plants after single and mixed virus-infection

E. Garzo, A. Moreno, and A. Fereres

Instituto de Ciencias Agrarias, CSIC, Madrid Spain.

(elisa.garzo@ica.csic.es)

S4-P3

Plant viruses are able to manipulate their host to modify the attractiveness and palatability of their vectors, in a way that enhance virus transmission (1-3). Virus infection alters plant biochemistry, which causes changes in emission of volatile organic compounds that modify the plant's relationship with other organisms including insect vectors and their natural enemies (4). Melon crops are commonly infected with more than a single virus, and mixed-viral infections are very common, but their impact on the behavior of their insect vectors is largely unknown. The aim of this work was to evaluate the response of non-viruliferous winged morphs of *Aphis gossypii* (Hemiptera: Aphididae) to volatile cues emitted by melon plants (cv Bazan) infected by the non-persistent (NP) cucumber mosaic virus (CMV, *Cucumovirus*) and/or the persistent (P) cucurbit aphid-borne yellow virus (CABYV, *Polerovirus*). Aphid response to volatiles was tested using a glass Y-tube olfactometer and the following treatments were compared: 1) healthy plant *vs* clean air (empty glass chamber); 2-3) single (CMV or CABYV) infected melon plant *vs* clean air; 4-5) single (CMV or CABYV) infected melon plant *vs* healthy plant; 6) mixed (CMV+CABYV) infected plant *vs* healthy plant. Forty replicates per treatment were compared. The results showed as previously reported that aphids were attracted most to volatiles emitted by CMV-infected plants. However, when the plants were infected by CABYV, no significant differences were found. Experiments with mixed-viral infected plants are underway. Aphid response to the volatiles emitted by single and mixed-viral infections on melon compared with heathy plants and its epidemiological implications will be discussed.

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Detecting virus-carrying *Xiphinema* spp. as an alternative to species identification of *Xiphinema* in trade

E. Everaert, N. Viaene, and K. De Jonghe

Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Merelbeke, Belgium.

(ellen.everaert@ilvo.vlaanderen.be) *S4-P4*

Several nematode species of the genus *Xiphinema* are regulated because they can transmit nepoviruses. Species identification is based on nematode morphology and is quite complex. Our study aims for a generic diagnostic procedure that detects harmful plant viruses in any *Xiphinema* specimen extracted from soil, as an alternative to identifying the nematode as a potential virus carrier. A selection of methods for nematode extraction from soil (Automated Zonal Centrifuging[1] and Flegg's Modified Cobb method[2]), homogenization (slicing, bead beating and bead beating with collagenase), RNA extraction (RNeasy Plant Mini Kit, CTAB and KingFisher MagMAX RNA Isolation Kit) and nepovirus detection (generic and specific nepovirus (q)PCR assays and MinION sequencing technology) are compared. *Xiphinema index, X. diversicaudatum* and *X. rivesi* are used as vectors of grapevine fanleaf virus (GFLV), *Arabis* mosaic virus (ArMV) and tomato ringspot virus (ToRSV), respectively. Nematode extractions from soil using the Automated Zonal Centrifuge resulted in the highest nematode yields. Assays using the bead beating technique showed a better homogenization than those using the slicing technique and preliminary tests returned a higher RNA recovery for RNeasy Plant Mini Kit extracted samples than for CTAB extracted samples. Methods for homogenization and RNA extraction, such as bead beating with addition of collagenase and KingFisher MagMAX RNA isolation, still need to be evaluated. When comparing available diagnostic assays, virus detection was only achieved with specific and not with generic nepovirus (q)PCR assays. Looking forward, MinION technology will be examined as a sensitive diagnostic tool for untargeted sequencing and could serve as a replacement for numerous single nepovirus (q)PCR testing.

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Bemisia tabaci Mediterranean cryptic species survey on soybean in Sao Paulo State (Brazil) and its interaction with cowpea mild mottle virus

F. Barreto da Silva[1], J. Uzan[1], J. M. Marubayashi[1], R. S. Raposo[1], C. C. Martines[1], M. R. Ribeiro-Junior[1,2], A. M. Nogueira-Portilho[1], M. A. Pavan[1], and R. Krause-Sakate[1]

[1]Departamento de Proteção de Plantas, Faculdade de Ciências Agronômicas, Universidade Estadual Paulista Júlio de Mesquita Filho, Botucatu, Brazil; [2]Institute of Biosecurity and Microbial Forensic – Oklahoma State University - Stillwater, Oklahoma, United States.

(felipe.barreto@unesp.br) *S4-P5*

The recent spreading and colonization of the whitefly *Bemisia tabaci* Mediterranean (MED) under field condition can cause important impact regarding the management of whiteflies in soybean (1). MED is considered more resistant to insecticides than *B. tabaci* Middle East Asia Minor 1 (MEAM1), the most predominant whitefly species in Brazil. Moreover, the carlavirus cowpea mild mottle virus (CPMMV) can be efficiently transmitted by MED and MEAM1 (2). We have surveyed *B. tabaci* species and CPMMV during the last three soybean seasons (2019, 2020 e 2021) in Sao Paulo State, Brazil. *B. tabaci* MED was detected in different places mostly co-occurring with MEAM1 and associated with infection of CPMMV on soybean plants. Transmission assays evaluating acquisition access period (AAP) and inoculation access period (IAP) by MED were also performed. Transmission efficiency of CPMMV by MED was determined using 5min to 24h AAP on infected soybean plants. After the acquisition, one single whitefly was transferred to a healthy soybean plant at the primary leaf stage for an inoculation access period of 24h. Five minutes of AAP was not sufficient to transmit CPMMV and the higher the AAP, the higher the transmission efficiency of CPMMV by MED. To determine the minimum IAP, insects were maintained for 24h on soybean infected plants, and one single insect was transferred to healthy soybean plants and maintained from 2min to 24h, ranging the IAP. CPMMV transmission efficiency was also higher with the increase of IAP. Comparative assays with MEAM1 are in progress and will permit us to determine which whitefly species is more effective in CPMMV transmission.

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Pre-infection of tomato plants carrying Sw-5 gene with tomato chlorosis virus does not seem to alter the infection with groundnut ringspot virus

H. D. Kraide[1], V. M. Camelo-García[2], G. M. Favara[1], F. F. de Oliveira[1], C. G. Ferro[1], E. Y. N. Carmo[1], E. F. B. Lima[3], A. B. Filho[1], and J. A. M. Rezende[1]

[1] Plant Pathology and Nematology Department, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, SP, Brazil; [2] United States Department of Agriculture, Agricultural Research Service, Salina, CA, USA; [3] Biological Sciences Departmente, Campus Amilcar Ferreira Sobral, Federal University of Piauí, Floriano, PI, Brazil.

(heron.kraide@usp.br) *S4-P6*

Tomato (*Solanum lycopersicum*) is one of the most planted vegetables in Brazil and many regions globally. Tomato crops can be affected by various pests and diseases, including those of a viral nature. Brazil's most critical virus diseases are caused by the begomovirus tomato severe rugose virus (ToSRV) and the crinivirus tomato chlorosis virus (ToCV), transmitted by *Bemisia tabaci* MEAM1. Infection with the orthotospovirus groundnut ringspot virus (GRSV) also occurs at a low incidence due to resistant hybrids carrying the *Sw*-5 resistance gene (1). Tomato plants can be naturally infected by one or more viruses, in which mixed infections can result in synergistic or antagonistic effects that could compromise virus control and plant yield. Under experimental conditions, tomato plants carrying the resistance gene and pre-infected with ToCV became susceptible to infection with the orthotospovirus tomato spotted wilt virus (TSWV) (2). This work evaluated the susceptibility of ToCV-pre-infected tomato plants cvs. Compack and Caeté, resistant to GRSV, to natural infection with this orthotospovirus in the field. Plants of cv. Santa Clara, susceptible to GRSV, were used as a control. For this, 50 plants, healthy and ToCV-pre-infected, of each cultivar were exposed and evaluated for infection with the orthotospovirus. The attractiveness of healthy and ToCV-pre-infected tomato plants to thrips was also evaluated on yellow sticky traps. For this, healthy and ToCV-pre-infected of cv. Santa Clara were transplanted in the field in three randomized blocks with 25 plants each. A yellow sticky trap was placed in the center of each block, where thrips were counted weekly. The results showed that, independently of the cultivar, ToCV-pre-infected tomato plants did not affect natural infection with the orthotospovirus GRSV. There was no difference in the number of different thrips species captured in the blocks containing healthy or ToCV-pre-infected tomato plants.

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New insights about the biology and structure of insect-transmitted plant viruses of the family *Secoviridae*

I. Ferriol[1,2], M. Byrne[3], A. Javed[3], N. Ranson[3], G. P. Lomonossoff[4], and J. J. López-Moya[1]

[1]Centre for Research in Agricultural Genomics (CRAG-UAB-UB-CSIC-IRTA), Spain; [2]Instituto de Ciencias Agrarias (ICA-CSIC), Spain; [3]University of Leeds, Leeds, England; [4]John Innes Centre (JIC), Norwich, England.

(iferriol@ica.csic.es) *S4-P7*

Members of the family *Secoviridae* are non-enveloped viruses with positive-sense ssRNA genomes, classified into eight genera; six with bipartite genomes (*Comovirus*, *Fabavirus*, *Nepovirus*, *Cheravirus*, *Sadwavirus*, *Torradovirus*) and two more with monopartite genomes (*Sequivirus* and *Waikavirus*). Most viruses within the family are transmitted by nematodes (such as nepoviruses) or insect vectors, including beetles (comoviruses), aphids (fabaviruses, sequiviruses, sadwaviruses, some waikaviruses and some torradoviruses), leafhoppers (other waikaviruses) and whiteflies (other torradoviruses). However, little is known about their mechanisms of transmission, requiring further studies on virion structure, retention site and other key aspects of the process. Reported structures for the virions are available for some comoviruses, cheraviruses and nepoviruses, leaving a gap in our knowledge of the structure of other members of the family. In this work, we analyzed structures of the fabavirus broad bean wilt virus 1 (BBWV-1), which is transmitted in a non-persistent manner by aphids (1); and the torradovirus, tomato apex necrosis virus (ToANV), which is transmitted in a semipersistent manner by whiteflies (2). BBWV-1 virions were purified from infected plants and the presence of each capsid protein was confirmed by western blotting and transmission electron microscopy (TEM). In the case of the torradovirus, we engineered a pEAQ-based construct to produce virus-like particles (VLPs) in *Nicotiana benthamiana* plants. The formation of VLPs was confirmed by TEM and western blotting using specific anti-CP antibodies. In each case, high-resolution structures of particles were determined using cryo-electron microscopy (Cryo-EM), revealing structural organisation of the icosahedral assembly. This structural information will be used to develop biotechnological applications, and to explore the mechanisms of virus transmission.

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Endosymbiont community structure of rice stripe virus-viruliferous *Laodelphax striatellus* (Hemiptera: Delphacidae) in Korea

M. Kwon, and E.-J. Kil

Department of Plant Medicals, Andong National University, Korea.

(ysx0007@naver.com)

S4-P8

Insects have a complex relationship with bacteria, but how they interact with their hosts has not been studied much. *Laodelphax striatellus* (small brown planthopper, SBPH) is polyecious insect that damages a variety of grain crops and is a carrier of plant viruses such as rice stripe virus (RSV) in rice. We investigated the endosymbiont community of non-viruliferous and RSV-viruliferous *L. striatellus* groups using 16S rRNA gene sequencing based on high-throughput sequencing technology in order to investigate how SBPH endosymbionts affect RSV infection. *Wolbachia* was commonly identified in the two different groups. The diversity of microbes and their composition were analyzed in this study, which can be served as a basis for the study of the virus-insect-endosymbiont interaction and the development of virus spread inhibitors.

A database on the transmission of plant viruses

D. Peters[1], P. van Vredendaal[2], and R.A.A. van der Vlugt[1]

[1]Laboratory of Virology, Wageningen University and Research, The Netherlands; [2]Wageningen Library, Wageningen University and Research, The Netherlands.

(rene.vandervlugt@wur.nl) *S4-P9*

Since their first descriptions, a prime focus in the research on plant viruses has been on the way they are transmitted. Understanding virus transmission mechanisms, routes and identifying virus vectors is crucial in understanding plant virus epidemiology and ecology and implementing adequate virus control measures. Over the last 100 years countless scientific papers have been published describing 'new' viruses, their host plants, their symptoms and means of transmission. Despite the fact that we now live in a digital age, the vast majority of these papers are not well known and therefor this often-important information remains hidden to the plant virology community. Over the last seven years the first author has studied a vast number of papers and other scientific information covering over 100 years of plant virus research. He has extracted relevant information on the transmission of well over 2500 plant viruses that have been described over the last century. This data not only cover the means of transmission (vegetative propagation and mechanical and/or vector transmission) but also the described vectors. In collaboration with the Wageningen University and Research Library we are currently working on developing a web-based tool to make this data publicly available.

The role of HCPro in the transmission properties of nine PVY field isolates from Tunisia

M. Makki[2], H. Sun[1], F. del Toro[1], K. Necira[2], F. Tenllado[1], F. Khouaja[2], and T. Canto[1]

[1]Department of Microbial and Plant Biotechnology. Margarita Salas Center for Biological Research. Ramiro de Maeztu 9, 28040 Madrid, Spain; [2]Laboratory of Molecular Genetics, Immunology and Biotechnology (LR99ES12). Faculty of Sciences. University of Tunis El Manar. Manar II, Tunis 2092, Tunisia.

(tomas.canto@cib.csic.es) *S4-P10*

In a previous work we had compared the virulence in *Nicotiana benthamiana* plants of nine PVY isolates sampled from different regions of Tunisia (eight from potato crops and one from pepper), under two controlled growing conditions: standard (26 °C, ~405 ppm CO_2), in which all nine isolates induced severe leaf curl, chlorosis and stunting; or climate change-associated (31 °C and 970 ppm CO_2) (1). We have now assessed their transmissibility by the peach aphid *Myzus persicae* under standard conditions. Seven isolates were highly transmissible and infection symptoms were similar in donor and recipient plants. However, the two remaining isolates displayed specificities: all recipient plants infected with potato isolate X1 displayed mosaic and mild curling, but no stunting. This suggests filtering of a viral subpopulation through the transmission bottleneck, and that its ability to be aphid-transmitted and to induce the novel symptoms are associated. Transmission of pepper isolate Kef Pep2 ranged from none in an initial test, to some in two additional tests. Sequencing of the isolate Kef Pep2 *HCPro* cistron in donor and recipient plants from these experiments showed that two HCPro species could be simultaneously present in donor plants, differing in only one amino acid at position 49, adjacent to the KITC motif (either **Y**KITC or **H**KITC). However, infected recipient plants expressed only HCPro with the **Y**KITC motif. The data on HCPro pre-transmission heterogeneity suggests that the **Y**49**H** substitution reported here for the first time could prevent transmission. We tested by BiFC whether the **Y**49**H** substitution would prevent HCPro dimers from attaching to the cell microtubules (MTs) under situations of osmotic stress, as had been reported for an adjacent **K**50**N** substitution, and found that it did not.

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Session

05

Other Vector-Borne Diseases

How does '*Candidatus* Liberibacter solanacearum' manipulate plant and insect immunity?

C. Tamborindeguy[1] and J. Levy[2]

[1]Department of Entomology, Texas A&M University, United Stated of America [2]Department of Horticultural Sciences, Texas A&M University, United Stated of America.

(ctamborindeguy@tamu.edu)

Keynote Lecture 5

'*Candidatus* Liberibacter solanacearum' (Lso) is an emergent bacterial pathogen of crops worldwide. In North America, two bacterial haplotypes, Lso A and Lso B, are transmitted by the potato psyllid, *Bactericera cockerelli*, to solanaceous plants, while other Lso haplotypes infect other plant families around the world. We have shown differences in virulence between these two haplotypes: Lso B causes more severe symptoms in tomato than Lso A. Similarly, we have identified difference in the interaction of each haplotype with the psyllid vector. We hope that this genetic diversity can help us overcome research limitations linked to Lso's fastidious nature and allow us to discovery key proteins involved in plant host and vector infection by Lso. We are using a comparative approach to unravel the molecular mechanisms of Lso interaction with host plants and the vector. We have identified over 100 putative Lso effector proteins. We have set-up a pipeline to identify and validate these Liberibacter effector proteins. This approach is yielding new knowledge about the mechanisms used by Lso to manipulate plant and insect immunity. Because commercially acceptable genetic resistance against potato psyllids or Lso has not been identified in potato or other solanaceous crops, the knowledge gathered through these studies will lead to novel approaches to control this and other related vector-borne pathogens.

Florida citrus growers' potential 'toolbox' for Huanglongbing (HLB) management: an alphabet soup (ISVs, PDIs, IPCs, NATI etc.)

O. Batuman[1], K. Britt[1], S. Kunwar[1], A. Redondo[1], and F. Alferez[2]

[1]Department of Plant Pathology, Southwest Florida Research and Education Center (SWFREC), University of Florida, Institute of Food and Agricultural Sciences (UF-IFAS), Immokalee FL, USA; [2]Department of Horticultural Sciences, SWFREC, UF-IFAS, Immokalee FL, USA.

(obatuman@ufl.edu) *S5-O1*

Huanglongbing disease (HLB; citrus greening), putatively caused by *Candidatus* Liberibacter asiaticus (CLas) and primarily transmitted by the insect vector Asian citrus psyllid (ACP; *Diaphorina citri*), has brought the citrus industry in Florida to the brink of collapse. Additionally, HLB continues to spread and threatens other production areas worldwide. Still, without a cure, HLB has been the most challenging disease to study or investigate effective management strategies. Our research goal is to develop an integrated pest management (IPM) for HLB control. To achieve our goal, we have detected several insect specific viruses (ISVs) in the ACP vector for potential biological controls, evaluated individual protective covers (IPCs) on citrus trees as physical barriers with 100% efficacy in preventing HLB over two years, and screened numerous therapeutics, including antimicrobials and plant defense inducers (PDIs), to delay CLas infection in young citrus trees. Furthermore, to effectively deliver these potential therapeutics into citrus phloem, we also developed and investigated a novel delivery method, NATI: needle assisted trunk infusion/injection. Although ISVs for biological control remain in infancy and newly identified PDIs and therapeutics provided good control against CLas infection, they have yet to go through the lengthy and expensive labeling process before becoming available for our growers. Nevertheless, the IPCs are now being commonly used on most new plantings by growers throughout Florida. Altogether, our ongoing results indicate that these IPM tools can be included in our growers' 'toolbox' in the near future and may provide effective control for HLB disease in citrus trees.

Vector biology, abundance, dispersal and temporal transmission dynamics shape *Xylella fastidiosa* epidemiology in Apulia

N. Bodino[1], V. Cavalieri[2], C. Dongiovanni[3], M. Saponari[2], and D. Bosco[1,4]

[1]IPSP-CNR, Torino headquarter, Torino, Italy; [2]IPSP-CNR, Bari Unit, Bari, Italy; [3]CRSFA Basile Caramia, Locorotondo (Bari), Italy; [4]DISAFA-University of Torino, Grugliasco (Torino), Italy.

(domenico.bosco@unito.it) *S5-O2*

Phenology, abundance and dynamics of spittlebug populations, together with transmission biology, are of major importance to outline the disease epidemiology of *Xyella fastidiosa* subsp. *pauca* in Apulian olive groves. The spread rate of *Xf* is mainly influenced by 1) the pathogen colonization of the host plant; 2) the acquisition of the pathogen by the vector from an infected plant, and its transmission to healthy plants; 3) the vector population dynamics and abundance; 4) the dispersal of the vector. In this contribution, we summarize experiments done to estimate 2), 3) and 4). Research was conducted in olive groves of the Apulia Region from 2016 onwards. The olive disease spread almost exclusively from olive-to-olive during the whole adult period of the main vector, *Philaenus spumarius*, lasting from May to October-November. The infectivity of the vector varies according to the season (higher in autumn vs early summer) (1) but other factors, such as insect abundance, survival and seasonal host-plant shifting (2,3), indicate a higher probability of transmission during early summer. According to mark-release-recapture experiments (4), the dispersal of *P. spumarius* is limited to some hundreds of meters throughout the whole year, although it can be influenced to a great extent by the structure of the agroecosystem. Since the observed spread of the disease in Apulia over the years was tens of time faster, a passive transportation of infected insects is very likely to explain the long-range spread. However, the knowledge of the active dispersal ability of the vector provides precious information for the management of new infection foci at the local scale.

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Elucidating the inoculation mechanism of *Xylella fastidiosa*

D. Cornara[1,3], A. Fereres[2], and R. Almeida[3]

[1]University of Bari Aldo Moro, DiSSPA, Italy; [2]ICA-CSIC Madrid, Spain; [3]Department of Environmental Science, Policy, & Management, UC Berkeley, USA.

(daniele.cornara@uniba.it) *S5-O3*

Despite decades of research efforts, a fundamental question on *Xylella fastidiosa* remains unanswered: how the fastidious bacterium is inoculated into the host plant by its insect vectors? Theoretical models and indirect evidences produced on sharpshooters have been recently challenged by an EPG-assisted transmission work on the meadow spittlebug *Philaenus spumarius*, where bacterium inoculation was associated with a non-stereotypical "spiking waveform", a behavior occurring within the first minutes when the stylets contact a xylem vessel. This waveform may represent a valve and/or muscles activity either directly pushing the bacterial cells toward the xylem vessel, or leading to an imperfect sealing of the pre-cibarium with the consequent leakage of fluid containing *X. fastidiosa* cells. The goal of the present work was to definitely prove the correlation between the "spiking waveform" and the inoculation of the fastidious bacterium, by using the best characterized pathosystem so far: *Graphocephala atropunctata X. fastidiosa fastidiosa*, and grapevine together with *P. spumarius*. Here we: i) report the results of our EPG-assisted transmission trials carried out with both the blue-green sharpshooter and the meadow spittlebug; ii) describe a theoretical framework on how the fastidious bacterium is inoculated into its host plant.

The immunodominant membrane protein (Imp) of Flavescence dorée phytoplasma interacts with gut proteins of insect vectors

L. Galetto[1], L. Bucci[1,2], M. Manfredi[3], S. Abbà[1], M. Rossi[1], D. Bosco[1,2], and C. Marzachì[1]

[1]Istituto per la Protezione Sostenibile delle Piante, CNR, Torino, Italy; [2]Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Grugliasco (TO), Italy; [3]Dipartimento di Medicina Traslazionale, Università del Piemonte Orientale, Novara, Italy.

(luciana.galetto@ipsp.cnr.it) *S5-O4*

Phytoplasmas are plant pathogenic bacteria that cause diseases and severe economic losses to crops worldwide. Phytoplasmas are transmitted by hemipteran insect species to the phloem of host plants, and they multiply in both hosts. As these unculturable bacteria are wall-less, the involvement of different cell membrane proteins in promoting pathogen internalization within the host cell has been hypothesized. Here we present the results on the interactions between the immunodominant membrane protein (Imp) of Flavescence dorée phytoplasma (FDp) and the insect vector proteins. FDp is a grapevine quarantine pest and a major threat to European viticulture. An optimized pull-down protocol combined with mass spectrometry was employed to identify host proteins obtained from dissected guts interacting with the synthetic Imp C-terminus domains of two FDp strains (16SrV-C and –D). The leafhopper species *Scaphoideus titanus* and *Euscelidius variegatus*, the FDp natural and laboratory vector, respectively, were the hosts of this study. Interacting protein fractions were trypsin-digested before mass spectrometry and identification by bioinformatic analyses. Five *E. variegatus* proteins interacting with Imp were further characterized by measuring expression of their corresponding transcripts in different insect tissues and in healthy vs infected insects. The five genes were silenced to evaluate the effects on phytoplasma acquisition. The specific silencing of two of them, namely legumain and natterin-4-like, resulted in a significant reduction of phytoplasma multiplication in insects upon pathogen acquisition compared to control insects. Since the epidemiology of FD is complex and involves different phytoplasma genotypes and insect vector species, the identification of genetic traits regulating transmission specificity pave the way to the possible disruption of the disease spread.

Investigation of the diversity of the destructive 16SrV phytoplasma group in grapevine, hazelnut and leafhoppers

Z. Kogej[1, 2], G. Seljak[1], T. Jakomin[1], J. Brodarič[1], A. Vučurović[1], M. Ravnikar[1], D. Kutnjak[1], and N. Mehle[1, 3]

[1]Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia; [2]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia; [3]School for Viticulture and Enology, University of Nova Gorica, Slovenia.

(zala.kogej@nib.si)

S5-O5

Grapevine Flavescence dorée phytoplasma of 16SrV group, is a quarantine pest in the EU and causes many problems in vineyards. In 2021, there were many new cases of infection with this phytoplasma in Slovenia and according to the regulations, several vineyards had to be destroyed. Grapevines can be infected with various isolates of 16SrV phytoplasma, some of which have been shown to cause epidemics because they can be spread very efficiently in vineyards by the American leafhopper (*Scaphoideus titanus*) (1). Other isolates, which cannot be transmitted by *S. titanus* and do not cause epidemics, can be transmitted from other hosts to grapevine by some other plant sap-sucking insects (2). In Slovenia, 16SrV phytoplasma was also detected in declining cultivated hazelnuts (3). In 2021, we observed numerous populations of the mosaic leafhopper (*Orientus ishidae*) in hazelnut orchards in Slovenia, and infestation with 16SrV phytoplasma was detected in 17% of 64 samples examined. Nucleotide sequence analysis of the *map* gene (4), which can be used to determine the map-FD genotype to distinguish between epidemic and non-epidemic phytoplasma isolates (2), was performed on total DNA extracted from 16SrV-positive samples of grapevine, hazelnut, and leafhoppers. In 95% of 16SrV-positive grapevines, we detected five different *map* genotypes, all of which were reported to be epidemic (2,5). The M54 genotype was the most widespread and was also detected in *S. titanus*. On the other hand, we never identified M54 in hazelnuts, but other epidemic genotypes detected in some Slovenian grapevines, such as M38, M50, and M122, were also found in hazelnuts and *O. ishidae*. Non-epidemic strains linked with grapevine were identified in only 8% of 16SrV-positive samples of hazelnuts. In a few samples of grapevine and hazelnut, we detected *map* genotypes that had not been previously described. To better understand the role of different genes in epidemic characteristics of different strains, we are working on genome assembly of 16SrV phytoplasma from both plant hosts. Knowledge of the similarity of isolates in grapevine and hazelnut will help us to properly plan further measures.

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Impact of insecticides in the feeding behaviour of *Philaenus spumarius* associated to the transmission of *Xylella fastidiosa*

C. Lago[1,2]; D. Cornara[1,3,4], S. A. Minutillo[4], A. Moreno[1,5], and A. Fereres[1,5]

[1]Instituto de Ciencias Agrarias (ICA), Consejo Superior de Investigaciones Científicas (CSIC), Calle Serrano 115dpdo, 28006 Madrid, Spain; [2]Departamento de Producción Agraria, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas (ETSIAAB), Universidad Politécnica de Madrid (UPM), Campus, Av. Puerta de Hierro, nº 2, 4, 28040 Madrid, Spain; [3]Department of Soil, Plant and Food Sciences, Entomological and Zoological Section, University of Bari Aldo Moro, Bari, Italy; [4]International Centre for Advanced Mediterranean Agronomic Studies - Institute of Bari (CIHEAM-Bari), Via Ceglie 9, 70010 Valenzano (BA), Italy; [5]Associate Unit IVAS (CSIC-UPM), Control of Insect Vectors of Viruses in Horticultural Sustainable Systems, Madrid, Spain.

(clara.lago@ica.csic.com) *S5-P1*

The recent emergence of *Xylella fastidiosa* in Europe is a major threat to agriculture, since the bacterium affects key crops such as olive, grapevine and almond. *Philaenus spumarius* was identified as the main vector in Europe[1]. Pesticides are essential, though controversial tools, in modern agriculture. Thus, studies on pesticides lethal and sub-lethal effects on insect vectors of plant pathogens are crucial to develop effective control strategies. In the present study, we evaluated the lethal and sub-lethal effects of six compounds (acetamiprid, deltamethrin, spinosad, sulfoxaflor, pyrethrin, kaolin) on *P. spumarius*. Moreover, we evaluated the impact of four compounds (acetamiprid, pyrethrin and kaolin) against the transmission of *X. fastidiosa* by *P. spumarius* under free-choice and no-choice conditions. Deltamethrin, acetamiprid and to a lesser extent pyrethrin altered the feeding behaviour of *P. spumarius*. Nevertheless, deltamethrin and acetamiprid were highly toxic against *P. spumarius*, while pyrethrin induced low mortality against the spittlebug. In contrast, spinosad, sulfoxaflor and kaolin exhibited low toxicity and did not significantly impact *P. spumarius* feeding behaviour. Under no-choice conditions, both pyrethrin and acetamiprid reduced the transmission rate compared to the control. On the other hand, pyrethrin was able to reduce transmission but acetamiprid failed do so under free-choice conditions. Overall, our results show that pyrethrin was the only product that reduced the transmission of *X. fastidiosa* both under free-choice and non-choice conditions. Therefore, *X. fastidiosa* control strategies solely based on the evaluation of pesticides acute toxicity could be ineffective in preventing the transmission and spread of the bacterium.

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Landscape complexity promotes the abundance of potential vectors of *Xylella fastidiosa* in Portuguese vineyards

I. Rodrigues[1,2], M. Villa[1], P. Baptista[1], and J. A. Pereira[1]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; [2]Departamento de Ingeniería Agrária, Universidad de Léon, Av. Portugal, n° 41, 24071 Léon, Spain.

(irodrigues@ipb.pt) *S5-P2*

Potential vectors of *Xylella fastidiosa* can play a key role in spreading this phytopathogen across the landscape. Therefore, understanding how the surrounding landscape influences the abundance of these insects is essential to assess the susceptibility of the agrosystems to *X. fastidiosa* in order to implement proper prevention measures. In this work, the community of potential vectors of *X. fastidiosa* was described, and the response of these insects' abundance to the landscape structure within a gradient of distances from the vineyards was analyzed. In 2018 and 2019, potential vectors were collected with an entomological sweep net in the canopy and vegetation cover of 35 vineyards distributed throughout Portugal (in July, September, and October). The landscape configuration and composition metrics were calculated within buffers constructed around each vineyard (750m, 1000 m, 1500 m, and 2000 m). The response of the abundance of the potential vector to the landscape variables along the sampling period at the different spatial scales was analyzed using a series of separated generalized mixed models. Tree species of xylem sap-feeding insects were collected in the vineyards: *Philaenus spumarius*, *Neophilaenus campestris*, and *Cicadella viridis*. Generally, the abundance of the potential vectors increased to the west of the country, showing higher values in the ground cover vegetation. The landscape complexity significantly increased the abundance of the potential vectors in all the spatial scales. High proportions of vineyards in the landscape also positively influenced the abundance of potential vectors in the larger scales (1500 m and 2000 m). Our results suggest that vineyards surrounded by more complex landscapes may be more susceptible to the emergence of *X. fastidiosa* epidemics in case of infection.

Acknowledgments

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Olfactory behavior of *Philaenus spumarius* and *Cicadella viridis* to cis-3-hexen-1-ol and cis-3-hexenyl acetate

I. Rodrigues[1,2], J. Benhadi-Marín[1], N. Rodrigues[1], P. Baptista[1], and J. A. Pereira[1]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; [2]Departamento de Ingeniería Agrária, Universidad de Léon, Av. Portugal, n° 41, 24071 Léon, Spain.

(irodrigues@ipb.pt) *S5-P3*

Cis-3-hexen-1-ol and cis-3-hexenyl acetate are green leaf volatiles (GLVs). Plants release them almost instantly upon mechanical damage or due to abiotic or biotic stresses. GLVs could function as an immediate and informative signal for many organisms in the plant's environment. They can repel or attract phytophagous insects and their natural enemies and induce plant defenses or prime plants for enhanced defense against phytophagous insects and pathogens. In this work, we evaluated the olfactory response of *Philaenus spumarius* and *Cicadella viridis*, a vector and a potential vector of the phytopathogenic bacterium *Xylella fastidiosa*, to cis-3-hexen-1-ol and cis-3-hexenyl acetate. We recorded 30 individuals of both sexes of *P. spumarius* and *C. viridis* for 20 min in a four-arm olfactometer. The cis-3-hexen-1-ol and cis-3-hexenyl acetate were tested at different concentrations (5, 10, 20, 30 µg/µL), and a stream of air and sunflower oil were used as controls. At the lowest GLVs concentration, the females of *P. spumarius* were significantly attracted by the two GLVs ($P<0.01$). When the individuals were exposed to the volatile compounds at 10 µg/µL, females chose more often the stream of air ($P<0.01$), whereas the males presented no significant differences in their choice ($P= 0.093$). The frequency of visits in each GLVs area significantly differed between sex except when the individuals were exposed to the GLVs at 10 µg/µL. Individuals of *C. viridis* did not show attraction for any GLVs in any concentrations. Low concentrations of cis-3-hexen-1-ol and cis-3-hexenyl acetate may attract *P. spumarius*. However, higher concentrations can saturate the olfactory sensilla affecting the choice. Regarding *C. viridis*, further studies are needed to understand better the role of GLVs in the behavior of this insect.

Acknowledgments

This work was supported by the EU H2020 Research Project XF-ACTORS "Xylella fastidiosa Active Containment Through a multidisciplinary-Oriented Research Strategy" (Grant Agreement 727987) and to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UID/AGR/00690/2020). Isabel Rodrigues also acknowledges the PhD research grant (2020.07051.BD) provided by FCT.

Association of '*Candidatus* Phytoplasma asteris' (group 16SrI) and '*Candidatus* Phytoplasma fraxini' with a new syndrome in potato crops in Colombia

L. Franco-Lara [1], C. A. Varela-Correa [1], G. P. Guerrero Carranza [1], and J. C. Quintero Vargas[2]

[1] Faculty of Basic and Applied Sciences, Universidad Militar Nueva Granada, Km2 via Cajicá - Zipaquirá, Cajicá, Colombia; [2] Instituto Colombiano Agropecuario (ICA), Carrera 68A N° 24B – 10 - Edificio Plaza Claro- Torre 3, Bogotá D.C., Colombia.

(Liliana.franco@unimilitar.edu.co) *S5-P4*

Cundinamarca is the major potato producer department of Colombia. Potato growers in Cundinamarca have reported a new syndrome affecting commercial potato fields. Two species of potato are cultivated in Colombia, *Solanum tuberosum* and *Solanum phureja;* both species show symptoms. The objective of this work was to test the association of the new symptoms with phytoplasmas. The presence of phytoplasmas was tested by nested PCR of the 16S rRNA gene, followed by Restriction Fragment Length Polymorphism (RFLP) or sequencing analysis (1). A survey was carried out in 22 municipalities of Cundinamarca. Of 152 symptomatic and non-symptomatic potato plants tested, 33,5% from five municipalities, were positive for phytoplasmas in infections with either '*Ca.* P. asteris', '*Ca*. P fraxini' or both phytoplasmas (mixed infections). Phytoplasmas were also detected by electron microscopy in symptomatic plants. The tubers of 45 field plants were harvested and planted in a greenhouse to track symptom development and phytoplasma transmission, from mother plants to daughter plants via tubers. Phytoplasmas of the 16SrI and 16SrVII groups in single or mixed infections were detected in 39 cases out of 45, and the same group of phytoplasmas was detected in mother and daughter plants in 32 plants. In seven mother plants phytoplasmas were not detected but their daughter plants were infected, or infected with the other group. Additionally, the insect vector *Exitianus atratus* (Hemiptera: Cicadellidae) transmitted '*Ca.* P. asteris' or '*Ca.* P. fraxini' to all 18 potato plants exposed to insects, generating the same symptoms of the field-infected plants. Therefore, symptoms including foliage yellowing, curly leaves with purple margins and distortions of the normal shape of the plant were associated with '*Ca.* P. asteris' and '*Ca.* P. fraxini' in Cundinamarca. Phytoplasmas of either species in single or mixed infections, produced indistinguishable symptoms in the infected plants. Projects CIAS 1926, 2740, 3110.

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In planta distribution of '*Candidatus* Phytoplasma asteris' (16SrI) and '*Candidatus* Phytoplasma fraxini' (16SrVII) infecting *Quercus humboldtii* trees in mixed infections

J. Lamilla[1], L. Franco-Lara [1], and Y. Arocha Rosete[2]

[1]Faculty of Basic and Applied Sciences, Universidad Militar Nueva Granada, Cundinamarca, Colombia; [2]Sporometrics Inc., Toronto, Ontario M6K 3J1, Canada.

(Liliana.franco@unimilitar.edu.co) *S5-P5*

Phytoplasmas are cell wall-less pathogenic bacteria. In *Q. humboldtii* urban trees in Bogotá, Colombia, a disease associated with phytoplasmas has been reported (1). In a survey conducted in 2017, 94% of the trees were infected with 16SrI and 16SrVII phytoplasmas in sinlge or mixed infections of both species. Due to difficulty to reach the upper branches, sampling for phytoplasma is biased towards lower branches, thus overlooking the presence of other phytoplasmas in different parts of the crown. Additionally, the distribution preference of '*Ca.* P. asteris' and '*Ca.* P. fraxini', in single or mixed infections in the different parts of the crown is unknown. Branches from the lower right, lower left, middle right, middle left, middle left, upper right and upper left of 12 *Q. humboldtii* trees were sampled. In ten trees mixed infections had been detected and two trees infected with only one phytoplasma were used as controls. Quantification essays of '*Ca*. P. asteris' and '*Ca*. P. fraxini' were performed by qPCR, using universal primers for phytoplasmas and species-specific TaqMan probes. For relative quantification the 18S gene of *Q. humboldtii* was used as a normalizer. Statistical analysis of the phytoplasmas titre and distribution were performed by ANOVA tests. No significant differences were observed in the concentration of '*Ca*. P. asteris' and '*Ca*. P. fraxini' in any crown stratum in trees infected with both phytoplasmas. Furthermore, the detected concentrations were similar when compared with trees infected with only one phytoplasma. In general terms, the two phytoplasma species were detected in all crown strata. In the middle right, middle left and upper right strata, '*Ca*. P. asteris' was detected in 8 trees, while '*Ca*. P. fraxini' was identified in 4 trees. In the lower strata, where sampling is usually performed, phytoplasmas were detected in only 6 of 12 infected trees. Project IMP-CIAS-3114.

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Preparedness for *Xylella fastidiosa* in Australia; understand biology, physiology and ecology or potential vectors

P. Trębicki[1,2]

[1]*The University of Melbourne, Melbourne, Australia;* [2]*Agriculture Victoria, Grains Innovation Park, Horsham, Australia.*

(piotr.trebicki@agriculture.vic.gov.au) *S5-P6*

Xylella fastidiosa is a destructive plant pathogenic bacterium, with several subspecies affecting many plant species including grapevines, almonds, peaches, apricots, and olives. It is xylem limited, and transmitted mainly by leafhoppers, froghoppers, sharpshooters and spittlebugs. Xylella and its exotic vectors have been identified by the Plant Health Committee as the number one National Priority Plant Pest for Australia, by New Zealand MPI as an 'unwanted organism' and by the European Commission as one of most dangerous plant bacteria worldwide. Our research aims to provide biosecurity agencies with tools and knowledge which can be effectively implemented to eradicate Xylella fastidiosa or prevent and suppress its spread if there is an incursion in Australia. This newly commenced project focuses on Xylella vectors, biology, physiology and ecology in targeted horticultural crops. Efforts, methodology, results, and its significance to Australian food production will be communicated and discussed.

Finding a suitable host plant for transmission trials and infectivity screening with *Xylella fastidiosa* and its vector *Philaenus spumarius*

S. Avosani[1], G. Cavallo[1], M.L. Vitale[1], M. Ripamonti[3a], N. Bodino[3], D. Bosco[4], V. Verrastro[1], and D. Cornara[1,2]

[1]CIHEAM-Bari, Valenzano, Italy; [2]Department of Environmental Science, Policy, & Management, UC Berkeley, USA; [3]Institute for Sustainable Plant Protection - National Research Council of Italy, IPSP-CNR, Torino, Italy; [4]Department of Agriculture, Forest, and Food Sciences (DiSAFA), University of Turin, Grugliasco (TO), [a]Present address: Luxembourg Institute of Science and Technology (LIST), Luxembourg.

(avosani@iamb.it)

S5-P7

Since the first report of *Xylella fastidiosa* in Europe, great efforts have been made for thoroughly characterizing bacterium-plant interactions and searching for sources of genetic resistance. On the other hand, the vector-bacterium-plant relationship remains poorly known. Finding a host plant suitable for the insect vector and highly susceptible to the bacterium, as well as easy to propagate and maintain, would be a crucial step forward for both research on transmission dynamics, and large-scale surveys aimed at screening the infectious status of xylem feeders in areas threatened by the bacterium. In our study, we evaluated the inoculation rate of *Xylella fastidiosa* by *Philaenus spumarius*, and spittlebug's survival on the host plant during the inoculation access period (4 days), on: *Catharanthus roseus* (periwinkle), *Ocimum basilicum* (basil), *Medicago sativa* (alfalfa), *Rosmarinus officinalis* (rosemary), *Lavandula angustifolia, Amaranthus retroflexus*, *Brassica rapa* subsp. *sylvestris* (field mustard), and *Helianthus annuus* (sunflower). Based on the results of the inoculation trials and survival assessment, *P. spumarius* probing and feeding behavior was evaluated through the EPG-technique on four of the eight initially selected hosts under screening: basil, sunflower, alfalfa, and periwinkle. Here we discuss the combined outcomes of out tests, and the practical application of our findings for both research on *X. fastidiosa* transmission, and early detection of new bacterium outbreaks.

Population genomics of the meadow spittlebug *Philaenus spumarius*, the main insect vector of *Xylella fastidiosa* in Europe

R. Biello[1], Q. Liu[1], S. T. Mugford[1], A. Stewart[2], C. Harkin[2], K. Lester[3], R. Cairns[3], M. Wilson[4], S. Conyers[5], D. Allen[5], D. De Marzo[5], G. Clover[1], T. C. Mathers[1], and S. A. Hogenhout[1]

[1]Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich, United Kingdom; [2]University of Sussex, Brighton, United Kingdom; [3]SASA, Edinburgh, United Kingdom; [4]National Museum Wales, Cardiff, United Kingdom; [5]FERA, York, United Kingdom.

(saskia.hogenhout@jic.ac.uk) *S5-P8*

The meadow spittlebug, *Philaenus spumarius* (Linnaeus, 1758) (Hemiptera: Aphrophoridae), is a xylem-feeding insect that vectors the bacterial pathogen *Xylella fastidiosa*, which has caused dramatic losses in a wide variety of crops and most recently severely damaged the olive production industry in southern Italy. *X. fastidiosa* is present also in other European countries but not in the UK. *P. spumarius* is native to the Palearctic region and was unintentionally introduced in other areas (e.g., the USA and New Zealand). The insect is considered a eurytopic and polyphagous species, tolerating a wide range of environmental factors and feeding on various plant species. To improve our knowledge of the evolution of this species and assess the genetic structure of UK and European populations, we first generated a chromosome-level assembly of *P. spumarius*. In addition, we re-sequenced whole genomes at 10x coverage of ~440 individuals from the UK and the rest of Europe. Across Europe, *P. spumarius* is split into three divergent mitochondrial lineages; two are found in the UK (one in the north and one in the south), whereas the third is predominantly found in southern Europe. Preliminary results from whole-genome sequencing data showed a weak genetic structure among the UK populations supported by isolation by geographical distance with evidence of admixture between the two lineages probably resulting from secondary contact. To investigate this further, we are analysing the rest of the European populations in order to describe global population structure and identify patterns of local adaptation to different habitats.

New sustainable approaches for interfering with vector-borne plant pathogens transmission

V. Zaffaroni-Caorsi[1], D. Cornara[2,3], D. Bosco[4], C. Marzachi[5], and V. Mazzoni[6]

[1]C3A Centro Agricoltura, Alimenti e Ambiente, University of Trento, Trento, Italy; [2]DISSPA, University of Bari Aldo Moro, Bari, Italy; [3]CIHEAM—IAMB International Centre for Advanced Mediterranean Agronomic Studies, Bari, Italy; [4]DISAFA, University of Torino, Grugliasco (TO), Italy; [5] Institute for Sustainable Plant Protection, CNR, Torino, Italy; [6]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy.

(valenzc@gmail.com) *S5-P9*

The increasing demand for safe and sustainably produced food is leading to the development of pest control tools and strategies alternative to pesticides, such as Vibrational Disruption (VD). VD has been mainly used to disrupt pest mating behaviour and for mass trapping. However, recent studies suggest the playback of intra-specific vibrational signals on the host plant might have relevant impact on insect feeding, thus possibly also on the behaviours associated with pathogen transmission. The main goal of this project was to evaluate whether and how VD might interfere with transmission of the phytoplasma *Chrysanthemum yellows* (CY) by the leafhopper *Euscelidius variegatus.* Here we present our preliminary results regarding: i) the characterization of *E. variegatus* vibrational communication; ii) EPG-assisted observation of infective and non-infective individuals leafhoppers feeding on CY infected and healthy plants “treated” with vibrations”; iii) transmission experiments conducted on plants “treated” with vibrations. The results of this study could help validating VD as an environmentally-safe strategy for the containment of vector-borne plant pathogens.

Session

06

Disease Management

Disease management in the omics era: Status and future prospects

H.R. Pappu

Department of Plant Pathology, Washington State University, Pullman, WA, USA.

(hrp@wsu.edu)

Keynote Lecture 6

Plant virologists have a reason to be boastful and self-congratulate as there are great success stories of successful management of economically important viral diseases in a wide range of agronomic and horticultural crops. Development of integrated management tactics that included modifying production practices, identification of resistant genes and their incorporation into existing cultivars or developing new cultivars, genetic markers for rapid screening for resistance, vector management, virus certification programs, application of epidemiological knowledge have greatly contributed to reducing the impact of many viral diseases. More recent approaches that proved promising include making use of RNAi such as topical application of dsRNA which was made possible through developing formulations that ensured the stability of the dsRNA for longer periods of time after application. Virologists were quick to apply CRISPR-based genome editing for generating resistance to a wide range of DNA and RNA viruses. Identification of susceptibility genes will likely enhance the utility of CRISPR in making susceptible crops become resistant. While commercialization of CRISPR-generated crops have already taken place in the US, this technology is likely to be subjected to same biosafety regulations as the GM crops in many other countries. With improvements in high throughput sequencing or HTS (=Next Generation Sequencing) and the bioinformatics tools to analyze the sequence data, a deluge of information on the transcriptomics of plant-virus interaction has been generated for a various virus-host combinations in the last decade. Transcriptome data provided important insights into the host responses to virus infection which is being used to identify markers for virus resistance – hastening the time it usually takes for this effort. Similarly, metabolomic studies have received renewed interest for their potential in developing biomarkers as tools for screening for resistance.

The interplay between Autophagy and defense hormone Salicylic acid shape disease during viral infection and contribute to host resistance.

A. Shukla, G. Hoffmann, N. Kushwaha, S. Gonzalez, D. Hofius, and A. Hafren

Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

(shukla.aayushi@gmail.com) *S6-O1*

Autophagy is a major conserved intracellular degradation pathway implicated in response to different stresses and environmental conditions in plants and other eukaryotes. Autophagy employs selective processes to recycle unwanted cytoplasmic components varying from specific proteins to entire organelles. It also plays an important role in plant-pathogen interactions including diseases caused by viruses. Both the virus and host plant try to utilize the plant autophagy process for their own benefit. The defence hormone Salicylic Acid (SA) can induce autophagy and promotes the early senescence phenotype of autophagy-deficient mutants. Notably, SA commonly promotes defense against plant viruses, but some viruses have been found to largely evade SA resistance responses. Here we show that, that autophagy contributes in resistance against several viruses by reducing virus accumulation in the infected *Arabidopsis thaliana* plants as well as decreases disease severity during viral infection. Moreover, we show autophagy is induced in *Arabidopsis thaliana* in an SA-dependent manner during infection with two positive-stranded RNA viruses – *Cucumber mosaic virus* (CMV) (1) and *Turnip crinkle virus* (TCV) (2). Evidence supports that CMV viral RNA silencing suppressor protein 2b and TCV viral RNA silencing suppressor protein P38 reduce SA-induced autophagy and may itself subject to autophagic degradation (1, 2). Our results show that autophagy and RNA silencing appear beneficial for viral infection through the synergistic promotion of the plant longevity, fecundity and viral seed transmission. Together, by using a diversity of viruses, we identified pro-viral and anti-viral autophagy-based mechanisms, their roles in virus pathogenesis and in future to utilize it for crop protection and disease management.

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Habitat manipulation for sustainable management of *Philaenus spumarius*, the European vector of *Xylella fastidiosa*

M. Morente[1, 2], M. Ramírez[1], D. De las Heras-Bravo[1], C. Lago[2], A. Benito[1], A. Moreno[2], and A. Fereres[2]

[1]Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Alcalá de Henares, Madrid, Spain. [2]Instituto de Ciencias Agrarias – Consejo Superior de Investigaciones Científicas (ICA-CSIC), Madrid, Spain.

(a.fereres@csic.es) *S6-O2*

The *Xylella fastidiosa* (Wells et al.) outbreak in Europe in 2013 causing severe epidemics in olives in southern Italy has led to investigate new ways to contain the disease. As there is no cure for infected plants, management of vector populations is one of the most effective strategies to contain the spread of *X. fastidiosa* in infected areas. Thus, we aimed to select plant species commonly used as cover crops in olive groves with specific properties to manage the population of *Philaenus spumarius* L. (Hemiptera: Aphrophoridae), the main vector of *X. fastidiosa* in Europe. The main goal was to select plant species that could be used for ecological infrastructures including a push-pull strategy to reduce the population size of *P. spumarius* in olive groves. We conducted a series of antixenosis and antibiosis assays using nymphs as well as oviposition preference tests for adults. We assessed the nymph's settling preference under non-choice and multiple-choice conditions and the developmental and mortality rate on ten candidate plant species. In addition, we monitored the egg masses and the number of eggs laid by adults on the leaf litter of the selected plant species. Results showed that *Anthriscus cerefolium* L. and *Diplotaxis tenuifolia* L. had a lethal and repellent effect on *P. spumarius* nymphs, while *Sinapis alba* L. could be used as an attract and kill (dead-end) trap plant. On the contrary, *Taraxacum officinale* L. and *Lavandula angustifolia* (Miller) (*X. fastidiosa*-susceptible) were among the most preferred species for nymphs. Furthermore, adults oviposited preferably on *Centaurea cyanus* L., *Sonchus oleraceous* L., *T. officinale* and *A. cerefolium* whilst *D. tenuifolia* and *S. alba* were the least preferred plant species for oviposition. In conclusion, *D. tenuifolia*, *A. cerefolium* and *S. alba* could be intercropped as cover crops in olive groves to provide ecological infrastructures under a push-pull strategy to control the main vector species of *X. fastidiosa*. It is also important to highlight that *T. officinale*, *L. angustifolia* and *C. cyanus* should be avoided on *X. fastidiosa*-susceptible crops because they are preferred hosts of *P. spumarius*.

Specificity of resistance and tolerance to cucumber vein yellowing virus in melon accessions and evidence for resistance-breaking associated with a single mutation in VPg

C. Desbiez[1], M-L. Domingo Calap[2], M. Pitrat[3], C. Wipf-Scheibel[1], G. Girardot[1], I. Ferriol[2,4], J. J. López-Moya[2], and H. Lecoq[1]

[1]Pathologie Végétale, INRAE, 84140 Montfavet, France; [2]CRAG, CSIC-IRTA-UAB-UB, Barcelona, Spain; [3]GAFL, INRAE, 84140 Montfavet, France; [4]ICA-CSIC, Madrid, 28006, Spain.

(cecile.desbiez@inrae.fr) *S6-O3*

Genetic resistance is a sustainable way of controlling plant viruses, but it requires the presence of resistance genes in the host germplasm. Besides, its durability can be reduced by resistance-breaking isolates, either pre-existing in the environment or emerging after the deployment of the resistance. In the case of cucumber vein yellowing virus (CVYV, a member of the genus *Ipomovirus*), an emerging virus on cucurbits in the Mediterranean Basin (1), few resistances are available in melon. The melon accession PI 164323 was found to display complete resistance to the strain CVYV-Esp, and melon accession HSD 2458 presented a tolerance, i.e. very mild symptoms in spite of virus accumulation in inoculated plants. The resistance is controlled by one dominant allele *Cvy-1[1]*, while the tolerance is controlled by a recessive allele *cvy-2* independent from *Cvy-1[1]*. Upon inoculation with eight geographically diverse CVYV isolates corresponding to the known molecular diversity of the virus (2), the resistance was found to be strain-specific since many CVYV isolates induced necrosis on PI 164323, whereas the tolerance presented a broader range. A resistance-breaking isolate inducing severe mosaics on PI 164323 was obtained. It differed from the parental strain by a single amino-acid change in the VPg-coding region. The effect on the mutation was confirmed by reverse genetics using a newly-obtained CVYV infectious clone (3). Competition experiments suggested a fitness cost of the resistance-breaking mutation in susceptible melon. Our results highlight the need to combine cultural practices and/or additional genes to develop a more durable control of CVYV than the use of the *Cvy-1[1]* allele alone.

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Advances in epidemiology and control of banana bunchy top virus disease pandemic in sub-Saharan Africa

P. L. Kumar[1]*, S. Ngatat[2], A.O. Adedeji[1], L. Nkengala-Asi[2], T. Alabi[1], T. Oviasuyi[1], A. Oresanya[1], C. A. Enoh[2], K.K.M. Fioboe[2], G. Mahuku[3], M. Zandjanakou-Tachin[4], O. Ogunfunmilayo[5], M. Adeime[6], M. Pooggin[7], M.L. Iskra-Caruana[7], and R. Hanna[2,8]

[1]International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria; [2]IITA, Yaounde, Cameroon; [3]IITA, Kampala, Uganda; [4]National University of Agriculture, Ketou, Benin; [5]NAQS, Ibadan, Nigeria; [6]IRAF, Libreville, Gabon; [7]CIRAD, Montpellier, France; [8]Center for Tropical Research, Institute of the Environment and Sustainability, University of California, Los Angeles, USA.

(L.kumar@cgiar.org) *S6-O4*

The banana bunchy top virus (BBTV, genus Babuvirus) is the most economically important plant virus affecting bananas (and plantain, *Musa* spp.) that poses a major threat to livelihoods and banana biodiversity in sub-Sharan Africa (SSA). Since the first report in the 1960s, the virus has spread to 17 countries in SSA, eight of those in the last decade. The virus is transmitted persistently by the banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae). In the absence of durable host resistance, the management of BBTV on a continental scale has been a major challenge. Traditionally, BBTV management relies on quarantine measures, cultural control, and seed certification schemes. These approaches were proven effective in large monocropping plantations, but their effectiveness in smallholder mixed farming conditions was unknown. To assess the feasibility and to develop fit-for-purpose management methods, we implemented an ALLIANCE strategy for controlling BBTD in SSA. Simultaneous efforts were made on mapping BBTV spread, and characterization of over 50 BBTV isolates from 10 countries, and the development of innovative technologies – including more sensitive diagnostic tools for virus detection in plants and vectors, remote sensing, and machine learning methods for banana and BBTD mapping – for BBTD surveillance and disease management. Evaluation of a large set of *Musa* germplasm in search of resistance to BBTV and banana aphid revealed a few tolerance genotypes. Observations on the effectiveness of interventions suggest a strong influence of socio-cultural aspects, especially the seed sourcing practices of male and female farmers. While one decade of our interdisciplinary efforts on BBTD management improved understanding of the virus and vector diversity and disease epidemiology, success on on-farm disease management was limited as BBTD management requires sustained interventions over the long term. Alternative strategies are necessary by integrating current knowledge and innovations and emerging technologies to develop robust BBTD management tactics appropriate for subsistence farming conditions in SSA. In this presentation, we will present key lessons from the management of BBTD in perennial plantations and recommendations for future tactics.

Integrated control of a polerovirus

J. A. Walsh[1], A. F.C. Greenslade[2], C. Sharp[3], A. Richardson[3], M. Williamson[2], S. F. Greer[1], A. Mead[2], K. Macleod[1], A. J. Hambidge[1], D. Katschnig[1], and G. C. Barker[1]

[1]*School of Life Sciences, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK;* [2]*Centre for Sustainable Pest and Disease Management, Rothamsted Research, Harpenden, AL5 2JQ, UK;* [3]*Allium and Brassica Agronomy Ltd., Kirton, PE20 1QQ, UK.*

(john.walsh@warwick.ac.uk) *S6-O5*

Turnip yellows virus (TuYV, *Polerovirus, Solemoviridae*) formerly known as beet western yellows virus is a widespread pathogen affecting the yield and quality of a broad range of crops, particularly vegetable and oilseed brassicas. It has a very broad host range, infecting many weed species. In the UK the major vector is the peach-potato aphid *Myzus persicae* (1) which transmits the virus in a persistent manner. The virus has been shown to reduce the weight yield of vegetable brassicas by up to 65% (2) and of oilseed rape by up to 30% (Nicholls, 2013). It has also been shown to cause tipburn of cabbage (4). We carried out an experiment over three years at two sites in the UK to investigate the potential of an integrated strategy for the control of TuYV in vegetable brassicas. The components of the strategy included reduced and informed insecticide treatments, partial plant resistance and planting date. TuYV incidence in the crop, weight yields, *M. persicae* numbers and the incidence of TuYV in *M. persicae* from yellow water traps and Rothamsted Insect Survey suction traps were determined during the growing seasons of the three years at the two sites. We are also investigating and exploiting a number of new sources of resistance to TuYV in *Brassica oleracea*, *Brassica rapa* and *Brassica napus* (5) and introgressing resistances into allotetraploid oilseed rape (*B. napus*) by resynthesis (6).

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Efficacy of the insecticide dimpropyridaz (AxaliON™) against the transmission of barley yellow dwarf virus (BYDV)

J. Sanz-Gomez[1], L. Velez[2], A. Moreno[2], G. Martin[3], and A. Fereres[2]

[1]BASF SE, Agricultural Solutions, Limburgerhof, Germany; [2]Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain; [3]BASF plc, Crop Protection Division Development Center, Woolpit, United Kingdom.

(jorge.sanz-gomez@basf.com) *S6-O6*

Barley yellow dwarf has been described as one of the most devastating viral diseases in cereals worldwide resulting in yield losses ranging from 5% to 80% (1, 2). The disease is caused by the barley/cereal yellow dwarf viruses (YDVs) which are luteoviruses transmitted by aphid vectors in a persistent circulative manner. In some European countries such as France and the United Kingdom, approximately 30% of the cereal sown area was treated from 2014 to 2018 with crop protection products against the aphid vectors transmitting YDVs (2). However, the ban of most of these products by the European Union and the lack of alternative solutions made evident the urgent need of innovation in this field. BASF has discovered and developed the active ingredient dimpropyridaz (AxaliON™), a pyridazine pyrazolecarboxamide insecticide with a novel mode of action for the control of piercing and sucking insect pests. Greenhouse experiments under controlled conditions were conducted to assess if AxaliON™ could interfere with the inoculation of BYDV by viruliferous aphids (*Rhopalosiphum padi*) landing on non-infected barley (*Hordeum vulgare*) plants (primary spread of the virus). The ability of the compound to avoid virus acquisition from virus-infected barley plants and subsequent transmission to non-infected plants was also tested (secondary spread of the virus). Furthermore, the efficacy of AxaliON™ against the spread of BYDV by aphid populations under field conditions was evaluated in barley fields in Europe following the methodology described by the European and Mediterranean Plant Protection Organization (EPPO). Our results indicate that the insecticide AxaliON™ can effectively reduce the primary and secondary spread of the virus under controlled conditions and can limit the incidence of the disease in the open field. In conclusion, AxaliON™ showed to be an effective alternative active ingredient against the spread of BYDV in barley.

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Use of glandular trichomes to control whitefly-transmitted viruses in tomato: modulation by natural enemies

C. Riahi[1], A. Urbaneja[1], M. Pérez-Hedo[1], R. Fernández-Muñoz[2], and E. Moriones[2]

[1]Instituto Valenciano de Investigaciones Agrarias (IVIA), Valencia, Spain; [2]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Málaga, Spain.

(moriones@eelm.csic.es) *S6-O7*

Whitefly (Hemiptera: Aleyrodidae)-transmitted viruses such as members of begomoviruses (genus *Begomovirus*, family *Geminiviridae*) and criniviruses (genus *Crinivirus*, family *Closteroviridae*) severely damage tomato (*Solanum lycopersicum*) crops in warm and temperate regions worldwide. A trichome-based resistance (WF-resistance) introgressed into cultivated tomato from the wild tomato *S. pimpinellifolium* was shown to be effective to control *Bemisia tabaci* and *Trialeurodes vaporariorum* whiteflies. Protection was based on antixenosis and antibiosis against the whiteflies, associated with the presence of acylsucrose-producing type-IV glandular trichomes. Whitefly-resistance was demonstrated to be effective to limit the spread of the begomovirus tomato yellow leaf curl virus (TYLCV) (persistently-transmitted) and the crinivirus tomato chlorosis virus (ToCV) (semipersistently-transmitted). Limited protection, however, was observed at early developmental stages of tomato plants because of reduced acylsucrose production levels. Nevertheless, early induction of type IV glandular trichome trichomes by using methyl jasmonate (MeJA) applications helped to increase resistance to whiteflies and to whitefly-transmitted viruses in young tomato plants. Biological control has been largely adopted to control pest infestations in tomato crops. The zoophytophagous predatory bug *Nesidiocoris tenuis* (Hemiptera: Miridae) is frequently used in biological control programs against insect pests including whiteflies in tomato, usually by pre-establishing populations during nursery stages. The phytophagy of *N. tenuis* was demonstrated to modulate jasmonic acid and salicylic acid signalling pathways in tomato. Interestingly, the inoculation of *N. tenuis* on WF-resistant tomato plants resulted into effective induction of type-IV glandular trichomes and acylsucrose-production, then preparing plants against whiteflies and whitefly-transmitted viruses. Thus, combining conventional breeding and biological control strategies in integrated pest management programs can provide an interesting alternative for effective control of whiteflies and whitefly-transmitted viruses in tomato.

Survival and disinfection of tomato brown rugose fruit virus on common glasshouse surfaces

A. Skelton, L. Frew, A. Fowkes, and A. Fox

Fera Science Ltd, York Biotech Campus, Sand Hutton, York, United Kingdom.

(adrian.fox@fera.co.uk)

S6-P1

Tomato brown rugose fruit virus (ToBRFV) has rapidly spread through the tomato growing regions of Europe, the Middle East, Asia and USA. As a seed and contact transmitted virus, good hygiene and biosecurity practices are paramount to prevent entry and spread of the virus. Previous studies have indicated that the length of time contaminated surfaces remain infectious may be influenced by the type of surface (1). The survival time of the virus (retention of infectivity) on common glasshouse surfaces was determined. The surfaces tested included skin, gloves, glass, aluminium, stainless steel, hard plastic, polythene and concrete. Each surface was contaminated with sap from ToBRFV positive plants, then swabbed at regular intervals for up to 180 days, and swabs were used to inoculate *Nicotiana tabacum* plants. The pathogen was infectious on skin and gloves for at least two hours. On the other surfaces the virus remained infectious for at least 28 days, and in some cases for at least six months. Therefore, there is high risk of re-infection following outbreaks. Hand washing was ineffective at removing the virus, even at one-minute with a combination of medicated soap and hand gel. Thermal inactivation of ToBRFV was determined at 90^{O}C for 5 minutes. The efficacy of disinfectants has previously been established on sap in solution (2). However, these studies do not report on treatment of glasshouse surfaces. Efficacy was influenced by both the active compound and the surface being treated. On glasshouse surfaces, products formulated with a mix of glutaraldehyde and quarternary ammonium compounds were most effective at inactivating the virus, both in terms of the rapidity and reliability on different surfaces after only 10 minutes exposure. Rough and porous surfaces, such as concrete, present a challenge for disinfection regimes. ToBRFV could still be detected by real-time RT-PCR following disinfection.

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Impact of dimpropyridaz (AxaliON™) on the feeding behaviour of aphid and whitefly vectors of plant viruses

A. Moreno[1], A. Herraiz[1], F.Marchal,J. Sanz-Gomez[2], and A. Fereres[1]

[1]Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, Madrid, Spain; [2]BASF SE, Agricultural Solutions, Limburgerhof, Germany.

(amoreno@ica.csic.es)

S6-P2

Hemipterans cause major economic losses in vegetable and field crops worldwide, not only because of the direct damage they cause, but also because their alimentary habits involve transmission of hundreds of plant viruses. Owing to the ban of their use and the development of resistances to some extensively used pesticides for control of sap-feeding pests, the discovery and development of new and alternative bioactive molecules are urgently needed. In this regard, BASF has developed dimpropyridaz (AxaliON™), a pyrazole carboxamide insecticide for the control of piercing and sucking insect pests. To understand how AxaliON™ and other active ingredients can impact the transmission of plant viruses by vectors, its effect on the feeding behaviour of two aphid species (*Myzus persicae* and *Rhopalosiphum padi*) (Aphididae: Hemiptera) and one whitefly species (*Bemisia tabaci*) (Aleyrodidae: Hemipera) was studied using the Electrical Penetration Graph (EPG) technique. Test plants (*Physalis floridana* for *M. persicae*, *Hordeum vulgare* for *R. padi* and *Solanum lycopersicum* for *B. tabaci*) were sprayed until run-off with the active ingredients in addition to an untreated control. Young adult insects connected to the EPG device were allowed to feed continuously on insecticide-treated and untreated plants for 8 h. A selected set of EPG variables was calculated and compared between treatments. Results indicate that the systemic compound AxaliON™ has anti-feeding properties and reduces the chances of aphids and whiteflies to feed from vascular tissues altering their feeding behaviour activities reducing the chances for inoculation and acquisition of persistently-transmitted phloem-restricted viruses.

Integrated pest management controls the spread of tomato leaf curl New Delhi virus in zucchini crops.

D. Janssen, M.M. Tellez, E. Rodriguez, A. Simón, M. Boulares, and L. Ruiz

Centro La Mojonera, IFAPA, Almeria, Spain.

(dirk.janssen@juntadeandalucia.es) *S6-P3*

The introduction and spread of tomato leaf curl New Delhi virus (ToLCNDV) in 2013 constitutes a major challenge for zucchini squash production in the greenhouses from South-east of Spain. Food safety legislation and consumer demands require sustainable control of the virus and its vector, the whitefly *Bemisia tabaci*. We have compared the effects of chemical, biological and integrated pest management treatments on the primary and secondary spread of the virus. Choice and no-choice experiments were conducted in greenhouse modules using zucchini plants that had preinstalled natural enemy *Amblyseius swirskii* and plants that had not. The oviposition from *B. tabaci* was compared, as well as the expression of symptoms and the presence of virus in the plants by probe-hybridisation. We also evaluated the role in the spread of the virus by *B. tabaci* adults that emerged from the pupae in infected plants in transmission experiments by their ability to infect healthy plants, and determined the viral loads in emerging adults and that of feeding adults by means of qPCR. The results showed that the use of preinstalled *A. swirskii* could not control the primary spread of the virus, but reduced the secondary spread significantly (75%). Treated plants had 90% reduced whitefly oviposition. Transmission experiments resulted in 20% infection when single emerging adults were transferred from ToLCNDV-infected to healthy plants, whereas we observed 96% infection when using feeding *B. tabaci* adults. Also, 80% of emerging adults had detectable virus, and 26% of these had viral accumulation similar to that in feeding adults. Greenhouse trials showed 45% ToLCNDV control using chemical treatments, 60% using biological control and 75% using a combination of both treatments. Taken together, we concluded that IPM strategy that combines the use of plants that have pre-installed *A. swirskii* from the nursery, and chemical treatments, that are compatible with natural enemies of the vector, and the best option to reduce significantly the spread of ToLCNDV in zucchini produced in the greenhouse.

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Secondary dissemination of tomato severe rugose virus (ToSRV) and tomato chlorosis virus (ToCV) in tomato fields under the effect of insecticides

F. F. de Oliveira, G. M. Favara, V. H. Bello, H. D. Kraide, C. G. Ferro, E. Y. N. Carmo, A. B. Filho, and J. A. M. Rezende

Plant Pathology and Nematology Department, Escola Superior de Agricultura Luiz de Queiroz, University of São Paulo, Piracicaba, SP, Brazil.

(felipe_oliveira@usp.br) *S6-P4*

In Brazil, tomato (*Solanum lycopersicum*) crops are affected by the begomovirus tomato severe rugose virus (ToSRV) and the crinivirus tomato chlorosis virus (ToCV), transmitted by *Bemisia tabaci* MEAM1. The control of both diseases is mainly based on resistant hybrids (only for ToSRV) and up to three weekly sprays of insecticides for vector control. Despite efficient vector control, infections with these viruses are frequent. Primary transmission with migrant viruliferous whiteflies has been reported as the primary mechanism of the spread of ToSRV and ToCV in tomato fields (1). However, as inoculum sources strong enough to sustain high incidences by primary dissemination alone are unlikely (1,2), secondary dissemination was hypothesized to occur, even with efficient vector control. This study aimed to evaluate the incidence of ToCV and ToSRV in experimental tomato fields sprayed alternately three times a week with the insecticides cyantraniliprole, acetamiprid, and flupyradifurone. Three areas denominated A, B, and C were used. Area A was 2600 m away from B and 3350 m from C, while B was 600 m away from C. The A and B area consisted of 200 tomato plants, of which 180 were healthy and 20 infected with ToSRV and ToCV, randomly interspersed. Only in area A, the tomato plants were sprayed. Five hundred adults of aviruliferous *B. tabaci* MEAM1 were released weekly in A and B. In C, 200 healthy tomatoes served to monitor the natural incidence of the viruses (negative control). Symptoms were evaluated weekly, and molecular tests were performed 70 days after transplanting to detect virus infection. The infection rates with ToSRV and ToCV in area B were 100% and 81%, whereas in area A, they were 9% and 11%, respectively. The C area showed no infected plants. Despite the relevant reduction, chemical control could not wholly prevent the secondary spread of the two viruses.

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A high-throughput image analysis method for assessing pepper quantitative resistance to Cucumber mosaic virus

J. Hirsch[1], M. Szadkowski[1], S. Piry[1,2], C. Lacroix[1], L. McLeod[3], B. Moury[1], and V. Lefebvre[3]

[1]Pathologie Végétale, INRAE, 84140 Montfavet, France; [2]CBGP, INRAE, CIRAD, IRD, Institut Agro, Univ. Montpellier, Montpellier, France; [3]INRAE, GAFL, F-84140 Montfavet, France.

(judith.hirsch@inrae.fr) *S6-P5*

Cucumber mosaic virus (CMV) is an economically important virus in pepper cultures worldwide. In the absence of curative methods, growing resistant cultivars is one of the main means of controlling plant viruses. Moreover, quantitative resistance is likely to be more durable than major gene resistance for controlling CMV in pepper. To gain insight into the genetic basis of quantitative resistance to CMV, we have explored natural variation in the response of pepper to CMV in order to perform a genome-wide association study (GWAS), focusing on the initial steps of infection in the inoculated leaves. A subset of 56 INRAE accessions belonging to the 423 accessions from the pepper core-collection of the G2P-SOL project (http://www.g2p-sol.eu/) were assessed for resistance to the N strain of CMV, which induces necrotic local lesions on mechanically-inoculated leaves. The percentage of leaf surface covered by local lesions was measured as a quantitative estimate of disease severity. A phenotyping method based on automated analysis of scanned leaf images was developed. Measurement of the leaf surface was automated using ImageJ software and was associated with accession code, plant number and leaf number using datamatrices and a dedicated R script. An analysis process using deep learning was set up to automatically detect lesions and estimate the total surface of lesions. Pepper plants infected by CMV showed a quantitative response, with pepper accessions displaying a continuum of resistance levels, from highly susceptible to highly resistant. The automated data acquisition and deep-learning pipelines make it possible to scale up the phenotyping process and analyze a much larger panel of accessions, as required in GWAS. By combining the phenotypic data reported here with the high-density genotypic dataset obtained on the G2P-SOL pepper core-collection, a GWAS will be conducted to identify Quantitative Trait Loci and candidate genes underlying early resistance to CMV.

Degeneration of Clean Virus-tested Sweetpotato Seed in High and Low Virus Pressure Areas at the Lake Zone, Tanzania

K.O. Ogero[1, 2*], J.F. Kreuze[3], M.A. McEwan[4], C.J.M. Almekinders[5], P.C. Struik[6], and R.A.A. van der Vlugt[2]

[1]International Potato Center (CIP), C/o Tanzania Agricultural Research Institute (TARI) Tanzania; [2]Laboratory of Virology, Wageningen University and Research (WUR), The Netherlands; [3]International Potato Center, (CIP) Peru; [4]International Potato Center, Regional Office for Sub-Saharan Africa (CIP – SSA), Kenya; [5]Knowledge Technology and Innovation Group, Social Sciences, Wageningen University and Research (WUR), The Netherlands; [6]Centre for Crop Systems Analysis, Wageningen University and Research (WUR), The Netherlands.

(k.ogero@cgiar.org) *S6-P6*

Viruses pose a major challenge to sweetpotato production at the Lake Zone, Tanzania. Dual infection with sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV) can cause up to 98% yield losses. Use of clean virus-tested seed disseminated through a formal decentralized system is among the appropriate strategies to address this. However, clean virus-tested seed can get reinfected once in the field and it is not known how it will perform if it is recycled alongside farmer-sourced seed for several seasons. We assessed performance of clean virus-tested seed and farmer-sourced seed of cv. Ejumula and cv. Kabode over five seasons to understand the trend in root yields, vine yields and virus incidences. To minimize rapid spread of viruses from farmer-sourced to virus-tested plants we used a split-plot design with source of planting material in main plots and the two varieties in subplots. The experiments were done in a high and a low virus pressure area. Clean virus-tested seed produced significantly higher root yields for cv. Ejumula in both high and low virus pressure sites. The root yields for cv. Kabode for both virus-tested and farmer-sourced seed were not significantly different. There was a general drop of yield across the seasons. SPFMV incidences increased over seasons for cv. Ejumula while SPCSV incidences reduced. The incidences for both viruses remained stable for cv. Kabode over the five seasons. Plants generated from clean virus-tested seed had lower incidences for both viruses compared to those from farmer-sourced planting material. This was significant for cv. Ejumula. The findings confirm that use of clean virus-tested seed can help reduce yield losses in sweetpotato especially for susceptible varieties. It also indicates that regular replenishment of clean virus-tested seed is more economical in high virus pressure areas and for more susceptible varieties.

Field biotest development with virus inoculation in sugar beet – efficacy of dimpropyridaz (AxaliON™) against *Beet mild yellowing virus* (BMYV) transmitted by *Myzus persicae*

M. Varrelmann[1], R. Hossain[1], C. Lachmann[1], and J. Sanz-Gomez[2]

[1]Institute of Sugar Beet Research at the University of Göttingen, Department of Phytopathology, Germany; [2]BASF SE, Agricultural Solutions, Limburgerhof, Germany.

(*varrelmann@ifz-goettingen.de)* *S6-P7*

In sugar beet cultivation, viruses from different families can cause considerable economic damage by inducing yellowing on leaves if environmental conditions foster the occurrence of insect vectors. Virus yellows in sugar beet is caused by different virus species. Monitoring has shown that *Beet yellows virus* (BYV), *Beet mild yellowing virus* (BMYV), *Beet chlorosis virus* (BChV) are common and widespread, while *Beet mosaic virus* (BtMV) is less prevalent. The aphid *Myzus persicae* is considered the main vector of these viruses which can cause yield losses amounting up to 40%. Virus vectors and the disease were properly controlled during the last three decades with neonicotinoid insecticides seed treatment. However, this chemistry was banned EU-wide in 2019 and alternative bio-active compounds are largely lacking. A field biotest has been developed to evaluate the efficacy of crop protection products against the transmission of BMYV and BChV in sugar beet. The biotest allowed to reach an infection rate of nearly 100% and a yield reduction of 30% by inoculating sugar beet plants within viruliferous *Myzus persicae* at a density of 4%. The suitability of the field biotest was proven by testing the efficacy of the insecticide dimpropyridaz (AxaliON™) against the transmission of BMYV. AxaliON™ is a pyrazole carboxamide insecticide developed by BASF for the control of piercing and sucking insect pests. The field biotest showed that AxaliON™ was able to reduce the disease incidence of BMYV from 86% (inoculated control) down to 6%. Additionally, the area under disease progress curve and the white sugar yield in the treatment remained comparable to the non-inoculated control. Results indicate that the field biotest is a solid method for efficient and practical evaluation of compounds against viruses spread in sugar beet. Furthermore, AxaliON™ showed to be an effective alternative compound against the spread of BMYV in sugar beet.

Reference

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Application of a reverse genetic system for Beet necrotic yellow vein virus to study Rz1 resistance breaking in sugar beet

S. Liebe[1], E. Maiss[2], and M. Varrelmann[1]

[1]Institute for Sugar Beet Research, Department Phytopathology, Holtenser Landstr. 77, D-37077 Göttingen; [2]Leibniz University Hannover, Institute of Horticultural Production Systems, Dept. Phytomedicine, Herrenhäuser Str.2, 30419 Hannover.

(varrelmann@ifz-goettingen.de) *S6-P8*

Beet necrotic yellow vein virus (BNYVV) causes Rhizomania disease in sugar beet, which is characterized by the abnormal proliferation of lateral roots leading to a significant decrease in sugar content and massive yield losses. The intensive usage of Rz1 as resistance gene has provoked the development of Rz1 resistance breaking strains. Several mutations in the pathogenicity factor P25 at amino acid positions 67-70 (AA67-70) as well as an additional RNA component from the P-type (RNA5) are associated with resistance breaking. However, experimental studies on resistance breaking populations and the associated mutations are missing. In an ongoing project we collected BNYVV populations in Europe for biological and molecular characterization. Rz1 resistance breaking was confirmed by bait plant tests in the greenhouse. The AA67-70 within P25 displayed large variability depending on the population. The presence of an additional RNA5 either from J or P type was also confirmed. Deep sequencing of selected populations revealed the presence of mixed infections with various BNYVV types. Finally, we applied a reverse genetic system to prove the resistance breaking ability of mutations in sugar beet. Here we could show that the replacement of AS67-70 indeed mediates resistance breaking as well as the presence of an additional RNA5. The results demonstrate the genome plasticity of BNYVV that allows the virus to overcome Rz1 resistance.

Development of *Agrobacterium tumefaciens* infiltration of infectious clones of grapevine geminivirus A directly into greenhouse-grown grapevine and *Nicotiana benthamiana* plants

Y.-W. Kuo[1], A. Bednarska[1], M. Al Rwahnih[1,2], and B. W. Falk[1]

[1]Department of Plant Pathology, University of California, Davis, CA 95616, U.S.A; [2]Foundation Plant Services, University of California, Davis, CA 95616, U.S.A.

(ywkuo@ucdavis.edu) *S6-P9*

Grapevine virus infectious clones are important tools for fundamental studies, but also because of their potential for translational applications for grapevine improvement. Although several grapevine virus infectious clones have been developed, there has been difficulty in directly infecting mature grapevine plants, and many of the viruses used still cause disease symptoms in grapevine plants making them less likely candidates for biotechnological applications in grapes. Here, we developed an improved *Agrobacterium tumefaciens* infiltration method that can be used to deliver DNA plasmids and viral infectious clones directly into ~20-40 cm-high (above soil) greenhouse-grown grapevine plants. We also developed infectious clones for two isolates of grapevine geminivirus A (GGVA): isolate Longyan – China (GenBank accession number: KX570611; GGVA-76) and Super Hamburg - Japan (GenBank accession number: KX570610; GGVA-93). Neither virus caused any obvious symptoms when inoculated to plants of grapevine varieties Colombard, Salt Creek, Cabernet Sauvignon, and Vaccarèse. However, the two GGVA isolates induced different symptom severity and viral titer in *Nicotiana benthamiana* plants. The two GGVA isolates used here were found to accumulate to different titers in different parts/branches of the infected grapevine plants. The GGVA infectious clones and the improved grapevine infiltration technique developed here provide new, valuable tools that can be applied to grapevine plants, possibly even for translational applications such as disease management and desired trait improvements.

Epidemiology and management of tomato spotted wilt virus in *Chrysanthemum morifolium* in South Korea

J. Y. Yoon[1], J. B. Yoon[2], M. H. Seo[2], and S. K. Choi[2]

[1]Graduate School on Plant Protection and Quarantine, Jeonbuk National University, South Korea; [2]Division of Horticultural and Herbal Crop Environment, NIHHS, RDA, South Korea.

(juyeon74@gmail.com) *S6-P10*

Tomato spotted wilt virus (TSWV) is one of most destructive viruses in vegetable and ornamental crop production worldwide. A greenhouse survey to determine the incidence of TSWV in *Chrysanthemum morifolium* Ramat. was conducted during the 2018 and 2019 growing seasons in South Korea. TSWV was detected using a double antibody sandwich-enzyme-linked immunosorbent assay, and positive results were confirmed using reverse transcription-polymerase chain reaction (RT-PCR). A total of 1569 chrysanthemum plants (70.77 %) tested positive for TSWV among 2217 symptomatic chrysanthemum plants collected from 16 greenhouses. In addition, 116 thrips (72.96 %; *Frankliniella occidentalis* Pergande) that contained TSWV were identified using RT-PCR from a total of 159 thrips collected from the greenhouses during the survey. A high incidence of viruliferous thrips may have played a role in TSWV occurrence in the chrysanthemum greenhouse. To develop a novel approach for thrips management, the effectiveness of a soil-dwelling predatory mite (*Stratiolaelaps scimitus* Berlese) and 45 essential oils (as bio-insecticides applied via foliar treatment) was assayed (1). Four essential oils (cinnamon oil, cinnamon bark oil, oregano oil, and thyme oil) were shown to be significantly toxic to eggs, larvae, and adults of *F. occidentalis*. For the combined treatment, individuals of *S. scimitus* (60/m2) were placed on the soil in the chrysanthemum greenhouses. Then, a mixture of the four essential oils was applied as foliar treatment at 4-day intervals. A very low incidence of thrips emerged as adults from the soil (1.2–8.5 %) in the combined treatment in the chrysanthemum greenhouses when surveyed twice per month, compared with the non-treated control or when conventional insecticide sprays were applied. The incidence of TSWV (0.93 %) in chrysanthemum treated with *S. scimitus* in conjunction with the mixture of four essential oils decreased significantly compared with that treated with chemical insecticides (32.05 %) and in the non-treated controls (84.85 %). Our findings contribute to the development of novel strategies to control TSWV disease in chrysanthemum plants; notably, the control of *F. occidentalis* using eco-friendly insecticides appears promising.

Reference

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Session

07

Climate Change

Anthropogenic climate change and its impact on interactions between viruses and plants

T. Canto

Department of Microbial and Plant Biotechnology, Margarita Salas Center for Biological Research, Ramiro de Maeztu 9, 28040 Madrid, Spain.

(tomas.canto@cib.csic.es)

Keynote Lecture 7

During the time that life has existed on Earth ambient conditions on the planet surface have varied enormously. Ambient variability has continued during the last ~250 million years in which co-evolution of new flowering plants with viruses and their vectors have created our present world. Currently, anthropogenic emissions leading to incremental alterations in the chemistry of the atmosphere are affecting the balance of heat that the planet traps or irradiates to outer space, gradually increasing the average temperature of its surface, and causing the so-called global warming. This anthropogenic warming is happening at an extremely fast pace in geological and evolutive times. Global warming drives in turn climate change, a generic term used to define the host of different alterations that it causes in the many and diverse local climates of Earth. The importance and potential reach of the changes in climate that are taking place or that are being predicted to take place is assessed and contextualized, with regard to historic and paleo records. Plants, viruses and their vectors, as well as humans and their crops are part of biological systems that will have to endure those changes (1). They will also be exposed to direct effects of alterations in the gas composition of the atmosphere, notably increasing ambient CO_2 levels. Ongoing studies are being made to investigate the effects of individual abiotic parameters, or of complex ambient changes, on those biological systems and on how they would respond to them.

Reference

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Milder autumns may increase risk for infection of crops with turnip yellows virus

V. Puthanveed[1], K. Singh[1,2], E. Poimenopoulou[1], J. Pettersson[1], and A. Kvarnheden[1]

[1]Department of Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden; [2]Division of Crop Protection and Plant Health, Crop Research Institute, Prague, Czech Republic.

(anders.kvarnheden@slu.se) *S7-O1*

As a consequence of climate change, milder autumns allow insects to be active for a longer period and they may spread viruses to newly sown winter crops. In autumn 2018, green peach aphids (*Myzus persicae*) were found unusually late in suction traps in southern Sweden and they presented a risk for infection of winter rapeseed (*Brassica napus*) with turnip yellows virus (TuYV; genus *Polerovirus*, family *Solemoviridae*). Infection of rapeseed may not result in obvious symptoms, but can still reduce crop yield with up to 30%. Therefore, a survey was carried out in spring 2019 with random leaf samples from 46 rapeseed fields (20 or 100 samples/field) in southern and central Sweden using DAS-ELISA. The results showed that TuYV was very commonly occurring, with TuYV being detected in all fields except one. In the counties of Skåne, Kalmar and Östergötland, the average incidence of TuYV-infected plants was 75% and reached 100% for nine fields. Sequence analyses of the coat protein gene revealed a close relationship between Swedish TuYV isolates and those from Europe as well as other parts of the world. Data from high throughput sequencing indicated the presence also of turnip yellows virus-associated RNA. Molecular analyses of nine plants of sugar beet (*Beta vulgaris*) with yellowing collected in 2019 revealed that two of them were infected by TuYV together with two other poleroviruses: beet mild yellowing virus and beet chlorosis virus. This is one of the first detections of TuYV in sugar beet and it may be a spillover from rapeseed. TuYV seems to develop into a new problem in Sweden, not only in rapeseed plants, but potentially also in other crops. Poleroviruses are prone to recombination and mixed infection with three poleroviruses in the same plant poses a risk for new polerovirus genotypes developing.

Modelling the effects of climate change on plant virus vertical transmission and prevalence

Á. Gutiérrez-Sánchez[1], M. López-Herranz[1], A. Cobos[1], T. Canto[2], and I. Pagán[1]

[1]Centro de Biotecnología y Genómica de Plantas UPM-INIA and E.T.S. Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid, 28223, Spain; [2]Departamento de Biología Medioambiental, Centro de Investigaciones Biológicas, CSIC, Madrid, 28040, Spain

(alvaro.gutierrezs@upm.es) *S7-O2*

Climate change has been predicted to have great impact on plant virus epidemics by, among other factors, enhancing between-host transmission. Most of the work on this subject focused on virus horizontal transmission through insect vectors. However, more than 25% of all known plant viruses are vertically transmitted through seeds. Seed transmission rate is determined by the level of virus multiplication, the speed of within-host movement, and by seed survival upon infection, all traits known to be affected by climatic factors. Thus, analyzing how climate change may alter virus seed transmission will provide relevant information to understand virus epidemiology. We studied the effect of climate change conditions (elevated CO_2 concentration, light intensity and temperature) on cucumber mosaic virus (CMV) and turnip mosaic virus (TuMV) seed transmission in *Arabidopsis thaliana* and on the survival of infected seeds. With this data, we parameterized a SIR-based model and we explored how the impact of climate change on seed transmission rate affected virus prevalence. Results showed that infection by both viruses favored long-term seed survival of CMV-, but not TuMV-infected, seeds under climate change conditions, and increased overall seed transmission rate of both viruses. When we applied this data to our SIR-based model, simulations indicated that climate change conditions impacted virus prevalence only when both seed transmission and survival are simultaneously enhanced. In sum, we provide evidence that climate change impacts plant virus seed transmission and seed survival, which may have important consequences for virus epidemiology.

Stability of the resistance conferred by *Sbm1* and *Sbm2* against soil-borne furoviruses in wheat in the context of climate change

K. Gauthier, C. Heidler, K. Poppendieck, and A. Niehl

Institute for Epidemiology and Pathogen Diagnostics, JKI, Brunswick, Germany.

(kevin.gauthier@julius-kuehn.de) *S7-O3*

Soil-borne wheat mosaic virus (SBWMV) and soil-borne cereal mosaic virus (SBCMV) are important cereal viruses worldwide. Two SBWMV strains (SBWMV-NY, SBWMV-N) and three types of SBCMV (SBCMV-O, SBCMV-C and SBCMV-G) exist, which differ in aggressivity in wheat. The viruses are transmitted by the soil-borne plasmodiophorid *Polymyxa graminis* (*P. graminis*). As soil contaminated by viruliferous *P. graminis* spores remains infectious over many years, the only control strategy against the viruses is the growth of resistant cultivars. Until now, only two resistance loci, *Sbm1* and *Sbm2*, effective against furovirus infection are known. They are thought to confer a translocation resistance to wheat, restricting SBWMV/SBCMV to the roots. The stability of the resistance conferred by *Sbm1* and *Sbm2* under changing climate conditions remains unclear. To better characterize SBWMV/SBCMV resistance with respect to climatic conditions, 13 genotypes differing in grade of resistance were sown under controlled conditions in field soil containing SBWMV-N. Eight conditions, testing a combination of three factors (temperature, watering, nutrients) at two levels each were tested. Eight plants per genotype and per modality were grown. Viral infection rates as well as virus and vector quantities were determined by ELISA and qPCR. Our results indicate that firstly, cultivar and culture conditions both influence infection rates; secondly, that virus quantities remain the same for every tested factor but vector quantities do not. Thirdly, we confirmed that translocation of virus into the upper parts occurred only seldom in resistant plants. Taken together, our results suggest that the resistance conferred by *Sbm1/Sbm2* is not exclusively a translocation resistance but has additional qualities. Moreover, environmental conditions have a high impact on the stability of the resistance. This work gives important new insights to furovirus epidemiology and contributes to defining conditions favoring infection or resistance.

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