APVW 2024

15th Australasian Plant Virology Workshop

Crowne Plaza Gold Coast
29 - 31 October 2024

WORKSHOP HANDBOOK

APPS

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WELCOME MESSAGE

From the Chair

Hello everyone!

Welcome to the 15th Australasian Plant Virology Workshop (APVW)

We're delighted to see such a diverse and engaged group of researchers, practitioners, and enthusiasts gathered to explore the latest advancements in plant virology and obligate pathogens.

As we gather on the ancestral land of the Yugambeh people, we acknowledge and pay respect to their enduring connection to this beautiful region.

The program features an exciting range of topics, from plant-virus interactions and ecology to new tools and technologies. We extend a warm welcome to our highly anticipated international and domestic keynote speakers: Dr Marco Incarbone, Dr Veronique Brault and Dr Edward Holmes, along with our wonderful presenters. Thank you all for your time and effort in preparing talks that make this workshop worthwhile. We encourage you to explore the abstracts booklet and program for a detailed overview of what's in store.

I would like to express my heartfelt thanks to my committee; Kathy Crew, Anthony James, Rebecca Roach, Andrew Geering and Flavia Bonora. Thank you so much for your support, encouragement, ideas and enthusiasm. You have all done an amazing job, and I certainly wouldn't want to have done this without you!

I would also like to extend my sincerest thanks to the event organisers, KAM events. Kate and Jenny have an incredible wealth of knowledge, experience and history in organising scientific conferences. We are grateful for your expertise! There is so much more to organising an event, and you both ensured that everything ran smoothly.

Finally, I would like to thank all of you— our delegates and presenters. We have a stimulating program planned, and we look forward to rekindling old friendships and forging new connections throughout our time together. Thank you for being here, and let's make this workshop a memorable and enriching experience for all!

Enjoy the conference!

Fiona

APVW 2024 Chair

Conference Managers - Kate Murphy and Jenny Lawler,

KAM Event Management, PO Box 318, Coolangatta Qld 4225.

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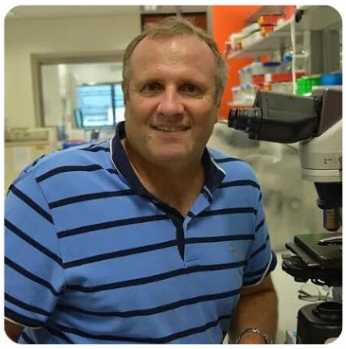
COMMITTEE

Fiona Filardo

Anthony James

Rebecca Roach

Kathy Crew



Andrew Geering

Flavia Bonora

APVW PROGRAM

Tuesday 28 October	
0830-1715	**Registration Open**
0930-1000	**Morning Tea**
1000-1200	**Session 1** **Sponsor:** QLD Department of Agriculture and Fisheries Chair: Andrew Geering, with co-chair: Bao Ton
1000 – 1015	**Fiona Filardo**: Opening. Acknowledgement to country
1015 – 1030	**Paul Campbell**: Tomato spotted wilt virus resistance breaking strains in Australia
1030 – 1045	**Satomi Hayashi**: Exploring the source of TYLCV resistance in *Nicotiana benthamiana*: a potential novel source of resistance for combatting a major disease in the field
1045 - 1100	**Solomon Maina**: Pea seed-borne mosaic virus genomes, reveals distinct lentil strain phylogroup; Biosecurity implications?
1100 – 1200	**R.E.F Matthews Lecture:** **Edward Holmes:** Redefining virus ecology and evolution using metagenomics
1200 - 1300	**Lunch** **Sponsor**: Destination Gold Coast
1300 – 1455	**Session 2 - Biosecurity & emerging viruses 1** **Sponsor**: Department of Agriculture of Forestry and Fisheries Chair: Kathy Crew, with co-chair: Eyal Zeira
1300 – 1330	**Andrew Geering**: The untold history of banana bunchy top disease
1330 – 1345	**Mignon de Jager**: Is phytoplasma titre correlated with symptom development in Banana Wilt Associated Phytoplasma disease in Papua New Guinea?
1345 – 1400	**Grant Chambers**: Updating diagnostics for graft-transmissible citrus pathogens
1400 – 1415	**Joanne Mackie**: *Comovirus viciae* genomes generated from historical isolates: Step 1 in designing new molecular diagnostics
1415 – 1430	**Murray Sharman**: Johnsongrass mosaic virus: resistance of maize in Australian and implications for preparedness for maize lethal necrosis
1430 – 1445	**Ruvini Lelwala**: Early detection of viruses and viroids in imported *Citrus* germplasm undergoing post-entry quarantine using a small RNA Sequencing (sRNASeq) workflow
1445 – 1450	Poster Presentation: **Marilyn Apa**: Distribution of begomovirus in Papua New Guinea (PNG)
1450 – 1455	Poster Presentation: **Madhav Jatin Chandarana**: Viruses associated with *Agaricus* mushrooms in Australia
1455 – 1525	**Afternoon Tea**
1525 – 1700	**Session 3 - New tools & technologies 1** **Sponsor**: Qiagen Chair: Monica Kehoe, with co-chair: Manda Wang
1525 – 1530	QIAGEN 3 min talk
1530 – 1545	**Lia Liefting:** The use of high-throughput sequencing for plant virus diagnostics at the New Zealand Ministry for Primary Industries
1545 – 1615	**Felipe de Felippes**: MicroRNA-induced gene silencing (MIGS): the tool of choice for silencing viral genes and to confer multi-virus protection in plants
1615 – 1630	**Mark Jackson**: Development of mild cross protecting strains of citrus tristeza virus through infectious clone assembly
1630 – 1645	**Tonny Kinene**: Tile amplicon sequencing: A comprehensive approach for potato virus Y (PVY) strain detection and characterization
1645 – 1715	**Nicole Thompson:** Tomato Brown Rugose Fruit Virus detection and emergency response in South Australia
1800 – 2000	**Welcome Reception**

Wednesday 30 October	
0830-1730	**Registration Open**
0845 – 1035	**Session 4 - Plant-virus interactions** **Sponsor**: QUT Chair: Anthony James, with co-chair: Madhav Chandarana
0845 – 0945	**KEYNOTE: Marco Incarbone**: Investigating the antiviral defences protecting plant stem cells and germline from infection
0945 – 1000	**Colleen Higgins**: Lettuce necrotic yellows virus subgroups elicit different responses in the model host *Nicotiana glutinosa*
1000 – 1015	**Roshni Rohra**: Grapevine leafroll-associated virus 3: molecular evolution, biological variation, and host response
1015 – 1020	Poster Presentation: **Sinethemba Nkosi**: CRISPR/Cas9 gene editing towards resistance to the Banana bunchy top virus in banana
1020 – 1035	**Robin MacDiarmid**: Talking about a (sexual) revolution in transmission terminology
1035 – 1100	**Morning Tea**
1100 – 1230	**Session 5 - Plant virus diversity & detection** **Sponsor**: GRDC Chair: David Lovelock, with co-chair: Wei-An (Vivien) Tsai
1100 – 1115	**Ben Congdon**: Clade divination phenotypic data facilitates epidemiological inferences from soybean dwarf virus sequence analysis
1115 – 1130	**Flavia Sarti Bonora**: Enhancing DNA and RNA extraction quality for viral detection in horticultural seeds
1130 – 1145	**Eyal Zeira**: Diversity of *Orthotospovirus impatiensnecromaculae* (impatiens necrotic spot virus; INSV) in Australia
1145 – 1200	**Julia Mulabisana**: Uncovering the diversity of viruses infecting leafy vegetables in South Africa, and their impact on the fresh weight of the leaves
1200 – 1215	**Ram B. Khadka**: Prevalence and incidence of lentil viruses in Nepal
1215 – 1220	Poster Presentation: **Nazanin Nazeri**: Investigating the correlation between cucumber mosaic virus (CMV) titre in narrow-leafed lupin tissues and seed transmission rates
1220 – 1225	Poster presentation**: Craig Webster**: Direct testing of potato tubers for plant viruses and implementation in a seed potato testing scheme
1225 – 1230	Poster Presentation: **Bao Ton**: A system for evaluation of transgenic turnip mosaic virus resistance in *Brassica napus* plants
1230 – 1330	Lunch **Sponsor:** AGRF
1330 – 1515	**Session 6 - Virus-vector interactions** **Sponsor**: Hort Innovation Chair: Bec Roach, with co-chair: Jingfeng Liang
1330 – 1430	**KEYNOTE: Véronique Brault**: Polerovirus, host plants and aphid vector: in the secret of a "ménage à trois"
1430 – 1445	**Narelle Nancarrow**: Transmission and host range of yellow dwarf virus species recently reported in Australia
1445 – 1500	**Mohammad Aftab**: Viruses in canola and aphids in western Victoria
1500 – 1515	**Rebecca Gough**: Unlocking biocontrol potential: Should we adopt *Botrytis cinerea* as a mycovirus model system?
1515 – 1545	**Afternoon Tea**
1545 – 1715	**Session 7** - Biosecurity & emerging viruses 2 Chair: Solomon Maina, with co-chair:Mignon de Jager
1545 – 1615	**Richard Davis & Lynne Jones**: How great passion, good will and a love for the tropics creates magic - the part that plant virus and virus-like diseases have played through 35 years of Northern Australia Quarantine Strategy plant health surveys
1615 – 1630	**Fiona Filardo**: What do lettuce chlorosis virus, yambean mosaic virus and an unknown cogu virus have in common?
1630 – 1645	**Shamila Abeynayake**: Small RNA-omics and Long-Read Sequencing Integrated Strategy for Characterisation of Novel Plant Viruses

1645 – 1700	**Candace Elliott**: Preparing for the transition of banana quarantine and diagnostics to Mickleham
1800 – 2100	**APVW Dinner Kurrawa Surf Club**
Thursday 31 October	
0830 – 1500	**Registration Open**
0930 – 1030	**Session 8** - Plant virus ecology, disease epidemiology & management 1 **Chairs**: Robin MacDiarmid, with co-chair: Nora Derbal
0930 – 0945	**Ben Congdon:** Field effectiveness of foliar systemic insecticides, a neonicotinoid-based seed treatment, and partial resistance to control turnip yellows virus in canola
0945 – 1000	**Wei-An (Vivien) Tsai**: Investigating resistance breakdown and alternative management strategies for tomato yellow leaf curl virus
1000 – 1030	***APVW Panel: Where to from here?***
1030 – 1100	**Morning Tea**
1100 – 1230	**Session 9** – New tools and technologies 2 **Chairs**: Flávia Bonora, with co-chair: Narelle Nancarrow
1100 – 1115	**Joshua Linn**: Digital PCR in plant virus detection: Advancing precision, sensitivity, and efficiency
1115 – 1130	**Chris Brosnan:** Nanoparticle-mediated delivery of VIGS vectors for protecting citrus from HLB
1130 – 1145	**Marie-Emilie Gauthier**: ONTViSc: a customisable nextflow pipeline for the identification and detection of viral genomes from Oxford Nanopore sequencing
1145 – 1200	**Dawit Kidanemariam**: Development of badnavirus infectious clones for research in taro and banana
1200 – 1205	Poster Presentation: **Nandita Pathania**: Exploring phages as promising biocontrol agents for managing bacterial diseases in plants
1205 – 1220	**Rebecca Roach**: Genome sequencing of phages infecting plant associated *Enterobacter* species
1220 – 1235	**Fiona Constable & Monica Kehoe:** High throughput sequencing, biosecurity and policy in plant virus diagnostics
1235 - 1330	**Lunch**
1330 – 1500	**Session 10** – Plant virus ecology, disease epidemiology & management 2 **Chair**: Nicole Thompson, with co-chair: Roshni Rohra
1330 – 1345	**Roger Jones**: Seed transmission of turnip mosaic virus to seedlings of *Brassica juncea*
1345 – 1400	**Jingfeng Liang**: Spray application of dsRNA targeting the CP of ZYMV reduces mechanical and aphid transmitted infection incidence in glasshouse grown *Cucurbita pepo*
1400 – 1405	Poster presentation: **Manda Wang**: RNA interference against the Green Peach Aphid, *Myzus persicae (Hemiptera)*
1405 – 1420	**Eloise Martin**: Managing potyviruses in cucurbit crops by host resistance
1420 – 1440	**Closing remarks**
1440	**Close**

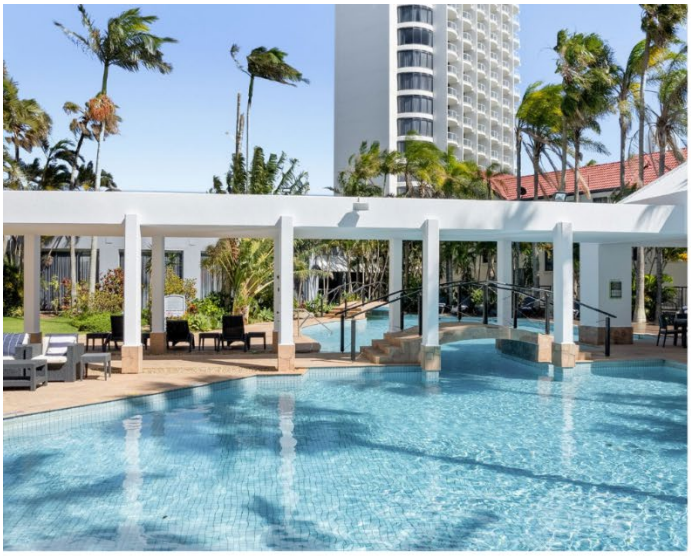
SOCIAL FUNCTIONS

Welcome Reception

Where: Poolside, Crowne Plaza
Time: Tuesday 29th 1800 - 2000

APVW Dinner

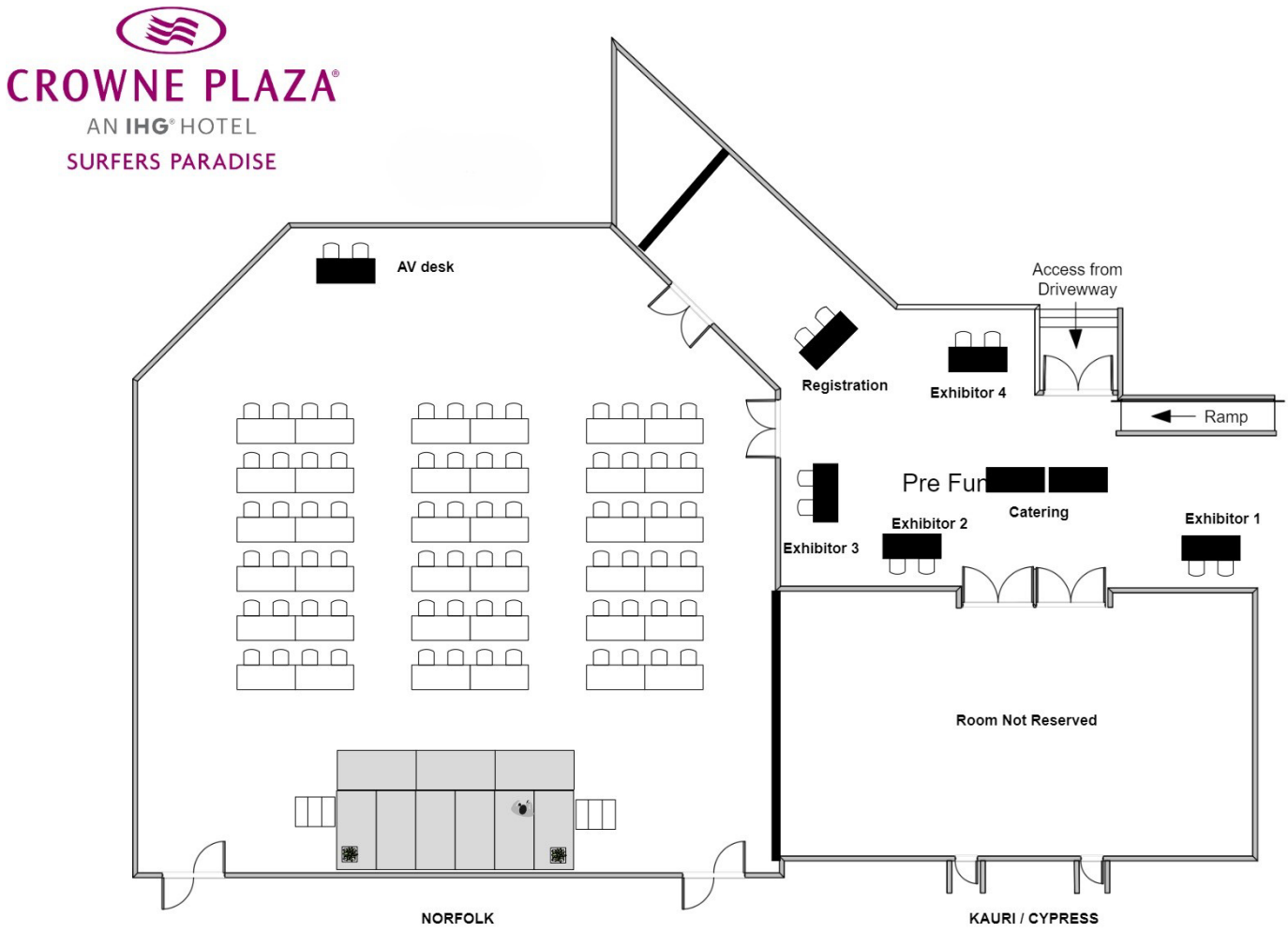
Where: Kurrawa Surf Club, 'Beachside' Old Burleigh Road, Broadbeach
Time: Wednesday 30th 1800 – 2100
Getting There: It is either a 20-minute walk along the beach path or tram from Florida Station to Broadbeach plus a 10 minute walk to the surf club. There is some parking at the Surf Club however it is limited and often full.



VENUE MAP

Norfolk Room

Located on the ground floor is the Norfolk meeting room.



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We manage biosecurity risks to Australia to protect our multi-billion-dollar agricultural industries, our environment and our way of life from exotic pests and diseases. The way we manage biosecurity risks is underpinned by good science, supporting informed decisions and policies. Across Australia, our scientists work hard to identify, assess and respond to plant and animal pests and diseases to keep our industries and country safe.

We employ a diverse range of people from a wide range of scientific disciplines,

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KEYNOTE SPEAKERS

Dr. Marco Incarbone

I obtained my PhD at the IBMP in Strasbourg, France, researching the molecular mechanisms mediating suppression of RNA interference by viruses in plants. After a short transitional postdoc in the Ritzenthaler lab, where I developed new techniques to isolate and characterize virus replication complexes, I moved to the Gregor Mendel Institute in Vienna, Austria, as a post-doctoral fellow. Here I joined the Mittelsten Scheid lab to investigate how plant stem cells and germlines efficiently prevent virus proliferation. These potent antiviral barriers are of crucial biological importance yet remains very poorly understood. I have recently established my lab at the Max Planck Institute of Molecular Plant Physiology in Potsdam, Germany, to broaden the study of stem cell and germline antiviral immunity

Dr. Véronique Brault

After two years as Deputy Director, Véronique Brault was appointed Director of the Joint Research Unit for Grapevine Health and Wine Quality at INRAE Colmar on 1 January 2020. As a researcher specialised in plant virology, she has been studying the molecular mechanisms of virus transmission by aphids for 27 years, cementing her status as a true expert in this field.

Professor Eddie Holmes

Edward Holmes is a world-leading evolutionary biologist whose research focuses on mechanisms for virus evolution and the processes by which RNA viruses jump species boundaries and spread in new hosts. He studies the emergence and molecular epidemiology of pathogens including hepatitis C, avian influenza and dengue fever. Holmes was elected a Fellow of the Australian Academy of Science in 2015 and of the Royal Society, London, in 2017.

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ABSTRACTS

Session 1

Tomato spotted wilt virus resistance breaking strains in Australia

Paul Campbell[1], Monica Kehoe[2], Craig Webster[2], Cherie Gambley[3], Denis Persley[1], Murray Sharman[1]

1. Department of Agriculture and Fisheries, Dutton Park, QLD, Australia

2. Department of Primary Industries and Regional Development, Perth, WA, Australia

3. University of Queensland, St Lucia, Queensland, Australia

Resistance to tomato spotted wilt virus (TSWV) in commercial tomatoes and capsicums are controlled by two single dominant genes, *Sw5b* and *Tsw*. The *Sw5b* was identified in *Solanum peruvianum* and has been introgressed into tomato lines for high level resistance against TSWV and other tospoviruses. TSWV resistant capsicum lines have the *Tsw* gene originally from *Capsicum chinense* 'PI' accessions. Resistance is displayed as a hypersensitive response, which prevents systemic spread of the virus. There has been the emergence of resistance-breaking strains of TSWV, both worldwide and occasionally in Australia. The *Sw5b* resistance-breaking strains exhibiting a conserved mutation in the *NsM* gene, the viral movement protein. Resistance-breaking of the *Tsw* gene has been identified as changes in the *NsS* gene (silencing suppressor), but no consensus has been reached on what changes contribute to the phenotype. We have sequenced TSWV isolates from across Australia from a variety of crops and alternative hosts, collected over the last 40 years with a number of these isolates well-characterised for *Tsw* resistance-breaking. We will present the current situation of resistance-breaking isolates in Australia.

Exploring the source of TYLCV resistance in *Nicotiana benthamiana*: a potential novel source of resistance for combatting a major disease in the field

Satomi Hayashi[1, 2], Jacqueline M Souvan[1], Julia Bally[1, 2], Felipe F Fenselau de Felippes[1], Peter M Waterhouse[1, 2]

1. Centre for Agriculture and the Bioeconomy, Queensland University of Technology, Brisbane, Queensland, Australia

2. Australian Research Council Centre of Excellence for Plant Success in Nature and Agriculture, Queensland University of Technology, Brisbane, Queensland, Australia

Tomato Yellow Leaf Curl Virus (TYLCV) poses a significant threat to tomato cultivation worldwide, capable of causing complete crop failure. The use of resistance varieties harbouring resistance gene (*Ty*) introgressed from wild-tomatoes has been most successful. However, with only a few resistance sources currently utilised in the field, they are in great danger of the rise of resistance-breaking virus strains. Therefore, identification of a new source of resistance that can be incorporated into the tomato breeding program is of high importance.

Here we investigated the nature and source of TYLCV resistance in the wild isolate of *Nicotiana benthamiana*, an Australian native plant closely related to tomato. The Queensland (QLD) isolate of *N. benthamiana* is near immune to TYLCV infection, whereas the LAB isolate; the isolate used in research around the world, is highly susceptible. We identified the orthologs of *Ty* genes in *N. benthamiana* and study their role in TYLCV resistance. Furthermore, mode of inheritance for the resistant phenotype was investigated using a population arising from the cross between LAB and QLD. Our results indicated that the resistance gene in QLD is single dominant in nature, and is different from previously described TYLCV resistance gene from tomatoes.

Pea seed-borne mosaic virus genomes, reveals distinct lentil strain phylogroup; Biosecurity implications?

Solomon Maina[1], Joop van Leur[2]

1. NSW DPI, Menangle, NSW, Australia

2. NSW DPIRD-Tamworth Agricultural Institute, Tamworth, NSW, Australia

High seed infection levels of pea seed-borne mosaic virus (PSbMV) have been reported in commercial seed lots in Australia. Sowing PSbMV infected seed stocks will result in multiple sources of inoculum within a crop early in the season. PSbMV can potentially spread rapidly from these initial sources as it is transmitted by multiple aphid species in a non-persistent manner. Lentils are vulnerable to PSbMV infection, but no seed infection has been found so far in commercial Australian lentil seed lots. PSbMV isolates are classified into four pathotypes (P1-P4) based on their reaction to a set of pea lines differing in resistance genes. Lentil seed-borne strains have been identified overseas and found to belong to the P2 pathotype. The lentil seed-borne strains are a major biosecurity threat to the Australian lentil industry. Previous studies on lentil seed-borne PSbMV strains have focussed on coat protein genes (CP). To understand differences between PSbMV strains isolated from pea and lentil seeds, we examined the full genomes of five PSbMV strains isolated from lentil seed kept at the Australian Grains Genebank (accessions originating from Ethiopia and Greece). Pathogenicity tests of the five isolates showed them to belong to the P2 pathotype. The results were compared to genome sequences of PSbMV strains available in GenBank. A distinct phylogenetic grouping consisting of these two of the new five full genomes together with the L2 type strain, originally isolated in 1977 from a Greek accession held in the USDA gene bank. This lentil phylogroup, revealed a 20% genetic difference with strains derived from peas. Further, their CP sequences were compared with other PSbMV CP sequences published in GenBank. The CP sequences of the new isolates grouped with CP sequences of strains isolated from Ethiopian lentil fields. The study forms part of the grains biosecurity research effort, to safeguard commercial Australian lentil industry.

Redefining virus ecology and evolution using metagenomics

Edward C Holmes[1]

1. The University of Sydney, St Ives, NSW, Australia

RNA viruses are diverse components of global ecosystems. Bulk RNA shotgun sequencing – metatranscriptomics – has transformed our understanding of the virosphere, providing a uniquely powerful means to describe the viral composition of any sample, and helping to reveal how viruses move across the human-animal interface and eventually emerge as new infectious diseases. However, the metagenomic identification of RNA viruses has traditionally been limited to those with sequence similarity to known viruses, such that highly divergent viruses that comprise the “dark matter” of the virosphere remain challenging to detect. Herein, I will show how metatranscriptomics, combined with advances in artificial intelligence (AI) technology that can integrate primary sequence and structural information to accurately and efficiently detect viral sequences, is providing new insights into fundamental aspects of virus evolution, ecology and emergence. I will use metatranscriptomics to identify the fundamental drivers of virus diversity and evolution at the scale of individual ecosystems, revealing the impact of host barriers to cross-species virus transmission. I will also show how a combination of metatranscriptomics and AI has led to the discovery of tens of thousands of novel RNA viruses, redefining our knowledge of the scale and composition of the virosphere. The RNA viruses newly identified were far more divergent than those described previously, and present in diverse ecological niches, including the air, hot springs and hydrothermal vents, varying dramatically in abundance between ecological types.

The untold history of banana bunchy top disease

Andrew DW Geering[1]

1. The University of Queensland, Dutton Park, QUEENSLAND, Australia

Nearly a century ago, a small laboratory and glasshouse just 30 kilometres south of where we meet today was the site of a research project that saved the Australian banana industry from almost certain destruction. The pathogen wreaking havoc was banana bunchy top virus (BBTV), and the team tasked with finding a solution for the disease consisted of Professor Ernest Goddard, Charles Magee and Henry Collard (the Bunchy Top Investigation Committee). In just 2 years, this small team of researchers uncovered the essential features of the epidemiology of the disease at a time when there was still conjecture as to whether viruses were really pathogens or just mishaps in the metabolism of the plant. This new knowledge gained by the research team allowed a very effective disease management program to be devised, which successfully rehabilitated the banana industry.

Most modern histories of banana bunchy top disease follow the written accounts of Magee, but he only provided a very narrow perspective. Bunchy top disease featured prominently in the local newspapers of this area such as the *Tweed Daily*, and these newspaper articles tell a very different story than that recounted by Magee. In this talk I will relive the history of bunchy top disease from the year it was discovered until the date of release of the final report of the Bunchy Top Investigation Committee. It is a story of human tragedy, unrecognised heroes, interstate rivalry, and egotistical scientists.

Reference

1. Geering ADW (2024) The untold history of banana bunchy top disease. Historical Records of Australian Science (in press).

Is phytoplasma titre correlated with symptom development in Banana Wilt Associated Phytoplasma disease in Papua New Guinea?

Mignon de Jager[1], Kathy Crew[1], Gou B Rauka[2], Aloma B Motamota[3], Richard Davis[4], Cecilia O'Dwyer[1], Andre Drenth[1], Lilia C Carvalhais[1]

1. Centre for Horticulture Science, Queensland Alliance for Agriculture and Food Innovation, Dutton Park, Queensland, Australia

2. Department of Agriculture, National Agriculture Research Institute, Lae, Morobe, Papua New Guinea

3. Department of Agriculture, The Papua New Guinea University of Technology, Lae, Morobe, Papua New Guinea

4. Department of Agriculture, Fisheries, and Forestry, Science and Surveillance Group, Biosecurity Plant Division, Cairns, Queensland, Australia

Banana wilt associated phytoplasma (BWAP) is associated with lethal yellowing in banana in Papua New Guinea (PNG) and the Solomon Islands. The banana wilt associated phytoplasma is also associated with Bogia coconut syndrome (BCS) in coconuts and lethal yellowing in other palms. Although the disease was reported in the early 1990s, the pathogenicity of the phytoplasma in the host has not been investigated. Most research regarding pathogenicity has traditionally focused on phytoplasma's that cause growth and flower abnormalities. Phytoplasmas are hypothesised to initially exist as endophytes and only become pathogenic once the titre in the host reaches a certain threshold. This has been investigated in crops such as grapevine, plum and apple, however this has not been investigated for BWAP. We sought to determine the relationship between symptom development and phytoplasma titre in banana hosts. Hence, we tested the hypothesis that BWAP titre is correlated with symptom severity in banana. Plants with BWAP symptoms were collected and the presence of phytoplasma confirmed using PCR based diagnostics. All banana plants were individually scored for symptom severity using a scale we developed to score disease severity from 0-3 based on presence and prevalence of certain visual symptoms. Real time PCR was conducted to obtain cycle threshold (Ct) values for banana tissues and to estimate phytoplasma titres. Correlation of symptom development to pathogen titre was determined by comparing Ct values of individual plant parts to the same plant part of different plants expressing different stages of disease severity. Asymptomatic plants were also included in the analyses. Our results suggest that BWAP titre

in banana hosts is correlated with disease progression. Future research will focus on identifying pathogenicity genes of the BWAP and the level of the expression thereof at different stages of disease development.

Updating diagnostics for graft-transmissible citrus pathogens

Grant Chambers[1, 2], Anna Englezou[1], Andrew Geering[2], Adrian Dando[1], Daniel Bogema[1], Nitin Mantri[3], Monica Kehoe[4], Tim Herrmann[5], Nerida Donovan[1]

1. Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW, Australia

2. Qld Alliance for Agriculture and Food Innovation, University of Queensland, Dutton Park , Qld, Australia

3. The Pangenomics Lab, RMIT University, Bundoora West, Vic, Australia

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A Hort Innovation funded project (CT21005 – 2022 to 2027) aims to ensure we are using the most specific, sensitive, and efficient detection methods available for endemic and exotic graft-transmissible citrus pathogens. The NSW Department of Primary Industries has an extensive pathogen collection, including endemic accessions in living plants. High throughput sequencing of accessions in the collection has allowed us to identify differences between Australian isolates and those found overseas. Diagnostic assays developed overseas for endemic citrus viruses and viroids do not always reliably detect Australian variants, therefore we have used the genomic information from Australian isolates to improve the specificity of these assays. We analysed citrus viroid VII quasispecies using amplicon sequencing and identified multiple viroid variant profiles in different tissues of the same plant; and all variants are successfully detected using the new diagnostic assay that we developed.

After validation or development of improved diagnostic tests, the recommended assays have been adopted by industry to test the Auscitrus budwood and rootstock seed supply trees, preventing the spread of disease to Australian nurseries and orchards. We have also used these assays to test samples from industry and government national surveys, providing evidence of absence for exotic diseases and knowledge around the prevalence of endemic viruses and viroids. For example, citrus tristeza virus has been detected in more than 60% of samples submitted. New viruses and viroids, not previously reported in Australia, have been detected in survey samples and reported. The discovery of citrus viroid VII prompted research on viroid transmission, distribution, plant responses to infection and co-infection with other viroids. The next conference of the International Organization of Citrus Virologists, planned for March 2025 in Mildura, Australia, will be organised by the project team to enhance scientific and industry engagement.

Comovirus viciae genomes generated from historical isolates – Step 1 in designing new molecular diagnostics

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Comovirus viciae (broad bean stain virus) (genus *Comovirus,* family *Secoviridae*) affects faba bean, lentil, field pea and common vetch, is readily seed-borne and is considered a biosecurity threat to the Australian pulse industry. It has previously been detected in post-entry quarantine screening of lentil and field pea plants using tissue blot immunosorbent assay screening, a serological technique that is impacted by strain variability and is generally less sensitive when compared to molecular methods. Design of molecular diagnostic assays for the fast and accurate screening, detection and identification of viral diseases requires quality genome sequences. There are only two publicly available sequences for BBSV; the full RNA2 polyprotein coding sequence and a 276bp RNA-dependent RNA polymerase partial coding sequence of the RNA1 segment. Transcriptome sequencing of historical *Comovirus viciae* (Broad bean stain virus) isolates has generated 30 full coding sequence genomes that include both RNA segments. These genomes will be lodged into the grains viral sequence database established

as part of the GRDC-funded National Grain Diagnostics and Surveillance Initiative, and will be used for future development of a specific molecular assay for screening of imported pulse seeds.

Johnsongrass mosaic virus: resistance of maize in Australian and implications for preparedness for maize lethal necrosis

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Johnson grass mosaic virus (JGMV) is widely distributed in the major perennial host Johnson grass (Sorghum halepense) in southern and central Queensland and north-west New South Wales.

Two strains of this aphid-transmitted potyvirus occur in Australia, the type strain (JGMV-JG) and the Krish -infecting strain (JGMV-K) which is able to infect grain sorghum hybrids/lines with the Krish resistance gene.

Although the economic impact of JGMV is currently low, the virus has caused sporadic severe losses in sweet corn, maize and grain sorghum over 30 years. A secondary threat is posed by the interaction of mixed infections of JGMV (and other maize-infecting potyviruses) and the exotic maize chlorotic mottle virus (MCMV) which can lead to maize lethal necrosis (MLN). Severe losses due to MLN have been reported from eastern and central Africa, Ecuador and China.

We used glasshouse and field studies to determine JGMV resistance status of commercially grown maize and sorghum. We used high pressure application of JGMV inoculum as a rapid and effective means for inoculating field plots. All ten commercial maize hybrids tested were resistant to both JGMV strains as were ten of 15 sweet corn hybrids. The resistance status of sweet corn and maize hybrids to JGMV was well correlated with published resistance levels to the related exotic maize dwarf mosaic virus (MDMV). All 15 sorghum hybrids were susceptible to JGMV-K while 10 of these hybrids were resistant to the type strain, suggesting the presence of the Krish resistance gene in their pedigree. We developed rapid LAMP assays for JGMV and MDMV to assist with monitoring for the exotic.

The high resistance of maize hybrids to JGMV determined in this study provides confidence of a high level of protection against maize lethal necrosis disease in the event of an incursion of maize chlorotic mottle virus into Australia.

Early detection of viruses and viroids in imported *Citrus* germplasm undergoing post-entry quarantine using a small RNA Sequencing (sRNASeq) workflow

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Every *Citrus* germplasm imported into Australia undergoes a minimum quarantine period of 24 months at the Department of Agriculture, Fisheries and Forestry's Post Entry Quarantine (PEQ) facility at Mickleham, Victoria. Upon arrival, material is propagated to form index/mother plants which are tested for a range of pathogens during either active growth or late stages of active growth. Viruses and viroids comprise most pathogen targets in *Citrus*, and are screened by visual inspection, ELISA, PCR and woody indexing. Currently, all *Citrus* imports must also undergo shoot tip grafting (STG), which is a technique used for disease elimination to ensure freedom from graft-transmissible diseases caused by viroids, viruses, phytoplasmas, phloem-limited bacteria, and diseases of unknown aetiology. The establishment of mother plants and, particularly, woody indexing and STG processes are time and labour intensive, often resulting in delayed market access to new citrus germplasm. Recently, sRNAseq was adopted as a primary screening tool for viral diagnostics in *Prunus*, *Rubus*, *Fragaria* and clonal grasses. The feasibility of utilising sRNAseq to detect viruses and viroids in imported *Citrus* budwood upon arrival was tested over two consecutive years. Cambium and bud samples taken from dormant budwood on-arrival, and cambium and leaf samples taken post-establishment during active growth, were screened through the PEQ sRNASeq workflow. Our results show equivalent and, in some instances, better sensitivity using sRNASeq in comparison to

existing methods for detection of viruses and viroids in *Citrus* plants. The use of sRNASeq has promising potential to streamline the *Citrus* testing workflow at the PEQ through reliable early detection, enabling selection of clean material for propagation and informing the downstream workflow.

Distribution of Begomovirus in Papua New Guinea (PNG)

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Begomovirus is a genus of plant viruses that threatens root and vegetable crop production in regions all around the world. In 2016, it was first detected in PNG on weeds during a joint surveillance in Sandaun Province. Over the years, real-time PCR has confirmed Begomoviruses in East Sepik, Central, Western Highlands, Madang, Manus and New Ireland Provinces. Detected begomoviruses were more prevalent on various weed hosts than root or crop vegetables. Sequence analysis and percentage identity from GenBank has identified two complete species including Sweet potato leaf curl virus (SPLCV) and Ageratum yellow vein virus (AYVV) on I. *batatus,* A. *conyzoides* and *Stachytarpheta* sp. Other novel and as-yet not fully characterized begomoviruses are present in tomatoes, pumpkin and non-crop hosts.

Virus strains of these species have been recorded between 2016 to 2023.The discovery of this virus occurrences in certain regions in PNG has potentially indicated the dissemination of these viruses to other areas of the country and draws the opportunity of detecting new Begomovirus species and or its' strains. Additionally, the negative impact of climatic change on virus transmission has demonstrated synergies between these pathogenic viruses and its' transmission agents. Whitefly transmitted geminiviruses-emerged in recent years as a major new threat to tropical crop production. With attentive surveillance and monitoring activities, more work needs to be directed to identifying begomovirus vectors and correspondingly update presence of new occurring species and their potential crop hosts.

Viruses associated with Agaricus mushrooms in Australia

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Two virus associated diseases are known to affect mushroom (*Agaricus bisporus*) production – La France disease and Mushroom Viris X disease (MVX). La France disease is characterized by bare patches on production beds, small sized mushroom caps, premature veil opening, and elongated and discoloured stipes. It is associated with Agaricus bisporus virus 1 (AbV1). Mushroom bacilliform virus (MBV) has also been reported from La France affected mushrooms, but it is not believed to contribute to the disease. Some of symptoms of La France disease are also observed in MVX affected mushrooms. Cap-browning distinguishes MVX from La France disease. MVX is associated with a complex of eighteen viruses. The bare patch symptoms in MVX are primarily associated with Agaricus bisporus virus 6 (AbV6) and the brown cap symptoms are correlated with Agaricus bisporus virus 16 (AbV16). The viruses are transmitted via hyphal fusion (anastomosis) between infected and uninfected mycelia or through infected basidiospores which can persist throughout the production facility.

There have been occasional outbreaks of the La France disease in Australia since 1969 and both AbV1 and MBV were detected. MVX has not been recorded. Between 2019 to 2024, 298 Australian mushroom samples with symptoms such as distortion and cap browning, were submitted to Crop Heath Services to test for viruses associated with La France disease and MVX: 89/298 of the samples tested positive for AbV6, 179/298 tested positive for MBV, and 82/298 samples tested positive for both. Neither AbV1 nor AbV16 were detected. This has led to speculation that a unique virus complex might be associated with mushroom virus diseases in Australia.

This project aims to better understand the risk of novel and known viruses associated with mushroom diseases. Diagnostic and management methods for these viruses will be developed. Early results of the study will be presented.

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The use of high-throughput sequencing for plant virus diagnostics at the New Zealand Ministry for Primary Industries

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The power of high-throughput sequencing (HTS) for virus diagnostics has been realised and is becoming a routine diagnostic tool worldwide. The Ministry for Primary Industries (MPI) Plant Health and Environment Laboratory have implemented two HTS technologies for different plant virus testing scenarios. The first is Oxford Nanopore Technologies (ONT) MinION for the detection of viruses in symptomatic plants. Examples of how ONT sequencing has greatly improved generic detection of viruses at MPI, such as mixed infections and new host associations, will be presented. The second utilises the higher sequencing depth of Illumina NovaSeq to determine whether plants in post-entry quarantine are free from specific viruses. Work to establish the minimum number of reads required to match the sensitivity of qPCR will be presented. The Illumina NovaSeq HTS procedure is now an accepted test method on the MPI import health standard for several commodities.

MicroRNA-induced gene silencing (MIGS): the tool of choice for silencing viral genes and to confer multi-virus protection in plants.

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RNA interference (RNAi), or RNA silencing, is an important mechanism of gene regulation and has a central role in protecting plants against virus infection. RNAi is mediated by small RNAs (sRNAs), which guide the multiprotein RNA-induced silencing complex (RISC) to recognise and, most often, cleave complementary RNA transcripts, leading to their degradation. Given its role in viral defence and the availability of several established methods to generate sRNAs against specific targets, RNAi has been successfully shown to provide plants with protection against virus infection. Viruses, however, are extremely flexible regarding changes to their genomic sequence and can rapidly accumulate mutations to avoid being targeted by sRNAs, resulting in loss of resistance. This limitation on the use of RNAi to confer protection against viruses can be minimized by targeting multiple sequences within the virus genome. In this scenario, mutations in the targeted sequences would have to occur simultaneously for the virus to “escape” the RNAi response. Unfortunately, the ability to target several genes simultaneously for silencing using the most popular methods to induce RNAi (such as artificial microRNAs or hairpin RNAs) is limited and/or time-consuming. MicroRNA-induced gene silencing (MIGS) is an efficient, yet, still poorly explored method to trigger RNAi in plants and stands out for its potential to trigger silencing of several sequences at the same time. Here we show that MIGS constructs targeting two or more viral sequences have a more severe impact on the virus replication, conferring better protection against the infection. We also show that the multi-targeting ability of a single MIGS molecule can be successfully used to confer protection to more than one virus, extending the scale in which RNAi can be used for plant protection.

Development of mild cross protecting strains of citrus tristeza virus through infectious clone assembly

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Citrus tristeza virus is a pathogen of citrus that is predicted to have caused the loss of over 100 million trees worldwide. While the CTV disease phenotype termed quick decline is now largely under control due to the use of resistant rootstocks, the phenotype termed stem pitting prevails and continues to affect yield and the productive life of citrus trees. CTV is a member of the complex closteroviridae family containing a large ~19.3 kB single stranded RNA genome. Being phloem limited, CTV is transmitted in the field by a number of aphid vectors and is readily transmitted during citrus propagation by grafting of infected budwood. In addition to a complex genome, multiple genotypes of CTV are known to exist, that can accumulate over time in infected citrus. The complex nature of field infections has made it difficult to link pathotype to individual genotypes, thus sequence signatures for disease causing variants are not known. One proven approach to protect citrus from damaging CTV strains is through cross protection, where mild isolates are purposely used to pre-infect citrus to prevent or delay severe disease. This phenomenon which is commercially exploited to protect grapefruit in Australia from severe stem pitting is only beginning to be understood, made possibly by advances in high throughput sequencing and assembly of CTV infectious clones. In this Hort Innovation sponsored project, we aim to develop our understanding of viral cross protection with a focus on developing or identifying mild CTV isolates that can protect against stem pitting in orange, which is thus far restricted in Australia to Queensland. For this, we will utilise the model plant *Nicotiana benthamiana* as an experimental host to fast track the detection or engineering of mild cross protective CTV variants for application in citrus.

Tile Amplicon Sequencing: A Comprehensive Approach for Potato Virus Y (PVY) Strain Detection and Characterization

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Potato virus Y (PVY) is a significant threat to potato cultivation worldwide, causing substantial economic losses. Traditional diagnostic methods for PVY often suffer from limitations in strain resolution, hindering effective surveillance and management strategies. Here, we present tile amplicon sequencing (TAS) as an innovative technique for the comprehensive detection and characterization of PVY strains.

TAS presents a significant advancement in the field of potato virus Y (PVY) diagnostics. By leveraging Oxford Nanopore technology and a comprehensive primer scheme, this technique allows for high-resolution sequencing of PVY viral genomes. One of its key advantages lies in the ability to sequence the entire viral genome and enabling thorough amplification and subsequent phylogenetic analysis to understand the evolutionary relationships between PVY strains.

Another advantage is the capacity to detect low-titre infections or viral strains that other methods might miss. Unlike conventional approaches to high throughput sequencing that often require ribosomal depletion, tile amplicon sequencing bypasses this step, simplifying the process and potentially reducing costs.

In conclusion, the data generated through TAS plays a crucial role in PVY surveillance efforts by providing detailed insights into the genetic diversity and distribution of PVY strains. This technique facilitates the development of more effective management strategies and thus improving biosecurity. Ultimately, the implementation of TAS is enhancing our understanding of PVY epidemiology and improving disease control measures in potato cultivation.

Tomato Brown Rugose Fruit Virus detection and emergency response in South Australia

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Tomato Brown Rugose Fruit Virus (ToBRFV) was detected in SA in August 2024, and an emergency response was initiated. As of writing, the emergency response is still underway with extensive tracing and surveillance taking place to determine the extent of the disease. The virus was found in the Northern Adelaide Plains area, a large hub for protected cropping and horticulture production.

ToBRFV is a tobamovirus which was first identified in Jordan in 2015 and has spread rapidly through Europe and the Americas. ToBRFV's main crop hosts are tomato, peppers (capsicum) and possibly eggplant; the main symptoms are mottling of leaves with browning of the edges and discolouration of fruit. The virus also can infect a number of weed species, including common weeds such as deadly nightshade. The virus is mechanically transmitted and has a very long persistence on hands, gloves (up to 2 hours), cutting implements, equipment and surfaces (up to 9 months). The virus is very hardy, being resistant to many disinfectants and with an inactivation temperature of 90 degrees Celsius. Research overseas is ongoing for remediation and treatment of the virus, however re-infection of previously infected premises is commonly reported. This talk will give an update on the situation of ToBRFV in SA, and discuss some of the issues encountered in the response.

INVESTIGATING THE ANTIVIRAL DEFENSES PROTECTING PLANT STEM CELLS AND GERMLINE FROM INFECTION

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Stem cells are essential for the development and reproduction of multicellular organisms, so their infection by pathogenic viruses must be prevented. In plants, a small group of stem cells harbored within the shoot apical meristem (SAM) generates all postembryonic above-ground tissues, including the germline cells. Many viruses do not proliferate in these cells, yet the molecular bases of this exclusion remain only partially understood. In addition, stem cell antiviral immunity is believed to contribute to the exclusion of many viruses from the plant germline and prevention of vertical transmission of infection from parent to progeny. We show that a plant-encoded RNA-dependent RNA polymerase, after activation by the plant hormone salicylic acid, amplifies antiviral RNAi in infected tissues. This provides stem cells with RNA-based virus sequence information, which prevents virus proliferation. Furthermore, we find RNAi to be necessary for stem cell exclusion of several unrelated RNA viruses, despite their ability to efficiently suppress RNAi in the rest of the plant. In parallel to this work we developed cutting edge live microscopy techniques to track virus movement in plant reproductive organs, which will allow unparalleled analysis of infection dynamics. We are currently investigating how virus vertical transmission through the germline is prevented in plants, which ensure many infections are not transmitted vertically from parent to offspring.

Lettuce necrotic yellows virus subgroups elicit different responses in the model host *Nicotiana glutinosa*.

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Lettuce necrotic yellows virus (LNYV) is endemic to Australia and New Zealand, with populations in both countries made up of two subgroups (SI and SII). Phylogenetic analyses have indicated that SII emerged more recently and has dispersed more rapidly than SI, to the point where SI appears to have become extinct in Australia. While symptoms don't differentiate infection by these subgroups, we hypothesise that SII impacts the host at the molecular level to favour its dispersal. We have used RT-qPCR, metabolome, and transcriptome analyses to identify molecular pathways of interest at 28 dpi. Initial data indicate common responses to infection by the LNYV subgroups as well as subgroup specific responses, supporting our hypothesis. RT-qPCR data showed increased accumulation of *RDR1*, *AOX1a*, and *NPR3* mRNAs in response to each subgroup, albeit at different levels. This suggests the host's response is associated with the salicylic acid pathway, but each subgroup impacts this to differing degrees. Specific metabolites have been identified through analysis of sugar, amino acid, organic acid, and fatty acid profiling. Transcriptome profiling supports these data as well as highlighting subgroup specific gene expression responses. Combined data suggest that SII influences amino acid and energy production to promote infection and, thus, dispersal. The complexities of the pathways involved are currently being analysed, and aspects will be presented. Findings of this study will make important contributions to our understanding of the complicated mechanisms of plant responses to virus infection, particularly infection by a cytorhabdovirus.

Grapevine leafroll-associated virus 3: molecular evolution, biological variation, and host response.

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Grapevine (*Vitis spp.*) is susceptible to ~100 viruses, among which grapevine leafroll-associated virus 3 (GLRaV-3) is the most economically destructive. GLRaV-3 infection is associated with grapevine leafroll disease which causes reduced quantity and quality of yield, necessitating costly management strategies. A potential long-term solution is GLRaV-3 mild strain(s) for cross-protection. GLRaV-3 comprises eight genetic groups associated with functional differences affecting virus-encoded suppression of RNA silencing (VSR) activity, virus load/distribution, and symptom onset/severity. Importantly, GLRaV-3 can infect *Nicotiana benthamiana*, a novel and RNAi-compromised host.

Molecular evolution of GLRaV-3 was explored *in field* with asymptomatic red berried grapevines (n = 15,000) across three growing seasons, and *in vitro* using *N. benthamiana* (n = 8 lines) with GLRaV-3 groups I, I+VI, VI, or VI+X across 10 serial passages over 20 months. *N. benthamiana*-evolved GLRaV-3 genotypes were characterised for p19.7 local VSR activity, *in planta* localisation, and viral/host transcriptomics.

In field candidate grapevines had GLRaV-3 infection and remained visually asymptomatic in each growing season (year 1, n = 21; year 2, n = 9; year 3, n = 3). Candidate mild strains were sequenced for CP, p19.6, p19.7, and p21 VSR genes which revealed conserved non-synonymous mutations in each VSR gene compared to a New Zealand reference isolate. *In vitro N. benthamiana*-evolved GLRaV-3 revealed 78 mutations across GLRaV-3 genotypes, most mutations (83%) were present at passage 10, reflecting adaptive viral evolution of GLRaV-3 to *N. benthamiana*. Compared to the original sequence the evolved GLRaV-3 group X p19.7 VSR activity was significantly reduced, attributed to Y78H (~1.5-fold less), and K103M (~4-fold less) mutations. Predicted *N. benthamiana* host response revealed potential activation of the salicylic acid defence response at passage 0 but no viral-associated response at passage 10.

Within the *in field* and *in vitro* plants, potential GLRaV-3 mild strain(s) exist which could have future application in cross-protection.

CRISPR/Cas9 gene editing towards resistance to *the Banana bunchy top virus* in banana

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The banana (*Musa acuminata*) plant is economically important in South Africa (SA) and a staple food in many developing African countries. The banana bunchy top virus (BBTV) is the most important virus of banana, and there is no natural resistance to the virus in any *Musa* spp. An outbreak of BBTV was reported in KwaZulu Natal in 2015. The *dynamin-related protein 1C* (*drp1C*), a gene associated with virus infection in other plants, was designated a candidate susceptibility gene towards developing resistance to BBTV in banana. RNA-seq data Illumina paired reads (40 million reads) were mapped to the *drp1C* reference gene sequence to assess allelic variation in the Cavendish Williams *drp1C*. Two single guide RNA (sgRNA) spacers within the gene were designed. Furthermore, two sgRNA spacers were designed to target the BBTV replication protein. The sgRNA were tested using an *in vitro* cleavage assay using Cas9 nuclease and CRISPR/Cas9 T-DNA expressing these sgRNAs were constructed. The efficacy of the constructs were evaluated by transient co-expression with target expressing plasmids in *N. benthamiana leaves*. Allelic variation results revealed the presence of two alleles. The designed guides were located in regions with polymorphisms, therefore two sgRNAs were designed to accommodate the polymorphism to knock out all copies the gene. *In vitro* cleavage assays showed the efficacy of the sgRNA to cleave the target. However, no mutations were detected using T7EI and restriction digestion assays in target DNA co-infiltrated with the T-DNAs. The results highlights the importance of ascertaining allelic variations in CRISPR studies especially in polyploid species and the significance of *in vitro* testing of guides. For this study the designed

sgRNAs cleaved the *drp1c* and DNA-R in vitro. Furthermore, the study showed the neccesity of further testing of the CRISPR/Cas9 T-DNAs in banana and not in tobacco. Though mutation was not induced, the study will contribute towards eludicating the BBTV-Banana interactions, CRISPR/Cas9 gene editing techniques and be a foundation on the ongoing development of cultivars resistant to BBTV using CRISPR/cas9 gene editing system.

Talking about a (sexual) revolution in transmission terminology

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In the context of intensive monoculture-based production of crops coupled with the trade of both pollen and seed (regional, national and international), there is an increasing risk (and reality) of rare virus transmission events that can result in new biosecurity incursions. Such events include seed transmission of viruses on seed coats, seed-borne transmission (e.g. within the seed endosperm but not embryo), and pollen transmission. Pollen transmission may be vertical via fertilisation by compatible pollen and virus transmission to the progeny seed or horizontal by compatible or incompatible pollen resulting in infected mothers of future generations of seeds. Crops with long flowering periods may enable horizontal incompatible pollen transmission followed by vertical transmission to seed within the same season. Alternatively, perennial crops may be infected by horizontal transmission and subsequent vertical transmission to their progeny from subsequent years. We discuss these seed and pollen transmission processes, their terminology (including more precise definitions – a revolution?), and what methods might be used to distinguish and manage the different modes of vertical transmission.

Clade divination – phenotypic data facilitates epidemiological inferences from soybean dwarf virus sequence analysis

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Soybean dwarf virus (SbDV; family *Tombusviridae*, genus *Luteovirus*, species *Luteovirus glycinis*), transmitted solely by aphids in a persistent manner, can cause significant losses in cultivated *Fabaceae*. SbDV isolates are categorised into four strains; YP, YS, DP and DS. Yellowing (Y) and dwarfing (D) strains differ in their symptom expression and host range. P strains are transmitted by *Acyrthosiphon pisum* (pea aphid), and S strains are transmitted by *Aulacorthum solani* (foxglove aphid). Genetically, Y and D strains separate into two clades at every genomic region except for the N-terminal region of the read through domain (N-RTD) in which P and S strains separate. SbDV diversity in Australia has yet to be significantly investigated and so complete genome sequences were obtained from 41 isolates infecting cultivated *Fabaceae* in two regions of Australia ('northeast' and 'southwest'). Phylogenetic analyses were conducted on the whole genome and the N-RTD of these sequences together with 50 sequences available on GenBank. At the whole genome level, isolates separated into D and Y clades. At the N-RTD level, with two exceptions, isolates separated into P and S clades. All south-west isolates were in the Y clade, whilst north-east isolates were in both the Y and D clades. All isolates sequenced were in the P group suggesting *Ac. pisum* is the primary vector of SbDV in these regions. Although biological evidence suggests S strain isolates are common in the southeast of Australia, just one previously sequenced Tasmanian isolate was available and was in the Y and S clades. Relevant biological data available for each isolate supported inferences made from phylogenetic clade. This study suggests that at least three of the four SbDV strains are present in Australia and is an example of the importance of continued collection of phenotypic data to provide more power to genetic analyses.

Enhancing DNA and RNA extraction quality for viral detection in horticultural seeds

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Imported seeds must meet Australian Biosecurity inspection and clearance requirements. Rapid, cost-effective diagnoses are needed to ensure imported seeds are free of seed-transmitted pathogens. Our project aims to develop an adaptable and reliable molecular diagnostics system to detect and mitigate the risk of introducing regulated pathogens into Australian horticultural production systems on seed, which is cost-effective and simple to implement. However, seeds are challenging for many molecular studies due to their high content of polyphenols, polysaccharides, and lipids, which can bind to nucleic acids, resulting in low yield and quality. Although there are published protocols claiming better seed nucleic acid extraction methods, none are applicable to high throughput analysis. We are attempting to create total nucleic acid extractions that are high quality, high yield, and high throughput to allow possible HTS analysis for infected seeds. To this end, investigations into the utility of SDS-based buffers were conducted extracting DNA and RNA from cucurbit seeds spiked with Cucumber Green Mottle Mosaic Virus. The improved protocols generated may contribute to rapid and sensitive diagnostics of viruses in horticultural seeds imported into Australia, reducing the risks of disease outbreaks in horticultural systems.

Diversity of *Orthotospovirus impatiensnecromaculae* (impatiens necrotic spot virus; INSV) in Australia

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Orthotospoviruses are among the most economically significant plant pathogens in the world at present. As of 2022, the genus *Orthotospovirus* includes 26 officially recognised species. In Australia, five species were reported to date: *Orthotospovirus tomatomaculae* (tomato spotted wilt virus, TSWV), *Orthotospovirus capsiciflavi* (capsicum chlorosis virus ; CaCV), *Orthotospovirus iridimaculaflavi* (iris yellow spot virus, IYSV), *Orthotospovirus impatiensnecromaculae* (impatiens necrotic spot virus; INSV) and pterostylis blotch virus (PtBV) which was found only in Queensland and described as a provisional species in 2022. INSV was found in NSW in 2010 in Begonia and back then it was reported to be successfully eradicated. In 2018 and 2019 it was detected again in NSW in lettuce. In Victoria it has never been reported so far. Between 2021-2023, during surveillance work for *Orthotospoviruses* and thrips, INSV was detected in ornamental plants in three different nurseries in Victoria and in one nursery in NSW, which is located close to the location where the first INSV was reported in 2010. The presence of this virus poses a high risk to the nursery industry, as the existence of its vectors (western flower thrips and onion thrips) and its host plants could lead to a wider spreading of the virus, and a negative cumulative effect when found in a co-infection with TSWV. The following work describes the diversity of INSV in the nursery sector in Victoria. A comparative phylogenetic analysis of the full genomes of INSV isolates found in lettuces and ornamental plants in Australia has been undertaken, examining the sequence variability of each of the short (s), medium (m), and long (l) genome segment sequences. The initial results suggests that there were four different incursions of INSV into Australia. An in-depth analysis of INSV diversity will be presented.

Uncovering the diversity of viruses infecting leafy vegetables in South Africa, and their impact on the fresh weight of the leaves

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Smallholder and emerging farmers of South Africa depends on leafy vegetables such as Swiss chard (Beta vulgaris), Brassica species (mustard spinach and rape), beet greens (Beta vulgaris and Amaranthus species for household consumption and income generation. Viruses belonging to the *Luteoviridae* and *Potyviridae* etc. were reported to infect these vegetables in other countries. However, their prevalence and impact is not known in South Africa. The study was aimed at identifying and genetically characterizing viruses affecting leafy vegetables production in the Gauteng Province of South Africa and to determine their impact on fresh and dry weight of Swiss chards and mustard spinach (*Brassica rapa*).

Samples were collected in Gauteng Province of South Africa (City of Tshwane, Randfontein and Germiston) using a W-pattern method of sampling, and symptomatic and non-symptomatic samples were collected. Samples were analyzed using reverse transcription polymerase chain reaction (RT-PCR), cloning and sequencing part of the genomes and the use of High Throughput sequencing to generate complete genome sequences of viruses detected. Effects of viruses on the fresh weight was determined by planting glasshouse trials with Swiss chard and mustard spinach and mechanically inoculate plants with virus treatments. Symptoms induced by virus treatments post inoculation was recorded and data (fresh weight) was collected and analyzed using SAS statistical software (SAS, 2014).

Molecular detection methods detected and confirmed viruses such as Turnip mosaic virus (TuMV), Beet mosaic virus (BtMV), Beet western yellows virus (BWYV), Beet chlorosis virus (BChV) etc. in samples collected. The fresh

weight of the virus treated mustard spinach was affected and significant difference was observed in trial 1. The fresh weight of Swiss chard was moderately affected by the virus treatment.

Viruses were detected and confirmed to reduce fresh weight of leafy vegetables, which indirectly affect the yield. These findings contributes to the development and implementation of control strategies, crucial for sustainable leafy vegetable production in the country.

Prevalence and incidence of lentil viruses in Nepal

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As one of the world's largest lentil producers, Nepal's lentil industry faces significant threats from biotic and abiotic factors. Fungal diseases are well known however the impact of viral diseases on lentil production is less clear. A comprehensive survey was conducted in 21 districts from six provinces and all agroecological regions of Nepal during the 2023-24 lentil growing season to document the presence of lentil viruses. Disease assessments were done in 94 fields using standard survey protocols. A 4911 random and 1785 systematic plant samples were collected and tested for the presence of viruses using tissue-blot immuneassay (TBIA); a battery of polyclonal (PAb) and monoclonal (MAb) antibodies were used. TBIA results showed that the most prevalent viruses affecting lentil were *Pea seed-borne mosaic virus* (*PSbMV*) (overall incidence in random samples was 12.24%) and luteoviruses (4.95%) that reacted with the broad-spectrum luteovirid MAb (5G4) and other MAbs produced against viruses belong to the genus *Polerovirus*, family *Solemoviridae*. This indicates that there are more luteoviral species in Nepal, which need to be verified by further molecular testing. Other viruses such as *Alfalfa mosaic virus* and *Cucumber mosaic virus* were detected at low incidence (less than 1%). All tested samples were negative to *Faba bean necrotic yellows virus, Chickpea chlorotic dwarf virus, Pea enation mosaic virus* and *Broad bean stain virus*. The high incidence of *PSbMV* highlights the necessity for seed testing in the lentil seed certification system. The widespread occurrence of viruses in lentils in Nepal has the potential to cause serious yield losses. The results of this survey will provide the basic information to support Nepal's lentil breeding program in developing virus resistant varieties and guide Nepal's seed certification authority in the enforcement of testing for seed-borne viruses in lentil.

Investigating the correlation between cucumber mosaic virus (CMV) titre in narrow-leafed lupin tissues and seed transmission rates

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Cucumber mosaic virus (CMV) is one of the most important diseases that can affect narrow-leafed lupin causing yield losses of up to 90%. In crops, the primary source of CMV infection is through infected seed with secondary spread facilitated by aphids (Jones & Congdon, 2023).

Consequently, it is crucial for growers to utilize CMV-free seed stocks. Although breeding companies are striving to develop lupin lines resistant to CMV seed transmission, they face substantial challenges. One major hurdle is the lengthy period from sowing to testing, which can result in the costly maintenance of susceptible lines. Developing methods for predicting the seed transmission rate prior to harvest would be advantageous to this process.

This study investigates the correlation between the CMV titre in various tissues of the narrow-leafed lupin and its rate of seed transmission. By identifying early predictors of transmission rates, this research aims to support breeding companies in efficiently selecting resistant lines, thus reducing the costs and time associated with bringing CMV resistant cultivars to growers.

Reference

Jones, R. A. C., & Congdon, B. S. (2023). Australian Cool-Season Pulse Seed-Borne Virus Research: 1. Alfalfa and Cucumber Mosaic Viruses and Less Important Viruses. *Viruses*. https://doi.org/10.3390

Direct testing of potato tubers for plant viruses and implementation in a seed potato testing scheme

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Seed potato testing schemes ensure the production of clean propagation material for use in ware and processing potato production, and their implementation ensures losses from plant viruses are minimized. Traditionally testing tubers has required cores of the tuber be treated with gibberellic acid and planted before testing the sprouts, which can take up-to 6 weeks to grow and be available for testing.

In contrast a method to directly test potato tubers for plant viruses offers several advantages over traditional grow out tests of tubers to screen tubers including speed, reduced labour and space requirements. Results can be returned to growers within a week, and larger numbers of samples can be tested as there is no requirement for glasshouse space for the grow out. Nucleic acid extractions of tubers can be pooled and tested by real-time PCR, which together reduces costs to growers and ensure accurate detection of any viruses which are present.

Such tests are also of great benefit during tracing and eradication efforts during incidents arising from novel virus detections. Rapid testing of potato tubers streamlines efforts to trace additional virus locations and can also allow producers to maintain market access by being able to demonstrate disease freedom. Where previously serological methods were used, the use of molecular methods is more flexible for further downstream testing, such as whole genome sequencing or additional surveillance.

The development and verification of a direct tuber testing method, and its implementation in a quality management system will also be presented.

A system for evaluation of transgenic turnip mosaic virus resistance in *Brassica napus* plants

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The disease caused by turnip mosaic virus (TuMV) infection in canola crops constitutes a persistent problem worldwide, reducing seed yield by up to 64%. RNA technology and RNA targeting CRISPR/Cas systems provide an attractive approach for introducing transgenic TuMV resistance into canola. To achieve this, a system for evaluation of transgenic TuMV resistance is required. Isolate 12.1 of TuMV's resistance breaking strain from Australia was recovered from silica gel desiccated leaf material and subcultured by sap inoculation to Chinese cabbage plants. When plants of two canola cultivars were germinated from seeds or regenerated *in vitro* both became systemically infected with isolate 12.1 at similar infection rates. Also, when isolate 12.1 inoculum was diluted in tenfold steps (10^{-1} to 10^{-6}), all plants that became infected showed similar patterns of systemic mosaic symptom development. At 20 days post inoculation, all inoculated plants developed symptoms following inoculation with 10^{-1} diluted infective sap preparations. The plant numbers infected declined with increasing inoculum dilution none becoming infected with a dilution above 10^{-4}. Days to first symptom appearance were fewer with the 10^{-1} inoculum (8-10 days) compared to those from the 10^{-3} and 10^{-4} inocula (12- 15 days). In addition, the relative virus titres from transformed ELISA A_{405} values measured virus concentration. In conclusion, the virus inoculation system devised here was suitable for use in the assessment of transgenic TuMV resistance in genetically modified canola plants. It consisted of: 1) extract sap from infected leaves of TuMV culture plants; 2. prepare a 10^{-1} sap extract dilution with inoculation buffer; 3. inoculate transgenic and non-transgenic canola plants at the 4-5 leaf stage, and 4. record days to first appearance of systemic symptoms, symptom severity and virus concentration in both types of canola plants, and whether any transgenic plants remain uninfected.

Polerovirus, host plants and aphid vector: in the secret of a “ménage à trois”

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There are many ways to study the mechanisms sustaining virus transmission by vectors. One can first study interactions in pairs, i.e., virus-plant, virus-vector or plant-vector interactions, before moving on to a more complex analysis of the tripartite interactions, by considering all three partners simultaneously. We have followed this progression during the last 30 years on the pathosystem consisting of poleroviruses, their host plants and aphids, as virus vectors. We have identified viral determinants required for polerovirus transmission, shown the role of phloem proteins in virus uptake, demonstrated the implication of proteins as virus receptors in the aphids. Finally, in recent years we have been studying viral manipulation, a fabulous coevolutionary process inducing plants and aphid modifications in order to promote polerovirus transmission. In the presentation, I will present you this long evolution that has led us to discover the intimate relationships between the three partners.

Transmission and host range of yellow dwarf virus species recently reported in Australia

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Yellow dwarf viruses reduce grain yield in cereals worldwide and are transmitted by aphids. YDVs are regularly found in cereals and grasses in south-eastern Australia, where the most common cereal aphids are *Rhopalosiphum padi* (*R. padi*, bird cherry-oat aphid), *Rhopalosiphum maidis* (*R. maidis*, corn aphid), *Metapolophium dirhodum* (*M. dirhodum*, rose grain aphid) and *Diuraphis noxia* (*D. noxia*, Russian wheat aphid). Several YDV species, such as barley virus G (BVG), cereal yellow dwarf virus RPS (CYDV RPS) and barley yellow dwarf virus PAS (BYDV PAS), have recently been reported in Australia, however, little is known about the epidemiology of these species in an Australian context. While BVG has been detected and reported in several countries, little information has been published about its transmission and host range. Additionally, *D. noxia* was found in Australia for the first time during 2016 and its ability to transmit Australian YDV isolates has not been examined. To address these knowledge gaps, glasshouse experiments were conducted to evaluate the transmission of BYDV PAS, barley yellow dwarf PAV (BYDV PAV) and BVG by *R. padi*, *R. maidis*, *M. dirhodum* and *D. noxia*, while the host range of BVG was also investigated. *R. padi* was the most efficient vector of both BYDV PAV and BYDV PAS while *R. maidis* was the most efficient vector of BVG. However, *M. dirhodum* was an inefficient vector of both BYDV PAV and BYDV PAS and *R. padi* was an inefficient vector of BVG. *D. noxia* did not transmit BYDV PAV, BYDV PAS or BVG. Host differences related to BVG infection were also observed and will be presented and discussed.

Viruses in canola and aphids in western Victoria

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Canola is one of the most important grain crops in Victoria, Australia, where it is found to be infected with viruses such as turnip yellows virus (TuYV), cauliflower mosaic virus (CaMV), and turnip mosaic virus (TuMV). TuYV is the most common and important virus infecting canola in the region, and green peach aphid (GPA) (*Myzus persicae*) is the main vector of this virus. As part of a regular surveillance program, samples were randomly collected from four winter canola crops in western Victoria during October 2023 and tested for TuYV, CaMV and TuMV by tissue blot immunoassay (TBIA) and TuYV incidences of between 66-96% were recorded. In addition, higher than usual summer rainfall in western Victoria resulted in the extensive growth of volunteer crop plants and weeds that can harbour GPA and TuYV between growing seasons (referred to as a 'green bridge'), potentially encouraging high numbers of virus-infected GPA early in the 2024 growing season. Therefore, samples were also randomly collected from eight paddocks containing volunteer canola, two summer canola crops and one forage turnip crop during February 2024. Samples were again tested by TBIA for TuYV, CaMV and TuMV. TuYV incidences of 18-98% were recorded while CaMV and TuMV were not detected in either October 2023 or February 2024. To further monitor for GPA and TuYV, yellow sticky traps have been installed on the fence-line of five canola paddocks and are being changed and examined fortnightly. Trapped aphids are being identified, counted, separated from the traps and tested for TuYV by RT-qPCR. These five canola crops are also being examined for aphids and tested for TuYV. Data will be presented on the aphid species present, along with the TuYV incidence in the green bridge, canola crops, and flying aphids, with special emphasis on GPA.

Unlocking biocontrol potential: Should we adopt *Botrytis cinerea* as a mycovirus model system?

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Botrytis cinerea, a phytopathogenic fungus affecting over 1400 plant species, including many crucial crops, presents significant agricultural challenges due to its widespread nature and resistance to fungicides. *B. cinerea* has one of the most numerous and divergent mycoviromes reported in scientific literature. In field incidence can be greater than 80% with many *B. cinerea* isolates hosting viruses of multiple species. This presentation advocates for the establishment of *B. cinerea* as a model organism for mycovirus research. We highlight its potential to unravel the complexities of mycovirus-fungus interactions and promote innovative biocontrol strategies. It is genetically malleable with an existing toolkit of mutants, universally distributed and therefore does not require physical containment, and has a vibrant international community with collections of field and laboratory isolates. Previously we identified a DNA mycovirus named botrytis gemydayirivirus 1 (BGDaV1) that reduces the growth rate of *B. cinerea.* Here, we demonstrate, BGDaV1-positive *B. cinerea* strains show significantly fewer and smaller lesions on leaf discs compared to BGDaV1-negative strains. However, biocontrol efficacy varied among BGDaV1-positive *B. cinerea* lineages that have different mycovirus backgrounds. The complex interspecies mycovirome and mycovirus-fungus interactions likely influence BGDaV1's ability to increase in titre within *B. cinerea* and reliably reduce fungal growth rate. Despite their promise, mycoviruses are

underexplored in biological studies, with a significant knowledge gap about how they interact with other mycoviruses and their host fungus. By adopting *B. cinerea* as a model system, the scientific community can deepen its understanding of mycovirus diversity, epidemiology, and cellular biology. This presentation calls upon researchers to collaborate and utilise *B. cinerea* to unlock the potential of mycoviruses for sustainable disease management.

How great passion, good will and a love for the tropics creates magic - the part that plant virus and virus-like diseases have played through 35 years of Northern Australia Quarantine Strategy plant health surveys

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This year, the Department of Agriculture, Fisheries and Forestry (DAFF) Northern Australia Quarantine Strategy (NAQS) clocks up 35 years of boots on the ground plant health surveillance across Australia's remote northern coastline. NAQS aims to protect Australia's plant and animal based industries and natural environment from biosecurity invasions posed by exotic pests and diseases present in the nation's northern near neighbours. Early detection of natural and human mediated incursions in a sparsely populated vast region lies at the heart of the strategy. A key pillar of the NAQS story has been joint agency surveillance with biosecurity scientists in the neighbouring nations to the north. This has providing 'over the horizon' insights into immediate threats, whilst also promoting natural synergies whereby NAQS staff gain hands-on experience with key target organisms and overseas colleagues benefit from close collegiate relationships and ready access to Australian and international diagnostic networks.

Domestically, the interaction between NAQS and First Nations communities has also been central to success. NAQS relies on the cooperation and goodwill of Aboriginal and Torres Strait Islander Traditional Owners and ranger groups. Today, the DAFF funded Indigenous Ranger Biosecurity Program engages over 60 ranger groups in fee for service activities to support biosecurity surveillance.

Within this narrative, plant viruses and virus-like diseases, specifically phytoplasmas and the causal agent of huanglongbing disease of citrus, have played a significant central role. This evolved from surveys in the early 1990s where adoption of tools to achieve meaningful diagnostics for these diseases were in their infancy, to today where high throughput sequencing (HTS) workflows are becoming the norm. In this presentation, some stories of investigative plant virology and phytoplasmology will be outlined.

What do lettuce chlorosis virus, yambean mosaic virus and an unknown cogu virus have in common?

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During a 2021 disease survey of the beautiful Northern Peninsular Area (NPA) of Australia with some talented Northern Australian Quarantine strategy (NAQS) staff and Indigenous rangers, a sample of calopo (*Calopogonium mucanoides*) or wild ground nut, was taken. This unsuspecting calopo plant was hanging out on a wire fence in Umagico hiding its secrets within. But a couple of plant pathologists noticed its wrinkled leaves and mild mosaic pattern which alerted them to something being a bit suspicious. The leaves of the calopo were taken to a not so secrete lab in Brisbane where testing revealed a poty virus was hiding. A general poty PCR revealed it to be yambean mosaic virus (YBMV) not previously reported in Australia. Don't worry, biosecurity was alerted! It could have all stopped there, but some pathologists need to know more (or don't know when to stop), so the calopo RNA was sent for high throughput sequencing and then the true secrets were revealed. This plant had not just one new incursion, but three! A plant virologist dream (or is it a nightmare?). The findings and implications will be discussed.

Small RNA-omics and Long-Read Sequencing Integrated Strategy for Characterisation of Novel Plant Viruses

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Plant viruses are harmful plant pathogens that cause over 20 billion USD in annual crop-losses world-wide. Rapid and accurate identification and characterisation of plant viruses are challenging due to the limited availability of viral genetic material in infected plant tissues. Over 300 different high-risk plant genera are imported into Australia annually via the national Post Entry Quarantine (PEQ) facility in Mickleham, Melbourne, where they undergo disease screening. Implementing multiple molecular assays that target individual viruses is becoming unsustainable for large-scale quarantine screening. Whilst small RNA-omics are transforming our capacity to detect plant viruses, the characterisation of novel viruses is still challenging due to the lack of whole genome coverage at low titre. Here, we report a small RNA sequencing (sRNA-Seq) and long-read sequencing integrated strategy for whole genome sequencing and characterisation of novel plant viruses. Our results suggest that this method is suitable for rapid and complete genome sequencing and characterisation of novel plant viruses. This method can be further improved by using probe-based target enrichment methods to pull-down the virus genome targets and uses random hexamers to target viruses with or without poly A tails, enhancing our capacity to resolve complete viral genomes to facilitate biosecurity risk assessment and decision making.

Preparing for the transition of banana quarantine and diagnostics to Mickleham

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Banana (*Musa* sp.) tissue cultures are currently imported and grown through post-entry quarantine (PEQ) in dedicated Queensland Department of Agriculture and Fisheries (QDAF) class 6.7 Approved Arrangement facilities, with a process management system approved by the Federal Department of Agriculture Fisheries and Forestry (DAFF). Importation of banana germplasm was recently resourced by two Hort Innovation projects, BA10020 and BA16001, which together imported thirty-three banana cultivars and met all facility, husbandry and testing costs. Now that these projects are completed, the costs of facility maintenance and accreditation fall to the banana industry. Due to the high on-going costs of maintaining accreditation of pathway-specific glasshouse and tissue culture facilities, alternative arrangements are being sought to transition the importation of banana germplasm through DAFF's national PEQ facility at Mickleham.

As a first step, growth trials of domestic *Musa* material at PEQ Mickleham were successfully completed, proving that the multi-commodity glasshouses there are fit for growing bananas. As a second step, DAFF PEQ staff travelled to QDAF laboratories at the Ecosciences Precinct in Brisbane, Queensland to develop their skills in banana pathogen detection during PEQ screening of imported banana germplasm. Dr Kathy Crew led the training covering the biology of banana viral and phytoplasma pathogens as well as practical training in the laboratory demonstrating specialist laboratory techniques such as virus purification, immunosorbent electron microscopy and multiplex immunocapture molecular assays for known banana viruses.

Further steps in transitioning banana diagnostics to Mickleham PEQ will include a reciprocal visit by Dr Crew to PEQ Mickleham for further training and troubleshooting, as well as side-by-side evaluation of current diagnostic methods and high throughput sequencing (HTS) of sRNA, for banana germplasm diagnostics.

Field effectiveness of foliar systemic insecticides, a neonicotinoid-based seed treatment, and partial resistance to control turnip yellows virus in canola

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Turnip yellows virus (TuYV) is a phloem-limited virus transmitted in a persistent manner primarily by *Myzus persicae* (the green peach aphid) and causes serious disease in canola *(Brassica napus*) worldwide. TuYV spread in canola is polycyclic; primary infection occurs when viruliferous *M. persicae* alates from outside the crop land and feed on uninfected plants which then become sources of infection for secondary spread as the subsequent generations of winged and wingless aphids growing on that plant move through the crop. In Australia, TuYV control is consciously or unconsciously attempted solely with insecticides either (i) prophylactically regardless of risk, (ii) reactively upon discovery of widespread *M. persicae* colonisation, or, less commonly, (iii) in a targeted manner based on risk information. A series of four field experiments were conducted to investigate the use of new foliar insecticides, a neonicotinoid-based seed treatment, and partial resistance to control TuYV in canola. Primary infection was simulated by transplanting TuYV-infected canola plants infested with *M. persicae* into the trial. Aphid counts and virus testing were conducted every two weeks after aphid introduction. Systemic insecticides applied to plots three days prior to aphid introduction limited subsequent *M. persicae* population growth but only modestly reduced TuYV incidence. Application of an insecticide two weeks after aphid introduction instead was more effective at restricting *M. persicae* population growth and significantly suppressed TuYV. The neonicotinoid seed treatment was modestly effective at restricting *M. persicae* population growth and failed to significantly reduce TuYV infection. In contrast to insecticidal strategies, the use of a partially resistant cultivar was highly effective at suppressing TuYV spread compared to a susceptible cultivar. This research suggests that (i) insecticidal strategies need to be highly effective at controlling *M. persicae* to significantly suppress TuYV spread, (ii) foliar insecticides applied during the early stages of viruliferous *M. persicae* colonisation are more effective than when applied before aphid incursion, (iii) commercial seed treatments are less effective at controlling TuYV than previously thought, and (iv) host resistance is an effective TuYV management tool that could reduce the reliance on insecticides and deserves further consideration in the Australian canola industry.

Investigating resistance breakdown and alternative management strategies for tomato yellow leaf curl virus

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Tomato yellow leaf curl virus (TYLCV), a single-stranded DNA virus in the Begomovirus genus, is transmitted by whitefly *Bemisia tabaci* in a persistent, circulative manner. This virus poses a significant threat to global tomato production. In Australia, TYLCV-Israel (TYLCV-IL) was first detected in 2006 in southeast Queensland and later spread to northern Queensland, causing substantial economic impact. Introducing TYLCV-resistant tomato hybrids containing the *Ty-1* resistance gene has effectively managed the virus in Queensland for several years. However, recently, resistance breakdown has been observed in Bundaberg. To investigate the factors contributing to this and explore alternative management strategies, PCR was conducted on samples collected from symptomatic plants of the TYLCV-resistant variety SV0215TH collected in Bundaberg in 2023. In addition, Sanger sequencing was conducted to obtain the genome sequence of the TYLCV 2023 isolate (AU23) for phylogenetic and severity prediction analysis using the machine learning software IML-TYLCVs. The results showed no detectable mixed infection of tomato leaf curl virus (ToLCV), tomato chlorosis virus, or ToLCV betasatellite. Although AU23 was predicted to be a mild isolate based on amino acid sequence, the phylogenetic analysis revealed that the pairwise distance of the nucleotide sequence of AU23 is far from that of the other Australian isolates retrieved from the NCBI virus database. Subsequently, spray-induced RNA interference (RNAi) was evaluated to protect tomato plants from AU23. Plants treated with dsRNA targeting the viral transcripts V1 and C3, combined with an

additive, showed reduced symptom expression and virus titre. Further research is needed to identify the major factors affecting the resistance breakdown, providing insights into developing new protection strategies for re-emerging viruses.

Digital PCR in plant virus detection: Advancing Precision, Sensitivity, and Efficiency

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Digital PCR (dPCR) is rapidly emerging as the new gold standard for nucleic acid quantification, surpassing traditional quantitative PCR (qPCR) in accuracy, sensitivity, and reproducibility. Its ability to provide absolute quantification without the need for calibration curves, coupled with superior resistance to inhibitors and enhanced detection of low-abundance targets, positions dPCR as a powerful took in plant virology.

Nanoparticle-mediated delivery of VIGS vectors for protecting citrus from HLB

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Recent years have seen a drastic increase in the incidence of Huanglongbing (HLB), having devastating effects on the citrus industry in North and South America. The potential spread of the disease presents a high biosecurity risk in countries including Australia. Candidatus Liberibacter asiaticus (CLas), the causal agent of citrus HLB is a phloem colonizing bacteria making it particularly difficult to target with traditional methods. Not only is resistance developing against antibacterial treatments, but the treatments are labour intensive, expensive and have negative impact on tree health. An exciting alternative, utilizing the plants natural immune system is siRNA-mediated or peptide mediated antibacterial treatments. The most efficient way of delivering these antibacterial treatments for a continual dose with minimal applications is the use of Virus Induced Gene Silencing (VIGS) vectors. Citrus tristeza virus (CTV) and citrus yellow vein-associated virus (CYVaV) represent exciting viral vectors as they are phloem localised, asymptomatic and cannot spread from tree to tree under natural conditions. While these vectors have yielded very promising results in combatting CLas, introducing them into the correct cells of the vascular system has been a significant hurdle. In order allow VIGS vector entry into plant cells, we utilised Layer Double Hydroxide (LDH) nanoparticles, which have been shown to efficiently deliver both RNA and DNA to multiple plant cells. We have shown that LDH can deliver plasmids to the citrus vasculature, and further that we can deliver DNA encoding VIGS to plant cells which can subsequently replicate to move throughout the plant. Together our results show enormous potential for the effective delivery of DNA encoding VIGS vectors for not only fighting plant diseases but multiple other potential uses in plant health and development.

ONTViSc: a customisable nextflow pipeline for the identification and detection of viral genomes from Oxford Nanopore sequencing

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Pathogenic viruses and viroids infecting plants can result in significant economic and ecological losses. Second-generation sequencing technologies and associated methods have revolutionised plant virus diagnostics because they are high throughput and sequence agnostic. Their limitations lie in their high-cost, labour intensiveness, bulkiness, and inability to unequivocally identify edge cases (e.g. low-titre novel viruses). In recent years, advances in third-generation Oxford Nanopore Technologies (ONT) have enabled real-time sequencing of long DNA or RNA sequences through portable sequencers and thus offered a new avenue for plant virus diagnostics. Long read

lengths permit easier reconstruction of complete or near complete viral genomes. Plant diagnostics laboratories might mainly focus on targeting specific pathogens (short or long amplicon sequencing), while some other groups might be interested in whole genome and metagenomic sequencing studies. Thus, different analytical approaches are required to analyse ONT data to tailor to different needs. Some laboratories might also first perform a direct read search on whole genome samples to quickly ascertain whether there is any viral signal in a sample. Finally, direct read and clustering approaches can recover viroid and low titre virus signal, where *de novo* assembly approaches are not suitable. To this end, we have developed ONTViSc (ONT-based viral screening), a nextflow pipeline that facilitates the detection of viruses from amplicon and metagenomic ONT data using different analytical strategies: 1) direct read classification using blast homology search and/or kmer search approaches; 2) reference guided mapping; 3) de novo genome assembly; and 4) clustering approach. End users can also pre-process, filter plant host sequences and assess the quality of the sequencing data. To demonstrate its utility, we successfully ran ONTViSc on published sequencing datasets as well as edge cases provided by collaborators. The bioinformatics tool was also able to reconstruct the genomes of novel plant viruses.

Development of badnavirus infectious clones for research in taro and banana

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Badnaviruses are double-stranded DNA (dsDNA) pararetroviruses in the genus *Badnavirus*, family *Caulimoviridae*, with a genome length typically of 7 to 8 kbp. Most possess three open reading frames (ORFs) on the sense strand of the virus genome while some members also encode a fourth ORF. Replication of these viruses occurs via reverse transcription of a greater-than-genome length RNA that serves as a template both for the translation of viral proteins and for reverse transcription to replicate the genome. The dsDNA genome facilitates the relatively straightforward preparation of infectious clones in plasmid vectors, which can be agro-inoculated into host plants to reconstitute an infection. These infectious clones are useful tools for investigation of host range, symptomatology, host plant resistance and can be modified as vectors for virus-induced gene silencing. We have prepared infectious clones of three badnaviruses, including taro bacilliform virus, which infects taro (*Colocasia* spp.) and two banana (*Musa* spp.)-infecting species, namely banana streak MY virus and banana streak CA virus. The infectious clones showed high infectivity in their respective host plants. Symptoms of infection in banana varied, with some accessions showing a significant delay in the time to develop symptoms.

Exploring Phages as Promising Biocontrol Agents for managing Bacterial Diseases in Plants

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Bacteriophages (phages) are beneficial viruses that infect and replicate within bacteria, presenting a promising agent for combating bacterial diseases in diverse ecosystems including agriculture. In recent years, there has been a growing interest in harnessing the potential of bacteriophages to control plant bacterial pathogens due to their specificity, efficacy, and environmentally friendly nature. In this study, we tried to recover lytic phages from local environmental samples; soil, irrigation water, rotted fruits, and organic manure with the aim to evaluate their presence and efficacy against soft rot pathogens. In total, eight lytic phages were isolated from ten environmental sources. Phages infecting *Pseudomonas* spp. were commonly recovered from a diverse range of environmental samples in comparison to *Pectobacterium brasiliense, Klebsiealle oxytoca* and *Dickeya fangzhongdai*. Enriched organic manures and rotted potato tuber assays were found to be effective (*in vitro*) and inhibited the growth of soft rot causing pathogens of potato soft rot, banana corm rot, bacterial soft rot of lettuce and leaf spot of parsley. Morphological characterization using electron microscopy (TEM) revealed that a *P. brasiliense* phage belongs to family Podoviridae and *Pseudomonas* sp. phages to Siphoviridae and Myoviridae. In conclusion, bacteriophages hold immense potential as sustainable and ecofriendly alternative for managing bacterial diseases in plants.

However, challenges still exist in scaling up of phage production, field application and molecular characterization of isolated phages. Continued research efforts are in progress to address knowledge gaps, optimize phage-based interventions, and integrating phage-based strategies into existing agricultural practices.

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Genome sequencing of phages infecting plant associated Enterobacter species

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Management of plant pathogenic bacteria has been complicated in recent years by a lack of options and chemical resistance in pathogen populations. Viruses that infect bacteria (Bacteriophages) are an emerging target for future biocontrol efforts of plant pathogenic bacteria. The use of bacteriophages in this capacity is still in its infancy, and many challenges exist at each stage of developing a viable biocontrol tool. After isolating phage particles, the next step is identifying and characterising a pure phage lysate. Recent work on bacteriophages of soft rot bacteria resulted in a collection of phage isolates with characterised activity and genome structure. A number of phages are described with complete genomes, generally within the Caudoviricetes. The challenges in effectively sequencing and describing phage genomes require specific strategies to overcome; sufficient phage particle concentration, purity and DNA quality are achieved through large propagations with precipitation and phenol/ chloroform extractions. A workflow using the Oxford Nanopore sequencing platform is presented here as a protocol for identifying and describing bacteriophages that have potential as biocontrol for plant pathogenic bacteria.

High Throughput Sequencing, Biosecurity and Policy in Plant Virus Diagnostics

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The goal of the High Throughput Sequencing Working Group (HTSWG), a working group for the Subcommittee on Plant Health Diagnostics (SPHDs) is to facilitate the implementation of high throughput sequencing (HTS) standards and guidelines for plant health diagnostics in Australian jurisdictions and inclusion in National

Diagnostic Protocols (NDPs). The uses of HTS in plant health diagnostics are many and varied. These cover anything from amplicon sequencing protocols to whole genome sequencing and even metagenomics. This means there is no one size fits all approach to assessing risk, meeting obligations under biosecurity at both state and federal levels and in developing new policy to ensure the results of HTS applications are reproducible, meaningful and relevant to our stakeholders. There is an increased risk of unintended consequences when complete sets of plant virus HTS data are uploaded to public databases owing to the fact they are often actually a plant metagenome, containing a wealth of other information which was never the original aim or target of the experiment. In this presentation, members of the working group hope to highlight the challenges with navigating the use of new methods in the plant virus diagnostics space where the policy and guidelines don't always keep pace with new developments in technology. We discuss, and challenge everyone to consider these potential unintended or incidental findings that could be lurking in your data.

Seed transmission of turnip mosaic virus to seedlings of *Brassica juncea*

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Turnip mosaic virus (TuMV) causes economically important diseases in *Brassicaceae* crops worldwide. In 2023, *Brassica juncea* (Indian mustard) cv. Tendergreen seedlings showing leaf vein clearing, mosaic and deformation, and plant stunting symptoms, were found growing amongst other seedlings in insect-proof glasshouse facilities. TuMV was detected when ELISA was used to test symptomatic seedling leaf samples. The seed lot sown was from New South Wales, Australia. This seed and seed lots from three other cv. Tendergreen sources were sown in trays inside Controlled Environment Rooms (CER's) from which virus cultures and other plants were absent. Seeds of four other *B. juncea* cultivars from different seed sources were sown alongside them. When grouped leaf samples from 2-3 week old seedlings were tested by ELISA (120 seedlings/seed source), TuMV was detected in 10% of seedlings from the original infected cv. Tendergreen seed source, but in none from the other seven seed sources. Further seeds from the infected *B. juncea* seed source were sown in trays. These trays were subdivided into two batches, one batch being placed inside transparent plastic boxes that completely excluded any possibility of seedling contamination by aphid vectors or contact. Each batch was placed in a different CER. When seedling leaf samples were grouped and tested by ELISA, in both instances TuMV was detected in 9% of seedlings (259 and 266 seedlings tested from inside or outside the plastic boxes, respectively). Absence of TuMV detection in the other seven seedling batches tested initially, combined with the 9-10% TuMV seed transmission rates found in all three batches of seedlings from the infected seed sample meant that any chance virus contamination of seedlings by aphid vectors or contact was absent. Therefore, TuMV seed transmission is confirmed in *B. juncea*. To prevent its dissemination in *B. juncea*, healthy seed stocks should be sown.

Spray application of dsRNA targeting the CP of ZYMV reduces mechanical and aphid transmitted infection incidence in glasshouse grown *Cucurbita pepo.*

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Exogenous application of double-stranded RNA (dsRNA) for protection against plant viruses has been demonstrated in a range of pathosystems. Here we adopt a spray on dsRNA application to induce RNAi protection against the *Potyviridae* family member, Zucchini Yellow Mosaic virus (ZYMV) in zucchini (*Cucurbita pepo*) plants by targeting the virus Coat Protein (CP) gene. We show significant protective efficacy against mechanically inoculated and aphid transmitted ZYMV in dsRNA-treated plants. Infection incidence in *C. pepo* plants treated with CP-dsRNA at 300 ng $\mu L^{-1}$and mechanically inoculated at 5 days post spraying was reduced by 60 – 80 % at 10 days post-infection. Furthermore, a significant reduction in infection incidence was observed for up to 15 days when challenged at 10- and 15-days post spray under glasshouse conditions. Infection incidence in viruliferous aphid challenged plants treated with 600 ng μL^{-1} CP-dsRNA at day 5 post spray was reduced by ~90 %. These assay results show that the spray-on application of dsRNA to induce RNAi against economically significant viruses affecting high value horticultural crops is a viable option in protected cropping environs.

RNA interference against the Green Peach Aphid, *Myzus persicae (Hemiptera).*

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Aphids are sap-sucking agricultural crop pests that cause significant fruit and fibre damage by infestation and transmission of plant viruses. Current means of aphid control rely on chemical pesticides, however a potential control method using double-stranded RNA (dsRNA) as a spray on technology to induce RNA interference (RNAi) is gaining traction. For spray-on RNAi to be effective against sap sucking insects, critical genes need to be identified that when targeted, disrupt homeostatic function, induce mortality, or significantly reduce insect fitness. Here, we show the ingestion of dsRNA via artificial diet (AD) assay, targeting several genes of the Green peach aphid, *Myzus persicae,* encompassing neuronal functioning; osmoregulation, probing/feeding behaviours or nucleic acid and protein metabolism either individually, in combination, or as a stacked construct induced mortalities ranging from 14 – 72%, 78 – 85% and 54%, respectively. Transcript level knockdown of the respective target genes ranged from 6.3% to ~54%, with inconsistent correlation observed when compared to mortality. Here we have identified several genes that may be targeted in a potential spray on RNAi based crop protection product against *M. persicae* infestation.

Managing potyviruses in cucurbit crops by host resistance

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Three potyviruses: Papaya ringspot virus(PRSV), Watermelon mosaic virus (WMV) ,Zucchini yellow mosaic virus (ZYMV) (family *Potyviridae*: genus *Potyvirus*) cause major economic damage to cucurbit crops in all States. Zucchini and melon crops are particularly susceptible.

PRSV dominates in Queensland and ZYMV in Western Australia. Insecticide applications have little effect on the rapid, non-persistent transmission of these viruses. Altering planting dates and crop hygiene can help but their influence is variable. Host resistance provides effective, economic control. Eighteen zucchini (*Cucurbita pepo*) varieties were recently assessed for virus resistance in a trial in south-east Queensland. All plants in two replicates were inoculated with PRSV at the cotyledon stage and the two replicates of the same varieties were not inoculated. Plants were assessed for symptom severity, total yields and yields of marketable fruit over five weeks with three harvests/ week. Four varieties were superior to the most widely grown virus tolerant variety in both inoculated and non-inoculated plots. Previous work has shown that varieties with good resistance to PRSV are also resistant ZYMV. Highly resistant zucchini varieties are commercially available and can largely prevent losses from potyvirus. Limitations identified in trial work include only average potential yields from some virus resistant varieties and mild leaf symptoms are not always well correlated with lack of fruit symptoms. Potyvirus resistant watermelon (Citrullus) and squash remain a challenge as no suitable material has been identified in greenhouse and field trials.