APVW 2018

20–23 February 2018
The Venue, Onetangi
Waiheke Island
New Zealand

THE 13TH AUSTRALASIAN PLANT VIROLOGY WORKSHOP

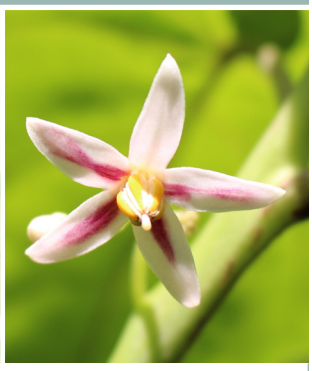
ABSTRACT AND PROGRAMME BOOK

Berry Co. growing innovation
The University of Auckland New Zealand | Science
New Zealand Wine Pure Discovery
Plant & Food Research Rangahau Ahumāra Kai

Thank you to our conference sponsors

APVW 2018





Welcome

Haere mai, haere mai, haere mai.

Welcome to New Zealand and the beautiful island of Waiheke which is our home together over the next few days. The name Waiheke means 'cascading waters', but it was actually given to the island by mistake. The original Māori name of the island was 'Te Motu-arai-roa', or 'long sheltering island'. We hope you enjoy relaxing for a while in the shelter of this island of beautiful beaches, stunning scenery, forest, vineyards and olive groves.

The 13th Australasian Plant Virology Workshop once again provides a wonderful opportunity to be in the company of people who a share common interest in everything plant virus and virus-like. We hope that you enjoy the environment here, that you have lots of fun, and that you have an amazing time sharing information and networking with your fellow virology colleagues.

A special thanks to several organisations for their support.

- University of Auckland, School of Biological Sciences (www.sbs.auckland.ac.nz) for sponsoring Kristiina Mäkinen to give the R.E.F. Matthews' Memorial Lecture
- BerryCo (www.berryco.co) for their sponsorship of Yannis Tzanetakis
- New Zealand Wine Growers (www.nzwine.com) for their sponsorship of Rodrigo Almeida
- Ministry for Primary industries (www.mpi.govt) for their sponsorship of Pascal Gentit
- Zespri (www.zespri.com) and Kiwifruit Vine Health (www.kvh.org.nz) for their sponsorship of Shaohua Wen
- NZ Citrus growers (www.citrus.co.nz) for their sponsorship of William Dawson
- EMBO (www.embo.org) for their sponsorship of Santiago Elena to give the Forster Memorial Lecture
- dnature (www.dnature.co.nz) for sponsoring lunches and student prize
- Australasian Plant Pathology Society (www.appsnet.org) for providing financial assistance and prizes to students
- Plant & Food Research (www.plantandfood.co.nz) for providing the support to organise this workshop
- The Venue (www.venuewaiheke.co.nz) for hosting the conference

Enjoy the workshop!

E hoa ma, ina te ora o te tangata
My friends, this is the essence of life

Contents

Organising Committee

Dr Lisa Ward (Co-Chair)
Ministry for Primary Industries

Dr Robin MacDiarmid (Co-Chair)
Plant & Food Research

Dr Colleen Higgins
Auckland University of Technology

Dr Kar Mun Chooi
Plant & Food Research

Samantha Edwards
Plant & Food Research

Dr Tracey Immanuel
Ministry for Primary Industries

Subuhi Khan
Ministry for Primary Industries

Dr Catia Delmiglio
Ministry for Primary Industries

Yvonne McDiarmid
Plant & Food Research

Emma Smith
Plant & Food Research

Field Trip Information

Itinerary – 23rd February 2018

0915 Pick up from The Venue and transfer to Goldie Vineyard

0930 Drop off at Goldie Vineyard

1045 Pick up from Goldie and commence a commentated scenic tour

1100 Visit Rangihoua Estate where the first olive oil in the North Island was produced. Rangihoua's award-winning olive oils have won many accolades since the first pressings. Here you will learn how the oil is produced and taste a range of different oils, including the peppery Greek-style Koreneiki, the Spanish-style Picual, or the Tuscan-style Frantoio, as well as island blends. Also taste their local honey and herb spread. Their Waiheke Blend recently won a gold medal and best in class at the LA County Fair.

1200 Visit Batch Vineyard, the highest vineyard on Waiheke Island with north facing sun-soaked hillsides of vines including terraces of traditional and elegant Alsace-style vines close to the winery. There are spectacular 360 degree views over the island and beyond to the gulf islands. Taste as range of their wine, including Syrah, Cabernet Sauvignon, Pinot Gris and Flora plus Batch Winery's sparkling wine Blanc de Blancs. – Here enjoy a kiwi high tea style lunch. Beverages own expense.

1345 Pick up from Batch

1400 Visit Tantalus Estate for a craft beer and wine tasting. This newly renovated winery within the vines offers a diverse experience of wine tastings, internationally inspired cuisine and handcrafted ales poured within close proximity of their state of the art brewery.

1500 Drop back to The Venue

Speakers

Dr David Teulon

Dr David Teulon is the Director of the Better Border Biosecurity (B3) research collaboration involving four Crown Research Institutes and one University Research Centre in New Zealand as well as MPI, DOC and representatives from the pastoral, horticultural/cropping and forestry sectors. B3 is the primary research provider for science-based plant border biosecurity solutions in New Zealand. David is also an Adjunct Professor at Lincoln University. He has postgraduate degrees in horticulture and entomology and has worked at research institutes or universities in the Netherlands, USA and Germany. He has a broad experience in plant protection and plant biosecurity having worked in fruit, vegetable and arable and forest systems specialising in thrips, aphids and psyllid pests. David co-leads the NZ US Invasive Species Working Group.

Dr Kristiina Mäkinen

Dr Kristiina Mäkinen is university lecturer in applied biochemistry at the University of Helsinki, Finland. Her research has focused on cellular and molecular biology of plant virus infection over 20 years. She has supervised several doctoral students and organized post graduate courses on plant virology e.g. recently on plant-pathogen co-evolution. Currently, her work focuses on elucidating the molecular mechanisms underlying potyvirus infection. She has been involved as a principal investigator with a number of multicenter, multi-investigator consortia including projects with sectorial institutes and industry partners, bilateral international projects in Plant Biotechnology (Indo-Finnish) and Sustainable Production and Products (French-Finnish), and participated in EU-funded programs (Intas and Erasmus Mundus Brave). She serves as a senior editor of Molecular Plant Pathology, an ad hoc reviewer of several international journals and an evaluator of grant proposals for many funding bodies abroad. She has co-authored around 70 journal articles and book chapters in respected international journals e.g. Plos Pathogens, Plant Cell, Plant J, J. Virol., and presented her research findings as an invited speaker in many international congresses like in Gordon Research Conference. More information can be found from https://tuhat.helsinki.fi/portal/en/person/krmakine and http://kristiinamakinen31.wixsite.com/plantvirology

Prof. Santiago F. Elena

My scientific interests are related with the evolutionary and systems biology of viruses. More precisely, on the mechanisms that generate, maintain and modulate the genetic variability of RNA viruses and their contribution to adaptation to novel hosts. Our approach to virus evolution combines experimental evolution, molecular phylogenomics and mathematical modeling. To unravel the mechanistic basis of adaptation of emerging viruses to novel hosts, we are also applying a systems biology perspective by which we characterize how the networks of interactions between host and virus factors evolve, resulting in the optimization of viral fitness in the novel host. Our model pathosystems are tobacco etch (TEV) and turnip mosaic potyviruses (TuMV) and their natural and experimental plant hosts.

Dr. Ioannis Tzanetakis

Dr. Tzanetakis studied Soil Science at the Agricultural University of Athens before moving to Oregon State University, to pursue a PhD in Molecular and Cellular Biology working on berry viruses. He continued with a postdoc focusing on translational enhancers of tymoviruses and bioinformatics analysis of coupled translation in eukaryotes. He is currently a Professor of Plant Virology at the University of Arkansas. He is a member of several national and international groups including the National Clean Plant Network, the International Council for the Study of Virus and other Graft Transmissible Diseases of Fruit Crops and the International Committee on Taxonomy of Viruses. His research focuses on the epidemiology and detection of plant viruses and particularly on the development of vertical pipelines starting from wet lab protocols to custom-designed bioinformatics tools for detection and discovery of pathogens using large scale sequencing.

Speakers

Pascal Gentit

After almost 20 years dedicated to the French fruit tree certification program at the CTIFL private institute, Dr Pascal Gentit is a Senior Scientist at the Plant Health Laboratory, ANSES, in France where he is in charge of the virology and phloem limited bacteria team and deputy head of the Virology, bacteriology and GMO laboratory. Member of the scientific committee of ICVF, he is a specialist in plant virus diagnosis and has almost 20 years of experience both in biological and molecular researches on plant viruses mainly on fruit trees. He contributed to more than 20 publications in peer reviewed journals and books in the field of virology. He is currently developing research programs on the detection of various regulated non quarantine and quarantine viruses by high throughput sequencing methods or by polyvalent degenerated PCR. Since 2012, he has also developed an important research program on the detection, the epidemiology and the genetic characterization of Candidatus liberibacters solanacearum.

Marc Fuchs

Marc Fuchs received his Master's and PhD degrees from University Louis Pasteur in Strasbourg, France. He joined the Department of Plant Pathology at Cornell University in 2004 with research and extension responsibilities on viruses of vegetable and fruit crops. Marc's program is translational, based on discovery-oriented research and the transfer of discoveries into practical applications. A major focus of his program is on virus-host-vector interactions with the ultimate goal of devising optimal management strategies. Marc is currently leading multidisciplinary team efforts on major grapevine virus diseases such as fanleaf, leafroll and red blotch, and on major vegetable diseases such as yellow spot in onion and mosaic in snap bean. Marc published over 100 peer-reviewed articles in international journals, 35 book chapters and review articles, and made multiple presentations at professional society conferences, as well as at growers meetings and conventions.

Rodrigo Almeida

Rodrigo Almeida is a leading scientist studying the biology and ecology of insect-transmitted plant pathogens. He is professor and ecologist of emerging infectious diseases in the Department of Environmental Science, Policy and Management at the University of California, Berkeley. Rodrigo is a Fulbright and Marie Curie Fellow, and recipient of the early career award from the American Phytopathological Society, among other awards. His team's current research areas are the ecology of emerging diseases, pathogen transmission biology, and insect-microbe interactions. In particular his research uses two pathogen-insect systems: the xylem-limited leafhopper-transmitted bacterium Xylella fastidiosa, and the mealybug-borne grapevine leafroll virus complex.

William Dawson

William Dawson is a world-renown scientist studying the molecular genetics of virus-host interactions and methods to control citrus diseases caused by virus and bacteria. He is an Eminent Scholar and the J. R. and Addie S. Graves Endowed Chair in the Department of Plant Pathology at the University of Florida, Citrus Research and Education Centre. William is also a current Fellow of the American Phytopathological Society and Fellow of the International Organization of Citrus Virologists. His current research program focusses on three related goals: to develop methods to manage diseases caused by citrus tristeza virus (CTV); to dissect the molecular mechanisms involved in replication and gene expression as a means to understand large RNA viruses; and to utilise the technologies developed with CTV to create a genetic tool for citrus improvement. Notably, a CTV-based transient expression vector is currently being trialled in-field as a tool to control the devastating citrus greening in Florida.

Programme

Tuesday 20 February 2018

13:30	**Registration (tea & coffee available till 3pm)**
14:45	**Welcome and housekeeping**
15:00 – 16:00	**REF MATTHEWS LECTURE** **Kristiina Mäkinen** Emerging picture of post-replication regulation of potato virus A RNA functions
SESSION ONE: VIRUS-HOST INTERACTION | Chair: Robin MacDiarmid and Brittney Caruana	
16:00 – 16:45	**PLENARY** **Bill Dawson** Evolving uses of the CTV vector
16:45	**Sarah Thompson** *Candidatus* Liberibacter solanacearum prophage excision
17:00	**Kar Mun Chooi** Knowing the enemy is half the battle: how virus genetic populations can influence management strategies
17:15	**Waqas Ahmad** Identifying potential viral suppressors of RNA silencing in the grapevine leafroll-associated virus-3
17:30	**Peter Palukaitis** Heterochromatin content in tobacco is regulated by RDR1 gene expression and both are altered by infection with potato virus Y
17:45^	**Zhi Xu** Microbial associations with the Tomato Potato Psyllid, *Bactericera cockerelli*
17:50^	**Eseul Baek** Interaction between transcription factors and resistance effectors in N gene tobacco infected with tobacco mosaic virus
17:55^	**Jasmine Marsh** The impact of drought and virus on wheat
18:30	**Welcome dinner – Kiwi BBQ at 'The Venue'**

Programme *continued*

Wednesday 21 February 2018

8:00	**Registration**
8:20	**Housekeeping**
SESSION TWO: DIAGNOSTICS AND ADVANCING TECHNOLOGIES | Chair: Lisa Ward and Solomon Maina	
8:30 – 9:15	**PLENARY** **Ioannis Tzanetakis** Modern diagnostic technologies and their impact on plant movement across borders
9:15	**Priyadarshana Ajithkumar** Diagnosis and genome analysis of lettuce necrotic yellows virus subgroups
9:30	**Brittney Caruana** Advanced genomics for improved disease resistance in potato
9:45	**Arnaud Blouin** Grapevine virus [insert a letter here]
10:00	**Brendan Rodoni** Next Generation Sequencing technology: a powerful research tool that is developing into a useful diagnostic tool
10:15	**Dan Cohen** Serotyping Grapevine leafroll-associated virus 3
10:30	**Morning tea (20 mins break)**
10:50	**Linda Zheng** Development and application of loop-mediated isothermal amplification (LAMP) assays for *Potato spindle tuber viroid* (PSTVd) and *Potato virus Y* (PVY)
11:05	**Stella Veerakone** First report of a citrivirus from *Nandina domestica*
11:20	**Roberto Barrero** Improving Border Biosecurity: Next generation sequencing for the detection of viruses and viroids in quarantined material
11:35^	**Yu bao Zhang** Developing virus assays to improve lily crop production in northwest, China
SESSION THREE: VIRUS EVOLUTION | Chair: Colleen Higgins and Priya Ajithkumar	
11:45 – 12:45	**EMBO LECTURE / FORSTER MEMORIAL** **Santiago Elena** Mechanisms of virus evolution and emergence
12:45	**Roger Jones** Potato virus S Isolates from the Andean Region of South America: Biology and Phylogenetics
13:00	**Colleen Higgins** Complete genome sequence of Colocasia bobone disease-associated virus, a cytorhabdovirus infecting taro
13:15	**Lunch (1 hour break)** **Student's lunch with plenary speakers** front deck of 'The Venue' (everyone else outside area)

Programme *continued*

SESSION FOUR: EPIDEMIOLOGY & VIRUS ECOLOGY | **Chair: Catia Delmiglio and Jacqueline Morris**	
14:15 – 15:00	**PLENARY** **Marc Fuchs** Ecological Insights into Grapevine Red Blotch Virus
15:00	**Piotr Trebicki** Greater virus incidence in wheat when subjected to elevated CO_2: a four-year field study
15:15	**Roger Jones** Zucchini yellow mosaic virus Epidemiology in the Ord River Irrigation Area: Aphid Vectors, Alternative Hosts, and Epidemic Development
15:30^	**Roy van den Brink** Preliminary investigation of seed transmission of Actinidia seed-borne latent virus
15:35^	**Craig Webster** Evaluation of disinfection methods against Cucumber green mottle mosaic virus and their suitability for managing it in cucurbits
15:40^	**Julia Cremer** Temporal-spatial dynamics of TYLCV and its vector in the dry tropics
15:45	**Afternoon tea (30 mins break)**
16:15	**Craig Webster** Development of alternative management strategies to control epidemics of zucchini yellow mosaic virus in the Ord River Irrigation Area
16:30	**Samantha Edwards** Modernising the terminology for pollen and seed transmission of plant viruses
16:45	**Narelle Nancarrow** The effects of preventative insecticide application on primary and secondary virus spread in wheat
17:00	**Robin MacDiarmid** Citrus tristeza mild strain and cross-protection in New Zealand citrus orchards
17:15^	**Piotr Trebicki** Devastating effect of viruses on a fenugreek crop and first detection in Australia
17:20^	**Piotr Trebicki** Incidence and distribution of viruses in pulse and canola crops: the main drivers behind disease outbreak
18:30	**Workshop dinner – Stoneyridge winery** Bus leaves 'The Venue' at 18:30

Programme *continued*

Thursday 22 February 2018

8:00	**Registration**
8:20	**Housekeeping**
SESSION FIVE: VIRUS-VECTOR INTERACTIONS | Chair: Subuhi Khan and Zhi Xu	
8:30 – 9:15	**PLENARY** **Rodrigo Almeida** Exploring the biology of *Grapevine leafroll-associated virus 3*
9:15	**Kar Mun Chooi** Retention and transmission of Grapevine leafroll-associated virus 3 by *Pseudococcus calceolariae*
SESSION SIX: BIOSECURITY AND EMERGING RISKS | Chair: Karmun Chooi and Shweta Shinde	
9:30 – 10:15	**PLENARY** **Pascal Gentit** Biosecurity in the European context : a multi-stakeholder management
10:15	**Morning tea (20 mins break)**
10:35	**Fiona Constable** Characterizing Melon necrotic spot virus isolates detected in Australia
10:50	**Catia Delmiglio** Seed testing in NZ – mitigating biosecurity risks
11:05	**Shaohua Wen** The progress of kiwifruit virus Research in China
11:20	**Solomon Maina** Sweet potato virus C and Sweet potato feathery mottle virus from Australian and East Timorese Sweet Potato Samples: Biology and Phylogenetics
11:35	**Grant Smith** Initial analysis of the draft genome of *Candidatus* Liberibacter ctenarytainae
11:50	**Jacqueline Morris** Comparative genomics of the Liberibacter genus
12:05^	**Steve Wylie** The virome of street flowers in Nannup, a small town in Western Australia
12:10^	**Lisa Ward** Identification of a new rose virus by NGS
12:15	**Lunch (45 mins break)**

Programme *continued*

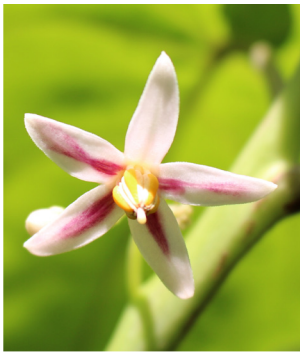
13:00 – 13:45	**PLENARY** **David Teulon** Plant viruses and their vectors at the border
13:45	**Joe Tang** Viruses infecting *Clivia miniata* in New Zealand
14:00	**Ralf Dietzgen** Detection and genetic diversity of alfalfa dwarf disease-associated viruses in lucerne pastures in Argentina and Australia
14:15	**Fiona Constable** *Cucumber green mottle mosaic virus* in Australia - current situation
14:30^	**Kathy Crew** Novel ampeloviruses from banana in south-east Asia
14:35^	**Paul Campbell** Papaya meleira virus comes to Australia
14:40^	**Lee Rabbidge** Discovery of a new Emaravirus in a New Zealand native species
14:45	**Afternoon tea (15 mins break & available through the closing)**
15:00 – 15:30	**APVW General Meeting and invitation to next meeting** **Student prizes** **Concluding remarks and farewell**

Note on talking times:

1. REF Matthews & Forster/EMBO lectures are 1 hour total (45 mins + 15 mins questions).
2. Plenary talks are 45 minutes total (30 mins talk + 15 mins questions).
3. Standard talks are 15 mins total (12 mins talk + 3 mins questions).
4. ^ denotes Quickfire talks that are 5 mins total (3 mins talk + 2 mins questions).

APVW 2018





ABSTRACTS

Emerging picture of post-replication regulation of potato virus A RNA functions

Dr Kristiina Mäkinen
University of Helsinki
kristiina.makinen@helsinki.fi

Swaranlok De, Andres Lõhmus, Maija Pollari, Shreya Saha and Kristiina Mäkinen

Faculty of Agriculture and Forestry, Department of Microbiology, P.O.Box 56, 00014 University of Helsinki, Finland

Potato virus A (PVA) is a positive-sense single-stranded RNA virus belonging to genus *Potyvirus*. Potyviruses cause significant crop losses worldwide. After amplification in replication complexes potyviral RNA (vRNA) is transferred to polysomes for viral protein production in a process aided by viral proteins helper component – proteinase (HCPro) and viral protein genome-linked (VPg). HCPro and VPg have several roles in robust PVA gene expression. We have found that HCPro i) induces compositionally unique transient RNA granules containing host proteins ARGONAUTE 1 (AGO1), VARICOSE (VCS) and eukaryotic initiation factor (iso) 4E (eIF(iso)4E) during PVA infection and proposed that this property is linked to its role in suppression of vRNA degradation; ii) has a role in translational enhancement along with VPg and proposed that it could have a role in relieving AGO1-mediated translational repression; iii) is required for PVA particle formation. Our data show that PVA VPg i) is pivotal for PVA translation, likely especially enhancing coat protein production late in infection; ii) counters RNA granule formation for the benefit of viral translation. To understand how the interactions of HCPro and VPg with the host cell proteins AGO1, AGO2, VCS and eIF(iso)4E function to promote PVA infection and particle accumulation we have characterized a VPg mutant deficient in eIF(iso)4E binding and two HCPro mutants. One of the HCPro mutants is deficient in AGO1-binding while the other mutation appears to weaken HCpro's potential interaction with VCS. The picture of post-replication regulation of PVA RNA functions arising from our recent results will be discussed.

Notes

Evolving uses of the CTV vector

Prof William Dawson
University of Florida
wodtmv@ufl.edu

William Dawson
University of Florida, Citrus Research and Education Center, Lake Alfred, FL USA

Some isolates of *Citrus tristeza closterovirus* (CTV), the largest known plus-strand RNA virus of plants, cause serious diseases of citrus. Reverse genetics of this virus has allowed a beginning of understanding of how the virus replicates and expresses its genes and how the virus causes diseases. This technology also allows the insertion of foreign sequence into the virus, which can be utilized as a transient vector to express foreign genes. The first use was to express the green fluorescent protein to allow visualization of the infection process in plants. Then it was designed as a tool for citrus improvement, to test expression or removal of genes in mature citrus. With the entry of huanglongbing (HLB) into the USA, it has been used as a screening tool for potential genes or peptides to manage the disease or to identify bacterial genes that induce disease. We then discovered that the vector could be used to control psyllid (HLB vector) reproduction on citrus by expressing sequences to induce RNAi suppression of the insect genes. With the development of CRISPR technologies, the CTV vector now is being used to induce RNAi to suppress and identify citrus gene targets for mutation. Because of its unusual stability (years), its ability to be rapidly deployed, and the desperation of the situation in Florida, the CTV vector is in the process of being used in the field to manage HLB.

Notes

Candidatus Liberibacter solanacearum prophage excision

Sarah Thompson
New Zealand Institute for Plant and Food Research, Lincoln
sarah.thompson@plantandfood.co.nz

Sarah Thompson[(1)(2)], Chris Johnson[(3)], Kerry Sullivan[(1)(2)], Charles David[(2)], Rebekah Frampton[(1)(2)], Falk Kalamorz[(1)(2)], Neil Gudmestad[(3)] and Grant Smith[(1)(2)]

[(1)] The New Zealand Institute for Plant and Food Research Limited, Lincoln 7608, New Zealand
[(2)] Plant Biosecurity Cooperative Research Centre, Bruce, Australian Capital Territory 2617, Australia
[(3)] Department of Plant Pathology, North Dakota State University, Fargo 58108, North Dakota, USA

Candidatus Liberibacter solanacearum (CLso) is an unculturable phloem-limited alpha-proteobacteria. Two of the five reported haplotypes (A and B) of CLso cause disease in solanaceous crops including potatoes and tomatoes. These haplotypes are vectored between plants by the tomato potato psyllid – *Bactericera cockerelli*. Currently the only control method is insecticide sprays to control the vector. Further understanding of the bacterial pathogen may lead to novel control methods. Whole genome sequencing of CLso has revealed the presence of prophages integrated into the bacterial genome. Mapping of Illumina sequence reads onto reference CLso genomes showed that some prophage regions had substantially different coverage than the core regions of the genome, suggesting that some prophage excise and circularise. Primers were designed at integration sites to explore the effect of host and heat on prophage integration and excision. This work provides tools to increase the understanding how prophage integration and excision may impact the complex interaction of the pathogen, plant, and vector.

Notes

Knowing the enemy is half the battle: how virus genetic populations can influence management strategies

Dr Kar Mun Chooi
The New Zealand Institute for Plant & Food Research Limited
karmun.chooi@plantandfood.co.nz

Kar Mun Chooi[1], Arnaud G Blouin[1][2], Daniel Cohen[1], Vaughn A Bell[3], Dion Mundy[4], Stephen Nobilo[5] and Robin MacDiarmid[1][2]

[1] The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand
[2] School of Biological Sciences University of Auckland; Auckland, New Zealand
[3] The New Zealand Institute for Plant & Food Research Limited, Havelock North, New Zealand
[4] The New Zealand Institute for Plant & Food Research Limited, Blenheim, New Zealand
[5] Waimarie Wines, Waimauku, New Zealand

Worldwide, grapevine leafroll disease is predominately caused by grapevine leafroll-associated virus 3 (GLRaV-3). It negatively influences vine growth and fruit quality. To control this economically important disease, the New Zealand wine industry reduces the risk of primary infection by planting virus-free vines, and secondary infection by removing infected vines. In red berry cultivars, diseased vines are identified by visual inspections for foliar symptoms (dark red inter-vein, green veins and rolling margins) or by ELISA testing white berry cultivars. While ELISA assays now detect all known GLRaV-3 genetic variants, little is known about how or if this variation influences spatio-temporal symptom expression across cultivars or the accuracy of visual inspections. A GLRaV-3 field trial was established in three New Zealand grape-growing regions (Auckland, Hawke's Bay, Marlborough) using four cultivars (Merlot, Pinot noir, Pinot gris, Sauvignon blanc) infected with one of three GLRaV-3 genetic variants (Group I, Group VI, NZ2). Twenty grapevines of each cultivar/variant combination were monitored for foliar symptoms over three growing seasons at each site. In the red berry cultivars, we observed significant spatio-temporal differences in symptom development based upon the GLRaV-3 genetic variant. To increase the probability of visually identifying an infected vine with a less severe genetic variant, or of detecting one with undeveloped foliar symptoms, the New Zealand wine sector recommends visual inspections be undertaken by trained personnel late in the growing season. Further research is underway to understand the physiological and biochemical effects caused by GLRaV-3 and its genetic variants on different grapevines cultivars.

Notes

Identifying potential viral suppressors of RNA silencing in grapevine leafroll-associated virus 3

Mr Waqas Ahmad
Plant & Food Research
Waqas.Ahmad@plantandfood.co.nz

Waqas Ahmad [(1)(2)], Heiko Ziebell [(3)], Kar Mun Chooi [(2)] and Robin M MacDiarmid [(1)(2)]

[(1)] The New Zealand Institute for Plant & Food Research Limited; Auckland, New Zealand
[(2)] School of Biological Sciences University of Auckland; Auckland, New Zealand
[(3)] Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut, Braunschweig, Germany

Grapevine leafroll-associated virus 3 (GLRaV-3) is an economically important ampelovirus from the family Closteroviridae and has been identified as a major causal agent associated with grapevine leafroll disease (GLD). GLD has caused major economic losses to the grapevine yield and quality globally as well in New Zealand. The GLRaV-3 species comprises a wide range of genetic variants that share less than 80% amino acid identity across their encoded proteins. GLRaV-3 has been reported to encode one virus-encoded suppressor of RNA silencing (VSR) protein (p19.7 kDa protein) showing local VSR activity. Yet Citrus tristeza virus, a relative of GLRaV-3 within Closteroviridae, has been reported to encode three VSRs with varying local and systemic VSR activities. To better understand the relationship between GLRaV-3 and its grapevine host we have sought to identify whether there are additional GLRaV-3 VSRs and to determine differences in VSR activity encoded by selected GLRaV-3 genetic variants. In our study, a wide range of assays are being employed to identify even weak VSR activity encoded by the GLRaV-3 genome. We demonstrate additional VSR activity of the p19.7 kDa protein and demonstrate that GLRaV-3 also encodes more than one VSR protein. The genetic variants of GLRaV-3 are shown to encode differential VSR activities and these appear to correlate with symptom development on grapevines.

Notes

Heterochromatin content in tobacco is regulated by *RDR1* gene expression and both are altered by infection with potato virus Y

Peter Palukaitis
Seoul Women's University
scripath1@yahoo.co.uk

Peter Palukaitis[(1)], Eseul Baek[(1)] and Ju-Yeon Yoon[(2)]

[(1)] Department of Horticultural Sciences, Seoul Women's University, Seoul 01797, Korea
[(2)] Virology Unit, National Institute of Horticultural and Herbal Science, RDA, Wanju 55365, Korea

Silencing of the *RDR1* gene, encoding RNA-dependent RNA polymerase 1, in *Nicotiana* species was shown previously to lead to less resistance to viral pathogens as well as insect pests. It also led to reduced expression of various defense genes. A subcellular ultrastructural examination of mesophyll cells in two transgenic tobacco lines in which *RDR1* gene expression was suppressed demonstrated an increase in heterochromatin aggregation and condensation in the nucleus, as well as shrinkage of the nucleus. These effects appeared to be linked, in part, to an increase in gene expression of *RDR2*, which is involved in RNA-directed DNA methylation (RdDM), as measured by qRT-PCR. Infection of *RDR1*-silenced transgenic tobacco by potato virus Y (PVY) led to an increase in *RDR1* gene expression, a decrease in heterochromatin content and aggregation, an increase in the diameter of the nucleus, and a reduction in *RDR2* gene expression. Thus, expression of the *RDR1* gene is able to negatively regulate the size of the nucleus, plus the content and aggregation of heterochromatin in tobacco mesophyll cells, while infection by PVY is able to decrease the content of heterochromatin, to the extent that it can increase the level of *RDR1* gene expression and decrease *RDR2* gene expression. RNA-seq analysis of 25 genes known to be involved in either RdDM or heterochromatin formation did not reveal clearly the nature of the genes regulated by RDR1, even though silencing of *RDR1* gene expression led to an increase in expression of retrotransposons, also reversed by PVY infection.

Notes

Microbial associations with the Tomato Potato Psyllid, *Bactericera cockerelli*

Zhi Xu
The New Zealand Institute for Plant & Food Research Limited
zhi.xu@plantandfood.co.nz

Zhi Xu[(1)(3)], Robin MacDiarmid[(2)(3)], Grant Smith[(1)] and Rebekah Frampton[(1)]

[(1)] The New Zealand Institute for Plant & Food Research Limited, Canterbury Agriculture & Science Centre, Gerald St, Lincoln 7608, New Zealand
[(2)] The New Zealand Institute for Plant & Food Research Limited, 120 Mt Albert Road, Sandringham, Auckland, 1025, New Zealand
[(3)] School of Biological Sciences, University of Auckland, 3A Symonds Street, Auckland 1010, New Zealand

The tomato potato psyllid (TPP, *Bactericera cockerelli* Šulc) is a pest that causes significant economic loss to the tomato and potato industries. Insecticide applications, sometimes fortnightly, are currently used for TPP control. This raises concerns that TPP will develop insecticide resistance despite the use of different chemistries. Insect viruses (e.g. Nucleopolyhedroviruses and Granuloviruses) have been used to control insect pests because they are nontoxic to humans, livestock, most beneficial insects, plants and the environment. To investigate the possibility of insect viruses for control of TPP, we need a thorough understanding of the viruses associated with this pest. We aim to classify the viral component of the TPP microbiome using DNA and RNA sequencing. TPP were collected using suction devices in New Zealand and Honduras. First, total DNA was extracted from individual insects using a Qiagen Blood & Tissue kit and the DNA sequenced using Illumina HiSeq. Analysis of the sequence data with Kraken, a metagenomic sequence classifier, revealed a diverse array of viral sequences associated with TPP. Most viral sequences belonged to the *Polydnaviridae*, *Podoviridae* and *Alloherpesviridae* families. Both the Honduras and New Zealand TPP DNA samples had similar virus diversity profiles. Total RNA and double-stranded RNA will also be isolated from TPP and sequenced, to investigate RNA viruses associated with TPP. This is the first survey to assess the viral component associated with TPP, as part of a larger study of the TPP microbiome.

Notes

Interaction between transcription factors and resistance effectors in *N* gene tobacco infected with tobacco mosaic virus

Dr Eseul Baek
Seoul Women's University
eseul@swu.ac.kr

Eseul Baek[1], Ju-Yeon Yoon[2] and Peter Palukaitis[1]

[1] Dept. of Horticultural Sciences, Seoul Women's University, Seoul, 01797, Korea
[2] Virology Unit, Dept. of Horticultural Environment, National Institute of Horticultural and Herbal Science, Rural Development Administration, Wanju, 55365, Korea

Hypersensitive resistance to tobacco mosaic virus (TMV) in tobacco is conferred by the *N* gene, which elicits the best characterized plant virus resistance response. Multiple responses that inhibit the infection of TMV are activated during this resistance, most of which are activated by salicylic acid (SA). One resistance effector, involving the synthesis of an inhibitor of virus replication (IVR), was shown to be independent of SA, as was the synthesis of another resistance factor, the transcription factor (TF) SHE1, an AP2/ERF TF. We found that SHE1 is upstream of and regulates IVR production. The kinetics of gene expression of various SA-activated, defense-related genes *AOX1*, *PR1*, *RDR1* and *IVR* in tobacco plants were examined by real-time RT-PCR, as were the kinetics of gene expression of *SHE1* and *MYB1*. The results indicate complex regulation of expression of the various defense genes, with silencing of *SHE1* or *MYB1* affecting the expression of genes in the other pathway. IVR and the TF SHE1 interacted with each other in the yeast two-hybrid system, as well as by bimolecular fluorescent complementation and co-immunoprecipitation, suggesting a feedback mechanism for expression of *IVR*. In addition, the SA-activated TF MYB1 interacted with SHE1, demonstrating cross-talk between the two pathways. The interacting domains between MYB1 and SHE1 were delimited, with that of SHE1 overlapping with its AP2/ERF DNA-binding domain. These data demonstrate interaction and complex regulation between different pathways involved in resistance to TMV in tobacco.

Notes

The impact of drought and virus on wheat

Jasmine Marsh
Agriculture Victoria
jasmine.marsh@ecodev.vic.gov.au

Jasmine Marsh, Narelle Nancarrow, Piotr Trebicki, Ashley Wallace and James Nuttall

Grains Innovation Park, 110 Natimuk Road, Horsham, Victoria, Australia, 3400

Under future climate predictions, drier conditions are expected across southern Australia, with a reduction in growing season rainfall for many arable crops. For crops grown under dryland conditions, plant available soil water is vital for buffering dry periods. An economically important disease of cereals worldwide is the *Barley yellow dwarf virus* (BYDV). The effects of water stress on crops is well documented, but studies on the combined effect of BYDV and water stress are less common. We examined the interaction between BYDV and water availability in wheat using a grey Vertosol soil in a glasshouse context, where three water regimes were imposed. Wheat was inoculated with BYDV using an aphid vector (*Rhopalosiphum padi*) at 3-leaf and mid-tillering phase and crop response monitored at six and eight weeks after sowing and at maturity. From the first sampling period, both leaf area and biomass were affected by the water regimes ($P < 0.05$) where compared with non-limiting water (high water), biomass was reduced by 28 and 33% for medium and low water treatment respectively. Leaf area was also reduced by 21 and 39% across this treatment comparison. For leaf area, a borderline significant reduction of 15% was observed for the infected plants compared with the control. Results from later sampling periods may show a widening of disease effects and determine if an interaction with water supply exists. These results will help our understanding of the complex interaction between host, vector and virus in an altered climate with increasing limited water availability.

Notes

Modern diagnostic technologies and their impact on plant movement across borders

Dr Ioannis Tzanetakis
University of Arkansas
itzaneta@uark.edu

Ioannis Tzanetakis

Department of Plant Pathology, Division of Agriculture, University of Arkansas System, Fayetteville, AR 72701, USA

High throughput sequencing (HTS) has revolutionized detection and discovery of plant viruses and viroids, with more than 200 new species identified in the last few years; affecting the movement of propagation material across state and country lines. As this robust technology is tested against conventional tools such as bioindicators, ELISA and PCR it has become evident that it will be the method of choice not only for research purposes but also for plant certification programs across the globe. This presentation will focus on the attributes of the different detection technologies and the effect of HTS in certification rules and regulations in the near future.

Notes

Diagnosis and genome analysis of lettuce necrotic yellows virus subgroups

Ms Priyadarshana Ajithkumar
Auckland University of Technology
priya.ajithkumar@aut.ac.nz

Priyadarshana Ajithkumar[1] and Colleen Higgins[1][2]

[1] AUT Roche Diagnostics Laboratory, School of Science, Auckland University of Technology, New Zealand
[2] Institute of Applied Ecology, Auckland University of Technology, New Zealand

Lettuce necrotic yellows virus (LNYV) is the type species of the genus Cytorhabdovirus, and causes a severe disease of lettuce. LNYV appears endemic to Australia and New Zealand, with a population consisting of two subgroups, I and II. Phylogenetic studies of the nucleocapsid gene and protein suggest that subgroup II emerged more recently than subgroup I and has dispersed rapidly. This may have led to the apparent extinction of subgroup I in Australia, an event that is yet to happen in New Zealand. Currently, no diagnostic test exists for identification of LNYV subgroups. Such a test would underpin greater understanding of the population structure in both countries, which is essential to understanding the origin of LNYV and how it is evolving. We will describe a diagnostic test we have developed for identification of LNYV subgroups. At present, only one complete LNYV genome is publicly available, which comes from an Australian subgroup I isolate. We have sequenced genomes from both subgroup I and subgroup II isolates from New Zealand with a view to understanding the molecular basis of the differences between isolates from each country as well as the reason for the rapid dispersal of subgroup II. We will report our initial findings.

Notes

Advanced genomics for improved disease resistance in potato

Ms Brittney Caruana
La Trobe University/Agriculture Victoria
brittney.caruana@ecodev.vic.gov.au

Brittney Caruana [1][2], Fiona Constable [1], Anthony Slater [1], Noel Cogan [1][2] and Brendan Rodoni [1][2]

[1] Agriculture Victoria Research, AgriBio, 5 Ring Road, Bundoora, Victoria 3083, Australia
[2] School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3086, Australia

Traditionally, potato breeding for desirable traits has relied heavily on phenotypic selection and extended field trials resulting in an 8-15 year long selection process. The introduction of marker-assisted selection has enabled the inheritance of several resistance genes to be predicted and rudimentary DNA markers have been developed and applied. These markers cannot predict highly polygenic traits and are often only near the gene, which leads to dissociation between the marker and trait resulting in incorrect genotypic information. To be able to genotype cultivars correctly and include highly polygenic traits, new marker systems are needed that deliver marker saturation across the genome to correlate the marker effects with the trait. This will allow faster selection of pathogen resistant parents to ensure plant health in disease prone areas. This project has implemented high-throughput transcriptome sequencing to genotype the Australian parental germplasm collection. The resulting genotypic data has been analysed for its applicability to replace a range of conventional DNA markers for disease resistance to a universal SNP based assay. This has the potential to enable improvement in all traits via genomic selection. The genotypic data has been used to identify candidate genes in genomic regions that are known to provide disease resistance and a candidate gene has been identified that could improve selection for introgressed PVY resistance. In parallel, quantification of virus titre has been assessed via molecular techniques to assist in developing advanced phenomic technologies for earlier detection to improve current diagnostic protocols.

Notes

Grapevine virus [insert a letter here]

Mr Arnaud Blouin
Plant & Food Research
arnaud.blouin@plantandfood.co.nz

Arnaud G. Blouin [1][2], Kar Mun Chooi [1] and Robin M MacDiarmid [1][2]

[1] The New Zealand Institute for Plant & Food Research Limited; Auckland, New Zealand
[2] School of Biological Sciences University of Auckland; Auckland, New Zealand

The use of High Throughput Sequencing (HTS) has had a massive effect on the rate of discovery of previously overlooked obligate parasites. The decrease of the technology cost combined with the uptake of the methodology by more diagnostic laboratories has resulted in an unprecedented level of detection of novel virus genomes. We have used three different HTS strategies on grapevine to identify viruses. Using small RNA, double-stranded (ds)RNA or total RNA sequencing we have detected two novel viruses from the genus Vitivirus. We have proposed the names grapevine virus G (GVG) and grapevine virus I (GVI). Both viruses are related to but distinct from grapevine virus E (GVE). From full genome analysis GVG is 54% identical (nt) to both GVI and GVE, and GVI shares 63% identity with GVE. With a modified method of dsRNA extraction using anti-dsRNA antibodies we performed a limited survey that revealed the presence of these viruses in multiple plants from a germplasm collection. GVG was also detected in commercial vineyards. In addition, a HTS survey identified an unusual strain of GVE from a commercial sample.

Notes

Next Generation Sequencing technology: a powerful research tool that is developing into a useful diagnostic tool.

Dr Brendan Rodoni
Agriculture Victoria Research
brendan.rodoni@ecodev.vic.gov.au

Brendan Rodoni [1], Roberto Barrero [2], Lisa Ward [4], Fiona Constable [1], Wycliff Kinoti [1], Linda Zheng [1], Rachel Mann [1] and Mark Whattam [3]

[1] AgriBio, La Trobe University, Agriculture Victoria Research, Australia.
[2] Centre of Comparative Genomics, Murdoch University, Australia
[3] Department of Agriculture and Water Resources, Australia
[4] New Zealand Ministry of Primary Industry

Next Generation Sequencing (NGS) is a powerful research tool for the detection and characterisation of plant viruses and viroids. Increasingly this technology is being applied in diagnostic labs to support pathogen detection and identification. Currently there are very few guidelines for diagnostic labs to follow to ensure accurate and reproducible results are generated when using NGS to support a diagnosis. For example, there are no formal guidelines for minimum standards or controls to be used within the diagnostic lab, there are no minimum standards required for external sequence providers and, when analysing NGS data, there are no guidelines for minimum requirements to identify a species or strain of a known or new pathogen. Strategies within Australia, New Zealand and globally to address these gaps will be discussed.

Notes

Serotyping Grapevine leafroll-associated virus 3

Dr Dan Cohen
Plant & Food Research
dan.cohen@plantandfood.co.nz

Daniel Cohen [1], Maher Al Rwahnih [2], Kar Mun Chooi [1], Arnaud G Blouin [1][3] and Robin M MacDiarmid [1][3]

[1] The New Zealand Institute for Plant & Food Research Limited; Auckland, New Zealand
[2] Department of Plant of Pathology, Foundation Plant Services University of California, Davis, USA
[3] School of Biological Sciences University of Auckland; Auckland, New Zealand

Grapevine leafroll-associated virus 3 (GLRaV-3) is most harmful virus to New Zealand vineyards, and the grafted grapevine standard was designed to ensure new planting material is GLRaV-3 free. GLRaV-3 status relies entirely on ELISA testing. It is therefore critical that the GLRaV-3 assay is sensitive and detects all known strains of GLRaV-3. We have previously reported differences of affinity of some of the GLRaV-3 strains detected in New Zealand against the original monoclonal antibody (mAbNY1.3) developed for its diagnostics. We here report the analysis of a range of antibodies for the detection of GLRaV-3 strains from NZ and USA. Selected GLRaV-3 strains were tested by either double or triple antibody sandwich ELISA using polyclonal antibodies for capture and three detection antibodies: the monoclonal mAbNY1.3, a polyclonal As63 or a newly developed monoclonal developed byDr Rowhani at Foundation Plant Services (FPS), University of California, Davis from recombinant protein. All GLRaV-3 strains were detected consistently with both the polyclonal As63 and the newly developed mAb, while mAbNY1.3 showed variable affinity among virus strains with weaker detection of the more genetically divergent New Zealand strains representative of Group VI and NZ2, and no detection of the USA strain GLRaV-3f. Despite the evidence for different GLRaV-3 serotypes between the Group I, Group VI, and GLRaV-3f, these results are reassuring that the commercially available ELISA reagents for GLRaV-3 are satisfactory for the detection of various virus strains since all ELISA kits rely on pAbs or a mixture between pAbs and mAb.

Notes

Development and application of loop-mediated isothermal amplification (LAMP) assays for *Potato spindle tuber viroid* (PSTVd) and *Potato virus Y* (PVY)

Linda Zheng
Department of Economic Development, Jobs, Transport and Reso
linda.zheng@ecodev.vic.gov.au

Linda Zheng [(1)], Fiona Constable [(1)] and Brendan Rodoni [(1)]

[(1)] Agricultural Research, Department of Economic Development, Jobs, Transport and Resources, Bundoora, VIC 3086, Australia

Loop-mediated isothermal amplification (LAMP) is a robust, sensitive and time-efficient method of detecting nucleic acid which requires only a single temperature incubation for nucleic acid amplification. The DNA polymerase used in LAMP is reported to be more tolerant towards sample matrix inhibitors compared to conventional thermocycling polymerase chain reactions (PCRs), allowing crude extracts of nucleic acids to be used for amplification. Once developed, LAMP tests are easy to carry out and are able to detect both DNA and RNA-based targets in under 40 minutes. Due to its simplicity, rapidity and robustness, LAMP has the potential to be used under field conditions, which is particular useful for plant pest surveillance programs. We report the validation of a LAMP assay for the detection of *Potato spindle tuber viroid* (PSTVd), a high priority emergency plant pest, and the first use of this LAMP assay in Australia for the successful on-site detection of PSTVd in tomatoes. We also report the development of a LAMP assay for the detection of *Potato virus Y* (PVY), one of the most economically important virus affecting commercial potato production. The PVY LAMP assay was designed to detect a range of PVY strains, including type “O” and “N”, as well as the “NTN” strain which is prevalent in the Australian potato industry. It is envisaged that the LAMP assays developed and validated in this study can be used by quarantine and surveillance officers for fast on-site detection of potato pathogens in the near future.

Notes

First report of a citrivirus from *Nandina domestica*

Ms Stella Veerakone
PHEL, Ministry for Primary Industries
stella.veerakone@mpi.govt.nz

Stella Veerakone, Lia Liefting, Joe Tang and Lisa Ward

Plant Health and Environment Laboratory, Ministry for Primary Industries, P.O. Box 2095, Auckland 1140, New Zealand

Nandina domestica (heavenly bamboo), is a flowering plant in the family *Berberidaceae*, native to eastern Asia. It is widely grown as a landscaping plant in many countries including New Zealand (NZ). Five viruses: *Alternanthera mosaic virus*, *Apple stem grooving virus*, *Cucumber mosaic virus*, Nandina stem pitting virus and *Plantago asiatica mosaic virus*, have been previously reported in *Nandina*. Here, we report for the first time, the identification of a citrivirus which is closely related to *Citrus leaf blotch virus* in the 5' terminal region of the genome but distinctly different at the 3' region. It was mechanically transmitted to *Nicotiana* species, although all of these infections were symptomless. Further testing of *Nandina* collected from across NZ revealed that this virus is widely established, however, only in the dwarf cultivar "firepower".

Notes

Improving Border Biosecurity: Next generation sequencing for the detection of viruses and viroids in quarantined material

Dr Roberto Barrero
Centre for Comparative Genomics, Murdoch University
rbarrero@ccg.murdoch.edu.au

Roberto A. Barrero [1], Jo Mackie [2] , Kathryn R. Napier [1], Lia Liefting [3], Subuhi Khan [3], Arnaud Blouin [4], Adrian Dinsdale [2], Lisa Ward [3], Matthew I. Bellgard [1] and Mark Whattam [2]

[1] Centre for Comparative Genomics, Murdoch University, WA 6150, Australia
[2] Department of Agriculture and Water Resources, Mickleham VIC 3064, Australia
[3] Ministry for Primary Industries, New Zealand
[4] The New Zealand Institute for Plant Food & Research Limited, Auckland, New Zealand

Detecting and preventing entry of exotic viruses and viroids is critical for protecting plant health worldwide. Existing post-entry quarantine (PEQ) screening protocols rely on time-consuming, ambiguous and costly biological indicators and molecular assays that require knowledge of infecting viral pathogens. Plants have developed the ability to recognise and respond to viral infections through Dicer-like enzymes that cleave viral sequences into specific small RNA products. We have implemented a Virus Surveillance and Diagnosis (VSD) toolkit using the open-access YABI analytical environment. The VSD toolkit assembled complete viral genomes using only a subset of small RNA reads (21-22 nt) in a range of plant species. We also found that using exclusively 24 nt RNA reads, full viroid genomes could be reconstructed. This method potentially identifies viruses that are integrated into the host plant genome. Comparison of the small RNA next-generation sequencing method with existing PEQ protocols show a strong correlation. The VSD toolkit detected viruses and viroids in quarantined samples more accurately than other computational approaches including VirusDetect and VirusFind. We envisage that the VSD toolkit will facilitate the surveillance and diagnosis of exotic viral pathogens in plants, insects, and invertebrates as these species share a similar antiviral response pathway.

Notes

Developing virus assays to improve lily crop production in northwest, China

Yu bao Zhang
Northwest Institute of Eco-Environment and Resources, CAS
zyubao@yahoo.com

Yubao Zhang[1][2], Zhongkui Xie[1], Yajun Wang[1], Zhihong Guo[1] and Le Wang[1]

[1] Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou 730000, China
[2] The New Zealand Institute for Plant & Food Research Ltd, Private Bag 4704, Christchurch, New Zealand

In Gansu, China oriental hybrid lily (*Lilium* spp.) is an important economic crop in the floricultural industry. Additional value is obtained from growing *Lilium davidii* var. unicolor bulbs, which are edible and have medicinal properties. Viruses commonly infecting lily include: lily symptomless virus (LSV), cucumber mosaic virus (CMV) and lily mottle virus (LMoV). These viruses usually co-infect lilies causing severe economic losses in terms of quantity and quality of flower and bulb production both locally and around the world. Reliable and precise detection systems have been developed for virus identification. A rapid multiple immunochromatographic strip (ICS) test was developed for the simultaneous detection LSV, CMV and LMoV. The multiple ICS tests are simple, fast, low cost, high sensitivity and specificity, and especially useful in the field, as well as in areas without laboratory facilities; We have also developed a quadruplex RT-PCR assay to simultaneously detect LSV, CMV, LMoV and using the lily 18S rRNA gene as an internal control. Furthermore, we have also developed a triplex immunocapture (IC) reverse transcription (RT) polymerase chain reaction (PCR) assay for the simultaneous detection of LSV, CMV and LMoV. The sensitivity of the triplex IC-RT-PCR system was 99.4%, 81.4% and 98.7% for LSV,CMV and LMoV, repectively. We are starting to use these three convenient and reliable detection methods now routinely for production of high health bulbs, large-scale field surveys or crop health monitoring of lily.

Notes

Mechanisms of virus evolution and emergence

Santiago F. Elena
Instituto de Biología Molecular y Celular de Plantas (IBMCP)
sfelena@ibmcp.upv.es

Santiago F. Elena[1,2,3]

[1] Instituto de Biología Molecular y Celular de Plantas (IBMCP), CSIC-UPV, 46022 València, Spain
[2] Instituto de Biología Integrativa y de Sistemas (I2SysBio), CSIC-UV, Paterna, 46182 València, Spain
[3] Santa Fe Institute, Santa Fe, NM 87501, USA

Experimental evolution offers biologists the “Beagle in a bottle”, a testing ground for evolutionary hypotheses. These hypotheses are tested by tracking of the laboratory evolution of microorganisms. One aspect that has received a good deal of attention in recent years has been the evolution of generalist and specialist pathogen strains, a process that is in the basis of viral emergence. Emerging viruses can be defined as the causative agents of infectious diseases whose incidence is increasing following its appearance in a new host population or whose incidence is increasing in an existing host population as a result of long-term changes in its underlying epidemiology. Over the years, we have been performing evolution experiments with tobacco etch (TEV) and turnip mosaic (TuMV) potyviruses that simulate the spill over from the reservoir to new hosts. A first group of experiments tackled the existence of trade-offs in TEV fitness and virulence among hosts species that differ in their degree of taxonomic relatedness with the reservoir [1,2,3]. In a second set of experiments, we explored the role of within-host species genetic variability for susceptibility in the extent of TEV and TuMV local adaptation and of virulence [5,6,8,9]. Experiments always result in a pattern of local adaptation, characterized by a higher infectivity and virulence on host(s) encountered during evolution. However, local adaptation not always pays a cost on the foreign hosts. Therefore, the expected cost of generalism, arising from antagonistic pleiotropy and other genetic mechanisms generating fitness trade-offs between hosts, could not be generalized and strongly depend on the characteristics of each particular pathosystem, specially on the genetic relatedness between new and reservoir host species [4]. Finally, we observed that selection for generalist or specialist strategies have a strong effect on the way virus alters the transcriptome of plants [7].

References

1. Agudelo-Romero P, et al. 2008 Infect. Genet. Evol. 8, 806-14.
2. Bedhomme S, et al. 2012. Mol. Biol. Evol. 29, 1481-92.
3. Bedhomme S, et al. 2013. BMC Evol. Biol. 13, 46.
4. Bedhomme S, et al. 2015. Curr. Opin. Virol. 10, 1-6.
5. Hillung J, et al. 2014. Evolution 68, 2467-80.
6. Hillung J, et al. 2015. Philos. Trans. R. Soc. B 370, 20140292.
7. Hillung J, et al. 2016. Sci. Rep. 6, 2490.
8. Lalić J, et al. 2010. Philos. Trans. R. Soc. B. 365, 1997-2007.
9. Lalić J, et al. 2011. PLoS Genet. 7, e1002378.

Notes

Potato virus S Isolates from the Andean Region of South America: Biology and Phylogenetics

Dr Roger Jones
University of Western Australia
roger.jones@uwa.edu.au

Franklin W. Santillan[1,2], Cesar E. Fribourg[2], Ian P. Adams[3], Adrian J. Gibbs[4], Neil Boonham[3,5], Monica A. Kehoe[6], Solomon Maina[7] and Roger A.C. Jones[6,7]

[1] Universidad de Cuenca, Cuenca, Azuay, Ecuador;
[2] Departamento de Fitopatologia, Universidad Nacional Agraria, La Molina, Lima, Peru;
[3] Fera Ltd, Sand Hutton, York, UK;
[4] Emeritus Faculty, Australian National University, ACT, Australia;
[5] Institute for Agrifood Research Innovations, Newcastle University, Newcastle-upon-Tyne, UK;
[6] Department of Primary Industries and Regional Development, South Perth, WA, Australia;
[7] Department of Agriculture and Environment and Institute of Agriculture, University of Western Australia, Crawley, WA, Australia.

The virulence of 11 *Potato virus S* (PVS) isolates from five Andean countries and one from Europe differed. Some infected *Chenopodium quinoa* systemically but others did not, so they belonged to biological strains PVS^A and PVS^O, respectively. PVS^A and PVS^O elicited similar foliage symptoms in wild and cultivated potato species. After graft inoculation, all except two PVS^O isolates were eventually detected in partially PVS-resistant cv. Saco, whereas clone Snec 66/139-19 developed systemic hypersensitive resistance with two isolates each of PVS^A and PVS^O, but a susceptible phenotype with the eight others. *Myzus persicae* transmitted all nine PVS^A, but none of the three PVS^O isolates. Seventeen new genomic sequences were obtained from seven Andean PVS isolates, seven isolates from Africa, Australia or Europe, and single isolates from *Solanum muricatum* and *Arracacia xanthorhiza*. Phylogenetic analysis of these genomes and 23 from GenBank revealed three major phylogroups. Two were predominantly South American (SAm) and evenly branched, and one non-SAm with a single long basal branch and many distal subdivisions. Least Squares Dating of the genomic sequences showed a basal PVS trifurcation at 1079 and 1055 CE (Common Era), and the divergence of the non-SAm lineage at 1837 CE. The *Potato rough dwarf virus/Potato virus P* cluster was sister to PVS, and diverged from PVS 5-7,000 years ago. This suggests a proto-PVS/PRDV/PVP first infected potatoes at least 5,000 years ago in South America, then around 1000 CE the present PVS population diverged there, but only one lineage spread elsewhere diverging worldwide in the 20th century.

Notes

Complete genome sequence of Colocasia bobone disease-associated virus, a cytorhabdovirus infecting taro

Dr Colleen Higgins
Auckland University of Technology
colleen.higgins@aut.ac.nz

Colleen M. Higgins[1][2], Nicolas Bejerman[3][4], Ming Li[1][2] , Anthony P. James[5] , Ralf G. Dietzgen[3] , Michael N. Pearson[6] , Peter A. Revill[5][7] and Robert M. Harding[5]

[1] Institute of Applied Ecology, Auckland University of Technology, New Zealand
[2] AUT Roche Diagnostic Laboratory, School of Science, Auckland University of Technology, New Zealand
[3] Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Australia
[4] Instituto Nacional de Tecnologia Agropecuaria Buenos Aires, Instituto de Patología Vegetal, Buenos Aires, Argentina
[5] Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, Australia
[6] School of Biological Sciences, The University of Auckland, New Zealand
[7] Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia

Bobone and Alomae are diseases of taro [*Colocasia esculenta* (L.) Schott] that are restricted to Solomon Islands and Papua New Guinea and whose aetiology remains unclear. Two rhabdovirus-like particles have been observed in infected taro plants; the smaller associated with taro vein chlorosis virus while the larger is associated with severe stunting and gall formation typical of Bobone/Alomae disease. The larger virus was named Colocasia bobone disease virus (CBDV). RNA- seq analysis of a Bobone infected taro plant from Solomon Islands that had the larger virus particles led to the discovery of a rhabdovirus genome. Analysis of this genome suggested it is a cytorhabdovirus. As we have not yet been able to establish that this virus causes Bobone disease, we have named this virus Colocasia bobone disease – associated virus (CBDaV) - now recognised as a species by the International Committee on Taxonomy of Viruses. The publication of the CBDaV sequence will now enable investigations into genome variability and virus distribution, and possibly the confirmation that CBDaV is in fact CBDV, the causative agent of Bobone and Alomae diseases. We will report our analysis of the CBDaV genome.

Notes

Ecological Insights into Grapevine Red Blotch Virus

Prof Marc Fuchs
School of Integrative Plant Science, Cornell University
marc.fuchs@cornell.edu

Marc Fuchs

Plant Pathology and Plant-Microbe Biology, School of Integrative Plant Science, Cornell University, New York State Agriculture Experiment Station, Geneva, NY 14456, USA

Red blotch disease was recognized a decade ago as a new threat to the grape and wine industry in North America. Grapevine red blotch virus (GRBV) is the causal agent of the disease and the type member of the genus *Grablovirus*, family *Geminiviridae*. This virus affects fruit maturity and ripening and its economic impact ranges from $2,213 to $68,548/ha over a 25-year lifespan of a vineyard. GRBV has a single-stranded DNA genome that codes for six open reading frames. Analysis of the spatiotemporal spread of GRBV in a 'Cabernet franc' vineyard revealed a 1-2% increased disease incidence annually from 2014 to 2017, although a higher rate (5-16% increase) was documented in a section of the vineyard with aggregated infected vines proximal to a riparian area. In this area, several free-living *Vitis* spp. infected with GRBV were identified along river banks. To identify vector candidates in the 'Cabernet franc' vineyard, insect sticky cards were set out over two consecutive years to show detection of GRBV in the majority of only four insect species: *Spissistilus festinus* (treehopper), *Colladonus reductus* (leafhopper), *Osbornellus borealis* (leafhopper), and *Melanoliarus* sp. (planthopper). Laboratory and vineyard studies revealed S. festinus as a vector of epidemiological significance. Experiments to determine the competence of the other three vector candidates at transmitting GRBV are under way. In spite of tremendous progress on the biology and spread of GRBV by *S. festinus*, research on the transmission mode, dynamics of spread in different vineyard ecosystems is needed to inform disease management strategies.

Notes

Greater virus incidence in wheat when subjected to elevated CO_2: a four-year field study

Dr Piotr Trebicki
Agriculture Victoria, Australia
piotr.trebicki@ecodev.vic.gov.au

Piotr Trębicki[1], Narelle Nancarrow[1], Nilsa A. Bosque-Pérez[2], Brendan Rodoni[3], Mohammad Aftab[1] and Glenn Fitzgerald[4]

[1] Agriculture Victoria, Horsham Centre, 110 Natimuk Road, Horsham, VIC 3400 AUSTRALIA.
[2] University of Idaho, 875 Perimeter Drive Ms 2339, Moscow, ID 83844-2339, USA.
[3] Agriculture Victoria, AgriBio, Centre for AgriBioscience, 5 Ring Road, Bundoora, VIC 3083 AUSTRALIA,
[4] Agriculture Victoria, 402-404 Mair St, Ballarat, Victoria, 3350, AUSTRALIA.

Vector transmitted plant viruses can cause substantial damage to the crop, lower the quality and yield and in the worst case, wipe out whole individual fields or areas. Due to the complexity of the mechanisms involved in vector transmitted viruses, predicting disease epidemics can be challenging as particular abiotic factors can have somewhat different effects on the vector, virus and the host. As changing climate,Driven by increasing atmospheric carbon dioxide (CO_2) concentration, can affect the vector, virus and host differently, understanding the individual effects and interactions between them is critical to sustain or increase future food production. Consequently, we conducted a study in a Free Air Carbon Enrichment facility under ambient and elevated CO_2 (550 ppm), to examine if and how CO_2 affects the natural incidence of barley/cereal yellow dwarf virus (B/CYDV). A range of wheat cultivars were tested using tissue blot immunoassay (TBIA) against four B/CYDV species during four years (2013-16). Virus incidence varied across years but BYDV-PAV was the most prevalent. Analysis across all cultivars, years and between ambient and elevated CO_2, showed a significant increase by 10.6% in virus incidence under eCO_2. The mechanism behind increased virus incidence under elevated CO_2 is not well understood but additional research and potential factors will be presented and discussed.

Notes

Zucchini yellow mosaic virus epidemiology in the Ord River irrigation area: Aphid Vectors, Alternative Hosts, and Epidemic Development

Dr Roger Jones
University of Western Australia
roger.jones@uwa.edu.au

Rebecca Clarke [1], Monica A. Kehoe [2], Sonya Broughton [2], Craig G. Webster [2], Brenda A. Coutts [2], Penny Goldsmith [3], Mark Warmington [4] and Roger A.C. Jones [2,5]

[1] Raitech Ltd., Kununurra, 6743, Western Australia.
[2] Department of Primary Industries and Regional Development, South Perth, 6151, Western Australia.
[3] Ord River District Cooperative, Kununurra, 7643, Western Australia.
[4] Department of Primary Industries and Regional Development, Kununurra, 7643, Western Australia.
[5] Institute of Agriculture, University of Western Australia, Crawley 6009, Western Australia.

In the tropical Ord River Irrigation Area (ORIA) in northwest Australia, irrigated cucurbit crops are grown in theDry season. They consist of pumpkin (*Cucurbita moschata* and *Cucurbita maxima*), melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*) plantings which suffer severe annual epidemics of the Southeast Asian zucchini yellow mosaic virus (ZYMV) strain. During 2015-2017, 11 large-scale surveys were undertaken to establish which aphid species occur, where ZYMV and vector aphids persist in the wet season (i.e. outside the growing season), and when vector aphids spread ZYMV during the growing season. All-year-round aphid trapping used sticky traps, and growing season data collection blocks of watermelon gathered information on aphid numbers and ZYMV incidence. Samples were sent to Perth for aphid identification (light microscopy and PCR) and virus testing (ELISA and RT-PCR). Six aphid species were found, three of which transmitted ZYMV, *Aphis gossypii, A. craccivora*, and *A. nerii*. In the wet season, *A. gossypii* survived mainly on home garden cucurbit crops, volunteer cucurbits, afghan melon (*Citrullus lanatus*), wild melon vines (*Cucumis melo* and *Cucumis picrocarpus*), milkweed (*Euphorbia hirta*), and native rosella (*Albemoschus ficulneus*); *A. craccivora* survived on legume crop plants in home gardens, *Sesbania* rootstocks used in sandalwood plantations and legume weeds; and *Aphis nerii* survived on calotrope (*Calotropis procera*). The virus reservoir was much smaller than the aphid reservoir, ZYMV being detected very rarely, and only in volunteer cucurbits, wild melon vines and home garden cucurbit plantings. The underlying factorsDriving ORIA's ZYMV epidemics will be discussed

Notes

Preliminary investigation of seed transmission of Actinidia seed-borne latent virus

Mr Roy van den Brink
The New Zealand Institute for Plant & Food Research Limited
Roy.VanDenBrink@plantandfood.co.nz

Roy van den Brink[1], Paul T Austin[1], Nicholas T Amponsah[1], Mary Horner[2] and Robin MacDiarmid[3]

[1] The New Zealand Institute for Plant & Food Research Limited, Palmerston North, New Zealand
[2] The New Zealand Institute for Plant & Food Research Limited, Havelock North, New Zealand
[3] The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand

Actinidia seed-borne latent virus (ASbLV) is a new-to-science virus, recently identified in imported seed within a New Zealand post-entry quarantine facility. ASbLV has not been associated with host symptoms and is assessed presently as a low-to-zero biosecurity risk. From the imported Actinidia families, some seedlings grown in germplasm collections within New Zealand were identified to have high seed infection rates (close to 100%). Actinidia seeds from one controlled cross between an ASbLV-infected male and non-infected female plants were analysed further to determine seed transmission biology. Testing of fifty Actinidia F1 seeds obtained from the cross, by RT-PCR, showed that 98% of the derived seeds inherited the virus. Cotyledons tested from 50 seedlings from the same cross showed 62% infection. Roots and leaves from the same seedlings tested positive or negative for ASbLV consistent with their cotyledon infection status. To verify these results and compare transmission from both male and female parents analysis of Actinidia F1 seedlings arising from crosses of ASbLV infected male or female siblings with non-infected plants is underway and will be reported. The preliminary results confirm that ASbLV is seed-borne in Actinidia, and indicates intriguingly that transmission from resulting seedlings may be significantly lower. The follow-up trial addresses the question as to whether both the male and female infected-parent plants can serve equally as infection sources.

Notes

Evaluation of disinfection methods against cucumber green mottle mosaic virus and their suitability for managing it in cucurbits.

Dr Craig Webster
Department of Primary Industries and Regional Development
craig.webster@dpird.wa.gov.au

Craig G. Webster [(1)], Monica A. Kehoe [(1)], Roger A.C. Jones (1,2) and Brenda A. Coutts [(1)]

[(1)] Department of Primary Industries and Regional Development, South Perth, 6151, Australia.
[(2)] Institute of Agriculture, University of Western Australia, Crawley, 6009, Western Australia.

Cucumber green mottle mosaic virus (CGMMV) emerged recently as a major problem in cucurbit production globally. Since it was detected in Australia in 2014, it spread to several cucurbit growing regions around the continent. CGMMV's high stability ensures its persistence in open field and glasshouse grown cucurbit plantings, including seedling production, making ongoing virus management essential in all three growing situations. Methods to reduce or eliminate this contamination are therefore required to help prevent spread to other locations and reduce its occurrence in CGMMV-infested areas. Here we report experiments that evaluated the effectiveness of a range of commonly available disinfectants and established their relative abilities to eliminate CGMMV infectivity. Disinfectants were mixed with infected sap and left for 1, 10 or 60 min before mechanically inoculating cucumber leaves. Household bleach (12.5 g/L solution of sodium hypochlorite) and skim milk powder (20% wt/vol solution) were the most effective agents at abolishing CGMMV infectivity. Other common disinfectants, including Virkon S (4% wt/vol) and pool chlorine (1% wt/vol), were also effective with low concentrations of viral inoculum, but tended to be ineffective when high concentrations of viral inoculum were present. Duration of treatment proved another important variable, with longer durations (up to 1 hr) increasing the efficacy of disinfection of CGGMV compared to shorter durations (1 or 10 min). Knowledge of the relative efficiency and length of treatment time required with each disinfectant is essential for their effective deployment in efforts to control the spread of CGMMV.

Notes

Temporal-spatial dynamics of tomato yellow leaf curl virus (TYLCV) and its vector in the dry tropics

Dr Julia Cremer
Department of Agriculture and Fisheries
julia.cremer@daf.qld.gov.au

Julia Cremer [1], Paul Campbell [1], Rebecca Roach [1], Visnja Steele [1], Siva Subramaniam [2], Vasanthaverni Sivasubramaniam [2], Chris Monsour [3], Tom Mullins [2], Denis Persley [1] and Cherie Gambley [4]

[1] Department of Agriculture and Fisheries, Ecosciences Precinct, Dutton Park QLD, Australia
[2] Department of Agriculture and Fisheries, 45 Warwick Road, Bowen QLD, Australia
[3] Prospect Agriculture, 34 Kelsey Road, Bowen QLD, Australia
[4] Department of Agriculture and Fisheries, Roessler Avenue, Applethorpe QLD, Australia

In 2011, TYLCV was first detected in tomatoes in Bowen, QLD, the major Australian winter production area. This presented an opportunity for monitoring the establishment, spread and impacts of the virus in the region. Plants were sampled from 2014 to 2017 on multiple blocks across three major producing areas in the district and virus incidence was determined by visual inspection and molecular indexing of 300 plants (bulked) at each block. Populations of Silverleaf whitefly (SLW), the vector for TYLCV, were also monitored for variation between virus levels and the proportion of viruliferous (i.e virus-carrying) SLW present in the crop. A time of infection trial performed under controlled conditions showed that early infection by TYLCV results in greater yield losses. Weed surveys identified several TYLCV host species, including *Solanum americanum* (black nightshade), *Amaranthus viridis* (green amaranth), *Datura stramonium* (thornapple), and *Trianthema portulacastrum* (giant pigweed), which may harbour the virus during non-cropping periods. Testing of seeds from TYLCV-infected plants indicates that the virus is not transmissible by seed, contrary to research by Kil et al, 2016. The widespread adoption of dual TYLCV and TSWV resistant hybrids in Bowen during the surveys allowed for the evaluation of virus incidences on properties where resistant lines were being cultivated alongside susceptible varieties. The prevalence of TYLCV within the district decreased almost 20% since the adoption of resistant varieties. The use of these hybrids appears to have reduced virus levels during the season with potential long-term benefits for diminishing non-crop TYLCV reservoirs due to reduced spread.

Notes

Development of alternative management strategies to control epidemics of zucchini yellow mosaic virus in the Ord River Irrigation Area

Craig Webster
Department of Primary Industries and Regional Development
craig.webster@dpird.wa.gov.au

Craig G. Webster [1], Brenda A. Coutts [1], Mark Warmington [2], Helena O'Dwyer [2], Rebecca Clarke [3], Sonya Broughton [1], Monica A. Kehoe [1] and Roger A.C. Jones (1,4)

[1] Department of Primary Industries and Regional Development, South Perth, 6151, Western Australia.
[2] Department of Primary Industries and Regional Development, Kununurra, 6743, Western Australia.
[3] Raitech Ltd., Kununurra, 6743, Australia.
[4] Institute of Agriculture, University of Western Australia, Crawley 6009, Western Australia.

Production of pumpkin (*Cucurbita moschata* and *Cucurbita maxima*), melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*) in the Ord River Irrigation Area (ORIA), East Kimberley, Western Australia is severely affected by annual epidemics of zucchini yellow mosaic virus (ZYMV) vectored by *Aphis gossypii, A. craccivora* and *A. nerii*. Traditionally, insecticides have been used in the ORIA to reduce aphid numbers and reduce ZYMV spread by aphids. However use of insecticides is ineffective commercially with total yield losses from ZYMV infection of late ORIA cucurbit plantings is still occurring. Replicated field experiments, conducted in the ORIA between 2015 and 2017, were undertaken to evaluate both insecticide applications and alternative agronomic methods of control of ZYMV epidemics, including the use of mineral oil sprays, non-host barriers, and planting into millet stubble groundcover. Appropriate control treatments were included, and the standard ORIA seed and foliar insecticide application practices used. The results indicated that sowing watermelon seeds into millet stubble groundcover significantly delayed (by up-to 4 weeks) both aphid arrival and the subsequent increase in ZYMV incidence. This delay proved sufficient in 2016 to significantly reduce marketable fruit yield losses due to the virus. In contrast, mineral oil applications and local commercial insecticide programs were both ineffective at controlling ZYMV, although insecticides controlled colonising aphids effectively. Sorghum barrier crops planted around the exterior of experiments were also ineffective at supressing ZYMV spread. Results from field experiments and the suitability of such methods for ZYMV control in cucurbit production will be discussed.

Notes

Modernising the terminology for pollen and seed transmission of plant viruses

Samantha Edwards
The New Zealand Institute for Plant & Food Research Limited
Sam.Edwards@plantandfood.co.nz

Samantha Edwards[1][2], Kar Mun Chooi [1], Michael Pearson and Robin MacDiarmid[1][2]

[1] The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand
[2] School of Biological Sciences University of Auckland; Auckland, New Zealand

Successful plant virus transmission ensures the continuation of a specific viral genotype, and the use of pollen and seed by a virus as a method of transmission ensures a continuation of susceptible host exposure. Only 15% of reported viruses are documented as pollen and/or seed transmissible although with continual improvements in molecular techniques and sequencing this number will undoubtedly grow in the years to come. With increased reporting it is essential that the terminology used to describe such viruses is concise, specific and uncomplicated - elements generally lacking in our current plant virology terminology. In addition, the recent identification of virus transmission by pollen to species which are pollen incompatible, introduces the potential for new hosts and new transmission terminology. In light of these factors we suggest for discussion a modernised and explicit terminology for pollen and seed transmission that highlights the biology of interactions in horizontal and vertical plant virus transmission. We will summarise the current mechanisms by which transmission is hypothesised to occur, with special consideration of virus localisation in pollen as a potential tool to predict transmission success and type.

Notes

The effects of preventative insecticide application on primary and secondary virus spread in wheat

Narelle Nancarrow
Agriculture Victoria
narelle.nancarrow@ecodev.vic.gov.au

Narelle Nancarrow[(1)], Mohammad Aftab[(1)], Grant Hollaway[(1)], Angela Freeman[(2)], Brendan Rodoni[(2)] and Piotr Trębicki[(1)]

[(1)] Agriculture Victoria, Horsham Centre, Horsham, VIC, Australia
[(2)] Agriculture Victoria, AgriBio Centre, Bundoora, VIC, Australia

Yellow dwarf viruses (YDV) such as barley yellow dwarf virus (BYDV), cereal yellow dwarf virus (CYDV) and maize yellow dwarf virus (MYDV) form a complex of economically important pathogens which affect cereal crops worldwide, resulting in significant yield and grain quality losses. YDVs are phloem-limited and are solely transmitted by aphids. The most common vector of YDV in Australia is the bird cherry-oat aphid (*Rhopalosiphum padi*). BYDV-PAV is the most abundant YDV in Victoria, Australia, although other YDV species are also commonly found. In 2016, a field trial was conducted in Horsham, Australia to investigate the effects of an insecticide application on the movement of viruliferous aphids and subsequent spread of BYDV-PAV in wheat. The trial consisted of a combination of insecticide and inoculation treatments applied to randomised, replicated field plots. Treated plots were sprayed with insecticide and three days later, wheat plants at the centre of selected plots were inoculated with BYDV-PAV using viruliferous *R. padi*. Before maturity, plants at the centre of each plot were tested for BYDV-PAV using tissue blot immunoassay (TBIA). Plants at varying distances from the centre of each inoculated plot were also tested using TBIA. At maturity, plants were harvested and assessments included plant height, biomass, grain yield and quality. Overall, insecticide application did not reduce primary virus infection but it did reduce secondary spread. The results and conclusions from this study, which include interesting patterns of virus spread as a result of aphid movement, will be presented and discussed.

Notes

Citrus tristeza mild strain and cross-protection in New Zealand citrus orchards

Dr Robin MacDiarmid
Plant & Food Research
robin.macdiarmid@plantandfood.co.nz

Katrin Pechinger [1], Kar Mun Chooi [2], Scott Harper [3] and Robin M MacDiarmid [1][2]

[1] School of Biological Sciences University of Auckland; Auckland, New Zealand
[2] The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand
[3] Clean Plant Center Northwest, Washington State University, USA

Citrus tristeza virus (CTV) is the most destructive disease of citrus worldwide. This graft- and aphid-transmissible closterovirus can cause severe disease, of which stem pitting (SP) represents its most economically important symptom in New Zealand. CTV possesses six phylogenetically distinct strains, named RB, T68, VT, T30, T3 and T36. Mild strain cross-protection (MSCP), a method of protecting plants from infection by a severe virus isolates by deliberate infection with a mild isolate of the virus, was assessed using two putative CTV mild isolates, termed 'Miho isolate' and 'Parent isolate'.MsCP trials set up in two commercial citrus orchards in 2008 (Taipa) and 2009 (Gisborne), comprised three Navel orange 'receptor' cultivars (Fukumoto, Navalina and Newhall) inoculated with either the Miho or the Parent isolate. CTV infecting 'donor' and 'receptor' trees as well as mandarin and Navel orange trees surrounding theMsCP trial plots ('neighbouring' trees) was characterised by quantifying SP and by determining each CTV strain identity via sequencing. The Miho 'donor' tree was found to contain RB, Parent 1 contained a mixture of RB, T68 and VT and Parent 2 contained RB and VT; none of the donor trees exhibited SP symptoms. Miho 'receptor' trees contained RB or T30 and remained largely symptomless. Parent 'receptor' trees contained either VT or a mixture of T68 and VT, and exhibited symptoms ranging from mild to severe SP. 'Neighbouring' trees contained predominantly T3 and remained largely symptomless. CTV symptomology was primarily dependent upon infecting CTV strain not upon the 'receptor' Navel orange cultivar.

Notes

Devastating effect of viruses on a fenugreek crop and first detection in Australia

Dr Piotr Trebicki
Agriculture Victoria, Horsham Centre, Horsham, VIC
piotr.trebicki@ecodev.vic.gov.au

Mohammad Aftab[1], Narelle Nancarrow[1], Angela Freeman[2], Jenny Davidson[3], Brendan Rodoni[2] and Piotr Trebicki[1]

[1] Agriculture Victoria, Horsham Centre, Horsham, VIC, Australia
[2] Agriculture Victoria, AgriBio Centre, Bundoora VIC, Australia
[3] South Australian Research and Development Institute GPO Box 397, Adelaide, Australia

Fenugreek (*Trigonella foenum-graecum*) is a multi-purpose, self-pollinated annual herbaceous cool season legume crop (family Fabaceae). It is cultivated worldwide, its leaves are used as a vegetable or as a herb and seeds are used as spice. A disease outbreak in fenugreek (cv. Sungold) in Mundoora, South Australia in 2016 was inspected, random and diagnostic samples were collected and tested for a range of viruses using tissue blot immunoassay (TBIA). Cucumber mosaic virus (CMV), pea seed-borne mosaic virus (PSbMV) and turnip yellows virus (TuYV) were detected. Selected samples that tested positive for CMV, PSbMV and TuYV with TBIA were also confirmed by RT-PCR. Ninety percent of the 5 hectare crop was dead. Various virus-like symptoms were observed: (i) non-symptomatic plants, (ii) red discoloration, (iii) mild red discoloration, (iv) yellow discoloration and tip necrosis. The level of infection in randomly collected samples reached 90%, 75% and 20% respectively for CMV, TuYV and PSbMV. These three viruses were also detected in self-sown field pea (*Pisum sativum*), lentil (*Lens culinaris*), burr medic (*Medicago polymorpha*) and barrel medic (*Medicago truncatula*) plants present in the fenugreek paddock. Additionally, transmission of TuYV from canola to fenugreek var. Sungold was examined in a glasshouse experiment by inoculating 133 fenugreek seedlings with viruliferous *Myzus persicae*. Four weeks post inoculation, 84% of inoculated plants tested positive for TuYV. This is a first report of fenugreek field infection by CMV, TuYV and PSbMV in Australia.

Notes

Incidence and distribution of viruses in pulse and canola crops: the main drivers behind disease outbreak

Dr Piotr Trebicki
Agriculture Victoria, Horsham Centre, Horsham, VIC
piotr.trebicki@ecodev.vic.gov.au

Mohammad Aftab [1], Narelle Nancarrow [1], Angela Freeman [2], Brendan Rodoni [2] and Piotr Trebicki [1]

[1] Agriculture Victoria, Horsham Centre, Horsham, VIC, Australia
[2] Agriculture Victoria, AgriBio Centre, Bundoora, VIC Australia

Viruses are a serious threat to pulse and canola production in Victoria and the rest of Australia. Pulse crops including chickpea, lentil, faba bean, field pea and lupin are frequently infected by alfalfa mosaic virus (AMV), bean yellow mosaic virus (BYMV), cucumber mosaic virus (CMV), pea seed-borne mosaic virus (PSbMV), bean leafroll virus (BLRV) and turnip yellows virus (TuYV), while TuYV is the most important virus infecting canola in Victoria. To understand the incidence and the extent of virus infection, pulse and canola crops from three geographic regions in Victoria (Mallee, Wimmera and South West) were randomly sampled and tested for the presence of viruses using tissue blot immunoassay. Spatial and temporal virus incidence varied across the regions and crop types. As virus epidemiology can be greatly affected by different climatic conditions, crop type and vector populations, this study, which targeted distinct regions based on temperature and rainfall gradients, will help us understand factors triggering disease outbreaks. Virus incidence data and its interpretation will be presented and implications for virus epidemiology discussed.

Notes

Exploring the biology of *Grapevine leafroll-associated virus 3*

Prof. Rodrigo P.P. Almeida
University of California
rodrigoalmeida@berkeley.edu

Rodrigo P.P. Almeida

Department of Environmental Science, Policy and Management University of California, Berkeley, CA 94720-3114

Grapevine leafroll-associated virus 3 (GLRaV-3) is considered the most important grapevine-infecting virus. Despite its economic relevance, the biology of this vector-transmitted virus remains poorly understood. We have been using an array of approaches to attempt to shed light into GLRaV-3-host-vector interactions. The role of virus infection on symptom development and fruit quality in field conditions will be discussed, as well as aspects of insect-virus interactions. Finally, the biology of GLRaV-3 infection of *Nicotiana benthamiana* for which we have data will be presented.

Notes

Retention and transmission of Grapevine leafroll-associated virus 3 by *Pseudococcus calceolariae*

Dr Kar Mun Chooi
The New Zealand Institute for Plant & Food Research Limited
karmun.chooi@plantandfood.co.nz

Brogan McGreal[1], Manoharie Sandanayaka[2], Kar Mun Chooi[2] and Robin MacDiarmid[1][2]

[1] School of Biological Sciences University of Auckland; Auckland, New Zealand
[2] The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand

The citrophilus mealybug, *Pseudococcus calceolariae*, transmits grapevine leafroll-associated virus 3 (GLRaV-3), an economically significant virus of grapevines. A previous study has shown no GLRaV-3 spread in an organic vineyard with high mealybug populations. To test the hypothesis that viruliferous mealybugs feeding on non-virus hosts such as groundcover plants lose GLRaV-3 therefore disrupting virus transmission, we first determined the retention and transmission of GLRaV-3 by *P. calceolariae* after access to white clover. Following a 5-day acquisition access period (AAP), *P. calceolariae* first instars retained GLRaV-3 for 4 days when fed on either an alternate host or GLRaV-3-free grape plants. In addition, GLRaV-3 was retained in second instar mealybugs and exuviae (cast-off exoskeleton of the first instar) after feeding on GLRaV-3 positive grapevine leaves shortly before moult. Furthermore, GLRaV-3 was still transmitted efficiently (40–60%) by *P. calceolariae* nymphs after access to white clover for up to 11 days post AAP; 90% transmission was achieved when no alternative host feeding was provided. The 11-day retention period, followed by transmission of GLRaV-3 to grapevines, is the longest retention period observed in mealybugs vectoring GLRaV-3 to date. Our results suggest that groundcover plants may act as a virus sink only if mealybugs settle and feed on them for more than 11 days. Furthermore, the retention of GLRaV-3 after moult suggests GLRaV-3 is transmitted in a circulative manner; further experiments are required to verify this finding.

Notes

Biosecurity in the European context : a multi-stakeholder management

Dr Pascal Gentit
ANSES - LSV
pascal.gentit@anses.fr

Marianne Loiseau, Isabelle Renaudin, Pascaline Cousseau-Suhard, Pierre-Marie Lucas, Aurélie Forveille and Pascal Gentit

ANSES-Laboratoire de la Santé des Végétaux (LSV), 7 rue Jean Dixméras 49044 Angers Cedex 01, France

Harmful plant pests and diseases are a major threat to biodiversity and have increased with the globalization in trade and travel. They can impact food safety, trade, market and the profit and sustainability of plant industries. Nowadays, biosecurity has emerged as major global issue and different approaches exist and might be listed like the one develop by the CRC in Australia. They have an interesting and basic definition of biosecurity that they define as a set of measures designed to protect crops from emergency plant pests at national, regional and individual farm levels. This is true for a situation like Australia, an island. But in Europe, the situation is more complex with almost 14000 kilometers of land borders which lead to an important pressure on its regulation. Safeguarding plant health is one of the European commission priority. The new plant health law will be in force at the end of 2019. It take the risk assessment as the corner stone of this new regulation. We will talk about how this approach is implement and how the different stakeholders are involved.

Notes

Characterizing Melon necrotic spot virus isolates detected in Australia

Dr Fiona Constable
Agriculture Victoria
fiona.constable@ecodev.vic.gov.au

Ellena Higgins[1], Linda Zheng[2], Wycliff Kinoti[2], Len Tesoriero[3], Grant Chambers[4], Brendan Rodoni[2] and Fiona Constable[2]

[1] School of Molecular Sciences, La Trobe University, Melbourne, VIC, Australia
[2] Agriculture Victoria Research Division, Department of Economic Development, Jobs, Transport and Resources, La Trobe University Campus, Bundoora, VIC, Australia
[3] Central Coast Primary Industries Centre, NSW Department of Primary Industries,Gosford NSW Australia
[4] Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW, Australia

The species *Melon necrotic spot virus* (MNSV) belongs to the genus *Gammacarmovirus* in the family *Tombusviridae*. It is transmitted by the fungus *Olpidum bornavanus* and can cause up to 60% loss in yield. MNSV is a quarantine pathogen of Australia, but there have been at least two incursions of MNSV in Australian watermelon and rockmelon crops since 2012. A Victorian outbreak in 2014 resulted in the complete rejection of rockmelon fruit at market due loss of quality at a cost to the grower of AU$2million. Seed is a pathway for introduction of MNSV into Australia and it has been detected in imported melon seeds. However theMsNV isolate from the Victorian rockmelon outbreak could not be detected in the seed lot used to produce the affected crop. In this study the genome sequences of six Australian MNSV isolates, which were detected during field outbreaks and in imported seed, were obtained using Sanger and next generation sequencing. A comparative bioinformatics analysis indicated that there were two distinct clusters of MNSV strains amongst the field isolates, suggesting that there were no links between the watermelon and rockmelon incursions. There was no link betweenMsNV isolates from any outbreak and the contaminated seed tested in this study, indicating that previously imported and untested seed from a different source or origin is likely to have caused the outbreaks in 2012 and 2014.

Notes

Seed testing in NZ – mitigating biosecurity risks

Dr Catia Delmiglio
Ministry for Primary Industries
catia.delmiglio@mpi.govt.nz

Catia Delmiglio[1], Lisa Ward[1] and Rose Souza Richards[2]

[1] Plant Health & Environment Laboratory, Ministry for Primary Industries, Auckland, New Zealand.
[2] Import & Export - Plants Group, Ministry for Primary Industries, Wellington, New Zealand

The emergence of new pests and diseases means that outbreaks are frequent, which increases the biosecurity risk for New Zealand (NZ). All consignments of seeds for sowing need to meet strict import requirements to prevent the entry of unwanted or new organisms into NZ, which if established, may damage agricultural or horticultural production, forestry, tourism, and affect trade in international markets. The Ministry for Primary Industries is continually reviewing emerging risks from seed and as a result, frequently updating biosecurity measures to mitigate those of high risk. In response to the biosecurity requirements for seed for sowing, the Plant Health and Environment Laboratory have developed methods to test seeds of Solanaceae, Cucurbitaceae, Apiaceae, and *Zea* species for viruses, viroids and liberibacters. The work presented gives an overview of the seed testing methods developed for NZ, and discusses the complexities and challenges encountered during method development.

Notes

The progress of kiwifruit virus research in China

Ms Shaohua Wen
Huazhong Agricultural University
whni@mail.hzau.edu.cn

Shaohua Wen, Yazhou Zheng, Xianxiang Wang, Chenxi Zhu, Zuokun Yang, Guoping Wang and Ni Hong

College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China

China is the site of origin of *Actinidia* spp. and is the largest commercial kiwifruit producer in the world, with *Actinidia chinensis* and *A. deliciosa* being the most widely cultivated species. Historically, virus-like diseases of kiwifruit have been observed but their causal agents have remained unknown. Since 2013, our group has carried out an extensive field investigation of virus and virus-like diseases in kiwifruit. We found that viral disease-like symptoms are very common, including chlorotic spots and ringspots on the leaves, and / or vein yellowing that are sometimes accompanied by leaf distortions. Using conventional RT-PCR complemented with next generation sequencing techniques for small RNAs and total RNAs, we identified eight viruses from kiwifruit plants grown in China. The four viruses *Actinidia virus A* (AcVA), *Actinidia virus B* (AcVB), *Citrus leaf blotch virus* (CLBV) and *Apple stem grooving virus* (ASGV), which have been reported previously in New Zealand and Italy as infecting kiwifruit, are also common in kiwifruit grown in China. A novel emaravirus, *Actinidia chlorotic ringspot-associated emaravirus* (AcCRaV), the first emaravirus reported infecting kiwifruit, has been identified in several *Actinidia* species sourced from several Chinese provinces. Two tospoviruses, tomato zonate spot virus (TZSV) and tomato necrotic spot associated virus (TNSaV), are newly identified viruses infecting kiwifruit in Yunnan and Guizhou provinces. Additionally, a candidate novel virus belonging to the *Closteroviridae* is molecularly characterized, and its genome is about 18900 nt and contains 12 ORFs. These results provide important information for kiwifruit viral disease control.

Notes

Sweet potato virus C and Sweet potato feathery mottle virus from Australian and East Timorese Sweet Potato Samples: Biology and Phylogenetics

Mr Solomon Maina
The University of Western Australia
solomon.maina@research.uwa.edu.au

S. Maina [1,2,3], M.J. Barbetti [1,2,3], O. Edwards (3,4), L. de Almeida [5], A. Ximenes [6], and R.A.C. Jones [2,3,7]

[1] School of Agriculture and Environment, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.
[2] UWA Institute of Agriculture, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia,
[3] Cooperative Research Centre for Plant Biosecurity, Canberra, ACT 2617, Australia.
[4] CSIRO Land and Water, Floreat Park, WA 6014, Australia.
[5] Seeds of Life Project, Ministry Agriculture and Fisheries, PO Box 221, Dili, East Timor.
[6] DNQB-Plant Quarantine, International Airport Nicolau Lobato Comoro, Dili, East Timor.
[7] Department of Agriculture and Food Western Australia, 3 Baron-Hay Court, South Perth

Sweet potato virus C (SPVC) and Sweet potato feathery mottle virus (SPFMV) isolates from sweet potato were examined for genetic connectivity between Australian and Southeast Asian viruses. East Timorese samples were on FTA cards. Australian shoot and tuberous root samples were planted in the glasshouse and scions graft-inoculated to *Ipomoea setosa* plants. Symptoms were recorded in infected sweet potato and *I. setosa* plants. RNA extracts from FTA cards and *I. setosa* leaf samples were subjected to high throughput sequencing, and complete genomic sequences (CS) of SPVC or SPFMV obtained (11 each). These sequences and their coat protein (CP) genes were compared with GenBank sequences. SPFMV clustered into phylogroups A and B (= RC(III)), and EA(I) and O(II)) within A. East Timorese sequences were in EA(I) and O(II), whereas Australian sequences were in O(II) and B(RC(III)). With SPVC, the CP phylogram was sufficiently diverse to distinguish phylogroups: A and B and 6 minor phylogroups within A (I-VI). East Timorese sequences were in I, whereas Australian sequences were in II, VI, and B. With SPFMV, Aus13B grouped with East Timorese sequence TM64B giving nt identities of 97.4% (CS) and 98.3% (CP), respectively, but the closest match was the 97.6% (CS) and 98.7% (CP) nt identity between Aus13B and an Argentinian sequence. With SPVC, the closest Australian match was the 99.1% (CP) between Aus4C and a New Zealand sequence, but the closest match between Australian and East Timorese sequences were 94.1% with Aus6a and TM68A (CS), and 96.3% with Aus55-4C and TM64A (CP).

Notes

Initial analysis of the draft genome of *Candidatus* Liberibacter ctenarytaina

Dr Grant Smith
New Zealand Institute for Plant and Food Research, Lincoln
grant.smith@plantandfood.co.nz

Sarah Thompson[1][2], Jacqueline Morris[2][3][4], Charles David[1], Rachel Mann[2][3][4], Brendan Rodoni[2][3][4] and Grant Smith[1][2]

[1] The New Zealand Institute for Plant and Food Research Limited, Lincoln 7608, New Zealand
[2] Plant Biosecurity Cooperative Research Centre, Bruce, Australian Capital Territory 2617, Australia
[3] La Trobe University, AgriBio, Bundoora, Victoria 3083, Australia
[4] Agriculture Victoria, AgriBio, Bundoora, Victoria 3083, Australia

Ctenarytaina fuchsiae (Aphalaridae), a native New Zealand psyllid, is found on Kotukutuku, *Fuchsia excorticata*, the world's largest fuchsia and an important nectar source for birds including the native Tui and Bellbird. A number of *Candidatus* Liberibacter species have been found associated with, or vectored by, psyllid species in the Psyllidae or Triozidae families, including the Asian citrus psyllid, *Diaphorina citri* and the tomato-potato psyllid, *Bactericera cockerelli*. Liberibacter 16S rRNA sequences were found in *C. fuchsiae* psyllids collected as part of a wider collection of psyllids in New Zealand. Illumina HiSeq2000 metagenomic sequences obtained from a single psyllid collected near Oxford in the South Island were *de novo* assembled using SPAdes. Liberibacter contigs were identified based on homology to the '*Ca*. L. solanacearum' ZC1 genome to produce a five contig 1.184 MB draft genome (excluding prophage regions). Initial phylogenetic analysis revealed that these sequences represented a distinct and unique lineage and '*Candidatus* Liberibacter ctenarytaina' was proposed as the specific name. This is the first Liberibacter species found to be associated with a psyllid species in the Aphalaridae family. Both ANI (average nucleotide identity) and AAI (average amino acid identity) analysis confirmed that '*Ca*. L. ctenarytaina' is a unique species in the Liberibacter clade. The assembled genome has a GC content of 36.3% with significant differences in locally collinear block synteny and orientation when compared with other Ca. Liberibacter genomes. An initial analysis of this bacterial genome will be discussed in the context of this emerging clade of putative plant pathogens.

Notes

Comparative genomics of the Liberibacter genus

Jacqueline Morris
La Trobe University and Agriculture Victoria
jacqui.morris@ecodev.vic.gov.au

Jacqueline Morris [1][2][3], Rachel Mann [1][3] , Rebekah Frampton [1][4], Mallik Malipatil [2][3], Brendan Rodoni [1][2][3] and Grant Smith [1][4]

[1] Plant Biosecurity Cooperative Research Centre, LPO Box 5012, Bruce, Australian Capital Territory, Australia, 2617
[2] Applied Systems Biology, La Trobe University, AgriBio, 5 Ring Road, Bundoora, Victoria, Australia, 3083
[3] Agriculture Victoria Research, Agriculture Victoria, AgriBio, 5 Ring Road, Bundoora, Victoria, Australia, 3083
[4] The Zealand Institute for Plant & Food Research Limited, Gerald St, Lincoln, New Zealand, 7608

Psyllids can vector species of *Candidatus* Liberibacter that pose serious threats to agricultural crops in the Solanaceae, Apiaceae and Rutaceae families, impacting production and trade when an incursion occurs. This predominantly unculturable bacterial genus now consists of nine phytopathogenic and endophytic species; *Liberibacter crescens* (Lcr), '*Candidatus* Liberibacter africanus' (CLaf), '*Ca*. Liberibacter americanus' (CLam), '*Ca*. Liberibacter asiaticus' (CLas), '*Ca*. Liberibacter caribbeanus' (CLca), '*Ca*. Liberibacter solanacearum' (CLso, synonymous to '*Ca*. Liberibacter psyllaurous', CLps), '*Ca*. Liberibacter europaeus' (CLeu), '*Ca*. Liberibacter brunswickensis' (CLbr) and '*Ca*. Liberibacter ctenarytainae' (CLct). Advancing technologies have allowed the increase of genetic information for the Liberibacter genus. Using new genetic data, a core-genome and pan-genome analysis has been conducted on this relatively undescribed genus. This will be the largest comparative genomics analyses performed for the genus and includes complete or high quality draft genomes. Understanding the genomic differences and similarities between species can provide insights into pathogenicity, host and vector specificity. In addition, this can inform both biosecurity preparedness and response management of Liberibacter-associated diseases.

Notes

The virome of street flowers in Nannup, a small town in Western Australia

Dr Steve Wylie
Murdoch University
s.wylie@murdoch.edu.au

Steve Wylie, Shu Hui Koh, Thao Thi Tran, Duy Quang Nguyen, Hua Li

Plant Virology - Plant Biotechnology Research Group, Western Australian Stage Agricultural Biotechnology Centre, Murdoch University, WA 6150, Australia

Nannup is a small mill town in southern Western Australia. It is located in Australia's global biodiversity hotspot. Nannup shire imports flower bulbs every year and these are planted in dense plots along the main road and in parks to beautify the town and attract visitors. Leaf samples collected from symptomatic leaves were analysed for RNA viruses. The complete or almost complete genomes of 10 virus isolates of nine species were identified. We discuss this finding and possible biosecurity implications.

Notes

Identification of a new rose virus by NGS

Dr Lisa Ward
Plant Health and Environment Laboratory, MPI, New Zealand
lisa.ward@mpi.govt.nz

Joe Tang, Lia Liefting, Stella Veerakone and Lisa Ward

Plant Health and Environment Laboratory, Ministry for Primary Industries, Auckland 1140, New Zealand.

Rose (*Rosa* spp.) is one of the most popular flowers with huge economic value worldwide. To date, about 20 viruses have been reported in rose. In December 2016, plants of *Rosa rubiginosa* showing leaf chlorotic patterns and stunting were observed on farmland in Central Otago, NZ. Symptomatic samples were submitted to the Plant Health and Environment Laboratory (PHEL) but none of the known viruses were detected using traditional diagnostic methods. Next generation sequencing (NGS) was used to test one symptomatic sample. A single contig containing 9,128 nucleotides was identified to be of viral origin following a BLAST search. Phylogenetic analysis of the sequence suggests this is a novel virus species within the family *Secoviridae*. The sequence obtained showed up to 33% amino acid identity to several other virus species within the *Secoviridae* family, with the closest match to *Bellflower vein chlorosis virus* and *Maize chlorotic dwarf virus* (both *Waikavirus*). Waikaviruses are transmitted by leafhoppers or aphids in nature, but cannot be transmitted by seed or mechanical means. Further work is needed to determine if this novel rose virus is a member of *Waikavirus* or represents a new genus. Using PCR primers designed to the NGS derived sequence, samples from a previous rose survey were tested for the presence of the new virus. Eleven of the 91 survey samples tested positive. The majority of the infected samples were from the South Island but two were from the North Island suggesting that this virus is established and widely distributed in New Zealand.

Notes

Plant viruses and their vectors at the border

David Teulon
Better Border Biosecurity (B3)
david.teulon@plantandfood.co.nz

David Teulon[1]

[1] Better Border Biosecurity, C\- New Zealand Institute for Plant & Food Research Ltd, Private Bag 4704, Christchurch, New Zealand

Plants in New Zealand and Australian natural and productive systems face continual threats from invasive alien species (invertebrates, pathogens, weeds), including plant pathogenic viruses. Perceived and actual threats are growing ominously due to increased trade and tourism and climate change. With a focus on New Zealand's biosecurity system, this presentation will explore the invasion of valued plant systems by plant viruses and their vectors, including their cumulative establishment over time, their economic, environmental, social and cultural impact, and contrast this with other invasive pathogens and insects. This will be placed in the context of current and new initiatives by government, industry and research institutions to reduce the rate of establishment and impact of plant pathogenic viruses and their vectors. Finally, it will explore some of the biosecurity challenges facing regulators and scientists, including plant virologists.

Notes

Viruses infecting *Clivia miniata* in New Zealand

Mr Joe Tang
Plant Health and Environment Laboratory, MPI, New Zealand
joe.tang@mpi.govt.nz

Joe Tang, Stella Veerakone and Lisa Ward

Plant Health and Environment Laboratory, Ministry for Primary Industries, Auckland 1140, New Zealand.

In New Zealand, a number of viruses, mainly from *Carlavirus*, *Potexvirus* and *Potyvirus* groups, have been found infecting some ornamental plant genera in the family Amaryllidaceae such as *Agapanthus*, *Amaryllis*, *Hippeastrum*, *Narcissus* and *Nerine* (Veerakone et al, 2015). *Clivia*, which is a genus in the Amaryllidaceae, has six recognized species and numerous interspecific hybrids. In New Zealand, virus-like symptoms have been noticed on *Clivia* by growers, however, no viruses have ever been reported in this genus anywhere in the world. Here, we report, for the first time, the identification of threes potyviruses, *Narcissus late season yellows virus* (NLSYV), *Narcissus yellow stripe virus* (NYSV), Snowdrop virus Y (SnVY) and one carlavirus, *Nerine latent virus* (NeLV), from *C. miniata* which is the most commonly grown species in New Zealand. Furthermore, a preliminary survey on 98 symptomatic and asymptomatic clivia samples revealed a strong correlation between symptoms and presence of a virus or multiple viruses. Among 66 symptomatic samples, 55 (83.3%) were found to be infected by NeLV, while the incidences of NLSYV, SnYV and NYSV were 13.6% (9 samples), 4.5% (3 samples) and 3.0% (2 samples), respectively.

Notes

Detection and genetic diversity of alfalfa dwarf disease-associated viruses in lucerne pastures in Argentina and Australia

Prof Ralf Dietzgen
The University of Queensland
r.dietzgen@uq.edu.au

Samira Samarfard[1], Nicolas E. Bejerman[2], Murray Sharman[3] and Ralf G. Dietzgen[1]

[1] Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St. Lucia, QLD 4072, Australia
[2] Instituto de Patología Vegetal, Centro de Investigaciones Agropecuarias, Instituto Nacional de Tecnología Agropecuaria, Córdoba X5020ICA, Argentina
[3] Department of Agriculture and Fisheries Queensland, Ecosciences Precinct, Dutton Park, QLD 4102, Australia

In 2010 a devastating disease of lucerne, named alfalfa dwarf disease (ADD) occurred in commercial fields in Argentina causing reduced seed yield. Next generation sequencing of ADD-infected lucerne plants in Argentina revealed the presence of a new cytorhabdovirus, alfalfa dwarf virus (ADV), the (+) RNA viruses, alfalfa mosaic virus (AMV), bean leafroll virus (BLRV) and alfalfa enamovirus 1 (AEV-1) and the DNA virus alfalfa leaf curl virus (ALCV). Due to the economic impact of ADD in Argentina and its potential threat to the Australian lucerne industry, we developed a set of diagnostic assays to determine the potential presence of ADD-associated viruses in Australia. In this study, lucerne leaves with virus-like symptoms were collected from seed crops in South Australia and fodder crops in Victoria and Queensland. A sensitive duplex RT-PCR was developed for detection of ADV and AMV. Similarly, the presence of BLRV and AEV-1 was determined by duplex RT-PCR and ALCV by PCR. Neither ADV, AEV-1 nor ALCV were detected in any lucerne samples we tested from 2015-17. However, AMV and BLRV were detected in some samples, with a high incidence of AMV in symptomatic seed production paddocks. ADV genetic diversity across Argentine lucerne regions was 1% or less indicating it may have arrived recently. Genetic diversity of AMV and BLRV in Australia was 2% or less, and isolates in both countries are also very similar. Ongoing epidemiological studies in Argentina will assist in the development of a biosecurity plan to prevent ADD from occurring in Australia.

Notes

Cucumber green mottle mosaic virus in Australia – current situation

Dr Fiona Constable
Northern Territory Dept of Primary Industry and Resources
fiona.constable@ecodev.vic.gov.au

Tran-Nguyen, L.T.T. [1], Lovelock, D.A. [1] , Constable, F.E. [2], Persley, D [3], Campbell, P [3], Finlay-Doney, M. [1], Coutts, B [4], Webster, C [4] and Dombrovsky, A [5]

[1] Department of Primary Industry and Resources, Northern Territory, Australia
[2] Department of Economic Development, Jobs, Transport and Resources, Victoria, Australia
[3] Department of Agriculture & Fisheries, Queensland, Australia
[4] Department of Primary Industries and Regional Development, Western Australia
[5] Department of Plant Pathology and Weed Science, Agricultural Research Organization, The Volcani Center, Israel

Cucumber green mottle mosaic virus (CGMMV) is a *Tobamovirus* that can infect cucurbit plants and is responsible for significant economic losses worldwide. CGMMV was first detected in Australia in the Northern Territory (NT) in 2014 and subsequently in Queensland (QLD) in 2015, Western Australia (WA) in 2016 and South Australia (SA) in 2017. In the NT, the virus was initially detected in commercial watermelons expressing severe mosaic and stunted foliar development. Additional infected cucurbit hosts included pumpkin, Asian cucurbit vegetables, squash and cucumber. CGMMV was also detected in weeds found near cucurbit production areas. These weed hosts included but are not limited to *Portulaca oleracea, Amaranthus viridus, Physalis minima, Solanum nigrum, Chenopodium album* and *Urochloa mosambicensis*. As a part of the biosecurity response, honey bee and hive products were also sampled and tested for the presence of CGMMV. Viable and infective CGMMV was identified from adult bee, pollen and honey. In early 2016, quarantine measures were lifted in the NT under a national CGMMV management program to allow market access and the introduction of auditable on-farm biosecurity protocols. Presently, CGMMV is endemic in the NT and WA and efforts are still underway in QLD and SA to contain CGMMV to infested properties. Research is being conducted in five key areas: 1) understanding the persistence of CGMMV in contaminated soils, 2) identifying alternative host range and CGMMV non-hosts, 3) identifying the role of honey bees in CGMMV epidemiology, 4) improving in-field diagnostics, and 5) developing extension activities including on-farm biosecurity protocols to develop management options for CGMMV. Findings from this national CGMMV project will be discussed.

Notes

Novel ampeloviruses from banana in south-east Asia

Kathy Crew
Queensland Department of Agriculture and Fisheries
kathy.crew@daf.qld.gov.au

Kathy Crew[1], Sébastien Massart[2], Maroua Hanafi[2], François Maclot[2], Matthew Webb[1], Andrew Geering[3], Benham Lockhart[4], Ines Van denhouwe[5], Nicolas Roux[6], Agus Sutanto[7] and John Thomas[3]

[1] Queensland Department of Agriculture and Fisheries, Ecosciences Precinct, Brisbane, Australia
[2] Laboratory of Integrated and Urban Phytopathology, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium
[3] The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Ecosciences Precinct, Brisbane, Australia
[4] Department of Plant Pathology, University of Minnesota, St. Paul, USA
[5] Bioversity International Transit Centre, KU Leuven, Heverlee, Belgium
[6] Bioversity International, Montpellier, France
[7] Indonesian Tropical Fruit Research Insitute, Solok, Sumatra, Indonesia

During indexing of banana germplasm, electron microscope (EM) examination revealed *Closteroviridae*-like particles in five accessions from south-east Asia. Samples were tested using degenerate PCR primers for three *Closteroviridae* genera. Closterovirus and crinivirus primers failed to give amplicons, but ampelovirus primers targeting the conserved HSP70 region allowed amplification of 580 bp products from two samples (Q6191 from Vietnam and Q6196 from Indonesia). Fragments were cloned and sequenced and the isolates shown to be 96.6-98.3% identical. Both isolates were 52% identical to the ampelovirus *Plum bark necrosis stem pitting-associated virus* (PBNSPaV; GenBank ID AIH06908), the best database match using BLASTp. A diversity of ampeloviruses or strains was suggested among the various accessions, as particles were differentially decorated with antiserum to the tentative ampelovirus Sugarcane mild mosaic virus (SMMV) in EM decoration tests. Moderate, little or no decoration was displayed by individual particles from the banana accessions, while SMMV particles were heavily decorated. Isolate Q6196 was analysed by NGS to seek further genomic sequence. Two complete ampeolovirus genomes were assembled. Virus 1 (total genome 13,239 nt) and virus 2 (13,224 nt) both have 8 ORFs. At the amino acid level, individual ORFs (1a, 1b/RdRp, 3/HSP70, 4/p61, 5/CP, 6) share 31-58% identity with each other, and both share 32-50% identity with PBNSPaV isolates, the nearest BLASTp matches, across the same ORFs. Partial sequence from Q6196 and other accessions implies the existence of further ampelovirus diversity. This work describes the first record of ampeloviruses from banana. Further research is needed to establish the symptomatology, vector (presumed to be mealybugs), and distribution of the viruses.

Notes

Papaya meleira virus comes to Australia

Dr Paul Campbell
DAF Queensland
paul.campbell@daf.qld.gov.au

Paul Campbell and Cherie Gambley

Department of Agriculture and Fisheries, 41 Boggo Road, Dutton Park, Queensland, Australia.

Papaya from Australian commercial production were submitted with weeping latex in 2014, suspected of papaya meleira virus (PMeV), a seed transmissible isometric virus first reported from Brazil in 1989. The virus accumulates in the lactifers of the plant, changing the structure of the latex particles, and increasing the pressure which causes the spontaneous exudation of the latex on the developing fruit. Initial characterisation of the dsRNA genome of the virus placed it as a novel virus, with the closest relatives similar to the fungus infecting Totiviruses. The disease was subsequently reported from Mexico in 2012, but the virus fragment recovered had no relation to the Brazilian strain. Australian fruit tested negative with the currently published diagnostic tests from Brazil and Mexico for PMeV, but dsRNA was found in the latex of the affected fruit indicating the presence of a virus. In 2016 the causative agent in Brazil was reassigned to an umbra-like virus, similar to the Mexican strain of PMeV, which is trans-encapsidated by the dsRNA virus previously detected. NGS of samples collected from 2014 and 2016 in Australia has detected two strains of an umbra-like virus similar to the Brazilian (69% amino-acid similarity in RDRP), Mexican (70%) and Ecuadorian (69.5%) strains. Outside of the coding region there is little similarity between the strains with only 29% to 42% nucleotide similarity in the ~1.9 kb 3' to the RDRP with no relationship with anything currently in Genbank.

Notes

Discovery of a new Emaravirus in a New Zealand native species

Mr Lee Rabbidge
Auckland University of Technology
lee.rabbidge@gmail.com

Lee Rabbidge[1] , Arnaud Blouin[2] , Colleen Higgins[1][3] and Robin MacDiarmid[2]

[1] AUT Roche Diagnostics Laboratory, School of Science, Auckland University of Technology, New Zealand
[2] The New Zealand Institute for Plant & Food Research Ltd, Auckland, New Zealand
[3] Institute of Applied Ecology, Auckland University of Technology, New Zealand

Emaravirus is a genus of viruses where the members' genomes are linear, negative sense, single stranded RNA. They are also multipartite, with genome segments contained within a single capsid. These viruses are likely transmitted by mites. Native trees growing on the Plant and Food Research site in Mt Albert, Auckland, were found to be displaying virus-like symptoms. Investigation of these plants by RNA-seq identified the presence of several sequences that appear to be segments of an Emaravirus-like genome. This putative Emaravirus is new to science, and importantly, present in a native species that has traditional importance for Maori. We will report our analysis of these sequences to date.

Notes

Notes

Thank you to our conference sponsors

SCIENCEEVENTS.CO.NZ/APVW