



8th Australasian Plant Virology Workshop

Lake Okataina
Rotorua
New Zealand

19-22 November 200



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Sponsored by



Welcome

Haere mai, haere mai, haere mai.

Welcome to New Zealand fellow plant virologists and researchers of virus-like organisms.

Welcome to this place of laughter, Lake Okataina.

Welcome to the Okataina Lodge which is our home together over the next few days.

The 8th Australasian Plant Virology Workshop is the first time we have met in NZ. We will have a fun and intellectually stimulating time here together. It is surprisingly thrilling to focus on plant viruses and virus-like organisms for several days with like minded people. We hope that you enjoy the environment here; the place, the science and the people.

This 8th Australasian Plant Virology Workshop provides an opportunity to formalise our relationship with our parent organisation, the Australasian Plant Pathology Society (APPS). A motion will be put forward to become a Special Interest Group of APPS so that we can take advantage of the legal umbrella of APPS for safety and financial matters, and to formally access a financial float from APPS for future Plant Virology Workshops. We would not be obliged to change the way we operate or to charge fees for membership. If we decide to become a special interest group we would call for a small team to represent the Plant Virology Group, formalize the relationship, and establish a website on the APPS homepage.

A special thanks to several organisations for their support.

- Ministry of Agriculture and Forestry (MAF) Biosecurity New Zealand (www.biosecurity.govt.nz) for sponsoring Rene van der Vlugt and Ricardo Flores
- The University of Auckland, School of Biological Sciences (www.sbs.auckland.ac.nz) for sponsoring Marilyn Roossinck to give the R.E.F. Matthews' Memorial Lecture
- The Bio-Protection Research Centre (www.bioprotection.org.nz) for providing financial assistance and prizes to students
- HortResearch (www.hortresearch.co.nz) for providing the support to organise this workshop
- Okataina Lodge (www.okatainalodge.co.nz) for hosting the workshop

The satchels were kindly gifted by CRC Biosecurity (www.crcplantbiosecurity.com.au) and have been adorned with kiwi icons made by two Auckland-based companies; badges custom-made by RedQueen (<http://www.redqueen.co.nz>), and pendants made by The Greenstone factory (Reagent Manufacturing, Kingsland, Auckland).

Thanks especially to Leonie Osborne for compiling the ever challenging registrations and to Frances Campbell for the organising the financial transactions. Thanks also to Elaine Chan and Sonia Lilly for providing chauffer services to and from Rotorua airport.

Enjoy the workshop!

The organisers

Robin MacDiarmid (HortResearch)

Mike Pearson (The University of Auckland)

Francisco Ochoa-Corona (Oklahoma State University and formally MAF Biosecurity)

Programme

Date	Activity	Time	Speaker	Title
Wednesday 19 November	Welcome and dinner	6pm		
	Opening of Workshop	7:30- 7:45pm		
	R.E.F. Matthews' Memorial Lecture Chair: Mike Pearson	7:45- 8:45 pm	Marilyn Roossinck	Virus evolution and ecology - Lessons from biodiversity inventories
	Mixer			
Thurs 20 November	Breakfast	7:30-9:00		
	New Diagnostic Methods Chair: Mai Hlaing Loh	9:00	Rene van der Vlugt	Plant virus diagnostics: something old, something new, something borrowed..... An overview
		10:00	Francisco Ochoa-Corona	Primer design: How we learn and do it in Australia and New Zealand
		10:20	Ting Wei	Detection of nepovirus subgroups A and B using primers with 5' flaps
		10:40	COFFEE	
	Chair: Murray Sharman	11:00	Linda Zheng	A pair of degenerate primers for potyvirus detection: from design to application
		11:20	Anastasija Chomic	Molecular Detection of the <i>Luteoviridae</i>
		11:40	Louise Winder	Detection of <i>Plum pox virus</i> using high resolution melt analysis
		12:00	John MacKay	Detection of grape viruses by multiplex real-time RT-PCR
		12:20	Sheila Mortimer-Jones	Development and validation of a high throughput, one-step,

				quantitative real-time RT-PCR assay for the simultaneous detection of PLRV, PVX, PVS and TSWV with a rapid RNA extraction method directly from bulked potato tuber samples
		12:40	Fiona Constable	Development and validation of diagnostic protocols for the detection of endemic and exotic pathogens of grapevines
		1:00	LUNCH	
	New tools and technologies for virus research Chair: Colleen Higgins	2:00	Arnaud Blouin	Generic method to identify plant viruses by mass spectrometry of their coat proteins
		2:20	Dave Greenwood	Tandem mass spectrometry as a tool for the identification of new virus infections in plants
		2:40	Robin MacDiarmid	siRNA sequencing for virus identification
		3:00	Mike Pearson	Can mycoviruses be used for the biocontrol of the plant pathogenic fungus <i>Botrytis cinerea</i> ?
		3:20	Barbara Boine	Molecular tools for studying the interaction between <i>Botrytis</i> and the viruses BVX and BCVF
		3:40	COFFEE	
	Virus-Like Organisms	4:00	Ricardo Flores	Viroids and viroid-host interactions
	Chair: Fiona Constable	5:00	Mark Andersen	Whole genome sequence and annotation of Candidatus <i>Phytoplasma australiense</i>
		5:20	Muhammad Saqib	Identification of phytoplasma that cause diseases of diverse plants in three isolated regions in Western Australia
		5:40	Lia Liefting	Liberibacter in New Zealand
		6:00		
	Kiwi Roast	7:30pm		
	Quiz night	After dinner	Restaurant	

Fri 21 November				
Breakfast		7:30-9:00		
	Virus biosecurity, quarantine and emerging threats Chair: Ros Lister	9:00	Murray Sharman	Distribution in Australia and seed transmission of <i>Tobacco streak virus</i> in <i>Parthenium hysterophorus</i>
		9:20	Denis Persely	Alternative hosts of two <i>Tospoviruses</i> in Queensland, Australia
		9:40	Dan Cohen	A simple protocol to obtain high-health grapevines
		10:00	Mai Hlaing Loh	Variant strains of <i>Bean leafroll virus</i> (BLRV); a cause for concern for international BLRV resistant breeding programs
		10:20	COFFEE	
	Chair: Benedicte Lebas	10:40	Brenda Coutts	Studies on the epidemiology of <i>Zucchini yellow mosaic virus</i> in Western Australia: patterns of spread, virus-tolerant cultivars, alternative hosts, and lack of seed transmission
		11:00	Kathy Parmenter	Viruses associated with rhubarb decline disease
		11:20	Paul Guy	Incidence and spread of viruses in white clover pastures: South Island, New Zealand
		11:40	Roger Jones	Epidemiology of <i>Wheat streak mosaic virus</i> in Australia
		12:00		
		12:20	LUNCH	
	Processes and	1:20	John Fletcher	Aims of workshop

	technologies for collections of viruses and virus-like organisms Chair: John Fletcher			
		1:30	John Thomas	Plant virus reference collections - a valuable resource
		1:45	Rene van der Vlugt	Plant virus collections in the Netherlands; their past and future
		2:00	Pooling and development of ideas	
		2:50	COFFEE	
	Plant-virus interactions Chair: Francisco Ochoa-Corona	3:10	Fiona Constable	Examining the effects of elevated CO ₂ and temperature on <i>Barley yellow dwarf virus</i> in wheat
		3:30	John Randles	The C-terminus of tomato leaf curl C4 is required for the movement function of this symptom- inducing protein
		3:50	Elaine Chan	Characterisation of plant protein kinase R
		4:10	Paul Guy	Integrated badnaviruses at large in the New Zealand flora
		4:30	Ralf Dietzgen	Towards protein interactome maps for plant rhabdoviruses
		4:50	Muhammad Saqib	Resistance to <i>Subterranean clover mottle virus</i> in <i>Medicago truncatula</i> and genetic mapping of a resistance locus
		5:10		
	Hell's Gate Experience	6:00		
	Supper at Lake Okataina	9:30		

Sat 22 November	Breakfast	7:00-8:30		
	Virus sequences and taxonomy Chair: Mark Andersen	8:30	Lee McMichael	Detection and characterisation of viruses from sweetpotato in Papua New Guinea and Queensland, Australia
		8:50	Julianne Biddle	Genetic diversity of Australian <i>Alfalfa mosaic virus</i> for an environmental risk assessment of genetically modified <i>Alfalfa mosaic virus</i> resistant white clover
		9:10	Scott Harper	The <i>Citrus tristeza virus</i> resistance-breaking strain in New Zealand and the South Pacific
		9:30	Joe Tang	Identification and characterization of <i>Hydrangea chlorotic mottle virus</i>
		9:50	Roger Jones	Phylogenetic analysis of <i>Bean yellow mosaic virus</i> isolates from four continents: relationship between the seven distinct groups found and their natural isolation hosts and geographical origins
		10:10	CLOSING	
			COFFEE	
			Farewell (with packed lunch)	

Posters

Chooi and Pong et al	Sequence variation in <i>Grapevine leafroll-associated virus-3</i> (GLRaV-3) and its affect on virus detectability
Muhammad Saqib	First full length sequence of <i>Bean common mosaic virus</i> from Australia
Zoila Perez-Egusquiza	Survey of viruses infecting <i>Allium</i> crops in New Zealand
Jason Shiller	Molecular detection of viruses in pollen
Benedicte Lebas	New plant viruses identified in New Zealand since 2007
John Fletcher	A survey of <i>Allium</i> diseases in New Zealand
Colleen Higgins	How is Dasheen Mosaic Virus evolving in the short term and long term? Are we witnessing evolution as it is happening?
Mike Pearson	The Effects of Botrytis Virus X on the fungus <i>Botrytis cinerea</i>
Sheila Mortimer-Jones	Diagnostic tools for the seed potato industry

R.E.F. Matthews' Memorial Lecture

Virus Evolution and Ecology—Lessons from Biodiversity Inventories

Marilyn Roossinck

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We have been analyzing RNA viruses from wild plants and fungi in two very different regions: Oklahoman tall grass prairie (low plant diversity); and Costa Rican dry, cloud and rain forest (very high plant diversity). The incidence of viruses differs dramatically between these two study sites, but the patterns of viruses are similar. Almost all of the viruses are only distantly related to known viruses, and most do not induce any obvious symptoms in their hosts. In fungal virus surveys we find evidence of relationships between plant viruses and those of their endophytic fungal symbionts. These studies have broad implications for the ecology of viruses and their hosts, as well as the deeper evolution of plant viruses.

Plant virus diagnostics: Something old, something new, something borrowed ... an overview

Rene van der Vlugt

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Diagnostics can generally be regarded as the methods available to detect and diagnose a particular pathogen. Plant virus diagnostics – ‘Finding the causal virus and recognising it’ (Bos, 1999) has come a long way since viruses were first recognised as unique disease-inducing entities at the end of the 19th century.

For a long time after their discovery, diagnosis and detection of plant viruses was mainly based on their biological properties like host range and typical symptoms. The development of specific antisera allowed introduction of new techniques like agglutination and later ELISA. Initially developed for research purposes these techniques quickly found their way into ‘the real world’ of plant health diagnostics.

Rapid advances in the field of molecular biology lead to the development of new molecular diagnostics notably hybridization- (‘Dot-blot’) and amplification-based methods (PCR). Initially developed in other areas like clinical diagnostics, these methods were soon adapted to and incorporated in plant health diagnostics, including plant viruses. This continues to date with an ever-increasing pace of development of new techniques developed in many different fields of research.

Many new technologies and platforms are available nowadays. The choice is wide and each new method looks even more promising. Their incorporation in and adaptation to plant virus diagnostics however poses many challenges.

Primer design. How we learn and do it in Australia and New Zealand.

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The selection and design of primer sequences with appropriate priming and thermodynamic characteristics for PCR-based diagnostics was initially achieved by visual means. However, visually searching for specific targets is time consuming and may require multiple rounds of reaction optimization of numerous candidate primer sets to ensure the assay is repeatable. Several primer design software packages have dovetailed with useful bioinformatic tools to speed the development of PCR assays in recent years. However, despite the number of software options available, primer design has remained a difficult area during incursion responses, emergencies and other agricultural biosecurity applications. Two surveys were conducted amongst 44 plant virologists and 21 other plant pathologists, during the 7th Australasian Plant Virology Workshop and the 16th Biennial Australasian Plant Pathology Conference in 2006 and 2007, respectively. The aim was to obtain insights about how primers are designed and how expertise in this area is gained and communicated between scientists. The survey results indicate that 47% of scientists use visual selection, 37% use software and 14% combine both methods. Regarding how the skills are gained, 7% had learnt during undergraduate or graduate education, 14% during postdoctoral research, 28% through colleagues and 22% were self learners. Twenty two per cent had combined more than one way for learning but none had learnt during workshops or conferences. Sixteen scientists self-ranked themselves as experts. The research results will be discussed in the light of the future training required to improve agricultural biosecurity responsiveness in the region.

Detection of nepovirus subgroups A and B using primers with 5' flaps

Ting Wei and Gerard Clover

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Generic PCR protocols were designed to detect nepoviruses in subgroups A and B using degenerate primers which amplified part of the RNA-dependent RNA polymerase (RdRp) gene. The sensitivity and specificity of the PCR protocols was improved by adding a 12-bp non-complementary sequence (flap) to the 5' termini of the forward, but not the reverse, primers. Using optimised PCR protocols for the two subgroups, a specific product (~340 bp and ~250 bp with subgroups A and B, respectively) was amplified from 17 isolates of five virus species in subgroup A and three species in subgroup B. The primers detect conserved protein motifs in the RdRp gene and it is anticipated that they will detect unreported or uncharacterised nepoviruses in the two subgroups.

A pair of degenerate primers for potyvirus detection: from design to application

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With 111 confirmed species and 86 tentative species recognised by the International Committee on Taxonomy of Viruses, the genus *Potyvirus* accounts for more than 10% of all plant viruses. Using potyvirus genomes and their deposition dates in Genbank, a system was developed to identify conserved sequences in the potyvirus genome and assess the stability of the conserved sites based on their sequence variability over time. All of the 17 conserved sites analysed in the study were found to have suffered consensus decay as our knowledge of potyvirus sequences accumulate over time, but the rates of consensus decay varied greatly between sites. The site with the smallest consensus decay is considered the most stable site in the potyvirus genomes and the best site to be targeted by group-specific primers for the detection of potyviruses.

To evaluate this theory, two primers were designed to target the most stable (NIB2F) and the 9th stable conserved site (NIB3R) in the potyvirus genomes. The breadth and specificity of the NIB primer pair was investigated and compared to two routinely used primer pairs in plant virus diagnostic labs. Reactions with the NIB2F and NIB3R primers successfully amplified a cDNA product of 350bp from all 40 virus isolates tested, three of which are potentially novel potyvirus species. It is possible that the NIB primer pair is capable of detecting virus isolates from all major clusters within the genus *Potyvirus*, with results that are better suited for use as a routine diagnostic assay.

Molecular Detection of the *Luteoviridae*

Anastasija Chomic¹, Michael Pearson², John Fletcher³, Gerard Clover⁴, Louise Winder⁵, John Hampton¹, Karen Armstrong¹

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The *Luteoviridae* (luteoviruses) is a family of aphid transmitted RNA viruses which can cause severe losses in economically important crops. Early detection of infection is a key factor in preventing the spread of luteoviral crop diseases in New Zealand. Luteoviruses which are not yet present in the country harbor a potential biosecurity risk and must be identified precisely to stop their spread. Up to now no universal detection and identification test for *Luteoviridae* has been available. Such systems are in demand for diagnostic purposes, especially for post-entry quarantine or border interception.

This project aims to develop a **Simple Detection System** for the *Luteoviridae* family (SDSL) based on amplification of one of the most conserved gene regions. Proof of concept was first delivered in 2003 using three luteovirus species. Current research has since shown that SDSL is able to detect 12 of 14 tried species of the *Luteoviridae*.

The proposed SDSL was tested for its suitability for Melting Curve Analysis (MCA). MCA uses the melting profile of the specific sequence and is far more rapid than direct sequencing as a species identifier. Current research has shown that MCA is able to distinguish most of the luteoviral species. Future studies will test the applicability of the more powerful method – High Resolution Melt, which is far more sensitive than MCA.

Taking into account the results of this research, SDSL offers the realistic and convenient test which is able to detect and identify luteoviral infection and could significantly enhance New Zealand biosecurity diagnostic capability.

Detection of Plum Pox virus using high resolution melt analysis

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Border biosecurity frequently requires the rapid and cost effective identification of many species of viral pathogens in plants. Traditional morphological techniques depend on the assessment of symptoms occurring in the host species, which are frequently cryptic.

Currently, many biosecurity identifications are achieved using DNA techniques, with DNA sequencing being the most popular. However recent developments with quantitative PCR (qPCR) have led to a technique of high resolution melt analysis (HRM). For this analysis, primers are used to produce a PCR amplicon in the presence of a fluorescent dye which becomes located between the strands of the newly formed DNA duplex. Following PCR, the amplicon is incrementally heated until the duplex melts, resulting in the release, and inactivation, of the fluorescent dye molecules. The temperature at which an amplicon melts is characteristic of the nucleotide sequence, and length, of the amplicon. When performed with high resolution, the melt temperature can be used to identify a pathogen.

In the current study, HRM is used to detect Plum Pox virus, a pathogen of importance to New Zealand's biosecurity.

Detection of Grapevine Viruses by Multiplex Real-time RT-PCR

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Over 50 viruses have been described as having grapevine (*Vitis* species) as a host. Of particular interest in New Zealand are the taxa closterovirus and vitivirus. However, even within these two viral taxa, there are more than a dozen distinct species of these viruses with more being classified regularly. As single-stranded RNA viruses, a further complexity is that each species has wide sequence diversity among isolates.

Molecular-based methods are widely-acknowledged as the most sensitive detection methods for these viruses, yet assay design and implementation requires very careful consideration given the sequence diversity described. Real-time RT-PCR has been previously described for the detection of a number of these grapevine viral species but current work in our laboratory has uncovered a number of limitations with some of these current assays.

Here we describe the design and testing of two internally-controlled, multiplex real-time RT-PCR panels for grapevine closteroviruses GLRaV-1, 2, 4, 5, 9, vitiviruses GVA, GVB and GVD as well as the foveavirus; rupestris stem pitting virus. Testing of these multiplex panels (and design of additional panels) is on-going. These - and future - panels will decrease screening time and costs for propagation material, quarantine screening and existing vineyards.

Development and validation of a high throughput, one-step, quantitative real-time RT-PCR assay for the simultaneous detection of PLRV, PVX, PVS and TSWV with a rapid RNA extraction method directly from bulked potato tuber samples

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Potato is important in Western Australia both for domestic food production and export. Four viruses diminish tuber yield locally, *Potato leaf roll virus* (PLRV), *Potato virus X* (PVX), *Potato virus S* (PVS) and *Tomato spotted wilt virus* (TSWV). A real-time multiplex, single tube RT-PCR assay for the detection of these viruses from potato leaves and tubers was developed using Cy5-, FAM-, JOE- and ROX-labelled TaqMan probes. The copy numbers for transcripts were quantified with a dynamic range of 8×10^1 to 8×10^9 copies of PVX and PVS, 1×10^2 to 1×10^{10} copies of PLRV and 1×10^3 to 1×10^{10} copies of TSWV. The inter-assay reproducibility was high, with a coefficient of variation (CV) of <2%. Total RNA was rapidly and efficiently extracted from bulked tuber samples for the reliable detection of one or more of the viruses. These data indicate that this high-throughput test is accurate and sensitive, and will provide a cost-effective diagnostic tool for the seed potato industry.

Development and validation of diagnostic protocols for the detection of endemic and exotic pathogens of grapevines

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We currently have a Grape and Wine Research and Development Corporation funded project for the development and validation of diagnostic protocols for grapevine viruses and some bacteria. Although biological indexing, ELISA and PCR are commonly used for the detection of grapevine viruses there have been few comprehensive, systematic studies to determine the reliability of these tests in comparison with each another. One of the main aims of this project is to identify diagnostic protocols for the detection of a range of endemic pathogens under Australian conditions.

To identify diagnostic protocols for endemic viruses we have established field trials in a two climates (Yarra Valley - cool climate; and Mildura - warm climate) in which Chardonnay and Shiraz grapevines have been inoculated with *Grapevine leafroll associated virus 2* (GLRaV-2), *Grapevine leafroll associated virus 3* (GLRaV-3), *Grapevine virus A* (GVA) or *Grapevine fleck virus* (GFkV). These trials are being used to determine the best time of year for the detection of viruses and the best tissue types. Preliminary results indicate that testing may be reliably conducted from late spring to early autumn for GLRaV-2, GLRaV-3 and GFkV. So far GVA has not been detected in any of the inoculated grapevines. Preliminary results also indicate that the PCR tests that we have developed are more sensitive than ELISA and should reduce the risk of obtaining false negative results.

Generic method to identify plant viruses by mass spectrometry of their coat proteins

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Virus detection has improved dramatically with the access to reliable serological and molecular tools. Many protocols have recently been developed for generic detection of a genus or even family by PCR or ELISA. However, a more universal approach to detect and identify most viruses across families is still required. On his website, Lane describes a minipurification protocol followed by sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE) as a universal plant virus detection tool (<http://lclane.net/minipur.html>). This method uses differential centrifugation to partially purify virions and SDS PAGE to visualise viral coat proteins and estimate their mass. In this project we have extracted putative coat proteins from the stained SDS gels and used high resolution mass-spectrometry to obtain peptide sequences.

We used the minipurification protocol for two known viruses (*Cucumber mosaic virus* and *Tomato spotted wilt virus*) and six unknown viruses, in *Nicotiana occidentalis*, and uninfected controls. Bands unique to the virus-infected material (between ~17 and ~41 kDa) were observed from SDS-PAGE. To identify these proteins, bands were excised and digested with trypsin prior to mass spectrometry. Analysis of the peptide masses against a virus database identified homology with known virus peptides. This method confirmed the two known viruses and identified successfully the six unknown viruses. The unknown viruses included two common viruses (*Alfalfa mosaic virus* and *Tobacco streak virus*), two new strains of known viruses (*Citrus leaf blotch virus* and *Ribgrass mosaic virus*) and, from conserved regions in their coat protein, two novel viruses (a *Potexvirus* and a *Vitivirus*).

Funded by FRST contract #C06X0710

Tandem Mass Spectrometry as a Tool for the Identification of New Virus Infections in Plants.

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For many years the analysis of viral coat proteins on sodium dodecyl sulphate polyacrylamide gels (SDS-PAGE) has been a useful diagnostic for determining the presence and partial characterisation of viral infections in plants thanks to the pioneering work of Les Lane. This approach often enabled a broad definition of any infectious agent but was really limited to known viruses by virtue of the size estimations of their coat protein(s). More recently the use of MALDI-Tof mass spectrometry has assisted in identifying viruses down to at least species level again where the organism has been reported is known, by using peptide mass fingerprinting (PMF) analysis following protease digestion of the coat proteins. Where viral sub classes have had their nucleic acid sequenced PMF analysis will often help resolve slight variations in the translated sequence reflected in changes in the mass of intact peptides. However when the peptides are themselves fragmented by collision induced dissociation inside a tandem or multistage mass spectrometer such as an electrospray ion trap or quadrupole Tof instrument, then the level of identification rigor is heightened considerably with the possibility of mutated or even novel viruses being uncovered from detailed coat protein analysis. This paper will outline the process involved with examples taken from our own laboratory.

Funded by FRST contract #C06X0710

Sequencing of small interfering RNAs (siRNAs) to identify plant viruses

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As a potential generic method to identify plant viruses we have utilised the plant defence activity of RNA silencing that produces uniformly sized small interfering RNAs (siRNAs) and next generation, massively parallel sequencing technology of Solexa, Illumina®. Leaf tissue was harvest from *Nicotiana occidentalis* indicator plants that were either; uninfected, infected with one of four known viruses, or infected with one of four unknown viruses. Low molecular weight RNA was isolated from the leaves and combined into three pools termed healthy, known infected and unknown infected. siRNAs from each of the three pools of RNA were excised from 15% denaturing PAGE gels and subjected to Solexa sequencing by the Allan Wilson Centre Genome Sequencing Service. The resulting ~22 million (total) sequences were subjected to trimming of adapters *in silico* yielding ~0.2 million unique sequences per pool. All sequences present in the healthy pool were then subtracted from the known infected and unknown infected pools leaving 255,306 and 118,602 unique sequences, respectively.

Bioinformatic mapping using the ELAND programme (proprietary to Illumina®) was used to align ~4,000 sequences onto one of the known virus genomes, a *Potexvirus*. Approximately 90% of the genome showed at least five-fold coverage thus demonstrating that the small RNAs were of viral origin. Contigs were assembled from unique sequences in the known infected pool using Velvet version 0.7.18 (Zerbino and Birney 2008) and Edena version 2.1.1 (Hernandez et al 2008). Some of these contigs identified the same *Potexvirus* by homology searching (Altschul et al 1997) and thus demonstrated the ability to identify a virus from assembled siRNA sequences.

Funded by FRST contract #C06X0710

Altschul, SF, Madden, T.L, Schäffer, AA, Zhang, J, Zhang, Z, Miller, W & Lipman, DJ (1997) *Nucleic Acids Res.* 25:3389-3402.

Hernandez D, François P, Farinelli L, Osterås M, Schrenzel J *Genome Research* 2008 18:802-9

Zerbino DR, Birney E 2008 *Genome Research* 18: 821-829

Can mycoviruses be used for the biocontrol of the plant pathogenic fungus *Botrytis cinerea*?

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The necrotrophic plant pathogen *Botrytis cinerea* is a major horticultural pathogen worldwide. Classical methods for this pathogen rely heavily on fungicides with their associated problems of resistance and chemical residues. Biocontrol offers an alternative approach and we are exploring the use of mycoviruses for this purpose. For successful exploitation of mycoviruses in this way they must have some deleterious effect against the target fungus and be able to spread and infect fungal populations in the field. From *B. cinerea* we have sequenced two filamentous ssRNA viruses (BVX and BCVF) belonging to the Flexiviridae and are assessing their effects on fungal fitness and pathogenicity. We have detected BVX and BCVF in *B. cinerea* isolates from several countries and have demonstrated that BVX is transmitted both through asexually produced conidia (>95%) and sexually produced ascospores (<50%). Natural viral transmission is presumed to occur mainly by hyphal fusion and vegetative incompatibility limits transmission by this route in *B. cinerea*, which has >66 vegetative compatibility groups. Although the viruses appear to have only minor effects on Botrytis it may prove feasible to use these viruses as gene vectors and produce infectious clones to alter their effects and transmissibility. BVX, BCVF are prime candidates for this approach as the Flexivirus *Potato virus X* has been successfully used as a vector for the expression of genes from a range of different sources in plants.

Molecular tools for studying the interaction between Botrytis and the viruses BVX and BCVF

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Understanding the nature of the relationship between viruses and their fungal hosts is critical in determining the ecological significance of mycoviruses and their potential usage as biological control agents. Two flexuous viruses belonging to the family Flexiviridae, Botrytis cinerea virus F (BCVF) and Botrytis virus X (BVX), from *Botrytis cinerea*, have previously been completely sequenced, providing the opportunity to examine their interaction with *B. cinerea* at molecular and cellular level. In order to study the virus-fungal interaction four basic tools were developed: (i) an efficient transfection protocol to introduce viruses into uninfected fungal isolates (ii) a transformation protocol to incorporate plasmid DNA into Botrytis, (iii) a consistent and reliable real-time PCR detection method for BCVF and BVX to study the effect of virus transfections, and (iv) an immunoassay for BVX to visualize the virus distribution and movement within the mycelia and also between compatible fungal strains. The key steps of each development will be discussed. These tools will enable the study of the relationship between the fungus and the mycoviruses at the cellular level.

Viroids and Viroid-Host Interactions

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Viroids are small (250-400 nt), circular, highly-structured RNAs able to infect plants and frequently induce specific diseases. In striking contrast with viruses, which encode proteins in their own genomes, viroids are non-protein coding RNAs, and therefore, they are extremely host-dependent for completing their infectious cycle. The approximately 30 known viroids are classified into the families *Pospiviroidae* (type species *Potato spindle tuber viroid*, PSTVd) and *Avsunviroidae* (type species *Avocado sunblotch viroid*, ASBVd). Members of the family *Pospiviroidae* and *Avsunviroidae* replicate in the nucleus and chloroplast, respectively. Viroid replication entails reiterative transcription of their circular genomes (to which the plus polarity is arbitrarily assigned) into head-to-tail (-) oligomers that, by themselves or after processing into circular RNAs, serve for a second RNA-RNA transcription round leading to (+) oligomers that are finally cleaved and ligated into the circular (+) forms. The three steps (RNA elongation, cleavage and ligation) are catalyzed by a DNA-dependent RNA polymerase forced to accept RNA templates, an RNase, and an RNA ligase, respectively. Remarkably, cleaving of the oligomeric RNA intermediates, and maybe ligation, is mediated in the *family Avsunviroidae* by hammerhead ribozymes embedded in both polarity strands. To invade distal plant parts, viroids move through the phloem assisted by host proteins. Recently, RNAs with the characteristic properties of the small interfering RNAs mediating RNA silencing have been identified in tissues infected by representative members of both viroid families, strongly indicating that viroids are inducers and targets (and perhaps suppressors) of the RNA silencing defensive response of their hosts.

Annotation of the Genome of “*Candidatus Phytoplasma australiense*”

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Phytoplasmas are bacteria that are associated with more than 600 plant diseases. As members of the class Mollicutes phytoplasmas lack bacterial cell walls and have genomes of c. 550-1350 kb – the small size being a result of genome reduction. Phytoplasmas have yet to be cultured *in vitro* limiting research into these enigmatic organisms. However the development of molecular techniques such as PCR and DNA sequencing have provided considerable insight into the nature of these pathogens.

In New Zealand “*Candidatus Phytoplasma australiense*” is associated with the four diseases, *Phormium* yellow leaf, *Cordyline* sudden decline, *Coprosma* lethal decline, and strawberry lethal yellows. It is also associated with several diseases in Australia including Australian grapevine yellows and papaya dieback. Phylogeographic analysis of the *tuf* gene indicates the population comprises three main lineages, one restricted to NZ, one to Australia, and one found in both countries. We have determined the complete genome sequence of a New Zealand isolate of “*Ca. P. australiense*” from a strawberry lethal yellows plant using the whole-genome shotgun method. The genome of “*Ca. P. australiense*” consists of a 959,779-bp circular chromosome as well as a 3,635-bp plasmid and is the largest phytoplasma genome that has been sequenced to date. Genomic dotplot analysis show that the “*Ca. P. australiense*” genome is not co-linear with two “*Ca. Phytoplasma asteris*” genomes that have been sequenced. Blast analysis of putative open reading frames (ORFs) identified a number that are not present in other genomes. Comparative analyses between previously sequenced phytoplasma genomes will be presented.

Identification of phytoplasma that cause diseases of diverse plants in three isolated regions in Western Australia

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Field trips to find phytoplasma-associated diseases in horticultural crops and native vegetation were made at Carnarvon and Kununurra in the Gascoyne and Kimberley regions, and in the Perth metropolitan area of Western Australia (WA). Phytoplasma-associated diseases were found in cultivated, wild and native plants and seemed relatively common in all three regions. Phytoplasma were confirmed to be associated with phytoplasma-like diseases of *Vigna radiata* (mung bean), *Rhynchosia minima* (jumby bean) and *Macropitilum atropurpureum* (siratro) in the Kimberly region and with *Lycopersicon esculentum* (tomato), *Solanum melongena* (egg plant) and *Carica papaya* (papaya) in the Gascoyne region. In Kings Park in Perth, phytoplasma-like symptoms were observed in the native woody plants *Allocasurina fraseriana* (western sheoak or casurina) and *Acacia saligna* (orange wattle). Polymerase chain reaction (PCR) and subsequent nested PCR with phytoplasma-specific primers confirmed the presence of phytoplasma in each host. The 16S rRNA and 16S-23S rRNA genes from these phytoplasma were sequenced and the sequences obtained submitted to GenBank. They were compared with those of other phytoplasma from WA reported previously. Despite the large distances between the three locations sampled and the considerable climatic differences, comparison of all phytoplasma sequences from WA suggests the presence of only two different types, 16SrII and 16SrXII. This work provides new knowledge on the extent and distribution of phytoplasma disease in WA, and indicates that native vegetation may act as a reservoir of infection for spread to horticultural and other crops.

Identification of a New *Liberibacter* Species Associated with Diseases of Solanaceous Plants

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In early 2008, a disease of glasshouse-grown tomato (*Solanum lycopersicum*) and capsicum (*Capsicum annuum*) was observed in Auckland, New Zealand. Affected plants are characterised by spiky, chlorotic apical growth, curling or cupping of the leaves, and overall stunting. Transmission electron microscopy revealed the presence of phloem-limited bacterium-like organisms in symptomatic plants. A range of universal and specific 16S rRNA PCR primers were used in different combinations on DNA extracted from healthy and symptomatic plants. One of the primer combinations produced a unique product from symptomatic plants only. Sequence and phylogenetic analysis of the 16S rRNA gene, 16S/23S rRNA spacer region, and the *rplK**AJL-rpoBC* operon revealed that although the bacterium shared high identity with 'Candidate *Liberibacter*' species it is distinct from the three *liberibacter* species previously described. This new *liberibacter* species of solanaceous plants has been named 'Candidate *Liberibacter solanacearum*'. With the development of a specific PCR diagnostic method, this new *liberibacter* was also detected in four additional solanaceous hosts, potato (*Solanum tuberosum*), tamarillo (*Solanum betaceum*), cape gooseberry (*Physalis peruviana*), and chilli (*Capsicum* sp.). The tomato/potato psyllid, *Bactericera cockerelli*, has been confirmed as the vector of 'Ca. L. solanacearum'. *B. cockerelli* was first discovered in an Auckland glasshouse tomato crop in May 2006, and is now established throughout the North Island and the top half of the South Island of New Zealand. A national survey of glasshouse-grown tomato and pepper, and packhouse-stored potato tubers determined that the *liberibacter* follows the same distribution in New Zealand as *B. cockerelli*. The *liberibacter* was determined to be graft-transmissible but not seed transmitted.

Subsequently published:

Liefting, L.W., Weir, B.S., Pennycook, S.R., and Clover, G.R.G. (2009).

'Candidate *Liberibacter solanacearum*', a *liberibacter* associated with plants in the family Solanaceae. *International Journal of Systematic and Evolutionary Microbiology* (in press).

Liefting, L.W., Sutherland, P.W., Ward, L.I., Paice, K.L., Weir, B.S., and Clover, G.R.G. (2009). A new 'Candidate *Liberibacter*' species associated with diseases of solanaceous crops. *Plant Disease* 93: 208-214.

Liefting, L.W., Ward, L.I., Shiller, J.B., and Clover, G.R.G. (2008). A new 'Candidate *Liberibacter*' species in *Solanum betaceum* (tamarillo) and *Physalis peruviana* (cape gooseberry) in New Zealand. *Plant Disease* 92: 1588.

Liefting, L.W., Perez-Egusquiza, Z.C., Clover, G.R.G., and Anderson, J.A.D. (2008). A new 'Candidate *Liberibacter*' species in *Solanum tuberosum* in New Zealand. *Plant Disease* 92: 1474.

Distribution in Australia and seed transmission of *Tobacco streak virus* in *Parthenium hysterophorus*

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Tobacco streak virus (TSV) has recently been reported from several important crops in central Queensland, Australia, including sunflower, mungbean, chickpea and cotton (Sharman *et al.* 2008). In recent years there have been important economic losses in sunflower and mungbean crops. However, until recently little was known about the causal strain of TSV or its key alternative hosts in the region. TSV was found to occur commonly in *Parthenium hysterophorus*, as symptomless infections, in central Queensland, across a large area infested with this highly invasive and prolific weed. Several isolates of TSV collected across the geographic range of *P. hysterophorus* were found to share identical coat protein sequence with each other and with TSV from crop plants in the same area. Seed transmission of TSV in *P. hysterophorus* occurred at rates of 6.8 to 48% and there was almost no change in this rate when *P. hysterophorus* seed was stored for up to 15 ½ months. These results indicate that *P. hysterophorus* is a key alternative host for the development of TSV disease epidemics in surrounding crops in central Queensland.

Sharman M, Thomas JE, Persley DM (2008) First report of *Tobacco streak virus* in sunflower (*Helianthus annuus*), cotton (*Gossypium hirsutum*), chickpea (*Cicer arietinum*) and mung bean (*Vigna radiata*) in Australia. *Australasian Plant Disease Notes* **3**:27-29.

Subsequently published

Sharman M, Persley DM, Thomas JE (2009) Distribution in Australia and seed transmission of Tobacco streak virus in *Parthenium hysterophorus*. *Plant Disease* **93**, 708-712.

Alternative hosts of two Tospoviruses in Queensland , Australia

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The tospoviruses (Genus: *Tospovirus*, Family: *Bunyaviridae*), *Tomato spotted wilt virus* (TSWV) and *Capsicum chlorosis virus* (CaCV) cause important diseases in capsicum, tomato and peanut in Queensland. As part of investigations into the management of these viruses, the alternative hosts of the two viruses have been examined.

TSWV infects a range of annual weed species including *Bidens pilosa*, *Sonchus oleraceus*, *Tagetes minuta* and several *Solanum spp.*. While these and other species can be locally important sources of virus during the cropping season they are often not well adapted to survival during harsh conditions of winter or summer. However, the introduced perennial species *Stachytarpheta jamaicensis* (Jamaican snakeweed; *Verbenaceae*) is commonly infected with TSWV in overgrazed pastures and disturbed areas, particularly in north and eastern Queensland. Surveys over five years indicate this species is likely to have an important role in the survival of TSWV, providing a virus source for thrips transmission into nearby susceptible vegetable crops. Recently, TSWV isolates from Jamaican snakeweed at several geographically separate locations have been virulent when inoculated onto capsicum cultivars having TSWV resistance conferred by the *Tsw* gene. This virulence appears to have occurred in the absence of selection pressure imposed by the *Tsw* gene.

Survey data indicates that CaCV has fewer weed hosts than TSWV. However, *Ageratum conyzoides* (Billygoat weed; *Asteraceae*) is a common and symptomless host of CaCV, widely distributed throughout some 1000 km of coastal Queensland.

Infection levels exceeding 50% have been found in random samples and high infection levels in tomato and capsicum crops are linked to the presence of infected *Ageratum*.

A simple protocol to obtain high-health grapevines.

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Many protocols have been used to eliminate viruses from grapevines including thermotherapy of intact vines, in vitro thermotherapy, chemotherapy, meristem-tip culture, and combinations of these techniques. Some viruses are relatively easy to eliminate, others such as *Rupestris stem pitting associated virus* (RSPaV) are more difficult. For this project 12 clones representing 10 grapevine cultivars infected with a range of known viruses or showing viral-like symptoms were selected. To develop a simpler protocol for high-health grapevine production, vines were subjected to temperatures up to 42°C for 16 weeks at the NZ Climate Laboratory, Palmerston North. Small nodal explants (2-4 mm) from expanding shoots were excised and placed into tissue culture at five times during thermotherapy. The resulting shoots were rooted and transferred to a greenhouse. Samples from untreated plants, tissue culture shoots and plants in the greenhouse were tested for the presence of *Grapevine leafroll associated virus* (GLRaV) 1, 2, 3 and 5, *Grapevine virus A* and *Grapevine fleck virus* using ELISA. Further samples from a selection of greenhouse vines that tested negative for these viruses, as well as infected control samples were sent for testing by RT-PCR to Linnaeus Laboratories in Gisborne, NZ. Fifteen RT-PCR tests for specific grapevine viruses as well as a generic closterovirus test were carried out on all samples. All viruses except GLRaV-3 and RSPaV were eliminated by very short periods of thermotherapy followed by tissue culture. Vines free of all viruses were identified from 11 of the 12 clones and only RSPaV was detected in the twelfth clone.

This research was funded by New Zealand Winegrowers.

Variant strains of *Bean leafroll virus* (BLRV); a cause for concern for international BLRV resistant breeding programs

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Luteoviruses, including *Bean leafroll virus* (BLRV, genus Luteovirus, family Luteoviridae), cause some of the most devastating crop losses in cool season food legumes, in some cases up to 95% (Makkouk *et al.*, 2003). Not only is germplasm sourced from the Central West Asia and North African region and incorporated into international resistant germplasm breeding programs, the Syrian BLRV strain is used to screen for potential BLRV resistant germplasm prior to sending it to Australia.

Discrepancies were found in serological and molecular results when screening for BLRV in faba bean samples collected during a survey conducted in Tal Kalakh, Syria, during March, 2008, when compared to Australian strains of BLRV. Tissue blot immunoassay (TBIA) (Makkouk and Comeau, 1994) analysis using broad-spectrum legume-luteovirus (5G4) and BLRV specific (6G4) monoclonal antibodies (Katul, 1992) identified BLRV in samples exhibiting yellowing, stunting and leaf cupping symptoms. The samples' RNA was extracted and further tested by Reverse Transcription PCR (RT-PCR) using three different BLRV-specific primer sets (Domier *et al.*, 2002; Ortiz *et al.*, 2005; Cavileer and Berger, 1994). The resultant molecular profile revealed differences between the BLRV isolate held at the ICARDA's Virology Lab, Syria, since 1995 and the Australian BLRV strain. The discrepancies found could potentially be a cause for concern when screening for resistant germplasm in international breeding programs. These results highlight the need to identify "type" strains of Luteovirid and generate an improved and more definitive detection method for BLRV.

Studies on the epidemiology of *Zucchini yellow mosaic virus* in Western Australia: patterns of spread, virus-tolerant cultivars, alternative hosts, and lack of seed transmission.

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Zucchini yellow mosaic virus (ZYMV) causes yield and quality losses in cucurbit crops worldwide. It causes severe losses every year in the two main cucurbit growing areas in northern Western Australia (Kununurra and Carnarvon) despite the 2-3 month annual break between cucurbit growing seasons. When seed collected from ZYMV-infected pumpkin fruit was germinated and the seedlings tested by ELISA, no seed transmission was detected in >4000 seedlings. In 2006-2008, surveys for alternative hosts of ZYMV involved testing more than 3500 weed samples from 20 different species collected from Kununurra and more than 1200 samples from 18 weed or wild native species from Carnarvon. ZYMV was detected in 3 samples of *Mukia maderaspatana* (family Cucurbitaceae) from 3 locations at Carnarvon, but not in any potential alternative hosts from Kununurra. The pattern of spread of ZYMV was examined in pumpkin plantings in which aphids spread the virus from internal or external infection foci. Spread to pumpkin was greater downwind than upwind of an internal source. When 25m wide fallow or non-host barrier of *Lablab purpureus* (family Fabaceae) separated external ZYMV sources from pumpkin plants, spread was smaller and more scattered with a non-host barrier than without. A field trial with 6 pumpkin cultivars (3 virus-tolerant and 3 susceptible) grown under high virus inoculum pressure showed that, although the virus-tolerant cultivars became infected, leaf symptoms were milder and infected plants were higher yielding with a greater proportion of fruit market-acceptable. These results were used to help validate an integrated management package for ZYMV in cucurbit crops.

Viruses associated with rhubarb decline disease

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In the eastern Australian mainland States, rhubarb crops are often affected by a severe yield decline disease, including symptoms of leaf mosaic or mottle, chlorotic and reddish or necrotic spotting. Viruses are generally associated and mixed infections are common, making assignment of field symptoms to particular viruses difficult. A novel virus, Rhubarb closterovirus (RCV), and *Cucumber mosaic virus* (CMV) were widely distributed in NSW, Victoria and Qld, while *Tomato spotted wilt virus* (TSWV) was found in Victorian and Qld crops and *Turnip mosaic virus* in NSW crops. *Cherry leafroll virus* alone was detected from South Australia. Additional isometric virions were frequently detected, including 40 nm (possible *Totivirus*) and 30 nm particles. TSWV can produce large chlorotic spots, reddish rings and vein mosaic. RCV is closely associated with chlorotic and necrotic spotting and chlorotic mottle symptoms.

Virus free rhubarb was produced by meristem tip culture, and field studies showed a high level of re-infection from adjacent infected plantings. After 22 months, virus was detected in 87% (RCV) and 37% (CMV) of plants. All symptomatic plants were infected with RCV but only a proportion with CMV.

The complete 14,642 nt genome of RCV has been sequenced, and includes 10 ORFs. Phylogenetic analysis places RCV in the aphid-transmitted *Closterovirus* genus and the genome organisation is similar to the type member *Beet yellows virus*, with the exception of an additional 18 kD ORF immediately 5' of the HSP70 in RCV. RCV was transmitted by the aphid *Aphis gossypii* and was found in the weed host curled dock (*Rumex crispus*).

Incidence and spread of viruses in white clover pastures: South Island, New Zealand

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White clover is of prime importance to the New Zealand economy. As well as being an important component in most grazing systems, its nitrogen fixing ability contributes to the nutritional value of pasture grasses and other agricultural species in a primary sector which accounts for 50% of New Zealand's total exports. With the steady influx of viruses into New Zealand (Pearson *et al.* 2006, Fig 1) it seemed timely to survey white clover pastures. Considering that Canterbury, Otago and Southland make up 50% of New Zealand's grazing, arable and fodder lands and the paucity of information on South Island pastures, we decided to concentrate on these regions.

Only one of 62 white clover pastures surveyed was found to be virus-free. Of the six viruses tested for *White clover mosaic virus* (WCMV) and *Alfalfa mosaic virus* (AMV) occurred, often at high incidences, on farms in every region. *Red clover necrotic mosaic virus* (RCNMV) and *Soybean dwarf virus* (SDV) occurred in over half the pastures while *Beet western yellows virus* (BWYV) and potyvirus infection was less common. There was a general reduction in virus diversity with increasing latitude with the most northern farms having greater species richness (all 6 viruses present) than those in Southland (1-3 viruses present). There was a significant relationship between the presence of AMV, BWYV, RCNMV, SDV and irrigation. Dairy farming also had a positive relationship with BWYV, RCNMV and SDV. WCMV increase was monitored in six pastures and incidence was observed to increase geometrically in young pastures. The relatively high incidence of RCNMV is in contrast to previous studies on white clover pastures and indicates that assessing this virus's effects on white clover is high priority.

Subsequently published in *Australasian Plant Pathology* 2009 **38**, 270-276.

Epidemiology of *Wheat streak mosaic virus* in Australia

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Wheat streak mosaic virus (WSMV) infection and infestation with its wheat curl mite (WCM; *Aceria tosichella*) vector were investigated at two sites in the low rainfall zone of the central grainbelt of south-west Australia. In the 2006 outbreak, after a preceding wet summer and autumn, high WCM populations and total infection with WSMV throughout a wheat crop were associated with presence of abundant grasses and self-sown 'volunteer' wheat plants before sowing the field that became affected. Wind strength and direction had a major impact on WSMV spread by WCM to neighbouring wheat crops, the virus being carried much further downwind than upwind by westerly frontal winds. Following a dry summer and autumn in 2007, together with control of grasses and volunteer cereals before sowing and use of a different seed stock, no WSMV or WCM were found in the following wheat crop within the previously affected area or elsewhere on the same farm. In the 2007 outbreak, where the preceding summer and autumn were wet, a 40% WSMV incidence and WCM numbers that reached 4,800 mites/ear at the margin of the wheat crop were associated with abundant grasses and volunteer wheat plants in adjacent pasture. WSMV incidence and WCM populations declined rapidly with increasing distance from the affected pasture. The alternative WSMV hosts identified in the grainbelt were volunteer wheat, annual ryegrass (*Lolium rigidum*), barley grass (*Hordeum* sp.), wild oats (*Avena fatua*), small burr grass (*Tragus australianus*), stink grass (*Eragrostis cilianensis*) and witch grass (*Panicum capillare*).

Published subsequently

Coutts, B.A., Strickland, G.R., Kehoe, M.A., Severtson, D.L. and Jones, R.A.C. (2008). The epidemiology of *Wheat streak mosaic virus* in Australia: case histories, gradients, mite vectors, and alternative hosts. *Australian Journal of Agricultural Research* 59, 844-853.

Coutts, B.A., Hammond, N.E.B., Kehoe, M.A. and Jones, R.A.C. (2008). Finding *Wheat streak mosaic virus* in south-west Australia. *Australian Journal of Agricultural Research* 59, 836-843.

Processes and technologies for collections of viruses and virus-like organisms

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The 23 October 2008 marked the 40th anniversary of the first deposit into the HortResearch (then DSIR) Plant Virus Collection. This collection was initiated by some of the internationally recognised giants in plant virology such as R.E.F. Matthews and his colleagues. This plant virus collection has 516 entries listed and similar collections exist in other organisations including Crop & Food research with around 400 entries and MAF with about 200. After some years of neglect virologists in HortResearch and Crop & Food Research are collaborating in a project to take stock of their collections to ensure their preservation, viability and purity. The project also plans to verify and update the records in an electronic form and establish a shared database.

To help guide us we are running this workshop with contributions from Rene van der Vlugt of Plant Research International who will speak on the Netherlands' national programme to re-vitalize their plant pathogen collection with its associated new database and web-interface programme. John Thomas from Department of Primary Industries and Fisheries Queensland will also speak on progress in developing the virus collection component of the Australian Pest and Disease Database.

Talk topics

Plant virus reference collections - a valuable resource

John Thomas

Plant virus collections in the Netherlands; their past and future

Rene van der Vlugt

Subsequently accepted for publication in *Australasian Plant Pathology* Dec 2009

Examining the effects of elevated CO₂ and temperature on *Barley yellow dwarf virus* in wheat

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The Intergovernmental Panel on Climate Change (IPCC) released their fourth assessment report in 2007 which concluded global warming is clearly occurring and that changes in the global climate system will continue into the future. These changes are expected to have major impact on agricultural systems, particularly as both CO₂ and temperature are expected to increase and more frequent severe weather events, such as drought, are expected to occur. As yet there is very little empirical data about the impact of elevated CO₂ and temperature on pest and pathogen populations and crop production. Consequently, predictions on the future of our major monoculture cropping systems such as wheat remain uncertain. The Department of Primary Industries Victoria, the University of Melbourne and the Australian Greenhouse Office have established a Free-Air CO₂ Enrichment (FACE) research facility at Horsham, Victoria, to study the effects of elevated CO₂ on wheat production in Australia. This facility is being used to study the effects of projected CO₂ concentrations (550ppm) under field conditions on *Barley yellow dwarf virus* in wheat. In addition to the FACE experiments a second study is being established in growth rooms to gather empirical information about the fecundity of BYDV in under elevated temperature. A third study will also be done to determine the ability of the BYDV vector, *Rhopalosiphum padi*, to acquire and transmit the virus under various climatic conditions.

The C-terminus of tomato leaf curl C4 is required for the movement function of this symptom- inducing protein

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The tomato leaf curl virus (ToLCV) C4 protein has been shown to be associated with the development of disease symptoms such as leaf curling and vein swelling. In addition over- expression of C4 in transgenic plants produces virus like symptoms. Recently, the C-terminal region of C4 has been shown to be required both for binding to a host plant shaggy-like kinase (SISK) and production of disease symptom. To investigate whether the C-terminus of C4 protein also has a role in movement, a truncated C4 with a deleted C-terminus was prepared. Tomato plants were inoculated with either both A and B DNA components of the bipartite geminivirus tomato leaf curl New Delhi virus (ToLCNDV) or DNA A together with an expression construct of either C4 or C4 mutant. All tomato plants inoculated with both components of the virus developed severe leaf curling 13-16 days post-inoculation (d.p.i.). A number of tomato plants co-inoculated with the infectious construct of DNA A together with the 35S:C4 construct showed mild leaf curling symptoms at 13–16 d.p.i. None of the plants inoculated with DNA A and the 35S:C4 mutant construct developed symptoms. Plants inoculated with DNA A alone lacked detectable levels of DNA A in the distal leaves when tested by dot blot hybridization and PCR at 13–16 d.p.i. In contrast, DNA A was detected in newly emerging leaves of a number of plants co-inoculated with DNA A and 35S:C4. These results suggest that the C-terminus of C4 is important for the movement function of this protein.

Progress in Characterising PKR, a Plant-Encoded and Double-Stranded RNA-Activated Protein Kinase

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During the infection of an RNA virus, replication occurs via a double-stranded (ds) RNA intermediate. As dsRNA is rare in a cell, these necessary replication intermediates of RNA viruses alert the host cell of a virus invasion. In turn, a signal cascade of defence mechanisms is activated, of which a sentinel is the dsRNA-binding protein, protein kinase R (PKR). In mammals, the expression and activity of this ~68 kDa protein has been extensively studied and it is induced to high levels by interferon treatment. Upon binding to dsRNA, PKR autophosphorylates and phosphorylates the protein translation initiator eIF2 α . This renders eIF2 α inactive, leading to the loss of protein translation and an inhibition of virus protein expression.

Recently, these hallmark activities of mammalian PKR have also been detected in plants, but no homologous sequence has so far been detected. Our aim is to identify plant PKR via a proteomics approach and then use the purified protein to determine the gene sequence. To this point, a number of purification techniques have been used, exploiting the known dsRNA and phosphate binding characteristics of PKR as well as separating the protein via its molecular size and charge properties. An activity assay has also been developed to detect the purification of PKR activity. Currently, transgenic plant lines containing knockouts or overexpressions of genes postulated to be involved in the PKR regulatory pathway are examined for their PKR activity. Our progress to date in identifying the plant-encoded PKR will be presented.

Integrated badnaviruses at large in the New Zealand flora

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Endogenous plant pararetroviruses (EPRVs) are the integrated counterparts of the members of the plant virus family Caulimoviridae. Despite lacking an integrase or long terminal repeats, integrated forms of these viruses are present in plant genomes. At some point in the past, episomal viral DNA integrated into the host genome through an illegitimate or homologous recombination event and gained access to reproductive cells. Subsequent duplication and propagation of the integrated virus gives rise to multiple copies of the viral genome. The initial integration event may be considered analogous to creating a molecular fossil of a virus circulating in a plant population at a particular time and in a particular place. Analysis of EPRV sequences in present day plant populations has the potential to yield information about the evolutionary history and geographic dispersal of the host population.

We have used specific PCR primers that amplify a 530 nucleotide sequence of badnavirus reverse transcriptase to screen New Zealand indigenous plants for badnavirus sequences and denaturing gradient gel electrophoresis (DGGE) to analyse the complex mixtures of products that have been formed. We found that band patterns are heritable and are related to the primary DNA sequence of the EPRV. Integrated badnavirus sequences are widespread in the New Zealand flora in 37 species (37/59) from 10 dicot families and the Poaceae (monocot). As well as being of virological interest these sequences have illuminated the reproductive biology of a native tree species and may be useful as markers for studies of the origin and diversity of New Zealand plants.

Towards protein interactome maps for plant rhabdoviruses

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Accurate and simultaneous determination of both protein:protein interactions and intracellular localization is critical for defining interactome networks related to a plethora of physiological processes that underlie plant:virus interactions. Here we report the construction and use of a series of plant binary vectors that permit the simultaneous determination of protein localization and interaction *in planta* using the methods of bimolecular fluorescence complementation (BiFC) and fluorescence resonance energy transfer (FRET). Additionally, we have generated transgenic *Nicotiana benthamiana* lines that express fluorescent protein markers targeted to nuclei, or the endoplasmic reticulum. We show that conducting BiFC assays in plants that express cyan fluorescent protein fused to histone 2B provide enhanced image quality and information over assays conducted without benefit of a subcellular marker. Taken together, the new combination of improved gene vectors and transgenic intracellular marker lines presented here offers powerful new tools to investigate protein and membrane dynamics in living plant cells. We will discuss the use of these novel tools in the context of mapping interactions for proteins encoded by *Sonchus yellow net virus* and *Potato yellow dwarf virus*, two members of the genus *Nucleorhabdovirus*, in comparison with *Lettuce necrotic yellows virus*, type-species of the genus *Cytorhabdovirus*.

Resistance to *Subterranean clover mottle virus* in *Medicago truncatula* and genetic mapping of a resistance locus

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Subterranean clover mottle virus (SCMoV), which causes an important disease of annual clover pastures, was inoculated to the annual pasture legume *Medicago truncatula*, a model legume species. Two hundred and nine accessions representing most of the core collection of *M. truncatula* from SARDI (South Australian Research and Development Institute) were inoculated with infective sap to determine their disease phenotypes. Forty two of these accessions remained uninfected systemically and so were potentially resistant to SCMoV. Accession DZA-315 developed a localised hypersensitive resistance reaction. In a mapping population from a cross between the susceptible parent A-17 and DZA-315, a total of 166 RILs were phenotyped for resistance and susceptibility to SCMoV. Resistant and susceptible lines showed parental phenotypic symptoms: 84 were susceptible and 82 were resistant suggesting presence of a single resistance (*R*) gene. The phenotypic data were combined with genotypic data (76 polymorphic molecular markers) for this RIL population to provide a framework map. Genetic analysis located a single SCMoV resistance locus on the long arm of chromosome 6. From existing maps of *M. truncatula*, most of the *R* genes located in this region are of the TIR-NBS-LRR type and occur in *R* gene clusters. A series of BACs that span the region of interest have been identified. These results provide a basis for fine mapping and identification of the SCMoV resistance gene.

Subsequently published.

Resistance to *Subterranean clover mottle virus* in *Medicago truncatula* and genetic mapping of a resistance locus. Muhammad Saqib, Simon R. Ellwood, Roger A. C. Jones, Michael G. K. Jones. *Crop and Pasture Science*, Vol. 60 No. 5 Pages 480 – 489. 2009.

Detection and characterisation of viruses from sweetpotato in Papua New Guinea and Queensland, Australia

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Sweetpotato (*Ipomoea batatas*) is a vegetatively propagated perennial plant, often grown as an annual. The species originated in the area of modern day Mexico, Ecuador and Peru. Sweetpotato is grown for its large storage roots which provide a staple crop in many developing countries, especially in the Pacific and African regions. In Papua New Guinea (PNG), approximately 60% of the daily calorie intake of the general population is from sweetpotato.

The Australian industry is based on orange-fleshed low dry matter cultivars with Queensland producing approximately 90% of the Australian crop.

Virus infection severely reduces yields and quality in many production areas.

As part of an ACIAR funded project on crop improvement of sweetpotato in highland production areas of PNG, cultivars from PNG were screened for virus infection by serological and molecular assays. *Sweet potato feathery mottle virus* (SPFMV) was present in most symptomatic plants while the potyvirus *Sweet potato virus G* was found in one cultivar.

In Queensland, SPFMV was the only virus detected in the main commercial cultivar Beauregard and in a collection of cultivars displaying a range of virus symptoms.

Phylogenetic analysis of SPFMV isolates from Australia and PNG will be presented.

Genetic diversity of Australian *Alfalfa mosaic virus* for an environmental risk assessment of genetically modified *Alfalfa mosaic virus* resistant white clover

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Alfalfa mosaic virus (AMV) infection of white clover can cause major economic losses to the dairy industry (Garrett, 1991). To reduce yield losses, genetically modified (GM) virus-resistant white clover expressing the RNA3 AMV coat protein gene have been developed and trialled in Australia. However, since white clover is naturalised or invasive in a wide range of high conservation-value plant communities in SE Australia (Godfree, 2004), transgenic white clover could pose a significant risk to native ecosystems in this region. The aim of our work was to determine the abundance, genetic diversity and evolutionary potential of AMV populations in white clover in potentially at-risk SE Australian ecosystems.

A total of 215 sites in NSW, the ACT and VIC were surveyed. White clover was present at 131 sites (61%) and AMV was detected in white clover at only 19 sites (15%). RNA3 was sequenced from 83 AMV isolates. Thirty-six haplotypes were identified (haplotype diversity; Hd = 0.914), with 49 polymorphic sites resulting in a nucleotide diversity (Pi) of 0.009. No evident difference in nucleotide diversity was found between community types but haplotype diversity was lowest in roadsides (Hd= 0.800) and highest in native plant communities of moderate (Hd= 1.00) and high (Hd= 0.859) conservation value. There was also minimal genetic structure of AMV populations across the study area and no differences in genetic diversity between collection regions. These data suggest that the evolutionary potential of AMV populations in response to the introduction of virus-resistance genes is limited in the environments studied.

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Godfree, RC, Chu, PWG, Woods, MJ (2004) *Australian Journal of Botany*, 52: 1-11.

The *Citrus tristeza virus* resistance-breaking strain in New Zealand and the South Pacific

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Citrus tristeza virus (CTV) is the most destructive viral disease of Citrus species, and results in the loss of tree vigour, stunting, dieback and possible death of the infected tree.

Nearly all citrus in New Zealand is grown on the *Citrus tristeza virus* (CTV) resistant rootstock *Poncirus trifoliata* or trifoliolate orange hybrids. However, these are susceptible to the resistance-breaking (RB) strain of CTV that was first found in Kerikeri in 1997. The genomes of five isolates of this strain, obtained from field sources by graft and aphid transmission were completely sequenced. Phylogenetic analysis against other CTV genotypes revealed that the RB isolates are distinct from other extant CTV genomes with an average 83.7% identity at the nucleotide level, being most similar to T36 (90.4%) from Florida and least similar to VT from Israel. Based on the genomic sequence data the RB isolates comprise a previously unreported genotype. The genomic sequences were used to develop markers to examine the incidence and spread of these isolates both in New Zealand and from sites across the Pacific. The RB genotype is present in New Zealand, where it is the dominant strain, and is also present in Western Samoa, Tahiti, and the Marianas. Sequence analysis of these isolates using a 700bp marker fragment show that the RB genotype is monophyletic, with nucleotide homology between isolates of approximately 96%. These data suggest that the RB isolates comprise a single and unique genotype that has remained stable as it spread across the Pacific. The implications for the breakdown of resistance and impact of this strain are discussed.

Identification and characterization of *Hydrangea chlorotic mottle virus*

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Hydrangea chlorotic mottle virus (HdCMV) was identified as a novel carlavirus species in Minnesota, USA in 2006 and a 1772 bp sequence covering partial open reading frame (ORF) 3 and complete ORF4-6 was reported (GenBank Accession No. DQ412999). In 2007, a viral disease was observed in Auckland, New Zealand, from a hydrangea plant (*Hydrangea macrophylla*) which caused leaf mottling and chlorotic spots. Carlavirus-like filamentous particles were observed by electron microscopy. The virus could be mechanically transmitted to a range of herbaceous indicator plants, and was detected using ELISA with antiserum raised against HdCMV. A partial sequence (3164 bp) of this isolate containing partial ORF1 and complete ORF2-6 showed 97% nucleotide identity to the published HdCMV sequence while their coat protein (CP) amino acid sequences shared 98% similarity. The CP amino acid sequence identity of HdCMV to other carlaviruses ranged from 49% to 76%. This is the first report of HdCMV in New Zealand but a survey during 2007-2008 suggested that the virus is widespread. The only carlavirus in hydrangea that is currently recognised by the International Committee on Taxonomy of Viruses is *Hydrangea latent virus* (HdLV). Further study is required to determine whether HdLV and HdCMV are in fact the same virus.

Phylogenetic analysis of *Bean yellow mosaic virus* isolates from four continents: relationship between the seven distinct groups found and their natural isolation hosts and geographical origins

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Genetic diversity of *Bean yellow mosaic virus* (BYMV) was studied by comparing sequences from the coat protein (CP) and genome-linked viral protein (VPg) genes of isolates from four continents. CP sequences compared were those of 17 new isolates and 47 others already on the database, while the VPg sequences used were from four new isolates and 10 from the database. Phylogenetic analysis of the CP sequences revealed seven distinct groups, six polytypic and one monotypic. The largest and most genetically diverse polytypic group, which had intra-group diversity 0.061 nucleotide substitutions per site, contained isolates from natural infections in seven host species. These original isolation hosts included both wild (four) and domesticated (three) species and were from monocotyledonous and dicotyledonous plant families, indicating a generalized natural host range strategy. None of the other five polytypic groups spanned both monocotyledons and dicotyledons and all contained isolates from fewer (1-4) species, all of which were domesticated, and had lower intra-group diversity (0.019-0.045 nucleotide substitutions per site), indicating host specialization. Phylogenetic analysis of the fewer VPg sequences revealed three polytypic and two monotypic groupings. These groups also correlated with original natural isolation hosts, but the branch topologies were sometimes incongruous with those formed by CPs. Also, intra-group diversity was generally higher for VPgs than CPs. A plausible explanation for the groups found when the 64 different CP sequences were compared is that the generalized group represents the original ancestral type from which the specialist host groups evolved in response to domestication of plants after the advent of agriculture. Data on the geographical origins of the isolates within each group did not reveal whether the specialized groups might have co-evolved with their principal natural hosts where these were first domesticated, but this seems plausible.

Published subsequently

Wylie, S.J., Coutts, B.A., Jones, M.G.K., and Jones, R.A.C. (2008). Phylogenetic analysis of *Bean yellow mosaic virus* isolates from four continents: relationship between the seven groups found and their hosts and origins. *Plant Disease* 92, 1596-1603.

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Poster Abstracts

Chooi and Pong et al	Sequence variation in <i>Grapevine leafroll-associated virus-3</i> (GLRaV-3) and its affect on virus detectability
Muhammad Saqib	First full length sequence of <i>Bean common mosaic virus</i> from Australia
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Sequence variation in *Grapevine leafroll-associated virus-3* (GLRaV-3) and its affect on virus detectability

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Grapevine leafroll-associated virus-3 (GLRaV-3) is an economically important virus that is found in most grapevine growing regions and is a serious disease throughout New Zealand vineyards. Sensitive and accurate detection of viruses is an essential component of any disease management programme, and variability within a pathogen population can compromise detection. For instance, diagnostic tests using a PCR-based diagnostic method targeting the HSP-90 gene have occasionally found difficulty in detecting GLRaV-3 from known ELISA positives. Therefore, our study investigated the potential sequence variation within GLRaV-3 in New Zealand, focusing on ORF1b, ORF5, and ORF6. Using RT-PCR, we have identified in New Zealand both of the GLRaV-3 strains that have full-length sequences available on Genbank (NY1 and GP18). In addition, preliminary SSCP results using cloned PCR products for fragments of the ORF1b (652 bp), ORF5 (300 bp), and ORF6 (527 bp) genes, suggest that there is sequence variation within these strains.

First full length sequence of *Bean common mosaic virus* from Australia

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In the agricultural region near Kununurra in the Kimberly region of Western Australia, *Bean common mosaic virus* (BCMV; genus potyvirus) was found infecting wild *Macroptilium atropurpureum* (purple bush bean, siratro) and *Phaseolus vulgaris* (borlotti bean, common bean). Whole genome sequencing of BCMV from *M. atropurpureum* was undertaken to provide the first full length sequence for this virus from Australia. Amplified PCR products were cloned and sequenced. The genomic sequence (10054bp) obtained was submitted to GenBank (Accession EU761198). This sequence and those of other BCMV sequences already on GenBank, were used to construct phylogenetic trees of (i) full length genomes (nucleotides), and (ii) coat protein (CP) sequences (amino acids). The results from the genomic and CP analyses indicate that the Australian BCMV isolate studied is closely related to BCMV isolates previously reported from the American continent.

Survey of viruses infecting *Allium* crops in New Zealand

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Surveys to identify virus diseases affecting garlic (*Allium sativum*), onion (*Allium cepa*) and shallot (*Allium cepa* var. *aggregatum*) were done in 2005 and 2007. The surveys covered two main growing areas in New Zealand, Pukekohe in the North Island and Blenheim in the South Island, with 17 fields surveyed in 2005 and 27 fields in 2007. Samples showing symptoms of infection such as yellow mosaics, stripes or distortion were collected and tested by ELISA and/or RT-PCR for the presence of *Allium* viruses. The survey in 2005 identified the following viruses: *Onion yellow dwarf virus* and *Leek yellow stripe virus* (genus *Potyvirus*); *Garlic common latent virus* and *Shallot latent virus* (genus *Carlavirus*); and *Garlic virus B*, *Garlic virus C* and *Garlic virus D* (genus *Allexivirus*). *Shallot virus X* (ShVX), *Garlic virus A* (GarV-A) (genus *Allexivirus*) and *Iris yellow spot virus* (IYSV) (genus *Tospovirus*) were identified during the survey in 2007. None of the samples reacted to *Shallot yellow stripe virus*, Sint-Jan's onion latent virus or *Tobacco rattle virus*. GarV-A, ShVX and IYSV had not been reported in New Zealand previously.

Published subsequently

Ward LI, Perez-Egusquiza Z, Fletcher JD, Wei T, Clover GRG.2009. A survey of viral diseases of *Allium* crops in New Zealand. *Australasian Plant Pathology* (Accepted)

Molecular detection of viruses in pollen

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Currently MAF requires pollen of plants which are hosts of regulated viruses to be imported into a quarantine facility where it is used to fertilise female plants. The resulting seeds are then collected, germinated and the emerging plants tested for viruses of concern. This process is costly and time consuming. A RT-PCR assay which could be used to test pollen directly for regulated viruses would reduce the costs and time associated with importation of pollen, providing New Zealand plant breeders with easier access to new germplasm. To evaluate the feasibility of such an assay, a test system was established by inoculating *Nicotiana glutinosa* plants with *Tobacco ringspot virus* (TRSV). Pollen from these plants was tested for virus infection using nepovirus primers as well as by pollinating healthy plants and testing their progeny. Although the virus was not transmitted to seed, all pollen collected from TRSV-infected plants tested positive for TRSV, demonstrating in principal that RT-PCR could replace current methods of testing pollen. Further work is underway to validate this method on other plant-virus systems.

New plant viruses identified in New Zealand since 2007

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MAF Biosecurity New Zealand's Plant Health Environment Laboratory (PHEL) is responsible for the identification of exotic pests and diseases in local and imported plants. Diagnostic tools used include transmission electron microscopy, herbaceous and woody indexing, serology and molecular tests. Since 2007, PHEL has identified 14 new viruses and two new virus-hosts in New Zealand. *Cucumber mosaic virus* was found in two new ornamental hosts (*Lobelia* sp. and *Phlomis* sp.). Seven new ornamental viruses were identified: *Hibiscus chlorotic ringspot virus*, *Hydrangea chlorotic mottle virus*, *Narcissus degeneration virus*, *Ornithogalum mosaic virus*, *Tulip virus X*, *Wisteria vein mosaic virus* and *Zantedeschia mosaic virus*. Six new viruses infecting vegetable crop were detected: *Carrot red leaf virus-associated RNA*, *Carrot mottle mimic virus*, *Garlic virus A*, *Iris yellow spot virus*, *Shallot virus X* and Sweet potato virus 2. One new virus infecting a horticultural crop was identified: *Strawberry mottle virus*.

A survey of *Allium* diseases in New Zealand

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To determine if un-detected regulated pests were present in New Zealand we completed a preliminary survey of viruses, bacteria and phytoplasmas in a representative group of 18 *Allium* spp. crops in Auckland and Marlborough in the summer of 2004-05. For each crop the entire field was walked in a 'W' pattern, and plants showing symptoms of bacterial, fungal and phytoplasmic disease were collected. One hundred leaves were randomly collected for virus assays and to estimate virus incidence. Bacteria and fungi were identified from laboratory assays, phytoplasma from PCR assay and viruses using ELSA and PCR. None of the *Allium* crops in Auckland and Marlborough were infected with phytoplasmas or regulated bacteria. Bacterial species detected included *Pseudomonas marginalis*, *Erwinia carotovora*, and *Pseudomonas viridiflava*. Onion white rot (*Sclerotium cepivorum*) was observed in one Auckland garlic crop and an onion crop with incidences of 1-3% and 5% respectively. In Marlborough, white rot was observed in a shallot and a garlic crop (5%) along with *Alternaria porri*, *Penicillium* spp., *Aspergillus* spp. and *Puccinia allii*, all at low incidence rates. The survey confirmed the presence of all *Allium* viruses previously recorded in New Zealand, and in some cases on new hosts. In particular, for the first time in New Zealand we detected the regulated virus GVA in *A. cepa*, *A. chinense*, *A. ascalonicum* and *A. sativum*; SMbLV in *A. cepa*, *A. ascalonicum* and *A. sativum*; and SYSV in *A. cepa*. Further work is continuing to confirm the suspected observations of regulated viruses OMbLV and SJOLV.

How is Dasheen Mosaic Virus evolving in the short term and long term? Are we witnessing evolution as it is happening?

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Dasheen mosaic potyvirus (DsMV) is probably the most important viral disease of a wide range of ornamental and edible aroids including *Colocasia* sp and *Xanthosoma* sp. This virus is especially common in tropical and subtropical countries causing significant yield losses of taro, a staple food of Maori and Pacific Island communities. The lack of proof reading during viral replication results in potyviruses existing as quasi-species where the dominant sequence(s) is determined by their concentration in the inoculum and selection pressure. This sequence variation allows the virus to evolve rapidly, firstly within a host plant where the dominant sequence may vary through time (short term evolution), between locations where the subtle genetic variations in host may influence the evolution of the virus, and between host species (both long term evolution). We wish to understand the short and long term evolution of DsMV and determine if all potyviruses evolve in a similar manner. We have compared DsMV CP sequences from a range of South Pacific isolates with glasshouse-grown isolates as well as public domain sequences. From this, we have identified three distinct phylogenetic groups: DsMV infecting aroids other than *Colocasia* and *Zanthosoma* spp; DsMV infecting *Colocasia* sp- and vanilla; and DsMV infecting both *Colocasia* and *Xanthosoma* spp. Isolates derived from a common ancestral sequence showed significant variation indicating that the DsMV genome can accommodate significant variation in the short term. Further, in the longer term, distinct DsMV sequences have appeared that are associated with particular hosts. The data also suggest that *Colocasia*-infecting DsMV may be evolving in different locations to infect vanilla.

The Effects of Botrytis Virus X on the fungus *Botrytis cinerea*

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The reports of impact from virulent viruses on their host has been well documented in scientific and medical literature. In fungi, hypovirulence from a mycoviral infection has also been reported as a conferred trait. However, reports of symbiosis or mutualism between fungal hosts and viruses are not as well documented.

Here, we investigate the effects of Botrytis Virus X on its host *Botrytis cinerea*. *In vitro* and *in vivo* experiments were performed on progeny from sexual crosses and also clonal parental strain REB705-1. These samples were made up of *B. cinerea* strains with and without a mycoviral "infection". Linear growth on Malt Extract Agar (MEA), sporulation counts, and sclerotia counts were used as methods for *in vitro* experiments. An apple rot experiment was used as an *in vivo* test.

The results from *in vitro* and *in vivo* experiments show differences for fungal growth. *Botrytis* containing BVX had better linear growth in culture than those without BVX. This was evident in both asexual strains and sexual progeny of *B. cinerea*. However, in apples, BVX negative *Botrytis* shows a more aggressive infection than BVX positive *Botrytis*. Finally there were no statistical differences from the sporulation and sclerotia count experiment between *Botrytis* containing BVX and those without BVX.

Diagnostic tools for the seed potato industry

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Potato is important in Western Australia both for domestic food production and export. Four viruses diminish tuber yield locally, *Potato leaf roll virus* (PLRV), *Potato virus X* (PVX), *Potato virus S* (PVS) and *Tomato spotted wilt virus* (TSWV). Current methods for detecting potato viruses in tubers usually require them to be sprouted first in the glasshouse and the shoots tested by ELISA; a time-consuming and costly procedure. A real-time multiplex, single tube RT-PCR assay for the detection of these viruses from potato leaves and tubers was developed using Cy5-, FAM-, JOE- and ROX-labelled TaqMan probes. The copy numbers for transcripts were quantified with a dynamic range of 8x10¹ to 8x10⁹ copies of PVX and PVS, 1x10² to 1x10¹⁰ copies of PLRV and 1x10³ to 1x10¹⁰ copies of TSWV. *In situ* hybridization and immunohistochemistry are being used on freshly harvested infected tubers of six cultivars to identify the distribution of PLRV, PVX, PVS and TSWV within them. Total RNA was rapidly and efficiently extracted from bulked tuber samples for the reliable detection of one or more of the viruses simultaneously. The assay is being validated in blind studies.

Published subsequently

Sheila M. Mortimer-Jones, Michael G.K. Jones, Roger A.C. Jones, Gordon Thomson and Geoffrey I. Dwyer. A single tube, quantitative real-time RT-PCR assay that detects four potato viruses simultaneously. *Journal of Virological Methods* (Accepted Jun 2009)

Notes

