9th Australasian Plant Virology Workshop

Melbourne Australia

16 – 19 November 2010

Sponsored by

Horticulture Australia

Victoria The Place To Be

CRC PLANT biosecurity

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Welcome

Welcome to the 9th Australasian Plant Virology Workshop (APVW). The organising committee would like to thank our fellow plant virologists and researchers of virus-like organisms for attending our virology workshop in Melbourne. This workshop provides a forum for plant virologists from Australia, New Zealand and the rest of the world to meet on a biannual basis and discuss plant virus and virus-like topics in a congenial environment, over four days.

Our theme for the workshop is “Plant viruses: Friend or foe?” We would like to build on the concepts introduced to this group at the 8th Australasian Plant Virology Workshop and consider the role of viruses as both pathogens and as beneficial organisms. The talks and posters for this workshop have been aligned with seven themes: Plant virus ecology and diversity; New and emerging viruses; Virus-like organisms; Plant virus diversity and detection; New tools and technologies; Plant host-virus interactions; and Plant virus epidemiology and climate change.

We have officially aligned the APVW with the Australasian Plant Pathology Society as a special interest group and we now have an official website aligned with APPS (http://www.australasianplantpathologysociety.org.au/). As part of the program we have also scheduled a one hour meeting to discuss the formalisation of the Australasian Plant Virology Special Interest Group (APV) and any issues that are relevant to our newly formed group.

We would like to extend a special thanks to several organisations for their support:

- Horticulture Australia Limited (HAL) for sponsoring this workshop
- Cooperative Research Centre for National Plant Biosecurity who are a platinum sponsor of this workshop, and providing the satchels
- Victorian Department of Primary Industries who sponsored Ulrich Melcher and Ko Verhoeven
- Qiagen as an exhibition sponsor, and
- The Jasper Hotel for their support in hosting the workshop.

The organising committee would especially like to thank the help and support of Chris Bottcher, Jo Mackie, Narelle Nancarrow, Mirko Milinkovic, Jo Luck, Sabine Perone, Mai Hlaing Loh and Mark Whattam for their help in assisting with the smooth running of this workshop.

We hope you enjoy the workshop and get a chance to enjoy the wonderful cosmopolitan city of Melbourne that we are lucky to call home.

Enjoy the workshop.

The Organisers

Brendan Rodoni
Fiona constable
Linda Zheng
<table>
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<tr>
<th>Time</th>
<th>Monday (15th)</th>
<th>Tuesday (16th)</th>
<th>Wednesday (17th)</th>
<th>Thursday (18th)</th>
<th>Friday (19th)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00 – 10:45</td>
<td>Workshop opening</td>
<td>9.00 – 9.30</td>
<td>Theme 2: New and Emerging viruses (cont)</td>
<td>Theme 5: New Tools and technologies</td>
<td>Theme 7: Plant Virus Epidemiology and Climate Change</td>
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<tr>
<td></td>
<td>Workshop opening</td>
<td>9.30 – 10.30</td>
<td>Theme 3: Virus-like organisms</td>
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<tr>
<td>10:45 – 1:15</td>
<td>Coffee</td>
<td>Coffee</td>
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<td>Coffee/Early Lunch</td>
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<tr>
<td>11:15 – 1:00</td>
<td>Theme 1: Plant Virus Ecology and Diversity (cont)</td>
<td>Theme 3: Virus-like organisms (cont)</td>
<td>Theme 5: New Tools and technologies (cont)</td>
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<tr>
<td>1:00 – 2:00</td>
<td>Lunch</td>
<td>Lunch</td>
<td>Lunch</td>
<td>Workshop closes</td>
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<tr>
<td>2:00 – 3:00</td>
<td>Poster session 1</td>
<td>APVW General Meeting</td>
<td>Poster Session 2</td>
<td></td>
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<tr>
<td>3:00 – 3:30</td>
<td>Coffee</td>
<td>Coffee</td>
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<tr>
<td>3:30 – 5:00</td>
<td>Registration for Workshop</td>
<td>Theme 2: New and Emerging viruses</td>
<td>Theme 4: Plant virus Diversity and Detection</td>
<td>Theme 6: Plant host-virus interactions</td>
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</tr>
<tr>
<td>5:31 – 8:30</td>
<td>Welcome reception</td>
<td>Organised tour/dinner</td>
<td>Free Evening</td>
<td>Official Workshop Dinner</td>
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## 9th Australasian Plant Virology Programme

<table>
<thead>
<tr>
<th>Date/Session</th>
<th>Time</th>
<th>Activity</th>
<th>Speaker</th>
<th>Title</th>
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<tr>
<td><strong>Monday 15/11/2010</strong></td>
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<tr>
<td></td>
<td>10:00 AM – 4:30 PM</td>
<td>Bioinformatics workshop</td>
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<td></td>
<td>3.30 – 5.30 PM</td>
<td>9th APVW Registration</td>
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<td></td>
<td>6:00 – 8:00 PM</td>
<td>Welcome Reception</td>
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<td><strong>Tuesday 16/11/2010</strong></td>
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<tr>
<td>Session 1</td>
<td>9.00 – 10.30 AM</td>
<td>Open Workshop</td>
<td>Chair: Brendan Rodoni</td>
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<td></td>
<td></td>
<td>Welcome Address</td>
<td>Graham Mitchell</td>
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<tr>
<td></td>
<td>10.30 – 11.15 AM</td>
<td>Coffee</td>
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<tr>
<td>Session 2</td>
<td>11.15 AM – 1.00 PM</td>
<td>Theme 1: Plant virus ecology and diversity (cont)</td>
<td>Chair: Brenda Coutts</td>
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<tr>
<td></td>
<td>11.15 – 11.45 AM</td>
<td>Oral presentation 1.2</td>
<td>Mike Pearson</td>
<td>Transmission and effects of <em>Botrytis virus X</em> and <em>Botrytis virus F</em> in <em>Botrytis cinerea</em></td>
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<tr>
<td></td>
<td>11.45 AM – 12.00 PM</td>
<td>Oral presentation 1.3</td>
<td>Ralf Dietzgen</td>
<td>Diversity of plant rhabdoviruses and evolutionary links to some multipartite negative-sense RNA viruses</td>
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<tr>
<td>Time</td>
<td>Session</td>
<td>Oral presentation</td>
<td>Speaker</td>
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<tr>
<td>12.00 – 12.15 PM</td>
<td>Oral presentation 1.4</td>
<td>Monica Kehoe</td>
<td>Sequence diversity of Australian <em>Zucchini yellow mosaic virus</em> isolates</td>
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<tr>
<td>12.15 – 12.30 PM</td>
<td>Oral presentation 1.5</td>
<td>Kieren Arthur</td>
<td>Genome analysis confirms uniqueness of the indigenous Australian <em>Velvet tobacco mottle virus</em></td>
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<tr>
<td>12.30 – 12.45 PM</td>
<td>Oral presentation 1.6</td>
<td>Stephen Wylie</td>
<td>Deep sequencing Australian native plant viruses</td>
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<tr>
<td>12.45 – 1.00 PM</td>
<td>Oral presentation 1.7</td>
<td>Colleen Higgins</td>
<td>Temporal and spatial analysis of <em>Dasheen mosaic potyvirus</em> genetic variability</td>
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<tr>
<td>1.00 – 2.00 PM</td>
<td>Lunch</td>
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**Session 3**

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<tr>
<th>Time</th>
<th>Session</th>
<th>Poster Session 1</th>
<th>Chair: Baozhong Meng</th>
<th>Title</th>
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</thead>
<tbody>
<tr>
<td>2.00 – 3.00 PM</td>
<td>Poster Session 1</td>
<td>Kar Mun Chooi</td>
<td></td>
<td>Sequence variation and the molecular detection of <em>Grapevine leafroll-associated virus</em>-3 (GLRaV-3) New Zealand isolates</td>
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<tr>
<td></td>
<td>Poster 1.9</td>
<td>Roger Jones</td>
<td></td>
<td>Potyviruses of wild and cultivated <em>Passiflora</em> spp. and legumes from Western Australia: biological properties and phylogenetic placement of coat protein sequences</td>
</tr>
<tr>
<td></td>
<td>Poster 1.10</td>
<td>Calum Wilson</td>
<td></td>
<td>Virus incidence within processing vegetable crops in Tasmania, and discovery of putative novel luteovirus</td>
</tr>
<tr>
<td></td>
<td>Poster 1.11</td>
<td>Nuredin Habili</td>
<td></td>
<td>Detection of grapevine viruses in wine grape vineyards in Thailand</td>
</tr>
<tr>
<td></td>
<td>Poster 1.12</td>
<td>Hanu Pappu</td>
<td></td>
<td>Biological characterisation of distinct strains of <em>Iris yellow spot virus</em></td>
</tr>
<tr>
<td></td>
<td>Poster 1.13</td>
<td>Nuredin Habili</td>
<td></td>
<td><em>Grapevine virus A</em> variants of group II are closely associated with Shiraz disease in South Africa and Australia and are also present in the USA</td>
</tr>
</tbody>
</table>
**Poster 2.10**
Hanu Pappu
Molecular characterization of *Bean leaf roll virus* and *Pea enation mosaic virus* from the Pacific Northwestern USA and development of ELISA assays for virus detection

**Poster 2.11**
Elizabeth Woo
Diagnostic techniques for detecting nepoviruses: *Cherry leafroll virus*, *Grapevine fanleaf virus*, *Strawberry latent ringspot virus* and *Tomato ringspot virus*

**Poster 2.12**
Mahmoud Khalifa
DsRNA elements and virus-like particles in *Sclerotinia sclerotiorum*

**Poster 2.13**
Kathy Parmenter
Passionfruit viruses in eastern Australia

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**3.00 – 3.30 PM**
Coffee

**Session 4**

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Speaker</th>
<th>Presentation Title</th>
<th>Additional Notes</th>
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<tbody>
<tr>
<td>3.30 – 3.45 PM</td>
<td>Oral presentation 2.1</td>
<td>Claude Bragard</td>
<td><em>Beet black scorch virus</em>, old endemic or emerging virus?</td>
<td></td>
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<tr>
<td>3.45 – 4.00 PM</td>
<td>Oral presentation 2.2</td>
<td>Roger Jones</td>
<td>Assessing the potential threat posed by spread of introduced and indigenous viruses to Australian native plants</td>
<td></td>
</tr>
<tr>
<td>4.00 – 4.15 PM</td>
<td>Oral presentation 2.3</td>
<td>Dan Cohen</td>
<td>Kiwifruit can be naturally infected by a wide range of viruses</td>
<td></td>
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<tr>
<td>4.15 – 4.30 PM</td>
<td>Oral presentation 2.4</td>
<td>Ramesh Chavan</td>
<td>Genome characterization of a <em>Tobamovirus</em> and a <em>Citrivirus</em> from kiwifruit</td>
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<tr>
<td>4.30 – 4.45 PM</td>
<td>Oral presentation 2.5</td>
<td>Arnaud Blouin</td>
<td>Novel vitiviruses infecting kiwifruit</td>
<td></td>
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<tr>
<td>4.45 – 5.00 PM</td>
<td>Oral presentation 2.6</td>
<td>Denis Persley</td>
<td>Current and potential viral diseases issues for the Australian vegetable industry</td>
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**6.00 PM**
Optional: Organised tour/dinner

Discover Melbourne CBD
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<thead>
<tr>
<th>Time</th>
<th>Session 5</th>
<th>Theme 2: New and emerging viruses (cont)</th>
<th>Chair: Stephen Wylie</th>
<th>Session 6</th>
<th>Theme 3: Virus-like organisms (cont)</th>
<th>Chair: Nicole Thompson</th>
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<tbody>
<tr>
<td>9.00 – 9.15 AM</td>
<td>Oral presentation 2.7</td>
<td>Nicole Thompson</td>
<td>Mosaic diseases of sugarcane in Indonesia: diagnostics and biosecurity implications</td>
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<tr>
<td>9.15 – 9.30 AM</td>
<td>Oral presentation 2.8</td>
<td>Cherie Gambley</td>
<td>Whitefly-transmitted viruses of Australian vegetable crops: endemic problems and exotic threats</td>
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<tr>
<td>9.30 – 9.45 AM</td>
<td>Oral presentation 2.9</td>
<td>Joe Tang</td>
<td><em>Strawberry latent ringspot virus</em> in New Zealand</td>
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<tr>
<td>Theme 3: Virus-like organisms</td>
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<tr>
<td>9.45 – 10.15 AM</td>
<td>Oral presentation 3.1</td>
<td>Ian Scott</td>
<td>Towards an understanding of the epidemiology of <em>Candidatus</em> Liberibacter solanacearum in New Zealand</td>
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<tr>
<td>10.15 – 10.30 AM</td>
<td>Oral presentation 3.2</td>
<td>Fiona Constable</td>
<td>Developing and validating molecular diagnostics for Liberibacter and Phytoplasmas for the Australian and New Zealand potato industry</td>
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<tr>
<td>10.30 – 11.00 AM</td>
<td>Coffee</td>
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<tr>
<td>11.00 – 11.45 AM</td>
<td>Oral presentation 3.3</td>
<td>Ko Verhoeven</td>
<td>Identification and epidemiology of pospiviroids</td>
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<tr>
<td>11.45 AM – 12.00 PM</td>
<td>Oral presentation 3.4</td>
<td>Paul Guy</td>
<td>RT-PCR of 50 year old RNA identifies <em>Peach latent mosaic viroid</em> in New Zealand</td>
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<tr>
<td>12:00 – 12:15 PM</td>
<td>Oral presentation 3.5</td>
<td>John Randles</td>
<td>A molecular comparison of Iranian and Australian <em>Peach latent mosaic viroid</em> isolates</td>
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<td>12:15 – 12:30 PM</td>
<td>Oral presentation 3.6</td>
<td>Mark Anderson</td>
<td>Genome comparison of two isolates of “<em>Candidatus Phytoplasma australiense</em>”</td>
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<tr>
<td>Time</td>
<td>Event</td>
<td>Speaker(s)</td>
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<tr>
<td>12:30 – 12:45 PM</td>
<td>Oral presentation 3.7</td>
<td>Kate Chamberlain</td>
<td>Gene expression and metabolite changes of tomato plants in response to <em>Candidatus</em> Phytoplasma infection</td>
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<tr>
<td>12:45 – 1:00 PM</td>
<td>Oral presentation 3.8</td>
<td>Ratana Sdoodee and Lucy Tran-Nguyen</td>
<td>Sugarcane white leaf disease – Thailand overview.</td>
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<tr>
<td>1.00 – 2.00 PM</td>
<td>Lunch</td>
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<tr>
<td>2.00 – 3.00 PM</td>
<td>APVW General Meeting</td>
<td>Chair: Brendan Rodoni</td>
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<tr>
<td>2.00 – 2.15 PM</td>
<td>Meeting agenda to be circulated</td>
<td>Mark Gibbs</td>
<td>Australian quarantine policy for nucleic acids of plants and plant pathogens</td>
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<td>3.00 – 3.15 PM</td>
<td>Coffee (shortened)</td>
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<tr>
<td>3.15 – 5.00 PM</td>
<td>Theme 4: Plant virus diversity and detection</td>
<td>Chair: Neena Mitter</td>
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<tr>
<td>3:15 – 3:30 PM</td>
<td>Oral presentation 4.1</td>
<td>Andrew Geering</td>
<td>Viruses for breakfast, lunch and dinner</td>
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<tr>
<td>3:30 – 3:45 PM</td>
<td>Oral presentation 4.2</td>
<td>Claude Braggard</td>
<td>Sugar beet soil-borne viruses – surprising “ménage a trios” combination</td>
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<tr>
<td>3:45 – 4:00 PM</td>
<td>Oral presentation 4.3</td>
<td>John Thomas</td>
<td>Diversity of <em>Tobacco streak virus</em> strains and first report of <em>Strawberry necrotic shock</em> virus in Australia&quot;.</td>
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<tr>
<td>4.00 – 4.15 PM</td>
<td>Oral presentation 4.4</td>
<td>Anthony James</td>
<td>Development of a rolling circle amplification-based assay for the detection and characterisation of <em>Banana streak virus</em>.</td>
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<tr>
<td>4.15 – 4.30 PM</td>
<td>Oral presentation 4.5</td>
<td>Sarah Jane Cowell</td>
<td>Elimination of viruses from elite kiwifruit germplasm</td>
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<td>Time</td>
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<tr>
<td>4.30 – 4:45 PM</td>
<td>Oral presentation 4.6 Merrin Spackman Spatial and temporal variation of pulse comoviruses using a novel PCR test</td>
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<tr>
<td>4:45 – 5:00 PM</td>
<td>Oral presentation 4.7 Hanu Pappu Genetic complementation between two viruses in an otherwise restrictive host</td>
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<tr>
<th>Time</th>
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<tr>
<td>6.00 PM</td>
<td>Free Evening</td>
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**Thursday 18/11/2010**

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<tr>
<th>Session 9</th>
<th>Time</th>
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<td></td>
<td>9.00 – 10.45 AM</td>
<td>Theme 5: New tools and technologies Chair: Dan Cohen Sponsored by Horticulture Australia Limited</td>
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<tr>
<td></td>
<td>9.00 – 9.45 AM</td>
<td>Oral presentation 5.1 Michael Kube Importance of genomics in phytoplasma research</td>
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<td>9.45 – 10.00 AM</td>
<td>Oral presentation 5.2 Anastasija Chomic New diagnostic tools for the <em>Luteoviridae</em></td>
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<tr>
<td></td>
<td>10.00 – 10.15 AM</td>
<td>Oral presentation 5.3 Narelle Nancarrow Development of a one-step multiplex RT-qPCR assay for the detection and quantification of CYDV-RPV in wheat</td>
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<tr>
<td></td>
<td>10.15 – 10.30 AM</td>
<td>Oral presentation 5.4 Sonia Lilly Identification and validation of reference genes for qPCR transcript normalisation of gene expression studies in virus-infected <em>Arabidopsis thaliana</em></td>
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<tr>
<td></td>
<td>10.30 – 10.45 AM</td>
<td>Oral presentation 5.5 Linda Zheng Use of a duplex quantitative one-step RT-PCR to measure rate of degradation for virus RNA isolated from FTA cards</td>
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<table>
<thead>
<tr>
<th>Time</th>
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<tr>
<td>10.45 – 11.15 AM</td>
<td>Coffee</td>
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<table>
<thead>
<tr>
<th>Session 10</th>
<th>11.15 AM – 1.00 PM</th>
<th>Theme 5: New tools and technologies (cont)</th>
<th>Chair: Denis Persley</th>
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</thead>
<tbody>
<tr>
<td>11.15 – 11.30 AM</td>
<td>Oral presentation 5.6</td>
<td>Neena Mitter</td>
<td>Artificial microRNAs-mediated resistance to <em>Tomato spotted wilt virus</em></td>
</tr>
<tr>
<td>11.30 – 11.45 AM</td>
<td>Oral presentation 5.7</td>
<td>Paul Guy</td>
<td>Rapid identification of a Tomato leaf curling virus using Mass Spectrometry</td>
</tr>
<tr>
<td>11.45 AM – 12.00 PM</td>
<td>Oral presentation 5.8</td>
<td>Hao Luo</td>
<td>Detection of plant viruses using one-dimensional gel electrophoresis and peptide mass fingerprints</td>
</tr>
<tr>
<td>12.00 – 12.15 PM</td>
<td>Oral presentation 5.9</td>
<td>Nuredin Habili</td>
<td>Detection of grapevine viruses by RT-PCR in vine leaves blot-dried with paper and stored at ambient temperature for over eight years</td>
</tr>
<tr>
<td>12.15 – 12.30 PM</td>
<td>Oral presentation 5.10</td>
<td>Fiona Constable</td>
<td>Molecular diagnostics for the detection of strawberry viruses</td>
</tr>
<tr>
<td>12.30 – 12.45 PM</td>
<td>Oral presentation 5.11</td>
<td>Dan Cohen</td>
<td>Using ELISA to indicate the presence of strain variants of <em>Grapevine leafroll-associated virus 3</em> in grapevines</td>
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<tr>
<td>12.45 – 1.00 PM</td>
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<td>Sharon van Brunschot</td>
<td>New technologies for monitoring begomoviruses and their whitefly vectors</td>
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<td>Poster 3.10</td>
<td>Nuredin Habili</td>
<td>A phytoplasma from subgroup 16Sr II is associated with little leaf of <em>Medicago arborea</em> (tree medic) in South Australia</td>
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<td>Poster 3.11</td>
<td>Nuredin Habili</td>
<td>Studies on the genetic variability of Lime witches’-broom phytoplasma in Iran</td>
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<td>Poster 5.14</td>
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<td>Hanu Pappu</td>
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<td>John Fletcher</td>
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<td>Poster 7.8</td>
<td>Mohammad Aftab</td>
<td>BWYV an emerging problem in pulse crops</td>
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<td>Poster 7.9</td>
<td>Mirko Milinkovic</td>
<td>Improved methods for detection and control of <em>Potato virus Y</em> (PVY) in potatoes</td>
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| 2.45 – 3.00 PM | Coffee (shortened) |

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<td>Peter Waterhouse</td>
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<td>Roger Jones</td>
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<td>Oral presentation 6.4</td>
<td>Robin MacDiarmid</td>
<td>Progress in characterising PKR, a Plant-encoded and Double-stranded RNA-activated protein kinase</td>
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<td>Hamish McLean</td>
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<td>Session 13</td>
<td>9.00 – 11.00 AM</td>
<td>Themed 7: Plant virus epidemiology and climate change</td>
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<td>9.00 – 9.40 AM</td>
<td>Oral presentation 7.1</td>
<td>Piotr Tribecki</td>
<td>“Wheat Curl Mite and its Role in the Transmission of <em>Wheat streak mosaic virus</em> in Australia”</td>
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<td>9.40 – 10.00 AM</td>
<td>Oral presentation 7.2</td>
<td>Adam Miller</td>
<td><em>Wheat streak mosaic virus</em>: alternative hosts, infection of wheat, oat and barley cultivars, seed transmission studies and spatial and temporal spread patterns</td>
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<td>10.00 – 10.15 AM</td>
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<td>Brenda Coutts</td>
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<td>10.15 – 10.30 AM</td>
<td>Oral presentation 7.4</td>
<td>Mark Schwinghamer</td>
<td>Studies on effect of climate factors on seasonal incidence, loss estimation, epidemiology and host plant resistance for <em>Peanut bud necrosis virus</em> of groundnut in north eastern Karnataka</td>
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<td>10.30 – 10.45 AM</td>
<td>Oral presentation 7.5</td>
<td>Gururaj Sunkad</td>
<td>Climate Change: potential impact on <em>Barley yellow dwarf virus</em> spread and occurrence.</td>
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<td>10.45 – 11.00 AM</td>
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<td>Workshop closes</td>
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Oral presentation 1.1

R.E.F. Matthews’ Memorial Lecture

Expanding concepts of plant viruses

Ulrich Melcher\(^1\)

\(^1\) Robert J. Sirny Prof. of Agricultural Biochemistry and Regents' Professor, Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater OK 74078 – 3035, USA
Presenter’s e-mail address: ulrich.melcher@okstate.edu

Plant viruses are generally regarded as highly undesirable pathogens of plants. Research of the Oklahoma-based Plant Virus Biodiversity and Ecology (PVBE) project suggests that this is a very narrow and prejudiced view of plant-associated viruses. The project collected specimens of over 500 plant species from a natural area in Oklahoma and tested them for sequence signatures of the presence of viruses. The results contribute to the expansion or revision of current concepts of plant viruses in many ways and support the following hypotheses. 1) Viruses are abundant in natural systems. 2) Viruses currently recognized are but a small fraction of the viruses in existence. 3) Viruses have not evolved to be pathogens. 4) Viruses from non-cultivated plants may differ from viruses adapted to cultivated plants. 5) Viruses are ancient parts of ecosystems. These concepts will be illustrated by PVBE project findings from among the families Tymoviridae, Totiviridae, Alphaflexiviridae, Comoviridae, Caulimoviridae, Tombusviridae and Virgaviridae. Overall, the concepts suggest that viruses play, and have played, important roles in stable ecosystems. Their contributions remain to be explored fully.
Oral presentation 1.2

Transmission and effects of *Botrytis virus X* and *Botrytis virus F* in *Botrytis cinerea*.

Michael Pearson\(^1\), Colin Tan, Barbara Boine

\(^1\)School of Biological Sciences, The University of Auckland, New Zealand. Presenter's e-mail address: m.pearson@auckland.ac.nz

The necrotrophic plant pathogen *Botrytis cinerea* is a major horticultural pathogen worldwide. The mycoviruses *Botrytis virus X* (BVX) and *Botrytis virus F* (BVF), which were originally isolated from a single *B. cinerea* isolate, are the type species of the viral genera *Botrexvirus* (*Alphaflexiviridae*) and *Mycocflexivirus* (*Gammaflexiviridae*), respectively. Both viruses were originally found in a single *Botrytis cinerea* isolate but have subsequently been detected in several *B. cinerea* isolates from a range of plant hosts and geographical locations worldwide. The conventional view of mycovirus transmission is that horizontal transmission occurs only via hyphal fusion and possibly sexual reproduction both of which are restricted in *B. cinerea* by incompatibility factors. However the presence of the two viruses in cultures from such diverse origins indicates that they have at some stage spread between *Botrytis* populations and we have demonstrated that between sexually compatible *B. cinerea* isolates BVX is transmitted to <50% of ascospores.

Comparison of virus infected and non-infected sexual progeny from the same parents indicated that the viruses have only minor effects on the *Botrytis* isolates from which they were first detected but their effect on previously uninfected isolates was more extreme. When *B. cinerea* BO5-10 was transfected with partial purified virus extracted from 3 different *Botrytis* isolates containing BVX, BVF, or both. BO5-10 strains transfected with BVF showed most severe phenotype changes like greatly reduced growth rate, unusual hyphae branching and lack of production of macrospores.
Oral presentation 1.3

Diversity of plant rhabdoviruses and evolutionary links to some multipartite negative-sense RNA viruses

Ralf G Dietzgen¹, Ee Ren Tan¹, Chun-Wei Allen Feng¹, Jade Yung-Chieh Hsu¹, Roger Mitchell¹, Neena Mitter¹, Colleen M. Higgins²

¹DEEDI Agri-Science Queensland, QABC and School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia QLD 4067, Australia
²School of Applied Sciences, Auckland University of Technology, Auckland, New Zealand
Presenter’s e-mail address: ralf.dietzgen@deedi.qld.gov.au

Rhabdoviruses are important pathogens of plants and vertebrates and many are vectored by insects in which they also replicate. Rhabdoviruses are enveloped negative-sense, single-stranded RNA viruses that are taxonomically classified in the family Rhabdoviridae, order Mononegavirales. Plant-adapted rhabdoviruses have been placed into the genera Cytorhabdovirus and Nucleorhabdovirus, based on their site of virion maturation in the cytoplasm and nucleus of infected plant cells, respectively. There are also over 75 putative plant rhabdoviruses identified chiefly by their distinctive bacilliform morphology and size, but many lack information on where in the cell they replicate and others may not even be rhabdoviruses. Polymerase amino acid sequence similarities of confirmed rhabdoviruses with those of plant-infecting viruses outside the Mononegavirales include viruses in the genera Ophiovirus and Varicosavirus and the unclassified orchid fleck virus.

We are using several approaches to gain sequence information from some of the less studied plant rhabdoviruses to assist in taxonomic placement and to study the evolution of lettuce necrotic yellows virus, the type species of the cytorhabdoviruses that occurs in Australia and New Zealand. 1. Nucleoprotein specific RT-PCR to identify LNYV subgroup evolution in time and space, 2. RT-PCR and degenerate primer sets designed from conserved motifs of the viral polymerase and 3. next generation 454 sequencing of purified virus preparations to obtain complete or near-complete viral genomes. This information will provide a more complete picture of rhabdovirus genomes, their diversity and phylogeny and may lead to the development of new diagnostic tools for biosecurity.
Sequence diversity of Australian Zucchini yellow mosaic virus isolates

Monica A. Kehoe\textsuperscript{1}, Brenda A. Coutts\textsuperscript{1}, Stephen J. Wylie\textsuperscript{2}, Craig G. Webster\textsuperscript{1,4} and Roger A.C. Jones\textsuperscript{1,3}

\textsuperscript{1}Department of Agriculture and Food Western Australia, Locked Bag No. 4, Bentley Delivery Centre, WA 6983, Australia,
\textsuperscript{2}State Agricultural Biotechnology Centre, Murdoch University, Perth, WA 6150, Australia,
\textsuperscript{3}School of Plant Biology, Faculty of Natural and Agricultural Sciences, University of Western Australia, Stirling Highway, Crawley, WA 6009, Australia,
\textsuperscript{4}Current address: United States Department of Agriculture-Agricultural Research Services (USDA-ARS), Fort Pierce, FL 34945, USA.
Presenter’s e-mail address: monica.kehoe@agric.wa.gov.au

Zucchini yellow mosaic virus (ZYMV) causes yield and quality losses in cucurbit crops worldwide. Between 2005 and 2010, leaf samples from symptomatic cucurbit plants were collected from five major cucurbit growing areas around Australia. The complete coat protein (CP) nucleotide sequences of 42 Australian ZYMV isolates, were compared to those of 100 other isolates from different countries. Phylogenetic analysis of all 142 complete CP sequences revealed the presence of three distinct clades, one of which (clade A) sub-divided into four sub-clades, I-IV. The Australian isolates fitted all within clades A and B, and within clade A into sub-clades A-I and A-II. The Australian isolates within clade B were all from one isolated location in north-west Australia (Kununurra), and had sequence identities of 85.66 - 89.13% to isolates from Singapore, Reunion Island and Vietnam. Isolates from the eastern (Queensland and Victoria) and central western (Carnarvon, WA) coasts of Australia fitted into clade A-I, with sequence identities of 95.94 – 98.92% to isolates from Europe and Japan. The three isolates from the Northern Territory fitted into clade A-II with sequence identities of 94.62 – 99.04% to others within this sub-clade, being closest to USA and Iranian isolates.

A multiplex Real-Time PCR was developed using dual-labelled probes which distinguished between the Australian isolates within the different clades or sub-clades. These findings show that Australian sequences grouped together according to collection location suggesting that there may have been at least three separate introductions of ZYMV. Also, once established in an isolated growing area few further sequence changes were evident indicating that new introductions occur rarely. It is difficult to determine how the virus was introduced into these areas given the lack of alternative hosts and the limited seed transmission found.
Genome analysis confirms uniqueness of the indigenous Australian *Velvet tobacco mottle virus*

Kieren Arthur¹ and John W. Randles¹

¹School of Agriculture, Food and Wine, The University of Adelaide, PMB1 Glen Osmond, SA 5064 Australia
Presenter’s e-mail address: kieren.arthur@adelaide.edu.au

*Velvet tobacco mottle virus* (VTMoV; genus *Sobemovirus*) occurs as a natural infection in *Nicotiana velutina* (Velvet tobacco), a native of the arid region of central Australia. VTMoV is distinguished by its narrow experimental host range and transmissibility by the mirid *Cyrtopeltis nicotianae* [Hemiptera; Miridae]. In the field, the virus, mirid vector and native host plant together comprise a unique plant virus pathosystem which is well adapted to its ecological niche, and independent of anthropogenic influences. The full genome sequence of VTMoV was attained using a genome walking strategy with both degenerate and specific primers. Its sequence and genome organization confirms that VTMoV is a unique sobemovirus and phylogenetic analysis groups it separately from other sequenced Australian sobemoviruses. This is consistent with the hypothesis that VTMoV is not a recently introduced sobemovirus, but rather a product of evolution within a unique Australian ecosystem, representing a novel plant virus lineage. Further investigations are being undertaken to determine the scope of genetic variation between isolates, and whether differences in sequence arise either from obligatory mirid transmission, or from the ability of particular isolates to support replication of the satellite RNA (also known as a virusoid). Further studies of the molecular ecology of VTMoV may provide insights into plant virus evolution in natural or ‘wild’ ecosystems.
Deep sequencing Australian native plant viruses

Stephen J Wylie1, Michael GK Jones

1Plant Virus Section, Plant Biotechnology Research Group, SABC
Murdoch University WA6150, Australia
Presenter’s e-mail address: s.wylie@murdoch.edu.au

Polyadenylated RNA from a plant of the Western Australian endemic legume Hardenbergia comptoniana and from a plant of the naturalised weedy species Passiflora cearulea was sequenced using Illumina Solexa technology. De novo assembly of the resulting 76 nucleotide reads revealed the complete genome sequences of two distinct isolates of Hardenbergia mosaic virus (HarMV), an isolate of Passionfruit woodiness virus, and an undescribed virus with identity to members of the family Betaflexiviridae. Overall sequence coverage was greater than 100-fold for each genome. The relative abundance of each virus was estimated. Possible interactions between the coinfecting isolates of HarMV, and relatedness of the novel virus to known viruses, are discussed.
Temporal and spatial analysis of *Dasheen mosaic potyvirus* genetic variability

Colleen M. Higgins\textsuperscript{1}, Wee-Leong Chang\textsuperscript{1}, Mary Cong\textsuperscript{1}, Annie Yuan\textsuperscript{1}, Nitish Anand\textsuperscript{1} & Michael Pearson\textsuperscript{2}

\textsuperscript{1}School of Applied Sciences, Auckland University of Technology, New Zealand. 
\textsuperscript{2}School of Biological Sciences, The University of Auckland, New Zealand. 
Presenter’s e-mail address: colleen.higgins@aut.ac.nz

Dasheen mosaic potyvirus (DsMV) is one of the most important viral diseases of ornamental and edible aroids. In particular, it can depress the yield of taro (*Colocasia* sp and *Xanthosoma* sp), a staple food of many Pacific Island communities. Being an RNA virus, DsMV exists as a population of sequences known as a quasi species. The profile of viral sequences that can occur within a plant can vary from one plant to another, with one sequence generally becoming dominant. The influences that cause a particular sequence to dominate, thereby driving the evolution of the virus, are unknown but the host and the conditions under which it is grown may be involved. We are interested in the genetic variation of DsMV and what role, if any, the host plays in influencing this evolution.

We have two questions we are attempting to answer in order to understand how DsMV evolves and if the host has a role in this process: 1. How does the DsMV coat protein (CP) sequence vary over time (temporal analysis)? 2. How does this sequence vary between hosts and between locations (spatial analysis)? To answer question 1, we are analyzing how the DsMV CP sequence changes as infection progresses within glasshouse grown *Colocasia* sp. To answer question 2, we are analyzing DsMV CP sequences of a range of isolates from both *Colocasia* sp or *Xanthosoma* sp. from different locations in the South Pacific. Our results suggest that DsMV can accommodate considerable variation in the short term and that over the longer term, the host does appear to play a role in which sequence becomes dominant.
Sequence variation and the molecular detection of *Grapevine leafroll-associated virus-3* (GLRaV-3) New Zealand isolates

K. M. Chooi¹, M. N. Pearson¹, and D. Cohen²

¹School of Biological Sciences, University of Auckland, P.O. Box 92019, Auckland, New Zealand
²The New Zealand Institute for Plant & Food Research Limited, Private Bag 92169, Auckland, New Zealand
Presenter’s e-mail address: kcho115@aucklanduni.ac.nz

*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. Results have shown that there is substantial genetic variability within the New Zealand GLRaV-3 population with the identification of both the New York (NY-1) and South African (GP18) isolates plus several other isolates that show significant variability compared to previously published sequences with nucleotide differences ranging from 5 to 22 %.

Based on this sequence data molecular detection protocols such as multiplex RT-PCR and quantitative real-time RT-PCR (qRT-PCR) were developed and used to screen field samples from around New Zealand. These protocols target several regions across the genome which allows for the detection and quantification of the main NZ isolates.
Poster 1.9

Potyviruses of wild and cultivated Passiflora spp. and legumes from Western Australia: biological properties and phylogenetic placement of coat protein sequences

B.A. Coutts¹, M.A. Kehoe¹, C.G. Webster²*, S.J. Wylie² and R.A.C. Jones¹,³

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²State Agricultural Biotechnology Centre, Murdoch University, Perth, WA 6150, Aus
³Plant Biology School, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia
*Current address: United States Department of Agriculture-Agricultural Research Services (USDA-ARS), Fort Pierce, FL 34945, USA.
Presenter’s e-mail address: roger.jones@agric.wa.gov.au

In 2005-2010, potyvirus infection was detected in Passiflora and legume species growing at eight widely separated locations in different climatic zones in Western Australia. The isolate collection localities were Broome, Carnarvon, Geraldton, Kununurra, Perth, Pemberton and Margaret River. Potyvirus isolates were obtained from *P. edulis* (passionfruit), *P. caerulea* (blue passion flower), *Vigna unguiculata* (cowpea), five naturalised wild species, *Passiflora foetida* (stinking passion flower), *Macroptilium atropurpureum* (siratro), *Clitoria ternatea* (butterfly pea), *Vigna trilobata* (wild mung bean) and *Rhynchosia minima* (Rhycho), and one native species *Hardenbergia comptoniana* (native wisteria). All samples were tested initially by ELISA using generic potyvirus antibodies.

When the complete coat protein genes of 33 potyvirus isolates were sequenced and their nucleotide sequences subjected to phylogenetic analysis, the isolates from legume species were *Bean common mosaic virus* (BCMV) (4), *Cowpea aphid-borne mosaic virus* (1), *Hardenbergia mosaic virus* (4), *Passiflora virus Y* (PaVY) (9), *Passiflora mosaic virus* (PaMV) (1), and one from *C. ternatea* tentatively named *Clitoria chlorosis virus* (CICV). The isolates from *Passiflora* spp. were *Passionfruit woodiness virus* (PWV) (8) and PaVY (5).

When isolates of PaVY (8), PWV (5), PaMV (1), and CICV (1) were inoculated to 13 plant species in six families, all infected *Nicotiana benthamiana* systemically. None infected *Cucumis sativus*, *N. glutinosa*, *Pisum sativum* or *Vicia unguiculata*. CICV, PaMV, 6/8 isolates of PaVY, and 1/5 isolates of PWV caused local infection in *Chenopodium* spp. CICV and 2/8 isolates of PaVY caused local infection in *Gomphrena globosa*. *Phaseolus vulgaris* plants became infected locally by CICV, PaMV, 8/8 isolates of PaVY and 3/5 of PWV. Plants of *Glycine max* were infected systemically by CaCV, PaMV, 6/8 isolates of PYV, and 0/5 isolates of PWV. *M. atropurpureum* became infected systemically by CICV, and 6/8 isolates of PaVY, including all four originally from legumes, but not by PWV, satisfying Koch’s postulates for one PaVY isolate originally from this host but not for CICV in which infection was symptomless. *P. caerulea* and/or *P. edulis* became infected by 5/5 isolates of PWV, and 4/8 isolates of PaVY, satisfying Koch’s postulates with PWV isolates from *P. edulis* (1) and *P. caerulea* (2).
Poster 1.10

Virus incidence within processing vegetable crops in Tasmania, and discovery of putative novel luteovirus

Susan Lambert, Alison Dann & Calum Wilson

1Tasmanian Institute of Agricultural Research, University of Tasmania
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The diversity of viruses within the processing and fresh market vegetable sector of Tasmania is relatively unknown. Over three seasons surveys were undertaken of legume (peas, green beans), and brassica (cauliflower, broccoli) crops.

The surveys involved random sampling (300 leaves) of plants across NW Tasmanian cropping districts for testing of a range of viral pathogens. Initial ELISA testing included, universal luteovirus, universal potyvirus, Alfalfa mosaic virus, Cucumber mosaic virus, Tomato spotted wilt virus, Subclover stunt virus (legumes only), and Cauliflower mosaic virus (brassicas only). Samples testing positive for universal tests were further screened for other specific viruses using ELISA (potyvirus) and genomic cDNA sequencing of the coat protein and P0 genes (luteovirus).

Most crops (80%) were found to have one or more of the tested viruses, however, the estimated level of infection within any crop was low (3%). Most viruses had been detected in Tasmania previously with the exception of Pea seed-borne mosaic virus (PSbMV) in pea crops in the first survey season, constituting a new record for Tasmania. However, incidence of PSbMV was associated with an imported seed lot and subsequent sampling seasons failed to find this virus again.

The most prevalent virus family recorded was the Luteoviridae (60% of all crops). Genomic sequencing revealed most isolates matched Turnip yellows virus (94-99%) (previously a synonym of Beet western yellows virus) coat protein and P0 gene sequences on GenBank. However, five isolates, from peas only, did not conclusively match any GenBank sequences. The coat protein gene sequences closest match was Cucurbit aphid-borne yellows virus (81-83%) and the P0 gene sequences matched Beet western yellows virus (95%). This result could indicate a new polerovirus previously unsequenced with similarities to other poleroviruses or a recombinant virus from different strains.

These results suggest the targeted virus disease do not pose any major threats to the productivity of the processed vegetable market but the prevalence of TuYV in particular needs to be monitored as this virus may cause significant crop losses in the future as has been found in the UK.
Detection of grapevine viruses in wine grape vineyards in Thailand

Nuredin Habili¹, Visooth Lohitnavy², Chaorai Kanchanomai³ and John W. Randles¹

¹Waite Diagnostics, Waite Campus. The University of Adelaide, URRBRAE, SA 5064, ²52 Moo 9 Phayyen, Pakchong, Nakornrachasima, Thailand. ³Hua Hin Hills Vineyard, 174/1-4 Viphavadee-Rangsit Soi 78 Viphavadee-Rangsit Rd. Sikun, Don Muang, Bangkok 10210 Thailand
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Thailand is not recognised as a major wine producing country, but it has the potential to become one. Over 80% of vineyards are allocated to table grape production. Currently, total wine production in Thailand is just over 1.1 million bottles a year. The Thai wine industry is dependent on the importation of popular Vitis vinifera varieties from temperate regions of the world. Newly planted vine material is being tested for performance in this tropical environment, and it is considered to be necessary to exclude viruses from these introduced vines as these pathogens could affect not only vine characteristics, but also initiate epidemics if new vectors are present in Thailand. For example, experience in various countries has shown that once infected material is established in the vineyard insect or other vectors of some viruses can spread infection to previously uninfected vine varieties. We have tested wine grape varieties imported into Thailand for virus infection. In February 2007 three vineyards in Nakorn Ratchasima were visited and 20 suspect samples and two healthy (including 13 Shiraz samples) were collected for processing. The samples were imported into Australia as extracts in 4 M guanidine hydrochloride. The varieties tested were Shiraz, Chenin Blanc, Tempranillo and the Brazilian rootstock IAC-572 Jales (V. tiliifolia x '101-14 Mgt') suitable for tropical regions. The viruses detected in samples by RT-PCR were, Grapevine leafroll-associated virus 1 (GLRaV-1), GLRaV-3, GLRaV-5, Grapevine virus A and Grapevine rupestris stem pitting associated virus. In May 2010 another 20 samples were sent from Hua Hin (14 were Shiraz samples). All Shiraz samples tested positive for Grapevine fleck virus variant B (GFkV-B) as well as GRSPaV, while 11 were also infected with GLRaV-1. GFkV-A was detected in 9 samples. The other infected samples in this group were V. vinifera cv. Colombard which had GLRaV-1 and GRSPaV and the Brazilian rootstock which had GRSPaV and GFkV-B. No GFkV was detected in Colombard. The following viruses were not detected in Thai material: GLRaV-2, GLRaV-9 and Grapevine fanleaf virus. None of the 23 samples tested for phytoplasmas using generic primers were positive. This is the first report of grapevine virus detection in Thailand.
Biological Characterization of Distinct Strains of Iris yellow spot virus

S. Bag\textsuperscript{1}, C.S. Cramer\textsuperscript{2}, H.F. Schwartz\textsuperscript{3}, and H.R. Pappu\textsuperscript{1}

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Thrips-transmitted \textit{Iris yellow spot virus} (IYSV) is an important limiting factor to the production of bulb and seed crops in the Pacific Northwestern USA (PNW) and elsewhere. While much is known about the molecular variability of IYSV isolates, little or no information is available on the biological variability and the possible existence of strains. Several IYSV isolates were evaluated for their phenotype on two indicator hosts, \textit{Datura stramonium} (a local lesion host) and \textit{Nicotiana benthamiana} (a systemic host) following mechanical inoculation. IYSV-infected onion plants were collected from commercial fields. Seedlings of both experimental hosts at four to six leaf-stages were mechanically inoculated. The initial symptoms of necrotic spots appeared in inoculated leaves after 8- to 9 DPI in both hosts; in \textit{N. benthamiana} the virus became systemic producing severe necrotic spots 15-20 DPI, gradually spreading to newly emerging leaves and ultimately killing the plants by 45-50 DPI. In the case of \textit{D. stramonium}, the virus remained confined to inoculated leaves and the leaves dried in 35-40 DPI. Based on various symptoms developed and the DPI resulting in plant death, virus isolates could be subdivided into severe and mild strains. Host response was evaluated based on the following scorable phenotypic parameters: appearance of symptoms on inoculated leaves days post inoculation (DPI), DPI for the appearance of systemic symptoms on younger, uninoculated leaves, severity of symptoms, and effect on plant growth and vigor. Based on these parameters, two distinct strains of IYSV were identified. The duration in DPI that was necessary to produce systemic symptoms and the subsequent death of inoculated plants varied between the mild and severe strains. In the case of the severe strain, systemic symptoms appeared 12 to 15 DPI and by 22 DPI, plants were severely infected and newly emerging leaves showed severe necrotic spots. By 50 DPI, inoculated plants died. The mild strain produced more benign symptoms as inoculated plants retained the vigor and optimal growth even after 60 DPI, with fewer new leaves showing systemic infection and the newly emerging leaves lacked symptoms.
**Poster 1.13**

*Grapevine virus A* variants of group II are closely associated with Shiraz disease in South Africa and Australia and are also present in the USA

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Shiraz disease (SD) is of great concern to the South African grapevine industry since it kills affected grapevines of noble cultivars such as Shiraz and Merlot, and is spreading naturally in vineyards. Symptoms of the disease include lack of lignifications in canes and abnormal function of cambium cells resulting in un lignified wood and enlarged, hypertrophic phloem cells. Canes of SD-affected plants do not mature and are rubbery in texture. The leaves are shed much later than in disease-free grapevines. Most grapevine cultivars and all *Vitis* rootstocks do not show the symptoms of SD, but it can be transmitted easily from these to SD-susceptible grapevines through grafting infected material and by the mealybug vector *Planococcus ficus*. Once susceptible plants show the symptoms of SD, they never recover and die within 3-5 years. A similar disease occurs in Australia, which has temporarily been named Australian Shiraz disease (AuSD). Infected vines in Australia do not die but their yield is greatly reduced. It has been found that a vitivirus, *Grapevine virus A* (GVA), is the major virus associated with the disease in both countries. Three divergent molecular groups of the virus (I, II, III) were identified in South Africa. Results showed that the variants of molecular group II are closely associated with SD. Primers flanking 293 nt of a highly variable segment of the replicase gene, specific to variant groups I and II were used in RT-nested PCR. Amplicons were cloned, analysed using single-strand conformation polymorphism (SSCP), and sequenced. We tested 15 samples of cv. Shiraz from South Africa, of which 6 had SD symptoms. Six samples from Australia with AuSD and 3 unknown cultivars from the USA were also tested. The results showed that the variants of the group II were associated with the 6 SD-affected plants in South Africa, while 8 of the 10 SD-free plants tested negative. Five of the 6 grapevines affected by AuSD from Australia and one unknown grapevine cultivar from the USA tested positive for the variants of group II.
Oral presentation 2.1

Beet black scorch virus, old endemic or emerging virus?

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Beet black scorch virus is a Necrovirus found in the eighties in China, later on in the USA and Europe. Questions have been raised about its spatial distribution as well as on the risk of development of the black scorch disease similarly as rhizomania, since the virus is soil-borne transmitted by Olpidium brassicae.

Based on extensive surveys in Iran, more than 60 positive samples out of 203 visited fields were analyzed. The diversity found within the targeted sequences obtained (coat protein gene, 3’UTR) compared to already known or published data support the hypothesis of a spread of the virus from Central Asia, therefore questioning the natural host range of the virus.

Result obtained using full length clones regarding the pathogenicity of the virus and the production of the “black scorch” symptom, using either local lesion or systemic host plants like Chenopodium quinoa, Beta macrocarpa and Nicotiana benthamiana will also be displayed.

The presence of numerous association of the virus with a satellite enhancing the symptom expression will be discussed as the structural conservation of the Barley yellow dwarf virus (BYDV)-like cap-independent translation element (BTE) lying in its 3’ untranslated region (UTR). Finally, the co-occurrence of BBSV with other soil-borne viruses like Beet necrotic yellow vein virus, Beet soil-borne and Beet virus Q will also be evoked.
Oral presentation 2.2

Assessing the potential threat posed by spread of introduced and indigenous viruses to Australian native plants

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Past surveys revealed that introduced, generalist viruses infect some native south-west Australian plant species. Their effects were investigated by inoculating 17 native species from eight families with 15 introduced and four indigenous viruses.

With introduced viruses, severe systemic symptoms involving marked stunting, apical necrosis or plant death developed with Cucumber mosaic virus (CMV) in Kennedia prostrata (scarlet runner); Tomato spotted wilt virus (TSWV) and CMV in Anigozanthos manglesii (mangles kangaroo paw); TSWV in Solanum symonii; Turnip mosaic virus (TuMV) in Trachymene coerulea (blue lace flower); and Alfalfa mosaic virus (AMV) in Gompholobium tomentosum (hairy yellow pea). Similar severe symptoms developed with indigenous viruses Clitoria chlorosis virus (ClCV), Hardenbergia mosaic virus (HarMV) and Passionfruit woodiness virus (PWV) in Kennedia coccinea (coral vine) and K. prostrata; and HarMV and PWV in Gastrolobium bilobum (heart leaf poison). Milder systemic leaf mottle, streaking and/or leaf deformation symptoms developed with introduced viruses Bean yellow mosaic virus (BYMV) in K. coccinea and G. bilobum; AMV in K. prostrata; Potato virus X (PVX) in A. manglesii; CMV in Hibbertia cuneiformis; AMV, CMV, BWYV and PVX in S. symonii; AMV, CMV and PVX in T. coerulea; and Wheat streak mosaic virus and Barley stripe mosaic virus in Stipa compressa. Similar symptoms developed with indigenous viruses ClCV and Passiflora virus Y in G. bilobum; and HarMV in H. comptoniana. When infection with introduced or indigenous viruses occurred in other combinations of virus and native plant, it was restricted to inoculated leaves, or, in one instance, caused only symptomless systemic infection.

In S. symonii, infection diminished shoot dry weight by 70-94% (TSWV), 34% (AMV) and 51% (AMV+CMV). With TSWV, CMV and AMV+CMV, fresh weight losses/fruit were 100, 40 and 47%, respectively. The CMV seed transmission rate was 0.4%. In A. manglesii, shoot dry weight losses were 100% (TSWV) and 53% (CMV), and in T. coerulea 100% (TuMV). In K. coccinea and K. prostrata shoot dry weight losses were 87-97% (ClCV), and in G. bilobum 96% (HarMV).

These results show that both introduced and indigenous viruses have the potential to decrease abilities of native species to compete and survive in natural plant communities.
Oral presentation 2.3

Kiwifruit can be naturally infected by a wide range of viruses

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Until recently there were no reports of plant viruses infecting Actinidia species. In 2003 Clover and colleagues reported the presence of Apple stem grooving virus in some quarantine plants imported from China. Since that time we have been characterizing a range of viruses transmitted to herbaceous indicators from a number of Chinese accessions of different Actinidia species imported as scionwood. We have identified isolates of Ribgrass mosaic virus and Actinidia-infecting strains of Citrus leaf blotch virus (CLBV) and two novel vitiviruses. The characterization of these viruses will be described in more detail by Chavan et al. and Blouin et al. (this meeting). In addition, we have also detected Alfalfa mosaic virus, Cucumber mosaic virus and a novel potexvirus related to Narcissus mosaic virus.

To date there do not appear to be any obvious virus problems in commercial kiwifruit crops. Most of the viruses we have detected are present at very low titre and/or are unevenly distributed in the kiwifruit plants. Most of these viruses are difficult to transmit mechanically from Actinidia leaf tissue. RNA isolation from kiwifruit leaves has been most successful using Sigma Spectrum Plant RNA kits and RT-PCR provides the best method for detection.
Oral presentation 2.4

Genome characterization of a Tobamovirus and a Citrivirus from kiwifruit

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Actinidia chinensis and A. deliciosa plants imported from China, held in quarantine, showed a range of viral-like symptoms, including mosaic, mottling, vein clearing, and chlorotic ring spots. The viruses could be sap transmitted in to Chenopodium quinoa, Nicotiana benthamiana, N. clevelandii and N. occidentalis. Transmission electron microscopy of the sap revealed the presence of ~300nm rod shaped tobamovirus-like particles in infected indicators ex A. chinensis and A. deliciosa and ~750-800nm flexuous particles in N. occidentalis ex A. chinensis.

The complete genome of the tobamovirus is 6311 nts long and the genome organization fits the crucifer-infecting sub group 3 tobamoviruses. The genome shared 86% nucleotide identity with published sequences of Turnip vein clearing virus and Penstemon ringspot virus across the whole genome and differed from the published genome of Ribgrass mosaic virus isolates Shanghai and Impatiens by ~20%. Phylogenetic analysis of all the four ORFs and the complete genome revealed clustering of the virus in sub group 3 tobamoviruses. ORF4, that encodes putative coat protein, had 98% amino acid similarity with Ribgrass mosaic virus Kons 1105, isolate R14 in the RMV cluster of sub group 3 tobamoviruses.

The complete genome of the citrivirus from Actinidia is 8860 nts long excluding the poly (A) tail, with three ORFs. It shows 76% nt similarity to Citrus leaf blotch virus (CLBV) across the whole genome, the putative replicase, movement and coat protein genes showing 78%, 95% and 92% amino acid identity respectively. Phylogenetic analysis of different ORFs and the complete genome with a range of viruses in the family Betaflexiviridae revealed clustering of the new virus with sequences of citrus-infecting CLBV and Dweet mottle virus but deviating as a distinct clade.
Oral presentation 2.5

Novel vitiviruses infecting kiwifruit

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Extracts from Actinidia chinensis leaves showing a range of viral-like symptoms, including vein clearing, interveinal mottling and chlorotic ring spots, were inoculated to herbaceous indicators and symptoms were observed on Nicotiana occidentalis. Infected indicators were used for a partial purification of viruses and a putative coat protein band of about 25 kDa was excised from a denaturing polyacrylamide gel stained with Coomassie Blue. High-resolution tandem mass spectrometry of the excised proteins digested with trypsin identified peptides similar to the coat protein of Grapevine virus B. Sequencing of the virus was undertaken with primers designed on conserved motifs of Grapevine virus B and others vitiviruses. It was subsequently discovered that the infected kiwifruit plants were co-infected with a second vitivirus.

The complete genomes of both viruses have now been obtained and the viruses have been tentatively named Actinidia virus A and Actinidia virus B. Their genomes are ~7600 and ~7400 nt, respectively, excluding the poly(A) tail and they share 60% identity across the full genome. The overall structure of these new viruses is typical of vitiviruses, with five ORFs, and a polyadenylated 3’ end. The putative replicase of Actinidia virus A has 66% amino acid identity to Actinidia virus B, 55% to Grapevine virus B, 47% to Grapevine virus A and 37% to Grapevine virus E. The ORF4 encodes for the putative coat protein of approximately 21 kDa for both viruses and is the most conserved gene of the vitiviruses. Actinidia virus A has 75% identity with Actinidia virus B, 68% with Grapevine virus B, 67% with Heracleum latent virus, 59% with Grapevine virus A and only 39% with Grapevine virus E.

Based on the molecular criteria for species demarcation in the family Flexiviridae, Actinidia virus A and Actinidia virus B are two novel, but related, vitiviruses.
Oral presentation 2.6

**Current and potential viral diseases issues for the Australian vegetable industry**

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The diversity of vegetable crops grown over several eco-climatic zones in Australia provides opportunities for the introduction and establishment of many plant viruses. Current and potential issues with virus diseases in vegetables will be discussed with emphasis on diseases caused by species within the genera *Tospovirus, Potexvirus, Tobamovirus, Potyvirus* and *Ophiovirus.*
Mosaic diseases of sugarcane in Indonesia: diagnostics and biosecurity implications.

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Sugarcane is a crop of major importance in Australia and Indonesia. BSES Limited is the principal provider of research, development and extension to the Australian sugarcane industry and biosecurity is an important component of BSES activities. An ACIAR funded collaborative project between BSES and the Indonesian Sugar Research Institute (ISRI) included an investigation of sugarcane mosaic diseases throughout the Indonesian island of Java.

Mosaic diseases of sugarcane can be caused by a number of viruses: the potyviruses Sugarcane mosaic virus (SCMV) and Sorghum mosaic virus (SrMV); Sugarcane streak mosaic virus (SCSMV); and Sugarcane striate mosaic virus (SCSMAV). Of these, SCMV, SrMV and SCSMV are the most prevalent throughout the sugarcane growing regions of the world. SCMV and SCSMV have been previously identified in Indonesia, while in Australia only one strain of SCMV (SCMV-A) and ScSMAV are found.

In 2008-2009 an extensive survey of sugarcane milling areas in Java, Indonesia, was carried out by ISRI. The location, sugarcane variety and disease symptom were recorded and 263 leaf samples taken for analysis by BSES. RNA was extracted and RT-PCR was used to identify the mosaic virus present in the samples: General potyvirus RT-PCR was used to detect any potyvirus and SCSMV specific primers were used to detect SCSMV. The RT-PCR was done without knowledge of the symptoms or sampling locations. Samples that tested negative were tested multiple times to ensure any virus was detected.

The results showed that the most prevalent mosaic virus in Java is SCSMV with >80% of the samples testing positive for this virus. Approximately 5% of the samples were infected with potyvirus, most of which were co-infected with SCSMV. Less than 1% of the samples were infected only with a potyvirus. Approximately 15% of the samples had no mosaic virus detected, and comparison with the symptoms revealed that these were either healthy (asymptomatic) controls or had very mild symptoms.

The potyviruses are being characterized by sequencing of the 3’ end of the virus, and preliminary analysis so far shows that the viruses are SCMV, but they do not have high sequence similarity to sugarcane infecting SCMV in neighbouring countries or the type strains of SCMV. Further work is being done and more sequencing will be required to fully characterize these viruses.
Oral presentation 2.8

Whitefly-transmitted viruses of Australian vegetable crops: endemic problems and exotic threats

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Australian vegetable crops are under siege from a range of whitefly-transmitted viruses and at threat of a number of exotic ones. The earliest estimates of significant losses in Australian vegetable crops from these viruses were in the early 2000’s with the introduction of Tomato torrado virus (ToTV) and Tomato yellow leaf curl virus (TYLCV). More recently, Beet pseudoyellows virus (BPYV) was detected in greenhouse cucumber crops in at least two states. ToTV appears to still be contained in the protected cropping area of Virginia, SA. A recent detection of TYLCV in northern NSW has expanded the known distribution of this virus beyond the south-east QLD tomato growing areas. Although detected in 1981 in Tasmania, BPYV has only had known significant impacts on crop production in recent years. Surveys for BPYV will continue in QLD, however, the virus is already known to occur in Virginia, SA, the Sydney basin and the mid-north coast of NSW around Coffs Harbor. All three viruses continue to cause economic damage to crops and management strategies for them are still in their infancy.

The diversity of whitefly-transmitted viruses is extremely wide, with species belonging to not only different genera but also different families of viruses and includes both RNA and DNA viruses. The three viruses affecting Australian vegetable crops belong to three separate genera in three distinct families; Begomovirus (Geminiviridae) transmitted by Bemisia tabaci, Crinivirus (Closteroviridae) transmitted by Trialeurodes vaporariorum and Torradovirus (Secoviridae) transmitted by both species. Control these whitefly species and of and of the viruses they transmit still is a huge problem where ever they exist worldwide.

In all three cases, these viruses went undetected in Australian crops for at least several growing seasons. This is of concern as the list of exotic whitefly-transmitted virus species which can affect vegetables is long and includes Begomoviruses, Criniviruses, Torradoviruses and Ipomoviruses. Some of these such as the Begomovirus, Cotton leaf curl Geizera virus, has a host in another large Australian agricultural industry, cotton. Several of these species can cause alteration of the pathogenicity of related viruses either through the suppression of gene silencing or by carrying additional genetic material such as DNA-β satellites. Early detection of these viruses will assist containment and management, and perhaps in some cases facilitate their eradication.
Strawberry latent ringspot virus (SLRSV) is widespread in many countries especially in Europe. The virus was thought uncommon in New Zealand, having only been recorded in sweet cherry (Fry and Wood 1973), flowering cherry (Everett et al. 1994) and Japanese plum (Richmond et al. 1998). However, this study reveals that SLRSV infects a much wider range of hosts. From 1999 to 2009, SLRSV was isolated from anemone (Anemone × hybridra), blackberry (Rubus sp.), impatiens (Impatiens walleriana), pepino (Solanum muricatum) and tibouchina (Tibouchina sp.) in the North Island of New Zealand. Particle morphology, host range and serological and molecular properties of these SLRSV isolates were described in this study. Phylogenetic analysis and host range indicate that these five isolates can be divided into two groups: impatiens / blackberry isolates share 99.6% nucleotide similarity in the coat protein gene sequence but only 85.4-85.8% to other isolates, and impatiens / blackberry isolates induce local and systemic symptoms on Nicotiana occidentalis but other isolates can not be mechanically transmitted to this herbaceous species. It is postulated that SLRSV may have been introduced into New Zealand on at least two separate occasions.

References:

Molecular Characterization of *Bean leaf roll virus* and *Pea enation mosaic virus* from the Pacific Northwestern USA and development of ELISA assays for virus detection

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The complete genomic sequence of one *Pea enation mosaic virus* (PEMV) isolate from Idaho (PEMV-ID) and one *Bean leaf roll virus* (BLRV) isolate from Washington State (BLRV-WA) were determined. PEMV-ID contained five ORFs. ORF4, which encodes the CP, shared a maximum identity of 98.9% with other members of PEMV while the least amino acid identity was seen with ORF1. Phylogeny tree based on CP sequences showed that PEMV-ID grouped with PEMV isolates UP58 and Germany. BLRV-WA contained five ORFs and ORFs 1 and 3 shared a maximum identity of 99.4% with respective ORFs of known BLRV isolates, while the least was with ORF-2. Antigen-coated plate (ACP) ELISA assay for the detection of PEMV was developed using antiserum raised against the recombinant coat protein (CP) of PEMV.
Diagnostic techniques for detecting nepoviruses: Cherry leafroll virus, Grapevine fanleaf virus, Strawberry latent ringspot virus and Tomato ringspot virus

Elizabeth N. Y. Woo, Michael N. Pearson, Gerard R. G. Clover

Nepoviruses are a genus of polyhedral plant viruses within the order Picornavirales, family Secoviridae and subfamily Comovirinae. The two other genera in this subfamily are Comovirus and Fabavirus but nepoviruses are mainly distinguished from these two genera by their ability to be transmitted through soil by free-living nematodes feeding on roots.

Nepoviruses cause economically important diseases in horticultural crops and are of serious concern to phytosanitary authorities worldwide. Four nepoviruses of particular concern in New Zealand are Cherry leafroll virus, Grapevine fanleaf virus, Strawberry latent ringspot virus and Tomato ringspot virus. New Zealand is considered free of many damaging strains of these viruses but there is some uncertainty due to a lack of systematic surveillance and biological knowledge. In addition, these viruses are regulated at the strain level but many tests do not differentiate strains, creating difficulties if these viruses are intercepted at the border.

To determine the ability of various polymerase chain reaction (PCR) tests to detect and differentiate different strains of the viruses, 64 nepovirus isolates representing twelve species and the three subgroups were acquired. Reverse transcription-PCR was conducted using twenty pairs of nepovirus primers, eleven generic and nine species-specific. The results of these studies are discussed.
DsRNA elements and virus-like particles in *Sclerotinia sclerotiorum*

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*Sclerotinia sclerotiorum* is a phytopathogenic fungus capable of causing significant yield losses in numerous crops in New Zealand and worldwide. Chemical control is increasingly fraught by fungicide resistance and chemical residues. An alternative innovative approach is to exploit mycoviruses as biocontrol agents.

Twenty two isolates of *S. sclerotiorum* were screened for the presence of double-stranded ribonucleic acid (dsRNA) as a preliminary indication for mycovirus infection. 50% of the screened isolates were found to contain dsRNAs of varying sizes and number of segments. The most interesting dsRNA elements are those of high molecular weight of 12 to 13 kb since dsRNA mycoviruses with the same genome size have been previously reported in infected pathogenic fungi *Cryphonectria parasitica*.

Attempts to purify virus-like particles from dsRNA-containing isolates were successful with only one isolate in which isometric VLPs of approximately 24-27 nm in diameter were seen, some appearing as paired-particles of almost the same morphology as geminiviruses.
Poster 2.13

**Passionfruit viruses in eastern Australia**

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Surveys of native and wild *Passiflora* species as well as commercial passionfruit cultivars (*P. edulis* and *P. edulis* f. sp. *flavicarpa*) were conducted to investigate virus susceptibility, distribution and prevalence. *Passionfruit woodiness virus* (PWV), *Passiflora latent virus* (PLV) and *Cucumber mosaic virus* (CMV) were previously reported to infect passionfruit and were identified in the survey. Two additional potyviruses were also found. *Passiflora virus Y* (PaVY) was characterised from symptomatic *P. foetida* plants and later found to be widespread in passionfruit. A third potyvirus was identified in passionfruit through generation of a second discrete amplicon in a generic potyvirus PCR. Sequencing of the additional fragment revealed *Clover yellow vein virus* (ClYVV) and this was confirmed by ELISA.

Commercial grafted cultivars in north Queensland, Bundaberg and northern New South Wales were commonly infected by all three potyviruses as well as PLV. CMV was additionally detected from one property. None of these viruses was seed transmitted in passionfruit. Within virus-infected commercial plantings, reinfection rates of virus-free passionfruit seedlings of 100% by all four prevalent viruses (PWV, PaVY, ClYVV and PLV) occurred within 7 months. Under these conditions, virus infection was not detected in *P. incarnata*, *P. coccinea*, *P. suberosa* and *P. herbertiana*. The following field infections of wild and native *Passiflora* were detected: PWV in *P. foetida*, *P. quadrifolia*, *P. quadrangularis*, *P. subpeltata*, *P. platylobax* and *P. semanii*; PaVY in *P. foetida*; ClYVV in *P. subpeltata* and *P. malformis*.

Passionfruit viruses were biologically purified from mixed infection passionfruit sources, except for ClYVV which could not be separated from PWV and does not appear to be mechanically transmissible to passionfruit from either passionfruit or other hosts infected singly or in conjunction with PWV. Plants infected with PWV alone displayed strong mosaic and leaf distortion symptoms on the older leaves, and chlorotic spots near the midrib on younger leaves. In contrast, infection with PaVY alone was associated with bright yellow spotting and leaf rugosity. CMV was associated with infrequent chlorotic spotting and mottle, but plants were often symptomless. Single infections with PLV were symptomless.
Oral presentation 3.1

Towards an understanding of the epidemiology of Candidatus Liberibacter solanacearum in New Zealand

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Since the 2008 discovery of Ca. Liberibacter solanacearum and confirmation of its role in disease in solanaceous crop species in the United States and New Zealand there has been an urgent need to gain knowledge about the biology and ecology of the organism. While the initial immediate threat of Liberibacter in NZ was to the tomato and capsicum export trade, the potato tuber symptom “Zebra Chip” caused by Liberibacter, and the recognised insect vector tomato psyllid (Bactericera cockerelli), are estimated to have caused economic losses to the NZ potato industry of approximately NZ$50 million per annum and increased spray costs of up to NZ$1200 per hectare.

A number of research initiatives are now gathering basic biological information about the pathogen and its insect vector. Molecular diagnostic techniques have been used to: identify potential alternate plant hosts of Liberibacter (34 species in 17 plant families), estimate the timing of infective insect flights (the first TPP to appear on yellow sticky traps carry Liberibacter), and identify non-insect mediated transmission as a potential disease risk (95% of Liberibacter positive mother tubers sprout and give rise to apparently healthy but infected plants and daughter tubers). The recent development of quantitative detection methods will improve understanding of these and other biological factors influencing the pathogen and its transmission and better inform management decisions.
Oral presentation 3.2

Developing and validating molecular diagnostics for Phytoplasma and Liberibacter pathogens of potatoes

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\textit{Candidatus} Liberibacter solanacearum (syn \textit{Candidatus} Liberibacter psyllaurous) is associated with serious diseases in several important solanaceaous crops including Zebra Chip of potato and psyllid yellows of tomato and capsicum. It has not been detected in Australia and is considered a high risk pathogen as it can have a significant economic impact on production of commercially grown solanaceaous hosts. To improve Australia’s preparedness in case of an incursion of this bacterium, a rapid, sensitive and validated diagnostic test is required. In addition to \textit{Ca.} L. solanacearum, an endemic phytoplasma, \textit{Candidatus} Phytoplasma australiense, has also been found in potato plants in New Zealand but its association with Zebra Chip disease is unclear. This phytoplasma is also endemic to Australia but has not been reported to infect potatoes. No extensive survey has been done to verify this observation. It is important to have validated diagnostic protocols in place for both \textit{Ca.} L. solanacearum and phytoplasmas to be able to react quickly to identify which pathogen is present and then respond to any suspected incursion.

We have a collaborative project for the validation of published and newly developed diagnostic tests for Liberibacter and phytoplasma detection in potatoes grown under Australian conditions. The project has three objectives:

1. Validate diagnostic protocols for \textit{Candidatus} Liberibacter solanacearum and phytoplasmas in Australian potato crops and identify baseline data of these pathogens in the absence of disease symptoms.

2. Improve preparedness of the Australian potato industry for an incursion of \textit{Candidatus} Liberibacter solanacearum and our ability to manage the associated diseases, especially through early detection.

Understand the incidence of phytoplasmas in Australian solanaceaous crops and their contribution to disease in the absence of \textit{Candidatus} Liberibacter solanacearum and its vector the Tomato potato psyllid (\textit{Bactericera cockerelli}, TPP).
Identification and epidemiology of pospiviroids

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Pospiviroids can cause serious diseases in potato and tomato. Since 1988 viroid infections have occasionally been detected in tomato crops in the Netherlands. To identify these viroid isolates unequivocally, two novel universal primer sets were designed for ‘Reverse Transcription-Polymerase Chain Reaction’ (RT-PCR), a sensitive technique enabling detection and identification of pospiviroids. Sequence analyses identified the viroids as Citrus exocortis viroid (CEVd), Columnnea latent viroid (CLVd) and Potato spindle tuber viroid (PSTVd). To find potential sources of infection, seed lots were traced and ornamental plants were screened for the presence of pospiviroids. The tracing studies did not reveal infections; however, the surveys revealed many new pospiviroid host plants as well as high infection rates for PSTVd in some ornamental species. Phylogenetic studies provided evidence that the PSTVd isolates from tomato originated from vegetatively propagated, solanaceous host plants. This conclusion was further substantiated by showing a high stability of predominant pospiviroid genotypes after mechanical pospiviroid transmission from ornamentals to potato and tomato. In addition, several experiments showed that mechanical inoculation is a likely way of pospiviroid transmission between these crops. In our studies, also a new pospiviroid from pepper was characterized, i.e. Pepper chat fruit viroid. This viroid can also infect potato, tomato and ornamentals. The results of above findings on pospiviroids are discussed in a broader context addressing diagnostic and epidemiological aspects as well as risk assessment in relation to quarantine measures.
Chamberlain et al. (1957) reported that a virus-like disease, peach calico, which caused a “brilliant yellow or papery white variegation of peach leaves” in a Central Otago nursery in 1948 was widespread throughout New Zealand. It was easily transmitted and impossible to heat treat however the rate of spread in the field was low.

By the late 1980s Peach latent mosaic viroid (PLMVd) was identified as the causal agent of peach calico disease. PLMVd is economically important: infection affects fruit quality, reduces the lifespan of trees and predisposes peach and other stone fruits to biotic and abiotic stress. PLMVd consists of a covalently closed circular RNA (335-342 nt). This RNA molecule, like all viroids, forms an extremely stable structure consisting of a series of short helicities and small loops.

In 1956 E.E. Chamberlain lodged a specimen entitled ‘Virus in fruit trees, Central Otago’ with the University of Otago Herbarium (OTA). The first four leaves show typical peach calico symptoms. Samples (4mg, 20mg) were taken from two leaves and homogenized with STE buffer and extracted using the Trizol method. Total RNA (1-5uL) was used to produce cDNA and amplified via PCR using the SuperscriptIII One-Step RT-PCR System (Invitrogen) and various two step systems. PLMVd specific primers complementary to positions 208-178 (RF-43) and identical to positions 199-225 (RF-44) of the PLMVd reference variant and primers specific for wheat rubisco mRNA were used at annealing temperatures of 55-60°C.

Amplicons were purified and then sequenced using the ABI 3730x1 DNA Analyser system. The PLMVd sequences showed 95-97% identity with peach calico variants of PLMVd and the rubisco sequences showed 100% identity with peach (Prunus persica) rubisco. Two step reactions with RT enzyme omitted produced no amplicons. These results confirm the presence of PLMVd in New Zealand and indicate that naked RNA may be preserved, under suitable conditions, for at least 50 years.

Previous studies showed that viral RNA associated with capsid protein may be preserved for 100 years. The fact that naked RNA may also survive indicates increased potential for preserved specimens to contribute to our understanding of virus and viroid evolution.
Oral presentation 3.5

A molecular comparison of Iranian and Australian *Peach latent mosaic viroid* isolates

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Viroids are infectious, pathogenic, small naked single-stranded circular non-coding RNAs which depend on host plant factors for their replication. Viroids replicate either in the nucleus (Family *Pospiviroidae*) or in the chloroplast (Family *Avsunviroidae*). We report the first identification of *Peach latent mosaic viroid* (PLMVd; *Pelamoviroid*; *Avsunviroidae*) in peach and plum samples from Iran, compare them at the nucleotide sequence level with an Australian isolate, and demonstrate the infectivity of one clone of the Australian isolate. During July and August 2009 samples were collected from peach and plum orchards in north-eastern Iran. The Australian isolate originated from nectarine in 1998 and was maintained in a grafted peach seedling in the greenhouse at the Waite Campus. RNA was extracted either with TRIZol or by silica capture. PLMVd was detected by RT-PCR, and amplicons which were slightly longer than full length were cloned and sequenced. Seven Iranian isolates, Genbank accession numbers HM185109 to HM185115, and two Australian sequences, HM185107 and HM185108, were compared. Phylogenetic analysis by the neighbor joining method showed that the Iranian isolates were distinct from the Australian isolates. The mechanical transmissibility of one Australian isolate [HM185107] was tested by high pressure injection combined with bark slashing using the following inocula: recombinant plasmid containing the PLMVd insert with a 9 nucleotide overhang; the PLMVd PCR product before cloning; total nucleic acids extracted from the peach infected with the viroid. Controls were patch-bark graft inoculated and uninoculated seedlings. Plants were maintained at 30°C with a 14h day. First symptoms were seen at three weeks on plants inoculated with recombinant plasmid, and at 6 weeks for the other treatments. Infection was confirmed by RT-PCR. Sequencing showed that the viroid recovered from the plasmid inoculated seedlings was the same as that of the original isolate. Selected inoculated trees are to be maintained to evaluate the infection phenotype associated with the Australian isolate.
Phytoplasmas are plant pathogenic bacteria that belong to the class Mollicutes. Bacteria in this group have no cell wall, and genomes in the range of 0.6 to 2 Mbp, the small genomes being a result of genome reduction. The inability to culture phytoplasmas in vitro has hindered research into these organisms, although interesting discoveries are being made as a result of whole genome sequencing. To date four phytoplasma genomes have been published, two belonging to “Candidatus Phytoplasma asteris”, one belonging to “Candidatus Phytoplasma mali”, and an Australian isolate belonging to “Candidatus Phytoplasma australiense”. Phytoplasmas belonging to “Ca. Phytoplasma australiense” are associated with more than six diseases in Australia and New Zealand. Although the rRNA genes of isolates within this species are almost identical, they can be divided into three groups according to variation in the protein coding tuf gene. We have sequenced the genome of a SLY isolate of “Ca. Phytoplasma australiense”, and compared it with the published genome sequence. The two isolates both belong to the same tuf gene group and a representation of housekeeping genes are 99% identical at a nucleic level. Despite this close similarity, there are significant variations in the genome organisation. The strawberry lethal yellows (SLY) isolate is 80 kbp larger than the Australian isolate, and there is variation in the gene synteny. This presentation describes factors associated with genome plasticity in “Ca. Phytoplasma australiense”.
Gene expression and metabolite changes of tomato plants in response to Candidatus Phytoplasma infection

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Phytoplasmas (Candidatus Phytoplasma) are a group of wall-less plant pathogenic bacteria that have been shown to cause over seven hundred diseases in several hundred plant species. The disease symptoms attributed to phytoplasmas include proliferation of roots and shoots, witches’-broom structures, virescence (green flowers), phyllody (flowers developing as leaves), yellowing, sterility, and in some cases death. As such, phytoplasmas are a significant challenge for agriculture worldwide. Tomato plants (Solanum lycopersicum) were graft-infected with phytoplasma from the 16SrII taxonomic group, and their development was monitored over a period of three months. Metabolite changes were analysed through use of gas chromatography-mass spectrometry, and it was found that the phytoplasma infected plants displayed increased levels of salicylic acid, a downregulation of amino acids, and altered carbohydrate metabolism. The effects of phytoplasma infection on the gene expression of tomato were also analysed using whole-transcriptome analysis. Major gene expression changes which were observed included an increase in the expression of PR proteins as well as altered phytohormone and carbohydrate metabolism. This research represents a first step towards an understanding of the physiological and biochemical effects of phytoplasma infection. This information has potential applications for future research into the biological control of these invasive pathogens.
Sugarcane white leaf disease- Thailand overview

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White leaf disease of sugarcane, Saccharum spp. and complex Saccharum hybrids is associated with the sugarcane white leaf phytoplasma (“Candidatus Phytoplasma oryzae” 16SrXI group, SCWL) (1, 2). The disease is predominantly found in Thailand, Taiwan, Sri Lanka and Japan. White leaf and grassy shoot disease of sugarcane are considered to be the most economically damaging of sugarcane phytoplasmas in Asia (3). Symptoms consist of abnormal tillering, bleached white or striped leaf blades and side shoots on the upper part of infected stalks. Plants which are severely diseased decline do not set fruit or produce millable canes. The disease is transmitted by leafhoppers, Matsumuratettix hiroglyphicus (2) and Yamatotettix flavovittatus (4). To date, SCWL phytoplasma or its vectors have not been found in Australia, however closely related phytoplasma strains have been detected in native grasses.

We report on the current SCWL disease situation in Thailand, new findings of a different phytoplasma associated with sugarcane white leaf disease and the availability of a SCWL field test kit.

References
Poster 3.9

Retention time of infectious Potato spindle tuber viroid (PSTVd) on common surfaces

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Potato growing areas of Australia are currently considered free of Potato spindle tuber viroid (PSTVd) and this pathogen is classified as an emergency plant pest (Category 3) under the Emergency Plant Pest Response Deed (EPPRD).

Three strains of PSTVd have been detected so far in the PSTVd outbreaks in Australia. The “Naaldwijk strain” was detected in glasshouse grown tomatoes in southern Western Australia (WA) in 2001 and a survey of field tomatoes in New South Wales detected a common European strain (PTVCGA). The last three detections in WA since 2004 have been of the “Chittering strain” of PSTVd and each instance the initial detection was in glasshouse or field grown tomatoes. It is unclear at this point what the source of PSTVd inoculum for these outbreaks was, by what pathway the pathogen reached the tomato crops and what the impact of these PSTVd strains was on tomato fruit yield and quality under Australian conditions.

Following an incursion of PSTVd in Australia an important aspect of the eradication program that follows is the cleaning and sanitizing of machinery and equipment used on the affected farm. However, some aspects of PSTVd epidemiology are poorly understood, including the stability of infectivity of PSTVd inoculum on different common surfaces such as those associated with machinery, mulches, staking and handling plants, eg. cotton, metal, plastic, unpainted wood, glass, rubber, string and leather.

In a preliminary experiment, fresh undiluted sap from PSTVd infected tomato plants was extracted and pipetted onto various surfaces (cotton, metal, plastic, unpainted wood, glass, rubber, string and leather) and left to dry out for 4 different time intervals (5 minutes, 1 hour, 6 hours and 24 hours). For each surface, after each time interval, the sap was rehydrated using distilled water and each extract was inoculated to five cv. Grosse Lisse tomato plants.

The outcome was that PSTVd contaminated glass, wood, plastic, leather and string surfaces were still infective after 24 hours; contaminated cotton was still infective after 6 but not 24 hours, and contaminated metal was still infective after 1 but not after 6 hours. The results for rubber were inconclusive will need to be repeated. The results to date indicate that further time periods (for example, 2 weeks, 1 month and 6 months) need to be incorporated into this work.
Poster 3.10

A phytoplasma from subgroup 16Sr II is associated with little leaf of *Medicago arborea* (tree medic) in South Australia

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*Medicago arborea* (tree medic) is a perennial leguminous shrub, which has potential as a fodder crop in the low rainfall zone of southern Australia. Some of the tree medics grown as border rows in the genetic resources nursery at the Waite Campus, South Australia, showed abnormal branches usually on one side of the plant with a bushy appearance, reduced cupped yellow leaves and short internodes. On January 25, 2010, five plants in the row were tagged and sampled for phytoplasma detection by PCR. Two samples from apparently healthy plants were also selected as negative controls. Total nucleic acids were extracted in 4 M guanidine hydrochloride followed by absorption to a silica matrix according to the protocol in the Qiagen Plant DNeasy Mini kit. Nested PCR was done using the P1/P7 primer pair as the external primers followed by a 1:10 dilution of the product and performance of the second round of PCR using R2F2n/m23sr as internal primers. Four out of the five symptomatic samples tested positive for phytoplasma sequence while both healthy controls tested negative. Similar symptoms were observed in a row of neighbouring young *M. arborea* shrubs. These plants were also sampled and tested positive using the above nested PCR. No positive signal was observed in the samples collected from a number of other legume species growing nearby and having some symptoms suggesting possible infection with phytoplasma. The PCR products from two plants (three from each plant) were sequenced either directly using the specific internal primers or cloned and sequenced using universal plasmid primers. Blast analysis of the sequenced products revealed that the phytoplasma belonged to the Faba bean phyllody phytoplasma group 16Sr II-D which clusters phylogenetically with Sweet potato little leaf and Tomato big bud phytoplasmas, all of which are members of *Candidatus Phytoplasma aurantifolia*. The phytoplasma sharing closest sequence similarity to the *M. arborea* phytoplasma (99%) was Ipomea phytoplasma (Accession No. EU168789) reported from Fiji.
**Poster 3.11**

**Studies on the genetic variability of Lime witches'-broom phytoplasma in Iran**

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*Candidatus* Phytoplasma aurantifolia is associated with Lime witches'-broom, a devastating disease, in southern Iran. The disease was first reported in 1997 but it is believed to have entered the country in the late 1980’s. The disease has been reported from India, Pakistan and Oman and it occurs in lime, grapefruit and Bakraee (*Citrus reticulata* hybrid). *Hishimonus phycitis* has been identified as its leafhopper vector. We aimed to see if the phytoplasma exists as one or more sequence variants. Samples from infected limes were collected in 2008 from Hormozgan, Sistan-Baluchestan and Kerman provinces. Initially, a PCR assay was carried out using P1/P7 as external primers and R16F2/R16R2 as internal primers. The nested PCR products (1250bp) from 25 collected samples were subjected to RFLP analysis using *Hae*III, *Eco*RI and *Msp*I. The RFLP profiles from selected isolates were similar indicating lack of heterogeneity among the isolates collected from various provinces. Since 16S rRNA spacer region gives a more precise genetic variability in phytoplasmas, we targeted this region by using the primer pair fP3/M23sr which gives an amplicon of 340 bp from five isolates that were selected from distant geographical regions. The amplicons from these five isolates were sequenced and no variability was observed. Finally we targeted polymerase beta III chain gene by using the primer pair pTBB14F/pTBB14R with a PCR product of 650 bp. The product was sequenced and again no variability was observed. The phylogentic analysis showed that these isolates were 99-100% similar in the deduced sequences and belonged to the same species in the Peanut witches-broom group as expected. It was concluded that the pathogen may entered the country from the same overseas source, confirming a previous report. In further analysis the following crop samples growing within lime orchards were tested for phytoplasmas: *Medicago Sativa*, *Trifolium pratense*, *Sesamum indicum*, *Alhagi camelorum*, *Solanum nigrum*, *Plantago major* and *Convolvulus arvensis*. Only a phytoplasma in *Sesamum indicum* with phyllody symptoms was detected. The 16S rRNA sequence of the phytoplasma in sesame showed 99% similarity to that of the lime isolates of LWBP. It is worth noting that sesame phyllody is an endemic pathogen in Iran.
Oral presentation 4.1

Viruses for breakfast, lunch and dinner

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Endogenous sequences from members of the *Caulimoviridae* and *Geminiviridae* have been identified in the genome of several plant species. These sequences are thought to result from illegitimate recombination events and are generally replication-defective. However, some endogenous caulimovirid sequences in tobacco, petunia and banana are capable of causing infection.

We have performed in silico analyses on nucleotide sequences from plant genome databases and reconstituted twelve full length and potentially replication-competent viral genomes from endogenous viral sequences embedded in the genomes of monocotyledonous and dicotyledonous plant species of the Brassicaceae, Euphorbiaceae, Fabaceae, Myrtaceae, Poaceae, Rutaceae, Salicaceae and Vitaceae. Sequence analyses show that the corresponding viruses belong to a new genus in the family *Caulimoviridae*, tentatively named Dionyvirus. Maps showing the distribution of the endogenous dionyviral sequences in the genomes of *Vitis vinifera*, *Prunus persica* and *Oryza sativa* have been produced and copy numbers determined. Dionyvirus-specific primers were designed and used for a PCR-based large scale screening of plant germplasm, which demonstrated that that the endogenous dionyviral sequences are widespread among plants of temperate, tropical and sub-arctic origins. The potential contributions of endogenous viral sequences to normal plant functions and to plant and virus evolution will be discussed.
Sugar beet soil-borne viruses – surprising “ménage à trois” combination

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Sugar beet is severely affected by protist-transmitted telluric viruses, with questions about the emergence of resistance-breaking isolates of the Beet necrotic yellow vein virus, a Benyvirus causal agent of rhizomania or reports of different disregarded soil-borne viruses, among which the Beet soil-borne virus and the Beet virus Q, two Pomovirus, and the Necrovirus Beet black scorch virus and its satellite.

The proportions of the different combinations recorded at several places in Europe (France, Belgium), Africa (Morroco) or in Asia (Turkey, Iran) will be presented. A total of up 800 soil samples have been collected and submitted to a standardized bioassay for the viruses and their vector Polymyxa betae. The heterogeneity of the viral distribution within the plant root was studied and lyophilisation of the samples was adopted as a mean to reduce variations between root RNA extracts used prior RT-PCR.

The analysis of the BNYVV P25 gene sequence shows various amino acid tetrad signatures at the position 67-70. The previously reported Alanine to Valine substitution linked to resistance breaking was not found in areas of high disease impact, contrary to the simultaneous co-occurrence of different BNYVV types within a single beet.

Numerous mix viral infections were encountered. The usefulness of bringing together reverse genetic approaches combining full length infectious clones for understanding their interaction in planta, together with studies of the virus diversity will be stressed.

Evidences for the rescue of BVQ by BNYVV, for a role of BBSV coat protein in the symptom production or for a link between BBSV 3’UTR and satellite association will be presented.
**Oral presentation 4.3**

**Diversity of Tobacco streak virus strains, and first report of Strawberry necrotic shock virus in Australia**

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*Tobacco streak virus* (TSV) is transmitted by thrips via infected pollen, and has recently caused serious losses in sunflower and grain legume crops in central Queensland. The virus was detected in 20 weed species in the region, including parthenium (*Parthenium hysterophorus*) and crownbeard (*Verbesina encelioides*). Isolates of TSV from affected crops had near-identical genome sequences to those from parthenium (Sharman *et al.* 2009), a symptomless host which is commonly infected in central Queensland. TSV is also symptomless and frequently found in crownbeard and ageratum (*Ageratum houstonianum*). The complete genomes of three genetically distinct strains of TSV were sequenced from these hosts. It appears that each strain is almost exclusively found in its “preferred” alternative host. The complete genomes of the three TSV strains had between 68% and 91% nt identity to the type strain of TSV from the USA. Interestingly, the ageratum-TSV strain was more closely related to the ilarvirus *Parietaria mottle virus* for RNA 1 and RNA 2 than it was to the type strain of TSV and may represent a new ilarvirus species. These newly characterised TSV genomes greatly increase the reported diversity of TSV strains.

An Ilarvirus from strawberry, thought to be a strain of TSV, was first detected in Queensland in 1979 (Greber 1979). The complete genome of the original Queensland isolate and partial genome of an isolate from Victoria were sequenced and shown to be isolates of *Strawberry necrotic shock virus* (SNSV). The SNSV strains from Queensland and Victoria have almost identical coat protein sequences to each other and to a strain from Mississippi (USA, GenBank AY363233), for which only coat protein sequence has been published. The complete genome sequence of the Queensland strain has between 93% to 96% nt identity to the published complete SNSV genome across the three RNA segments. SNSV may have originally entered Australia into Queensland via strawberry breeding lines from the USA and was subsequently moved to Victoria. SNSV has not been found in field crops in Australia since the early 1980s suggesting it is now absent due to the clean runner scheme.

Development of a rolling circle amplification-based assay for the detection and characterisation of *Banana streak virus*

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Banana streak disease (BSD) is caused by a group of badnaviruses named Banana streak viruses (BSVs). Although there are many symptoms associated with BSV infection in banana (*Musa* sp.), the most prominent symptom is the presence of chlorotic and necrotic flecks on the leaves, which run parallel to the vascular tissue. Despite attempts to control the disease using cultural practices such as clean planting material, BSD is still widespread in many countries. Four species of BSV are currently recognised by the ICTV, with each species having 57-60% sequence identity across the complete genome. An additional 15 species have been proposed based on the detection of badnavirus sequences in banana, however these have not been completely characterised. The significant genetic and serological diversity amongst banana-infecting badnaviruses has complicated efforts to develop a diagnostic test which can detect all isolates of BSV. An additional challenge is presented by the presence of integrated badnavirus sequences within the host plant genome of species in the genus *Musa*. Several of these integrated sequences are identical to their episomal counterparts. Further, the activation of BSV from integrated sequences has been demonstrated and complicates efforts in breeding, *in-vitro* germplasm maintenance and production of virus-free planting materials through tissue culture, as all three lead to activation of the integrated sequences. We have developed an assay using rolling circle amplification (RCA) for the detection of badnaviruses in banana. This RCA-based method provides two major advantages for detection of BSVs, i) it is not sequence specific and can therefore amplify a much wider range of sequences than conventional PCR based tests and ii) it only amplifies episomal circular DNA and is not confounded by the presence of genome integrated sequences. Using samples collected in Uganda and Kenya, we have demonstrated the utility of this method for the detection of at least nine species of BSV, six of which were not previously characterised. We have now completely characterised the genomes of these six previously uncharacterised species following sequencing of RCA-amplified DNA.
Elimination of viruses from elite kiwifruit germplasm

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The discovery of several viruses in \textit{Actinidia} spp. (kiwifruit) has potential impacts on future importation of kiwifruit germplasm into New Zealand. Inhibiting the efficiency of access to new germplasm may consequently compromise the development of new cultivars. The time and cost involved in importing new virus-free material to replace virally infected germplasm, even if this can be found, is excessive. It is therefore important that viruses are eliminated from infected germplasm lines in order to realize the full potential of this material, and prevent the introduction and spread of viruses. As part of a high-health program, protocols for the elimination of specific viruses found in kiwifruit are being developed using \textit{in-vitro} culture of \textit{Actinidia} spp. (and \textit{Nicotiana occidentalis} as a model system) in combination with anti-viral chemicals and/or thermotherapy.
Oral presentation 4.6

Spatial and Temporal Variation of pulse Comoviruses using a novel PCR test

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Broad Bean stain comovirus (BBSV) is seedborne in a range of temperate pulse species and has been identified in Africa, Asia, Europe and the Middle East. BBSV is listed as a quarantinable pathogen in Australia and is actively tested for in post-entry quarantine services using standard serological tests. In the post-entry quarantine service at Horsham, BBSV has been identified regularly in field pea and in some lentil seed over several years. Seed has been tested from diverse countries around Europe and the Middle East, some of which were multiplied in ICARDA, Syria.

Primers were developed from conserved regions of an alignment of available comoviridae coat protein amino acid sequence. Primers do not amplify Broad Bean True Mosaic Comovirus, which is also serologically distinct from BBSV. Thus they can be used as a diagnostic test independent of the serological test currently used. Variation in the nucleic acid sequence of 26 samples taken over four years and 11 countries was largely at the third base position of the codon. The amino acid UGMPA consensus tree clusters all samples collected from field pea as largely identical separating from the samples collected from lentil. The nucleotide UGPMA consensus tree further breaks down the field pea cluster into smaller clusters grouping countries of origin together suggesting that there is population structure within a single strain of the virus on field pea and that a different strain may be found on lentil.
Oral presentation 4.7

Genetic Complementation between Two Viruses in an Otherwise Restrictive Host

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Tospoviruses cause serious diseases in several important crop plants. The genome of tospoviruses consists of three RNAs, large (L), medium (M) and small (S). The L RNA is organized in negative sense orientation, whereas M and S RNAs are in ambisense. The S RNA codes for a non structural protein (NSs) in sense direction which was shown to function as viral suppressor of gene silencing in plants. We used datura (\textit{Datura stramonium}) as a differential host for two distinct tospovirus species, \textit{Iris yellow spot virus} (IYSV) and \textit{Tomato spotted wilt virus} (TSWV). Following mechanical inoculation of Datura, TSWV causes systemic infection, whereas IYSV infection of Datura remains localized to inoculated leaves. We demonstrate that, in a mixed infection, the silencing suppressor NSs is expressed at a much higher level as compared to single infection in inoculated as well as systemic leaves. The systemic symptoms produced by TSWV in the presence of the IYSV silencing suppressor were more severe than those caused by TSWV infection alone. Even though the IYSV infection remained limited to the inoculated leaf, it was able to facilitate increased expression of TSWV NSs indicating complementation between two distinct tospovirus species.
Oral presentation 5.1

Importance of genomics in phytoplasma research

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In contrast to other Mollicutes, it was not possible to cultivate phytoplasmas in cell-free media so far. This fact is severely handicapping phytoplasma research. Molecular analysis of phytoplasma genes and genomes is thus an essential means to improve our knowledge on evolution, taxonomy, metabolism, pathogenicity and diagnosis of these bacteria. The completely identified genomes of four phytoplasma genomes including those of ‘Candidatus Phytoplasma asteris’ strains OY (line OY-M) and AY-WB [1, 2], ‘Ca. P. australiense’ [3] and ‘Ca. P. mali’ [4] provided valuable insights into the genetic environment of these bacteria. However, these strains represent only a relatively small part of phytoplasma diversity. Nevertheless, the sequence information provided significant insights in chromosome organization, such as circular and linear forms, the variation in genome size, and the reduced metabolic capabilities [5]. The loss of genetic resources obviously indicates an adaptation to plant and insect hosts. The essential dependence of phytoplasmas from their hosts is indicated by the common transport systems. Variations in important pathways such as glycolysis highlight differences in the central metabolism, in which the original function was probably lost [4]. It seems likely that nutrient import and secretion by phytoplasmas affects host metabolism and are thereby pathogenicity factors. The majority of genes involved are part of the core gene set, which can be distinguished from other parts of the genomes characterized by duplication, rearrangement and integration events. The integration of complex transposons (PMUs) [2] [6], prophage-related elements [7] and plasmid sequences [2][8] results in an increase of the genome size and stands in contrast to genome condensation processes. The importance of plasmid-encoded proteins for virulence could not be evaluated so far [9] but influences vector transmissibility of some species at least [10]. Examples of integration events can be identified in each genome examined. The genome data suggest that the integrated genetic material is, on the one hand, of significant importance for the phytoplasmas but probably also an accident resulting from a high pressure of integration events.

Because phytoplasma pathogenicity is still poorly understood, we are currently, in cooperation with B. Schneider and E. Seemüller, determining the complete genome sequence of mild strain 1/93 of ‘Ca. P. mali’. By comparing the data of this strain with those of virulent strain AT, information on phytoplasma pathogenicity can be expected. In the current strain 1/93 sequencing work, a combination of traditional Sanger sequencing and pyro-sequencing (454 Life Sciences, Roche) is used in the first round. In the ongoing data polishing the sequencing of PCR products and improving the overall data quality by additional complete genome coverage with sequencing by synthesis derived reads (Illumina) is included. Preliminary data indicate deletions or lacking of insertions in strain 1/93. Deletions are mainly assigned to absent phage-derived integrations. Determination of elements involved in pathogenicity remains problematic due to the high portion of proteins with unknown function and the genome instability. Candidates of secreted virulence factors such as
the immunodominant proteins occur in the different phylogenetic branches [11]. Symptom associated proteins such as Tengu of OY-M [12] or proteins interacting with the host such as SAP11 of AY-WB [13] are not shared within all four genomes. Virulence factors common to various phytoplasmas have not been identified even if they show similar symptoms. In consequence, two scenarios are conceivable: virulence factors may be part of the shared phytoplasma gene set including the conserved proteins of unknown function or they are part of the dispensable genome. Arguments for both options are presented.

References:
Oral presentation 5.2

New diagnostic tools for the Luteoviridae


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This study describes the detection of 13 Luteoviridae species with RT-PCR using three separate sets of low degeneracy generic primers to amplify a 68, 75 and 129/156 bp region of the Luteoviridae coat protein gene respectively. Species detected and discriminated by sequence analysis include all members of the genus Luteovirus (Barley yellow dwarf virus-PAV, BYDV-PAS, BYDV-MAV (129 and/or 156bp amplicons), Soybean dwarf virus [SbDV], Bean leafroll virus [BLRV] (68bp amplicon)) and eight of nine species from the genus Polerovirus (Beet western yellows virus[BWYV], Beet chlorosis virus [BChV], Beet mild yellowing virus [BMYV], Turnip yellows virus[TuYV], Potato leafroll virus [PLRV], Cucurbit aphid-borne yellows virus [CABYV], Cereal yellow dwarf virus-RPV [CYDV-RPV] (68bp amplicon) and Sugarcane yellow leaf virus (75bp amplicon)). These primers were not able to detect Carrot red leaf virus, Sweet potato leaf speckling virus (both belong to unassigned Luteoviridae) and Pea enation mosaic virus - 1 (genus Enamovirus). A synthetic positive control containing all primer sequence priming sites was designed to facilitate this method as a generic tool for use with plant material infected with any of the above species.

Furthermore a two step real-time RT-PCR melting curve analysis was investigated as a more rapid adaptation of the method above for the detection and discrimination of nine species, SbDV, BLRV, BChV, BMYV, BWYV, CYDV-RPV, CABYV, PLRV and TuYV. Melting temperature and shape of the melting peak were analysed for the 68 bp and 148 bp coat protein gene amplicons using SYBR® GreenER™ fluorescent dye. Specific melting peaks with unique melting temperature were observed for all species using the 68 bp amplicon, but not with the 148 bp amplicon. Due to the high variability of sequences for some members of this family, a range of melting temperatures was observed between different isolates of CYDV-RPV and TuYV. Nevertheless, discrimination was achieved for SbDV, BLRV, BChV, BMYV, CABYV and either PLRV or BWYV. Melting curve analysis, as used here, is a faster and more discriminatory alternative to gel electrophoresis of end-point PCR products for the detection and discrimination of these species.
Oral presentation 5.3

Development of a one-step multiplex RT-qPCR assay for the detection and quantification of CYDV-RPV in wheat

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Cereal yellow dwarf virus-RPV (CYDV-RPV) is a phloem-limited polerovirus (family Luteoviridae) that is primarily transmitted by the oat aphid (Rhopalosiphum padi) and occasionally by other aphids. CYDV-RPV is associated with yellow dwarf disease, one of the most economically important groups of diseases of cereals worldwide. As part of DPI Victoria’s climate change and vector-borne disease program, the CYDV-RPV/R. padi virus-vector system is being used to study the potential impact of increasing atmospheric CO₂ levels and temperature on virus infected wheat. We aim to quantify the titre and movement of CYDV-RPV in planta under simulated projected climate conditions in order to model the future impact of yellow dwarf diseases on wheat yield.

To aid this research, we have developed a specific one-step multiplex quantitative real time reverse-transcription PCR (RT-qPCR) assay using dual-labeled fluorescent hydrolysis probes to accurately measure CYDV-RPV titre in wheat. The multiplex assay combines PCR assays for CYDV-RPV and two reference wheat genes (Cell division control protein, AAA-superfamily of ATPases and ADP-ribosylation factor) which are reported to be stably expressed at different temperatures and in different tissue types. The reference genes are used to normalise the variation between samples that can contribute to inaccuracies in quantifying the target of interest, such as the amount of RNA extracted and efficiency of the RT and PCR steps, so that the target can be accurately compared between samples. To develop this assay we screened five previously published potential reference genes using SYBR-based RT-qPCR assays and these genes were ranked according to the stability of their expression using two different tools, GeNorm and NormFinder. The two highest-ranking reference genes were selected for inclusion in the multiplex RT-qPCR assay. To quantify the virus a standard curve was created using in vitro transcribed CYDV-RPV RNA (cRNA).

Validation of the performance of the two reference genes after various temperature and CO₂ treatments is currently being undertaken using the multiplex RT-qPCR assay. If reference gene validation is successful, this assay will be used in future experiments to monitor and evaluate the effect of CO₂ and temperature on CYDV-RPV in wheat plants.
Oral presentation 5.4

Identification and validation of reference genes for qPCR transcript normalisation studies in virus infected Arabidopsis thaliana

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Real-time quantitative PCR (qPCR) of cDNA has become a standard method for in-depth studies of gene transcript accumulation. However, the qPCR method can only identify genuine variation that is biologically significant when transcript quantities are accurately normalised to an appropriate reference. As such the selection of reference genes for normalisation has become an essential consideration when designing qPCR experiments. Validated reference genes are of particular importance when using qPCR to measure both viral and cellular gene transcript accumulation in the context of viral infections as viruses can interfere with host cell pathways that include housekeeping genes that have traditionally been used as reference genes. We describe a systematic evaluation of candidate reference genes of Arabidopsis thaliana ecotype Columbia-0 (Col-0) infected by a range of viruses to identify the most reliable reference genes for transcript quantification by qPCR. Twelve genes were selected for transcript stability studies as assessed by qPCR of cDNA prepared from Arabidopsis leaf tissue infected with one of five plant viruses (Cauliflower mosaic virus, Tobacco mosaic virus, Tomato spotted wilt virus, Turnip mosaic virus or Turnip yellow mosaic virus). The F-Box family protein (F-BOX), sand family protein (SAND), and elongation factor 1-alpha (EF1α) gene transcripts showed the most stable accumulation, whilst a traditionally used reference gene, Actin8, showed the least stable accumulation as measured by the geNorm statistical algorithm. The data provided here furnishes plant virologists with reference genes to consider for normalisation of qPCR derived gene expression in virus infected Arabidopsis and will be beneficial to the selection and design of primers targeting orthologous genes in other plant species.
Use of a duplex quantitative one-step RT-PCR to measure rate of degradation for virus RNA isolated from FTA cards

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FTA card technology was primarily designed and used for the transport of deoxyribonucleic acids. RNA can be stored on FTA cards, but the length of storage before degradation occurs is unknown. A quantitative reverse-transcription PCR assay was developed to assess the degradation rate of the RNA stored on FTA cards over time. Results from the study will benchmark the application of FTA cards for the storage of plant RNA and determine the usefulness of FTA cards to support active surveillance and incursion management programs. If feasible, this method could be used for the safe movement of plant RNA across quarantine borders.
Artificial microRNAs-mediated resistance to *Tomato spotted wilt virus*

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Thrips-transmitted tospoviruses are one of the most economically important viral pathogens of numerous vegetable, legume and ornamentals and are considered to impact crop production on a global scale to the tune of US $1 billion per year in lost yield. *Tomato spotted wilt virus* (TSWV), the type member of the *Tospovirus* genus has a wide host range (more than 900 plant species) that includes numerous crops and many weeds. Due to the complex epidemiological association of virus, host plants and thrips vectors, developing effective and durable virus control strategies has been a challenging endeavor.

We are developing artificial microRNAs (amiRNAs) for introducing resistance to TSWV. Micro RNAs are expressed in the host plants from non-protein coding genes and are processed by Dicer-like enzymes to produce mature miRNAs (21-24 nt) which guide post-transcriptional gene silencing in a sequence-specific manner. The biogenesis of mature miRNAs is not affected if several nucleotides are changed in the mature miRNA sequence. We have modified an *Arabidopsis thaliana* miR159 precursor to express artificial miRNAs (amiRNAs) targeting viral mRNA sequences encoding the nucleocapsid protein (N) and the silencing suppressor (NSs) genes of TSWV. Transient expression of amiRNAs in *Nicotiana benthamiana* has confirmed expression of virus-specific amiRNAs as well as the ability of the amiRNA constructs to confer resistance to TSWV. We are currently investigating the efficiency of these constructs to impart virus resistance in a stable transgenic system with Arabidopsis and tomato. The amiRNA-based resistance has been shown to be active even at lower temperatures unlike siRNA-based resistance and offers an effective approach to prevent breakdown of resistance in the field by expressing 2 to 3 amiRNAs targeting different essential regions of a virus. In addition, broad spectrum resistance to several viruses may also be obtained by co-expression of appropriately designed amiRNAs.
Oral presentation 5.7

Rapid Identification of a Tomato Leaf Curling Virus using Mass Spectrometry

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Tomato plants (cv Sub Arctic Plenty) showing leaf curling, crumpling, purpling and stunting (but not yellowing) were collected from Waitati, East Otago NZ.

Samples were tested for *Alfalfa mosaic virus*, Begomoviruses, *Cucumber mosaic virus*, Nepoviruses, Potyviruses, Tospoviruses and *Tomato bushy stunt virus* infection by ELISA, using kits (Agdia, Adgen, Loewe) in accordance with the manufacturer’s instructions.

Total nucleic acids and RNA were purified from leaf tissue using the phenol-chloroform and Trizol methods respectively. Total nucleic acids extracts were used in PCR in attempts to amplify Begomovirus, Curtovirus, *Tomato leaf curl virus*, *Tomato yellow leaf curl virus* and Phytoplasma fragments of various sizes. RNA extracts were used in RT PCR in attempts to amplify Carls-, Clostero-, Potex- Poty-, Tospo- and Tricho-viruses.

Frozen leaf material was homogenized in phosphate EDTA buffer containing reducing agents, extracted with chloroform, precipitated using PEG 8000 and further purified using differential centrifugation. The final high speed pellets (and other fractions) were resuspended in Laemli loading buffer and analysed using PAGE. A ~28kD band was cut from stained gradient gels (10-20% acrylamide) for analysis by mass spectrometry (MS).

Proteins of interest were digested in-gel using trypsin and the resulting peptides were subjected to MALDI tandem Time-of-Flight (TOF/TOF) MS. MS data was searched against the NCBI nr database using the Mascot search engine (www.matrixscience.com).

Although the symptoms displayed by infected plants resembled those of *Beet curly top virus* (Curtovirus) or other Geminiviridae no gemini or other viruses were detected using ELISA or PCR. In contrast purification and MS rapidly identified the ~28kD band as the Mr = 28.8kD N protein of *Tomato spotted wilt virus*. The significantly identified peptides covered 40% of the N protein target sequence. Examination of negatively stained purified preparations in the electron microscope revealed tospovirus-like virus particles 75nm in diameter.

The speed and utility of using Mass Spectrometry to identify and help characterize *Tomato spotted wilt virus* will be compared with a less successful attempt to identify *Cocksfoot mild mosaic virus* to discuss the pro’s and con’s of these methods.
Oral presentation 5.8

Detection of plant viruses using one-dimensional gel electrophoresis and peptide mass fingerprints

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A generic assay to detect and partially characterize unknown viruses from plants was developed. Proteins extracted from virus-infected and uninfected plants were separated in one dimension by SDS polyacrylamide gel electrophoresis. Differentially-expressed protein bands were eluted after trypsin digestion and resulting peptide fragments separated according to their mass by matrix-assisted laser-desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry. Resulting peptide mass fingerprints (PMF) were compared with those in protein databases. The assay was used to identify four known viruses: the potyviruses Zucchini yellow mosaic virus and Turnip mosaic virus, an alfamovirus Alfalfa mosaic virus and a cucumovirus Cucumber mosaic virus. It was also used to identify a virus that manifested symptoms in wild Cakile maritima plants, tentatively identified as Pelargonium zonate spot virus (PZSV) (genus Anulavirus) by its PMF, and then confirmed by nucleotide sequencing. The detection of PZSV constitutes a first record of this virus in Australia and in this host. It is proposed that this rapid and simple assay is a useful approach for analysis of plant samples known to harbor viruses that could not be identified using antisera or nucleic acid-based assays.
Detection of grapevine viruses by RT-PCR in vine leaves blot-dried with paper and stored at ambient temperature for over eight years

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In January 2002 virus infected leaves of *Vitis vinifera* cv. Limberger and cv. Shiraz clone PT23 showing typical leafroll symptoms with reddening and green veins were collected from a vineyard in the Adelaide Hills, South Australia. Total nucleic acid (NA) was extracted in 4 M guanidine hydrochloride followed by absorption to a silica matrix based on the protocol in the Qiagen Plant RNeasy Mini kit. The RT-PCR assay was used to test for the following 11 grapevine viruses: *Grapevine leafroll-associated virus* 1 (GLRaV-1), GLRaV-2, GLRaV-3, GLRaV-4 GLRaV-5 GLRaV-9, *Grapevine fanleaf virus*, *Grapevine virus A* (GVA), *Grapevine rupestris stem pitting-associated virus* (GRSPaV) and *Grapevine fleck virus* variants A and B. Three viruses, i.e. GLRaV-1, GVA and GRSPaV were detected in both varieties in 2002. The leaves were air-dried inside a lab book at ambient temperature until July 2010 when the dried leaf veins (20 mm) were subjected to the above NA extraction method and re-tested for all viruses. The three viruses found in 2002 were again detected. The Limberger vineyard was re-sampled and re-assayed in July 2010 and the same three viruses only were detected indicating that no other viruses had spread naturally into this variety during this 8 year period. In another experiment, leaves of cv Grenache growing at the Waite vineyard at Urrbrae, South Australia, were tested for all 11 viruses in 2008 and gave a positive result for GLRaV-2 and GRSPaV. The Grenache leaves were air-dried on a pin board and re-tested in 2010 for all viruses. Again the same two viruses were detected. Grapevine is known to be high in phenolic compounds which interfere with PCR reactions. It appears that the silica based method recovered sufficiently intact viral RNA from air-dried tissue for its detection by the RT-PCR assay. As GRSPaV is known to be present in almost all grapevine varieties, it is a potential candidate for testing the longevity of viruses in old grapevine herbarium specimens. The viruses we have studied here have helical symmetry, and may be vulnerable to physical breakage. It would be interesting to test the longevity of detectable virion RNA in viruses with icosahedral symmetry.
Oral presentation 5.10

Molecular Diagnostics for the Detection of Strawberry Viruses

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The supply of high-health, certified strawberry runners throughout Australia is dependent on the collections of high-health nucleus plants maintained in Victoria and Queensland. Both collections are tested annually in spring using a biological indexing method of petiole grafting candidate tissue onto sensitive indicator species for several virus associated diseases. Advances in molecular techniques have been published for detection of strawberry viruses. To assist in implementation of the protocols that we developed for these viruses into the certification schemes we created a “dummy” nucleus of virus infected strawberry varieties that were maintained in a similar manner to the industry nuclear collection in Victoria. The dummy nucleus in 2008/09 and 2009/10 was tested by biological indexing and PCR to give confidence that the PCR tests will work in this scenario. In 2008/09 two varieties (3 replicates of each) were inoculated with three virus combinations and tested monthly during the growing period for viruses. The 2008/09 nucleus plants were used to generate the nucleus plants for 2009/10, which were also tested monthly during the growing season.

PCR testing of the 2008/09 nucleus collection showed that viruses may not be reliably detected in strawberry plants during the first six months post-inoculation. Reliable detection was only achieved during the following season. PCR testing revealed that the rate of virus transmission from mother to daughter plants can reach 100% for most viruses. The results of virus testing of both the 2008/09 and 2009/10 dummy nucleus plants during the 2009/10 season suggest that October, March, April and May are the most reliable months for virus detection.

Our results indicated that biological indexing is less reliable for virus detection than PCR techniques as many of the inoculated indicators that were expected to show symptoms were symptomless. PCR testing revealed that multiple viruses can be transmitted and that it was difficult to associate symptoms with specific viruses. However, the PCR tests were also not 100% reliable because there were very few months in which viruses were detected in all known infected nucleus plants. This highlights the importance of using both molecular ad biological tests for certification of high health planting material.
Oral presentation 5.11

Using ELISA to indicate the presence of strain variants of *Grapevine leafroll-associated virus 3* in grapevines

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The most damaging virus infecting grapes in New Zealand is *Grapevine leafroll-associated virus 3* (GLRaV-3). Infection by GLRaV-3 is particularly serious with red grape cultivars because of uneven ripening of grapes and reduced anthocyanin production. It has been recognized for many years that there is considerable strain variation in many of the viruses infecting grapes, such as *Grapevine fanleaf virus*, grapevine virus A, *Grapevine leafroll-associated virus 1* and *Grapevine leafroll-associated virus 2*. However, there appeared to be less variation in GLRaV-3 (Turturo et al. 2005). Most primers used to detect GLRaV-3 have been designed from the genomic sequence of the NY1 isolate (Genbank AF037268) and one of the most widely used antibody kits used to detect GLRaV-3 (Bioreba) was prepared against the NY1 strain.

Some vines infected with GLRaV-3 appear to be infected with either a low titre of virus or a strain of GLRaV-3 that has low immunoreactivity to the Bioreba reagents. We also found that RNA extracts of some grapevines did not give PCR products using primers that were thought to be reliable. There is increasing evidence of the presence of more divergent strains of GLRaV-3 such as EF508151 and EF445655, and from Chooi et al. (this conference), which can only be detected using specific primers.

We have recently compared the apparent titre of GLRaV-3 in a range of cultivars and clones in a germplasm collection using either Bioreba reagents or polyclonal rabbit antibodies combined with an anti-rabbit-AP conjugate for detection in a TAS format. The ratio of the apparent titres from these reagents can be used as a guide to indicate the presence of GLRaV-3 strains that differ in immunoreactivity to the NY1 or GP18 strains.

Oral presentation 5.12

New technologies for monitoring begomoviruses and their whitefly vectors

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Whitefly-transmitted begomoviruses (family Geminiviridae) cause economically important diseases of many dicotyledenous food and fibre crop plants worldwide. Epidemics of begomoviruses are increasing in frequency due to the capacity of these viruses to rapidly evolve, the globalisation of trade, and the worldwide dispersal and polyphagous nature of the efficient whitefly vector Bemisia tabaci (B biotype).

In 2006, the exotic begomovirus Tomato yellow leaf curl virus (TYLCV) was detected for the first time in Australia in commercial tomato plantings surrounding Brisbane, the Lockyer Valley and Bundaberg (Queensland). The introduction of TYLCV has had a significant economical impact on tomato production, with disease incidence in some areas reaching 100%.

To limit further spread of TYLCV within Australia and to detect new incursions of exotic begomovirus species, rapid, efficient and reliable diagnostic strategies are required. Multiplexed real-time PCR and novel microsphere-based suspension arrays are being investigated to improve the efficiency and accuracy of routine plant virus detection in Australia.

Multiplexed TaqMan™ real-time PCR diagnostic assays for the detection of the endemic begomovirus species Tomato yellow leaf curl virus and Tomato leaf curl virus, in addition to assays for the detection of genetic groups of the vector B. tabaci (specifically those known as the endemic “B” and exotic “Q” biotypes), have been developed. Together, these assays provide a flexible diagnostic toolkit for the detection and differentiation of begomoviruses and their vectors in Australia. These assays will facilitate monitoring of the spread of TYLCV and ToLCV, and the potential incursion of exotic begomoviruses and genotypes of B. tabaci.

The Luminex® platform is being investigated for high-throughput nucleic acid detection of plant viruses and vectors in Australia. The xTAG™ system utilises a proprietary universal tag system allowing the simultaneous analysis and reporting of up to 100 different reactions in a single reaction vessel. We propose using this technology for the detection of endemic and exotic begomovirus species and B. tabaci genetic groups, utilising a hierarchical assay design for the detection of DNA-A, DNA-B, DNA-α and DNA-β satellite molecules. This hierarchical assay design will facilitate the identification of both known and unknown (or newly evolved) begomovirus species and B. tabaci genetic groups.
PCR assays for the detection of members of the genus Ilarvirus and family Bromoviridae

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Bromoviridae is one of the most important plant virus families and the genus Ilarvirus is the largest in the family. Members of the genus infect a wide range of hosts and several species cause serious yield losses in important crops. The use of generic primers is an important tool in the diagnoses of plant viruses, especially for quarantine and certification programmes. Commercially available generic Ilarvirus primers are expensive and cloning of the PCR product is required prior to sequencing.

An RT-PCR assay for the universal detection of members of the Bromoviridae family and a specific Ilarvirus assay have been developed. The RT-PCR can be completed in a one-step assay and products can be sequenced directly.

The specificity of the Bromoviridae primers, which target the helicase motif of RNA1, was analysed and verified in silico for all members in the family. The assay was also demonstrated to detect all species tested including members of the Alfamovirus, Anulavirus, Bromovirus, Cucumovirus and Ilarvirus genera.

The Ilarvirus primers, targeting the RNA-dependent RNA polymerase gene in RNA2, were able to detect 32 isolates of 17 Ilarvirus species. No cross reaction was observed with healthy plants nor with virus isolates from other genera in the family.
Development of a one-step multiplex RT-qPCR assay for the detection and quantification of PVY\textsuperscript{NTN} in potatoes

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PVY is the type member for the genus \textit{Potyvirus} (family Potyviridae) and there are three main strains including PVY\textsuperscript{O}, PVY\textsuperscript{C} and PVY\textsuperscript{N}. PVY\textsuperscript{NTN} is a sub-group of PVY\textsuperscript{N} which causes Potato Tuber Necrotic Ringspot Disease (PTNRD) and can lead to significant economic losses to growers. Since their discovery, PVY\textsuperscript{NTN} and PTNRD have spread into many potato growing countries including Australia where they were first detected in 2003. Attempts to eradicate PVY\textsuperscript{NTN} in Australia were not successful. PVY\textsuperscript{NTN} now appears to be spreading rapidly in the cultivar “Atlantic”, which is the main crisping variety in Australia. PVY\textsuperscript{NTN} appears to be mechanically transmitted in Australia, adding to the difficulty growers have to control spread of PVY\textsuperscript{NTN}.

This project aimed to develop a multiplex quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay for the detection and accurate quantification of PVY\textsuperscript{NTN} in potatoes. The assay incorporates two reference genes that are stably expressed in potatoes and are used to normalise the variation in RNA quantity and quality between samples so that PVY\textsuperscript{NTN} can be accurately measured and compared across all samples. Four reference genes were assessed for stability in PVY\textsuperscript{NTN} infected and uninfected potato leaf tissue (cv. Atlantic) using a SYBR Green RT-qPCR assay, geNorm and NormFinder. The β-tubulin and Cyclophilin reference genes were most stably expressed and were selected for further development of the multiplex RT-qPCR assay.

Primers and dual-labeled fluorescent hydrolysis probes were then designed for these two genes using Primer3. The primers were analysed for interference and primer-dimer formation in Oligo Analyzer. Once the primers and probes were optimised individually, the individual RT-qPCR assays for the two normalisation genes were then combined into one assay with an RT-qPCR assay that has been developed for PVY\textsuperscript{NTN}. To quantify the virus a standard curve was created using in vitro transcribed CYDV-RPV RNA (cRNA). The assay will be validated using PVY\textsuperscript{NTN} infected and uninfected potato plants at different growth stages, from different tissue types.

If successful, the multiplex RT-qPCR assay will be used to monitor the titre and movement of PVY\textsuperscript{NTN} in infected plants to assess cultivars for resistance and susceptibility.
Oral presentation 6.1

Virus-induced gene silencing and its applications in plant functional genomics

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As a result of the advanced DNA sequencing technologies, entire genome sequences of an increasing number of plant species have been obtained. In this era of post-genomics biology, we are faced with the daunting task of assigning functions to a huge number of genes. Traditionally, elucidation of gene functions has been accomplished through treatment with chemical mutagens, radiation, and insertional mutagenesis through T-DNA and transposons. However, these methods are time-consuming and labour-intensive, and often inefficient for large-scale applications. In recent years, numerous plant viruses have been developed as Virus-Induced Gene Silencing (VIGS) vectors and their potential as a powerful tool for the elucidation of plant gene functions has been quickly recognized. VIGS is based on the newly discovered and widespread phenomenon that infection of a plant with a recombinant virus carrying a plant nuclear gene or gene segment triggers the activation of a cytoplasmic RNA silencing machinery, which degrades both the viral RNA and the homologous plant RNA. Consequently, the inoculated plant will exhibit a loss of function phenotype, thus providing clues to the function of the gene. VIGS system is advantageous over traditional functional genomics approaches as it is much more straightforward and faster, and overcomes issues related to functional redundancy. At present, the most successfully used VIGS vectors include those derived from Tobacco rattle virus and Potato virus X for dicotyledonous plants, and Barley stripe mosaic virus and Brome mosaic virus for monocotyledonous species. In this communication, I intend to give a brief account on the current state of utilizing VIGS technology for plant gene function studies and discuss the different technical aspects pertaining to the successful use of this technology. Prospects of VIGS system for gene discovery and functional studies in the woody plant grapevine will also be discussed.
Oral presentation 6.2

Poleroviruses and Luteoviruses, their origins and interactions

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Members of the family Luteoviridae are phloem-limited and aphid transmitted plant viruses with a single, positive sense, non-polyadenylated RNA. Based on differences in the RNA-polymerase protein (RdRp) and structural proteins, the family is divided into three genera: Luteovirus, Polerovirus and Enamovirus. The P0 gene of one member of the Poleroviruses has been shown to encode an F-box -like protein that suppresses the plant defense RNA silencing pathway and the existence of suppressor proteins in members of the luteovirus genera is unknown. We are exploring the existence, identity and mechanism of action of suppressor proteins in members of the family Luteoviridae. We are also investigating the origins of Australian cereal yellow dwarf virus which appears to be a member of a much more exclusive group of poleroviruses than previously thought.
Oral presentation 6.3

Occurrence of *Potato virus X* strain groups and resistance genes, and phylogenetic placement of coat protein genes of Australian isolates

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Infection of potato with *Potato virus X* (PVX; genus *Potexvirus*) causes tuber yield losses of 5-20\%. Its four strain groups are defined by phenotypes induced when isolates are inoculated to cultivar differentials carrying hypersensitivity genes *Nb* or *Nx*. Group 1 strains overcome neither gene, group 2 strains *Nx* only, group 3 strains *Nb* only, and group 4 strains both genes. Andean isolate HB overcomes both and also extreme resistance gene *Rx*. On phylogenetic analysis, coat protein gene (CP) sequences of PVX isolates fell into two major clades, I and II, with clade II dividing into sub-clades II-1 and II-2. PVX strain groups 1 and 3 have been found in Australia but no sequencing of Australian isolates had been undertaken. Also, although *Nx*, *Nb* and *Rx* sometimes occur in parental lines used in potato breeding programs, knowledge of their occurrence in cultivars is usually lacking.

Three Australian PVX isolates were inoculated to potato cultivar differentials carrying genes *Nx*, *Nb* and *Rx*, and shown to belong to strain groups 1 (WA1) or 3 (XK3, TAS3). Isolate XK3 was sap-inoculated to 25 potato cultivars and one breeding line grown in Australia, and graft-inoculated to the six cultivars that remained uninfected. The hypersensitive resistance phenotypes obtained in two cultivars and one breeding line originally bred in Australia, two cultivars from Europe and one from North America revealed presence of *Nx*. Extreme resistance phenotypes identified *Rx* in another four cultivars bred in Australia, and in one each from Europe and North America.

The CP nucleotide sequences of 11 PVX isolates from Western Australia, Queensland or Tasmania were obtained and compared to those of 74 others. Strain group information was available for two Australian isolates and 26 others. On phylogenetic analysis of CPs, clade I contained all 11 Australian sequences, and their distribution within it suggested five separate introductions. Overall, Clade I isolates were from six different continents, but those in sub-clades II-1 and II-2 were only from Europe or the Americas, respectively. Clade I contained isolates in strain groups 1, 3 and 4, and sub-clades II-1 and II-2 isolates in strain groups 2 and 4.
Oral presentation 6.4

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

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Viruses constantly challenge plants; in response, plants have evolved a number of defence systems that protect them or reduce the severity of the virus invasion. Translational inhibition, an innate antiviral defence mechanism that is utilised by, and most studied in, mammals, is proposed also to exist in plants. This mechanism is activated by the presence of double-stranded (ds) RNA, a necessary replication intermediate of RNA viruses, and relies on the phosphorylation of the α subunit of the eukaryotic initiation factor (eIF) 2 by the dsRNA-dependent protein kinase R (PKR). Phosphorylation of eIF2α results in the global inhibition of translational initiation in the host cell, and subsequently blocks the infection of the virus.

Initial evidence supports the presence of a plant-functional homolog of PKR (pPKR), but no homologous sequence has so far been detected. Our aim was to identify pPKR via a proteomics approach using Arabidopsis thaliana, whereby the protein was first enriched as determined by the correlation of its activity, and then sequenced. The identification of pPKR would provide evidence for the presence of a novel antiviral defence mechanism in plants.

The immobilised eIF2α peptide assay was developed to allow the rapid detection and quantification of eIF2α phosphorylation activity in vitro. Flower buds from young Arabidopsis plants had the highest eIF2α phosphorylation activity and ds but not single-stranded RNA activated this pPKR activity. Size exclusion chromatography was used to enrich for pPKR from Arabidopsis, and mass spectrometry analysis of proteins associated with kinase activity identified a number of RNA binding proteins and protein kinases, including calcium-dependent protein kinases (CPKs). The assessment of ions on putative pPKR activity revealed that eIF2α phosphorylation was activated in the presence of calcium. A statistically significant decrease in eIF2α phosphorylation activity was seen in a CPK T-DNA insertion line and also a line overexpressing the plant-encoded inhibitor of PKR, p58IPK. These two plant lines also had similarly stunted phenotypes. These findings provide evidence for a CPK as the putative plant-functional homolog of PKR.
Oral presentation 6.5

The role of subgenomic RNA 3 in Luteoviral defence

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The Luteoviridae are a family of phloem-restricted, positive-sense ssRNA viruses that are transmitted exclusively by aphids. Classified into three genera – Luteovirus, Polerovirus and Enamovirus – the Luteoviridae encompass approximately 20 viruses of varying homogeneity.

Luteoviridae are the most abundant and economically important cereal crop virus, causing substantial global yield losses. Polerovirus encodes a silencing suppressor protein, P⁰, which specifically targets AGO1 for ubiquitination. The degradation of AGO1 severely disrupts the plant’s anti-viral defence mechanism, allowing the virus to replicate freely.

Despite remarkably similar infection symptoms between Luteovirus - Barley yellow dwarf virus and Polerovirus - Cereal yellow dwarf virus, Luteovirus do not encode any known silencing suppressor protein/s. Sequencing and northern blot analyses have revealed however that most Luteoviruses express at high levels, a unique subgenomic RNA, termed sgRNA3. Although sgRNA3 has no known function, it is possible that this non-coding RNA disrupts the host anti-viral defence mechanism. The ability of sgRNA3 to suppress host silencing in a transient expression system is being investigated.
Poster 6.6

Suppression of RNA silencing is required for lethal systemic cell death induction by *Clover yellow vein virus* in pea

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In plants, RNA silencing acts as an effective antiviral defense. To counteract RNA silencing, many plant viruses encode suppressors of RNA silencing, including the helper component-proteinase (HC-Pro) of potyviruses. Our prior studies showed that the D-to-Y mutation at amino acid position 193 in HC-Pro (HC-Pro-D193Y) drastically attenuated the virulence of *Clover yellow vein virus* (CIYVV) and RNA-silencing suppression activity (RSS) of HC-Pro-D193Y was significantly reduced. Here, we show that expressions of heterologous suppressors of RNA silencing, including *Tomato bushy stunt virus* p19 and *Cucumber mosaic virus* 2b, fully and partially complement the function of HC-Pro-D193Y for virulence expression (induction of lethal systemic cell death, LSCD) in pea, respectively. Furthermore, p19 expression restored the ability for the CIYVV D193Y mutant to activate the hypersensitive response-like defense pathway, which is systemically activated in wild-type CIYVV infection. Our results suggest that RSS activity of HC-Pro is critical for induction of LSCD by CIYVV in pea.
Three distinct caulimoviruses have been reported from dahlia (*Dahlia variabilis*): *Dahlia mosaic virus* (DMV), *Dahlia common mosaic virus* (DCMV) and an endogenous plant pararetrovirus (DMV-D10). Based on sequence comparisons and promoter prediction programs, the putative 35S promoter regions from these three viruses were identified. The promoter regions were independently cloned into pCAMBIA1281Z. All constructs were introduced into *Agrobacterium tumefaciens* by electroporation, and agroinfiltrations were done into *Nicotiana benthamiana*. The activity and strength of the putative 35S promoter was determined by transient expression of the beta-glucoronidase gene (GUS). Results from the quantitative GUS assays demonstrated that DMV, DCMV and DMV-D10 promoters were active in expressing the GUS and their relative strengths varied from one another. The highest GUS activity was by the DCMV promoter followed by those from DMV and DMV-D10.
Oral presentation 7.1

Epidemiology of *Tobacco yellow dwarf virus* and insights into the feeding physiology of its principal leafhopper vector

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*Orosius orientalis* (Matsumura) (Hemiptera, Cicadellidae) is an important leafhopper vector of many phytoplasma and virus diseases in Australia, including tobacco yellow dwarf, legume little leaf, tomato big bud, lucerne witches broom, potato purple top wilt and Australian lucerne yellows. Despite its economic importance, until recently little was known about *O. orientalis* population dynamics, feeding behaviour, host-plant interactions, nutritional requirements and epidemiology of pathogen transmission/acquisition. As a result, current control measures against the leafhopper are less than effective.

In an attempt to develop alternative and more efficient management strategies, we have been investigating the epidemiology of *Tobacco yellow dwarf virus* (TbYDV) in bean and tobacco plant. We identified the primary vector of TbYDV and in detail studied the vectors population dynamics, interactions with host and non host plants and developed \textit{in vitro} and \textit{in planta} systems to study the insects feeding behaviour and characterised its nutritional requirements. We have now developed artificial diet bioassay system for *O. orientalis* and demonstrated the antimetabolic activity of two plant lectins (snowdrop lectin and wheat germ agglutinin). In addition, we have used an electrophysiological approach, the electrical penetration graph (EPG), to monitor vector probing behaviour, both \textit{in vitro} and \textit{in planta}. This is the first study, to demonstrate an artificial diet for *O. orientalis*, identify potential feeding inhibitors and examine the feeding behaviour on host, non-host plant and artificial diets.
Wheat curl mite and its role in the transmission of wheat streak mosaic virus in Australia

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The wheat curl mite (WCM), Aceria tosichella Keifer, is an eriophyoid pest of cereals, and the vector responsible for the transmission of wheat streak mosaic virus (WSMV). To date, detections of WSMV have been confirmed in all states and territories of Australia, except the Northern Territory, and it is now evident that the entire Australian wheat belt is at risk of WSMV infections. Given the increasing prevalence and spread of WSMV in Australia, there is a need to understand the biology, ecology and genetics of the wheat curl mite, the primary vector of this virus.

Using a variety of molecular tools we have shown that Australian wheat curl mite consist of two genetic lineages likely to represent distinct species. These lineages occupy similar distributions, occurring throughout the entire Australian wheat belt, and are often found in sympatry. Using a series of laboratory experiments we found only one WCM lineage capable of transmitting WSMV virus under controlled conditions. These findings were further substantiated via assessments of mite presence and relative abundance at infected sites in the field. Using microsatellite markers we have shown that wheat curl mite likely reproduce by haplodiploidy and show limited genetic structuring suggesting the species has high dispersal potential. These results have implications for the management of WCM and WSMV within Australia.
Oral presentation 7.3

Wheat streak mosaic virus: alternative hosts, infection of wheat, oat and barley cultivars, seed transmission studies and spatial and temporal spread patterns

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Wheat streak mosaic virus (WSMV) causes severe yield losses in wheat crops when summer and autumn conditions are conducive to the build-up of its wheat curl mite (WCM) vector and WSMV is present in grass hosts and volunteer cereals. WSMV is seed-borne at low levels in wheat. A number of alternative WSMV hosts have been identified. However, whether perennial grasses act as alternative hosts in Western Australia is unknown, or if WSMV is seed-borne in annual grass hosts or oats and barley.

Annual and perennial grasses were grown from seed and sap inoculated up to four times with WSMV. Species that remained healthy were reinoculated using WCM. Annual grasses, including barley grass (Hordeum sp.), brome grass (Bromus sp), annual ryegrass (Lolium rigidum), bearded oats (Avena barbata), Panic grass (Panicum sp) and blowfly grass (Briza maxima), all became infected. Seed was collected from infected plants and seedlings tested by ELISA, no WSMV detected in barley grass (>5000 seedlings), annual ryegrass (>5000), bearded oats (>1000), Panicum (>500) and brome grass (>3000) and blowfly grass (>100). Possibly, however, low seed transmission levels might have detected if more seedlings had been available. None of the perennial pasture or wild grasses inoculated, including Rhodes grass (Chloris sp), tall fescue (Festuca sp), Phalaris grass (Phalaris sp), perennial ryegrass (Lolium perenne) or African lovegrass (Eragrostis curvula), became infected following multiple sap and WCM inoculations.

All 11 wheat cultivars sap inoculated with WSMV became infected. Oat cvs Swan and Bandicoot became infected readily. However, when barley was sap inoculated, cvs Baudin and Yagan remained uninfected following multiple inoculations, whereas cv. Stirling became infected. When plants of barley cv. Stirling, wheat cv. Calingiri and oat cv. Swan were grown, inoculated and maintained at 18°C or 26°C, there was no difference in the number of plants that became infected at either temperature. Also, when their seeds were germinated, most of those from plants grown at 26°C were shrivelled and did not emerge. No WSMV was detected in any of the seeds tested.

Data from field experiments on WSMV infection sources and temporal and spatial spread patterns will also be described.
Oral presentation 7.4

Virus control in chickpea—special considerations

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Chickpea (\textit{Cicer arietinum}) is presently the most valuable cool season grain legume grown in Australia. Compared to other broadacre crops, it is colonised poorly by aphids but nevertheless incurs significant losses from aphid-transmitted viruses. Spread is thought to be by migrant alatae that feed just long enough to transmit viruses in either nonpersistent or persistent manners. The implications of little or no colonisation need to be considered in making virus control recommendations. Control strategies that target secondary spread by colonising apterae are expected to be ineffective. There is no evidence for efficacy of seed or foliar applied aphicides, and consequently aphid monitoring and forecasting cannot be used a basis for aphicide application. Use of virus free seed has been shown to be effective for controlling \textit{Cucumber mosaic virus} (CMV) in chickpea Western Australia, but efficacy has not been reported for \textit{Alfalfa mosaic virus} (AMV) and this strategy is not applicable to non-seed transmitted luteoviruses. Both AMV and luteoviruses are generally more damaging than CMV in eastern Australia where most chickpea is grown. In this paper we evaluate the above-mentioned and other commonly cited control strategies for the Australian chickpea industry. Present control options are inadequate. The best options are simply best agronomic practices: retaining standing stubble, using optimal sowing rates and times, and controlling in-crop and fallow weeds. Chickpea genotypes with relatively low infection incidence (tolremicity) appear to have potential for resistance breeding.
Oral presentation 7.5

Studies on seasonal incidence, loss estimation, epidemiology and host plant resistance for *Peanut bud necrosis virus* of Groundnut in North Eastern Karnataka

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Peanut bud necrosis disease (PBND) caused by *Peanut bud necrosis virus* (PBNV), transmitted by *Thrips palmi* Karny is an economically important and destructive disease of groundnut in north eastern Karnataka. The continuous monitoring indicated that the first appearance of disease was noticed between 15-27 days after sowing. The studies also revealed that the disease was severe not only in rainy season but also in post-rainy. The disease adversely affected the growth and yield parameters of groundnut and the considerable reduction was recorded in PBND plants infected at 30-50 DAS. The epidemiology of the disease provides information on effect of host plant resistance on disease incidence. The present findings indicated that epidemic development reached a plateau before the complete maturity of the crop in all the genotypes tested. The epidemic development ended up independently of the disease pressure and resistance level of host genotype.

Management of viral diseases is a difficult task and cultural practices and host plant resistance play an important role. Studies revealed that sowing of groundnut crop during June (Rainy) and November (Post Rainy) was very effective in reducing the incidence of peanut bud necrosis disease which in turn reflected on higher yields. The biochemical parameters viz., total sugar, phenol, ortho-dihydroxy phenol and protein were more in resistant genotypes than susceptible. The intensive screening of groundnut entries at hot spot location resulted in the identification, development and registration / release of few resistant genotypes. Three resistant genotypes viz., R-8808, R-2001-2 and R-2001-3 were released for cultivation among the farmers of the region. Further, eight PBND resistant genotypes viz., NRCGCS-77 (National ID: IC-0582472), NRCGCS-85 (IC-0582473), NRCGCS-86 (IC-0582474), NRCGCS-21 (IC-0583387), NRCGCS-83 (IC-0583388), NRCGCS-124 (IC-0583389), NRCGCS-180 (IC-0583390) and NRCGCS-222 (IC-0583391) have been registered and deposited at the National Gene Bank (National Bureau of Plant Genetic Resources (NBPGR), New Delhi.
Oral presentation 7.6

Climate change: potential impact on Barley yellow dwarf virus spread and occurrence

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The Department of Primary Industries Victoria, the University of Melbourne and the Department of Climate Change have established a Free-Air CO\textsubscript{2} Enrichment (FACE) research facility at Horsham, to study the effects of elevated CO\textsubscript{2} on wheat production in Australia. This facility is being used to study the effects of elevated CO\textsubscript{2} (550ppm) under field conditions on wheat, the Barley yellow dwarf virus (BYDV), as well as biology of its aphid vector Rhopalosiphum padi (Hemiptera, Aphididae). Results from studies on wheat plants conducted at the FACE facility show changes in C:N ratio, increase in plant height, biomass, number of tillers, and surface area in response to elevated CO\textsubscript{2}. However, variable field conditions have proved difficult for studying the impact of elevated CO\textsubscript{2} on BYDV and its vector, therefore in addition to the FACE facilities, controlled environment growth chambers are being used to study the physiology and feeding behaviour of \textit{R. padi} and its ability to acquire and transmit BYDV under various climatic conditions and CO\textsubscript{2} concentrations. Results from the FACE facility and growth chambers will be described and presented. Potential ecological and epidemiological consequences will be discussed.
The impact of *Turnip mosaic virus* (TuMV) on rare and endangered native *Lepidium* spp. in the South Island, New Zealand

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Cook’s scurvy grass (*Lepidium oleraceum* agg.) is an endangered species of native Brassicaceae to New Zealand that is considered at risk of becoming extinct. Virus-like disease symptoms were observed in a newly established plant of *L. oleraceum* at Stony Bay, Banks Peninsula, Canterbury, New Zealand. *Turnip mosaic virus* (TuMV) was subsequently identified as the cause (Fletcher et al. 2008). TuMV was also detected in plants with virus-like disease symptoms observed in a collection of rare native *Pachycladon* (Brassicaceae) species held at Lincoln, Canterbury, New Zealand.

A survey was undertaken of seven isolated South Island sites where *L. oleraceum* and other *Lepidium* species grow. TuMV was detected in approximately 20% of plants at two of the seven sites. *Cauliflower mosaic virus* (CaMV) was also detected at three sites with up to 50% incidence at one site. There was also some evidence of possible infection by *Beet western yellows virus* (BWYV). The poster reports survey results and possible implications of virus infections for the survival of endangered species.
Poster 7.8

**BWYV an emerging problem in pulse crops**

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In the last five years *Beet western yellows virus* (BWYV) has become an increasing problem in Victoria, South Australia and New South Wales. Pulse surveys conducted in 2006 and 2007 found high incidences of BWYV in Victoria, South Australia and NSW, including some sampled lentil, chickpea and field pea crops with levels of virus up to 79%. In 2009, a chickpea survey was conducted in eastern Australia. In Victoria and southern NSW, all sampled crops were infected with BWYV and within crop virus incidences ranged from 3-69%. In South Australia and Northern NSW, 58% and 36% of sampled chickpea crops respectively were infected with BWYV and the within crop virus incidences ranged from 1-29%. During these surveys, ten BWYV host weed species were found, five of which were perennials. In 2009, in southern Victoria (Western District), 14 summer mustard crops were surveyed for viruses and aphids. Six crops were infected with BWYV and cabbage aphid was found colonizing seven out of 14 crops. Due to the severity of BWYV in eastern states there is a need to develop management strategies for this virus. The predictive model developed by the Department of Agriculture and Food Western Australia (DAFWA) for BWYV in canola will be validated in Victoria and SA and modified for predicting BWYV in pulses in conjunction with DAFWA staff. The development of a decision support system for advising on the risks of BWYV to pulse and canola production will be assessed. Studies on the biology of BWYV and the epidemiology of BWYV and its aphid vectors are being undertaken.
Improved methods for detection and control of *Potato virus Y* (PVY) in potatoes

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*Potato virus Y* (Potyvirus: *Potyviridae*) (PVY) has been recorded in all states of Australia and is an emerging problem for the south east Australian potato industry. In 2003 symptoms consistent with potato tuber necrotic ring spot disease were observed in potato tubers in Victoria and a tuber necrotic strain of PVY (PVY\(^{NTN}\)) was identified as the causal agent of the disease. Since 2003 there has been a steady increase in the incidence of PVY\(^{NTN}\) in several potato growing districts. It has become apparent that the current practices in potato seed certification and disease management are proving to be ineffective in limiting the spread of this virus.

Field work completed in 2008/09 demonstrated that current sampling strategies of collecting fully expanded leaves from field grown plants reliably detect *Potato leafroll virus* (PLRV), *Tomato spotted wilt virus* (TSWV) and *Potato Virus S* (PVS) by ELISA. However, the ELISA test was less reliable in detecting PVY in potato leaves collected from the field, particularly towards the end of the growing season. These results indicate a need to certify seed potatoes based on post harvest tuber testing. Currently the “grow-on ELISA” test is the most widely used post harvest tuber test for seed potato certification in Australia. This test however is time consuming and as such is not widely adopted by industry.

We report the development of an RT-PCR method for the detection of PVY that can be carried out directly on tubers. Comparative serological, molecular and biological analysis has revealed that the different PVY strains or their recombinants may vary in their distribution within a potato tuber. Therefore, to maximize the chances of detecting PVY in potato tubers, the best tuber sampling methods and RNA extraction techniques have been identified. Our results indicate that the direct RT-PCR test on infected tubers is more sensitive than the grow-on ELISA test for the detection of PVY, PLRV, TSWV and PVS.
APWV General Meeting agenda

Australian quarantine policy for nucleic acids of plants and plant pathogens

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Australia is free of many plant pathogens that cause significant disease in other countries. To maintain this status, it is important to be able to detect and identify the foreign pathogens. To this end, research is needed to develop and verify diagnostics. The importation and containment of high priority organisms for research was discussed at the Plant Health Committee in 2008, but some issues were not resolved. Without an agreed national approach, the Biosecurity Services Group is unlikely to allow import of samples of significant foreign pathogens, including those on Plant Health Australia’s High Priority Pests list.

Nucleic acids (NAs) extracted from plant pathogens pose much lower risks than cultures or plant samples. Importing NAs may facilitate research. A recent refinement of policy requires quarantine containment of certain NAs, with permit conditions depending on estimates of the level of risk.

NAs of most known cellular pathogens pose no appreciable risk and applications are likely to be granted for in vitro use in standard laboratories. NAs of cellular pathogens on a restricted list may be permitted into QC1 quarantine approved laboratories.

The NAs of some plant viruses and most viroids are infectious when host plants are inoculated. For this reason higher level quarantine is required for all viroids and the NAs of virus species not present in Australia or prescribed or declared in a State or Territory. NAs extracted from foreign field collected samples may contain infectious virus or viroid NAs and therefore, also require quarantine, as do NAs from unidentified organisms.

NAs from healthy plants may be imported for in vitro or in vivo use outside quarantine with a permit. In vivo use of NAs from infected plants or from plant pathogens may present some risk if the pathogen is a virus or viroid and imports may not be permitted or quarantine may be required.
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