Hort Innovation

Final Report

Area wide management of vegetable diseases: viruses and bacteria (Qld report)

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Area wide management of vegetable diseases: viruses and bacteria (Qld report) (VG16086)

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Public summary

VG16086 'Area wide management of vegetable diseases: viruses and bacteria' delivered its overall outcome for industry in providing updated, contemporary management strategies for important viral and bacterial diseases affecting Australian vegetable growers. These diseases impact producers nationally, collectively costing millions of dollars each year. Multiple project activities over the four-year period combined to produce the management strategies. This included national annual disease surveys to capture current disease concerns, review of past disease research and new research through multiple management trials and genetic diversity studies of pathogens and vectors. The trials were conducted in multiple districts for known highly impacting diseases (e.g mosaic of zucchini and viruses of capsicums) and for pectobacterium affecting zucchini which was identified through early surveys as an emerging concern. These activities also generated a priority list of endemic and exotic diseases of concern for the Australian vegetable industry. Additionally, the project delivered a second important outcome through improved biosecurity preparedness to six key exotic threats for the Australian vegetable industry. Contingency plans and factsheets were prepared for three exotic virus groups and three bacterial diseases.

Specific industry impacts and benefits from this project include:

- Improved resilience to manage viral and bacterial diseases (for both field and protected cropping systems)
- Increased capacity for disease management through a reconnected national network of experts in the fields of virus and bacteria diseases. This also included training four PhD students, two in plant virology and two in plant bacteriology and industry stakeholders through extension activities
- Improved diagnostic capability nationally which provides faster results for producers when seeking confirmation of causal agents for disease outbreaks. Twenty-three assays were circulated and validated through the national diagnostic services, including 16 for viruses and 7 for bacteria. Accurate and fast diagnostics assists with early disease management interventions
- Delivery of four hands-on pest and disease identification training workshops
- Improved understanding of the potential of seed as a pathway for endemic and exotic pathogens to affect Australian production. All stakeholders including growers, seed companies, biosecurity agencies, nurseries, AusVeg and Hort Innovation participated in two workshops focused on this topic
- A disease priority list to inform on future R, D&E investment for crop protection

Future industry and benefits from this project include:

- Future project on seed pathways: AS21007 'Streamlining diagnostics for horticultural seeds imported into Australia.' was tendered by Hort Innovation in June 2022
- Extension of research findings to improve management of viruses affecting lettuce and brassicas: recommendation for proposed project developed
- Extension of research findings on pectobcaterium in zucchini to develop management strategies for this emerging disease: recommendation for proposed project developed
- Conclusions from research highlights a need for new-age bactericides to reduce bacteria within infections and thus decrease disease spread and impact, current products attempt prevention of infection and regularly fail: recommendation for a proposed project developed

Multiple resources were generated for the industry and available through the Hort Innovation website. These include:

- A guide to understanding and managing bacterial diseases affecting Australian vegetable crops
- A guide to understanding and managing virus diseases affecting Australian vegetable crops
- Nine specific factsheets on virus diseases affecting vegetable crops
- Six contingency plans
- Priority list of endemic and exotic pathogens

Keywords

Area wide management, thrips, aphids, leafhoppers, potyvirus, orthotospovirus, polerovirus, *Pseudomonas*, *Xanthomonas*, *Pectobacterium*, contingency plans, begomovirus, tobamovirus, diagnostic protocols, disease management, capsicums, lettuce, brassicas, zucchini

Introduction

Area wide management (AWM) historically has been applied to management of insect pests. It also has potential for controlling plant diseases, particularly those with aerial dispersal mechanisms such as insect-vectored viruses, insect-vectored bacteria and wind dispersed bacteria and fungi. This type of management is contrasted with traditional management essentially by scale and co-ordination, where control tactics are applied over a broad area, incorporating multiple premises to maintain pest populations below economic impact levels. AWM strategies for virus diseases need to consider factors such as vector management and vector migrations, development of insecticide resistance, alternative hosts of the viruses and their vectors, genetic diversity of viruses and the use of host resistant lines. Similarly, for bacterial diseases, development of resistance to bactericides, bacterial survival during non-cropping seasons, genetic diversity of pathogen populations and the use of host resistant lines require consideration. Additionally, management of public areas within a district is important to success. Monitoring of weather and its influence on virus vector and bacterial populations is an important tool for developing and implementing disease management.

All these factors are best considered at an area wide scale rather than on an individual property or block basis as the actions of one grower will impact neighboring producers throughout the district. For example, overuse of a single mode of action chemical to control the insect vectors or bacterial pathogens may lead to the rapid development of insensitivity by that organism, and subsequent loss of effectiveness of that chemical. Similarly, widespread use of single gene host plant resistance can result in selection of more aggressive strains of viral or bacterial pathogens which can avoid the resistance, hence rendering that resistance ineffective. The research delivered in this project was aimed at prevention or minimization of these types of complications to disease management. To underpin AWM, effective diagnostics with short sample processing times are needed, as is a comprehensive understanding of pathogen genetic diversity. Extension activities were aimed at promoting the concept of area wide management plus delivery of advice on a range of management options and education on how to identify diseases.

The capacity to provide pathology and entomology support to industry by Australian research providers is increasingly stretched through multiple exotic incursions of pest and diseases in recent years. These incursions require rapid responses by skilled staff to provide accurate and timely identification, expert advice on the potential spread of the organism and the likelihood of successful eradication. To address this diminishing capacity, the project has invested in early career scientists through direct employment and mentoring of others. It also invested in four PhD students. The project further addressed biosecurity threats to the Vegetable industry through improved preparedness. This was through development of contingency plans for six key exotic threats and the development of improved diagnostic protocols for identification of bacterial and viral pathogens affecting vegetables. It also reviewed entry pathways for exotic and endemic diseases including seed and potential risk mitigation strategies such as seed disinfestation and/or pathogen testing.

The specific aims delivered by the project were:

1. Development of management strategies to address high priority viral and bacterial diseases affecting vegetable crops. This includes thrips-, aphid- and whitefly-transmitted viruses leafhopper-transmitted phytoplasmas and management of thrips, whitefly and aphids as pests. The second major focus is the management of foliar bacterial diseases. Targeted crops for the research include Solanaceae, cucurbits, Apiaceae, brassicas, Asian vegetables and lettuce.
2. Development of effective, innovative and rapid diagnostics for key viral and bacterial pathogens. These diagnostic tools will be developed with a view to adoption by growers and/or consultants for in-field testing and will underpin management strategies.
3. Improve preparedness of the vegetable industry to key viral and bacterial exotic threats through contingency planning and increased awareness.
4. Increase capacity for crop protection RD&E within Australia through investment in PhD students, recent post-graduates, and early career scientists.

Methodology

Data on pathogen detection: geographical distributions

Surveys were completed to monitor the distribution and diversity of viruses, insect vectors and bacteria in vegetable crops. This included both field and protected crops. As seasonal weather conditions can vary greatly, the activity was spread throughout the project life span to increase likelihood of detecting all major pathogens. Data for this activity was supplemented through collation of sample results from formal government diagnostic services associated with the project.

Seasonal activity of insect vectors and disease

The seasonal activity of insect vectors, virus and phytoplasma was monitored in several sites over several seasons. This included:

- Leafhopper and phytoplasmas in the Granite Belt, QLD
- Thrips and orthotospoviruses in the Dry Tropics, QLD
- Thrips and orthotospoviruses in the Bacchus Marsh, Vic
- Vector trapping and virus monitoring in Carnarvon, WA
- Aphid and virus monitoring near Perth, WA

Queensland: Leafhopper and Phytoplasma monitoring

Capsicums and tomatoes are produced in the Granite belt from November through May depending on the occurrence of frost at both ends of the growing season. The more temperate weather conditions allow continuous production over the hotter Christmas and New Year period. For the first season, crop surveys in the district commenced in October 2019 and continued through February and April 2020. Surveys recommenced for the new cropping season in October 2020, however, during this 2020-21 season, dry conditions, a shortage of labour and poor prices brought an end to production in the study blocks in early March. In addition to commercial crops, nearby riparian areas and headlands were monitored for potential alternative phytoplasma hosts and vectors. Surveys of weeds continued from March 2021 through the non-production period, with a focus on blue heliotrope as this was identified in the earlier surveys as regularly hosting leafhoppers.

Monitoring involved counts of leafhoppers plus collection of individuals for potential species identification. Five broad species groupings of leafhoppers were collected as Morphospecies 1 through 5. Morphospecies 1 potentially represents *Orosius argentatus* and *Orosius orientalis* that are morphologically difficult to distinguish, and both share the common name of 'common brown leafhopper'. These two species are known vectors of phytoplasma. Morphospecies 4 possibly contains further potential vector species, such as *Austroagallia torrida* (spotted leafhopper) and *Batrachomorphus angustatus* (large green jassid). Crop and weed species were also surveyed for phytoplasma disease symptoms. Representative symptomatic samples were collected to determine the identity of the phytoplasma.

Queensland: Thrips and orthotospoviruses

Monitoring for the two orthotospoviruses, *tomato spotted wilt virus* (TSWV) and *capsicum chlorosis virus* (CaCV) and their thrips vectors was completed in the dry tropics, QLD near Bowen and Gumlu. This is a tropical wet-dry production area with a distinct break in cropping over the summer. Crops are normally planted in about March and run through to October or November depending on the weather. Monitoring was a combination of regular data collection at two geographic locations, one in Bowen and the other in Gumlu, disease surveys to estimate virus incidence and diagnostic samples sent in from growers. The data collected at the two regular monitoring areas were thrips on sticky traps and thrips in capsicum flowers. Morphological identification of the thrips species is very difficult, so numbers from this monitoring isn't strongly linked to vector species. Additionally, more intensive surveys were done from late 2021 through to June 2022 to identify key weed species as environmental sources of the viruses through the wet season. Monthly crop surveys were also done from April to June, 2022 due to a significant outbreak of orthotospovirus in Gumlu to Bowen capsicum crops.

Victoria: Thrips and orthotospoviruses

Vector monitoring for thrips and the incidence of *tomato spotted wilt virus* (TSWV) in lettuce crops was conducted in Bacchus marsh (Victoria) from September 2019 to March 2020. Blue and yellow sticky traps were used for monitoring and to determine the efficiency of both colours to attract thrips. Four traps (2 blue and 2 yellow traps) were placed in each monitoring block and changed fortnightly. The incidence of TSWV was assessed in three areas of the blocks by counting the number of symptomatic plants near each trap in 4 rows of approximately 120 plants at the end of each thrips monitoring fortnight. Symptomatic plants were occasionally tested by RT-PCR to confirm TSWV.

Vector trapping and virus monitoring in Carnarvon, WA

The Carnarvon horticultural district produces capsicum, tomato, pumpkin, zucchini, and eggplant crops from May to December each year, while melons and other non-vegetable crops are grown the remainder of the year – leading to near year-round production of virus and insect hosts. Monitoring commenced in Nov. 2018 and continued to the end of the project. A series of 8 traps were placed on vegetable producing farms located across the district, with an additional trap located near the town center. The effectives of different colored traps for monitoring thrips (yellow or blue sticky traps) were determined in 2019 with both types of traps used.

The traps were changed fortnightly, and numbers of the key disease vectors (aphids, whiteflies, thrips, and leafhoppers) were made on the traps. During peak aphid flights in Sept. 2020 a series of aphids were removed from traps and sequenced to confirm their identity. Surveys of vegetable virus and phytoplasma diseases were made throughout the project on key vegetable crops and weeds in the district.

Aphid and virus monitoring near Perth, WA

Carrots are grown under irrigation year-round near Perth and suffer from outbreaks of *Carrot virus Y*, which are spread by multiple species of aphids. Monitoring of aphids on traps located near carrot crops commenced in Dec. 2016 and continued through the end of the project and included counts of aphids off traps fortnightly as well as morphological identifications made from leaf samples from nearby crops and weeds. Levels of virus infection were determined by testing of random leaf samples taken from adjacent crops, while reservoirs of the virus were made by sampling weed and volunteers (sprouted from previously unharvested blocks). During peak aphid flights the identity of aphids on sticky traps were determined by sequencing.

Disease management trials

A series of management trials were completed to investigate strategies for disease control. This included potyviruses affecting zucchini, potyviruses affecting brassicas, and orthotospoviruses affecting capsicum/chilli. In addition, several pot and field trials were conducted to evaluate survival of *Pectobacterium* spp. to inform on potential management strategies for control of soft rot in zucchini and brassicas. The trials evaluated:

- Genetic tolerance to potyviruses affecting zucchini
- Management of potyvirus in zucchini without genetic tolerance
- Cover crop and biofumigant brassicas as hosts of potyviruses affecting commercial brassica crops
- Management of viruses in brassicas with insecticides
- Glasshouse trial of *Pectobacterium* and *Xanthomonas campestris* pv. *campestris* in Wombok
- *Pectobacterium* spp. Causing crown rot in zucchini

Data on pathogen and vector diversity

Knowledge of the molecular and biological diversity of pathogens and their insect vectors is highly valuable for disease management and pathogen diagnostics. Understanding the genetic diversity assists with evaluation of crop host genetic resistances, development of molecular crop protection products (e.g RNAi), potential for insecticide resistance and in targeted specific diagnostics. Understanding diversity in epidemiology of the pathogens and ecology of the insect pests allows more targeted disease management. For example, knowledge of alternative hosts, environmental sources and seasonal triggers provide key targets for reducing pathogen or vector populations during non-crop windows and thereby reducing risk of primary transfers into early crops.

Investigation of diversity of the vector and pathogens groups involved a suite of molecular, morphological, pathogenicity and biochemical analyses. Molecular characterisation used new technologies to generate whole genome sequences of

the pathogens and multi-locus sequence analyses (MLSA) for the insect vectors. The morphological, pathogenicity and biochemical analyses was done where appropriate to underpin molecular identifications, identify virus recombination events and evolution of new pathogens and vector associations. This work was collaborative, involving pathogen collections in multiple states and regions, and analyses done at the different collaborating institutes. This collaborative approach also fostered technical transfer between the different groups and provided opportunities for mentoring less experienced staff.

Targets for the diversity study were:

- Orthotospoviruses including tomato spotted wilt virus (TSWV), capsicum chlorosis virus (CaCV), Impatiens necrotic spot virus (INSV) and Iris yellow spot virus (IYSV)
- Beet pseudo yellows virus (BPYV)
- Genetic diversity of phytoplasma (PhD student Bianca Jardim)
- Genetic diversity of *Pseudomonas syringae* pv. *syringae* affecting cucurbits (PhD student Noel Djitro)
- Genetic diversity of viruses in weeds (PhD student Joanne Mackie)
- Diversity of leafhoppers in the Granite Belt, QLD
- Diversity of thrips vectors in the Dry Tropics, QLD

Extension: Grower forums and industry engagement; Factsheets; and Benchmarking/Case studies

The major extension activities for the project were:

1. Grower forums and industry engagement. This was a combination of organized in-person meetings and workshops, distribution of information via newsletters, webinars and informal discussions on-farm.
2. Factsheets. These were a series of documents prepared on single topics to provide detailed awareness of specific diseases. They were prepared for both endemic and exotic diseases.
3. Benchmarking and case studies. The original plan for this work was to generate baseline data on disease management at the beginning of the project, during the project and at the end of the project. Early in the project it was evident that generating baseline data from existing practices and monitoring this over time was not an effective or efficient use of time and resources. Therefore, activities were changed to generation of baseline data through research, refining the potential management strategies and then delivering field demonstration trials to extend the information.

Contingency plans on key exotic pathogens

Development and publication of contingency plans was done in collaboration with Plant Health Australia and AusVeg Biosecurity. The plans were developed to allow upload onto the newly designed contingency plan portal. Technical information on the exotic pests was compiled and uploaded. Other information pertinent to industry or biosecurity regulations to be completed by others with expertise in those fields.

Diagnostic protocols field tested, and Ring-testing of diagnostic assays completed

A proposed diagnostic protocol for validation and ring-test system was developed. *Cucumber green mottle mosaic virus* (CGMMV) in leaf/fruit tissues was used as the model and up two ELISAs, four endpoint RT-PCR assays and three RT-qPCR assays were tested by seven laboratories. The network of laboratories for this included the main diagnostic services in each state and the NT. Multiple diagnostic protocols developed, optimised or discovered during the project were disseminated around this network. A second proficiency/ring-test was developed, in collaboration with the project *CMI C02897/C02755: Reducing constraints on the timely implementation of PCR tests for imported vegetable seeds for sowing,* to assess the detection of CGMMV, kyuri green mottle mosaic virus (KGMMV), zucchini green mottle mosaic virus (ZGMMV), cucumber fruit mottle mosaic virus (CFMMV) and melon necrotic spot virus (MNSV) in seed.

Recommendations for seed disinfestation and indexing

To derive recommendations for seed disinfestation and indexing, a comprehensive literature and web search was completed. Additionally, two seed pathway workshops were held with industry, biosecurity agencies, seed companies and researchers. All information gained through this process has contributed to the recommendations. Hort Innovation tendered an RFP for seed pathway improvements in June 2022.

Results and discussion

Data on pathogen detection: geographical distribution

Surveys were completed to monitor the distribution and diversity of viruses, insect vectors and bacteria in vegetable crops. This included both field and protected crops. Surveys were completed in most districts, however, COVID restrictions did impact on this activity in all areas. A summary of the surveys and pathogens detected is provided in Appendix 1.

Key findings from this activity were:

- Many pathogen detections are seasonal and area specific (e.g CaCV mostly in QLD, CMV mostly in WA and NSW, INSV only in VIC and NSW)
- Some pathogens are very widespread affecting many commodities and areas (e.g TSWV and *Pseudomonas* spp.)
- Insect vectored virus disease outbreaks are linked closely with seasonal influences, particularly where weather influences environmental hosts of the viruses and the insects (e.g rainfall)
- New pathogen detections – viruses and bacteria detected in vegetable crops where previously not known to cause disease (e.g new tobamovirus in snake gourd in NT)
- Bacterial diseases are sporadic and closely linked with wet weather, where impacts are significant (e.g single block outbreak of xanthomonas in brassicas cost the grower $400K in direct losses alone in 2021)
- Multiple examples of bacterial disease outbreaks most likely linked to contaminated seed
- Emergence of *Pectobacterium* spp. affecting zucchini as an under-reported disease
- Phytoplasma was very common in the Northern Territory
- Northern Territory has specific pest and disease concerns which are quite different to other areas in Australia

The information gained through these surveys helped formulate key information for the two management guides published from the project. These results allowed comprehensive reference tables to be included whereby each major growing district has a list of most found bacterial or viral diseases for each key commodity grown in that district. This provides a quick reference guide for growers or agronomists wishing to check what is likely to occur in their region on their crops.

Seasonal activity of insect vectors

The seasonal activity of insect vectors, virus and phytoplasma was monitored in several sites over several seasons. A summary of information collected from seasonal activity monitoring is provided below and for further details refer to Appendix 2. Results from these monitoring activities were used to inform management recommendations provided in the 'Guide to understanding and managing virus diseases of vegetables'.

The overall outcome for industry from these activities is highlighting the variability between different regions and seasonal variation within a region and thus a need to build local knowledge through regular monitoring. Virus and phytoplasma disease outbreaks are highly site specific. As the insect vectors vary in their dominance due mostly to seasonal conditions so do the likely dominant disease. Regular monitoring can also identify high-risk planting windows where disease is likely for more seasons than not. To better manage disease, improved understanding of seasonal variations and ongoing monitoring is recommended. Building good local knowledge, preferably on-farm, linked with weather will allow more informed predication of likely disease outbreaks. This allows proactive management, if possible, for example, removal of key weed hosts or applying product to reduce insect populations on those weeds, if ongoing rain is forecasted which prolongs the weeds presence in the area. This reduces risk of transfer of viruses and phytoplasmas into newly planted crops.

Queensland: Leafhopper and Phytoplasma monitoring

The results from monitoring leafhoppers and phytoplasma are very important for industry as it is the first comprehensive research study conducted on these diseases affecting Australian vegetable crops. Prior to this, there was very little knowledge on what spreads phytoplasmas in vegetables or where the vectors are likely to reside outside cropping periods. Importantly, identification of the common brown leafhopper (*Orosius argentatus*) in association with phytoplasma disease outbreaks will hopefully counteract much of the misinformation circulating on what spreads

phytoplasma. In the monitoring district, spread of the pathogen has been blamed on Rutherglen bug and green jassids, despite no scientific basis for these claims. It is hoped, with reporting of the accurate data collected in this project, the misinformation will now stop, and growers can apply appropriate management strategies targeted at the known cause.

Additionally, the results show phytoplasma diseases and leafhopper populations vary a lot during the season and are not closely linked to each other. The peak in activity of the disease observed in December 2020 was unrelated to the peak in leafhopper numbers. For industry, this means use of insecticides to manage phytoplasma disease would not be effective as it would be difficult to predict when to apply insecticides. Further results identified blue heliotrope as an important 'out of season' host for both the leaf hoppers and the phytoplasma. Management of this key weed will provide useful control of phytoplasma.

Queensland: Thrips and orthotospoviruses

Tomato spotted wilt virus (TSWV) and *capsicum chlorosis virus* (CaCV) are both known to occur in the dry tropics production area of QLD and cause disease in capsicum and chili. TSWV is normally the predominant orthotospovirus present in this district and caused significant impacts to tomato crops until resistant varieties were introduced. Since the commencement of this project in 2018, it has become evident that CaCV has also become an economically impacting disease in this area. Similarly, in the Bundaberg district, although both are present, CaCV is the dominant virus. There is no known crop-host resistance to CaCV. The reported vectors of CaCV are *Thrips palmi* (melon thrips)*, Frankliniella schultzei* (tomato thrips) and *Microcephalothrips abdominalis* (composite thrips) (Persley et al. 2006). Both *F. occidentalis* (Western flower thrips, WFT) and *F. schultzei* vector TSWV. An understanding of the vector populations in relation to virus dominance is very important to manage disease, particularly as WFT requires more expensive chemicals for control. The two viruses also have very different host ranges, thus the environmental sources of the virus between cropping seasons are different. For TSWV it is well known that Jamaican snakeweed (*Stachytarpheta jamaicensis*) is a very important and widespread weed host for TSWV. At the start of the project the weed hosts of CaCV in this district were unknown. Blue billygoat weed (*Ageratum houstonianum*) is the known important weed host of CaCV in Bundaberg but it does not occur in the dry tropics.

Thrips monitoring in sticky traps was somewhat effective to generate broad data on potential vector flights, however, the lack of accurate identification complicates interpretation of the data. The trapping showed there was no significant difference in the use of blue versus white sticky traps and that there were big differences in seasonal variation of thrips during the study and this also varied between location. Virus incidences at these locations was low during the project, except for 2018 where incidences were moderate, and the virus was mostly TSWV. This meant attempts to correlate thrips numbers with virus incidence was not possible from this monitoring activity.

During 2022, regular virus incidence counts were done on a property in Gumlu and one in Bowen, in close collaboration with the agronomists from those businesses. Virus incidences ranged from <1% to about 10% on these properties. Results from a single survey of a reported badly affected crop near Gumlu showed virus incidence of 50%. CaCV was detected in almost all samples collected from these crops, with TSWV only rarely found and not until June surveys. Additionally, thrips monitoring in crops were completed but very low insect numbers were collected from any of the capsicum crops. The most was detected in an organic crop, but these were still quite low.

Weed surveys commenced in November 2021 and continued through to June 2022. New weed hosts for CaCV were detected and in some instances tomato thrips were present on the weeds. Molecular and morphological methods identified the key weed as *Praxelis clematidea*.

Surveys of the weeds and capsicum crops for thrips failed to detect WFT in Bowen or Gumlu. This species was however, detected in capsicum crops near to the townships of Ayr and Clare in July surveys but in significantly lower numbers than tomato thrips.

These results are very important for industry as they highlight a significant change in virus dominance in this district. Capsicum has resistance genes for TSWV, but none are commercially available for CaCV. It also shows that despite good thrips management in crop, virus diseases can still occur at economically damaging incidences. The 2022 growing season was unusual as there was continual and sporadic rainfall into winter, whereas normally the wet season is finished by early March. This continued moisture has resulted in ongoing weed presence well into the production window and thus its probable the virus incidence is from continual primary spread via ongoing thrips migration from the weeds. Similar disease outbreaks occurred in this district in tomato with TSWV during the 2014 season due to late rainfall, economic

impacts during that season were significant. A switch to TSWV-resistant tomato varieties has prevented further outbreaks. The results showing dominance of tomato thrips over WFT is also important for managing this as a pest in the district.

A summary of this research was provided to industry stakeholders on the 27th of July in Gumlu. Over 20 participants attended the presentation, which was facilitated by Nutrien, Ayr.

Reference: Persley DM, Thomas JE, Sharman M (2006) Tospoviruses—an Australian perspective. Austral Plant Pathol 35:161–180. https://doi.org/10.1071/AP06015

Vector trapping and virus monitoring in Carnarvon, WA

Monitoring of virus vector populations in Carnarvon during the project highlighted the variability in the dominance of the major insect groups studied. This in turn results in variability of which major virus disease is likely to be present.

Thrips were the most caught vector in the district and were almost continually present on the traps. Peak catches of thrips were variable throughout the study period with the maximum number recorded in Sept (2019), Dec. (2020) and March (2021) with over 100 insects per trap. Only the tomato thrips (*Frankliniella schultzei*) and western flower thrips (*F. occidentalis*) were found on vegetable crops. A major outbreak of *tomato spotted wilt virus* (TSWV) in capsicum occurred in June to Sept. 2020, when thrips levels were high.

Whitefly were also commonly seen in the district; however, no whitefly transmitted diseases have been recorded so their presence is of low concern to the region. The whiteflies caught at several properties were identified as *Bemisia argentifolia* (formally MEAM-1 or B biotype of *B. tabaci*). Leaf hopper of unknown species were detected throughout the project, these can spread phytoplasma diseases which were seen on several vegetable crops (capsicum, pumpkin, eggplant). While this disease was commonly found it rarely exceeded 1% incidence in the crop so was of low economic impact.

Of most concern were aphid numbers and the spread of virus diseases such as *zucchini yellow mosaic virus* (ZYMV; seen in all years of the project) and *cucumber mosaic virus* (CMV; seen in capsicums in 2021). Aphids were present in two major waves (Feb. 2019 and Sept. 2020) in events that were linked to rainfall in the district. Their numbers were also highly variable amongst traps on different farms, however during the periods of peak numbers there were present on nearly all traps. The identity of 192 aphids caught flying over vegetable crops were determined. Of these, the green peach aphid (*Myzus persicae*) was the most detected (>40%), while the majority of the remainder were turnip, oat or blue green aphids. All aphids detected (except the rice root and *Hyperomyzus carduellinus*) were able to transmit ZYMV, while the melon aphid (the only aphid which regularly colonizes cucurbits) was only rarely detected (2.6%). This means that monitoring cucurbit crops via leaf inspections will miss the large majority of aphids which can spread the virus and trying to control virus infection by spraying systemic insecticides will largely fail (as the non-colonizing aphids transmit the virus before being killed by insecticides).

Aphid and virus monitoring near Perth, WA

Monitoring of aphids and virus near Perth, showed some seasonal consistency during the period of study and highlights a high-risk planting window for carrots. Aphids were first seen in April (2019 or 2021) or May (2020), following first rains in the area. Numbers increased rapidly and peaked in September of each year. They had dropped markedly by October and were not observed on traps for the remainder of the year. Young carrots planted during this window each year developed high incidences of virus (near 100%). Management of aphids in these crops was primarily through systemic insecticides.

A total of 318 caught aphids the majority were the oat aphid (*Rhopalosiphum padi*) and green peach aphid (*Myzus persicae*) with 8 other species representing approximately 25% of the aphids caught. For several species of aphids, the efficiency of the aphid to spread *Carrot virus Y* was determined and from these numbers *M. persicae* was identified as the vector contributing the most to the epidemics. This knowledge can help in targeting sprays to key areas, as the grass colonizing aphids (*R. padi* and *R. maidis*) do not contribute significantly, while the legume and brassicae colonizing aphids (*M. persicae*, *L. pseudobrassicae* and *A. kondoi*) together contributed >98% of virus spread. It was also noted the percentage of *M. persicae* changed on traps, generally increasing in both absolute number caught and percentage of the population over time.

Disease management trials

The major outcomes from the disease and pathogen trials are provided below. Key findings are captured in the two disease guides published from the project. Further details on the trials are provided in Appendix 3.

Genetic tolerance to potyviruses affecting zucchini

Potyvirus infection is a major limiting factor to zucchini production in many Australian production areas. *Papaya ringspot virus* (PRSV) type W is the main virus species found in Qld while *zucchini yellow mosaic virus* (ZYMV) dominates in WA. Surveys in the Swan Hill/Mildura area (Victoria) several years ago identified *watermelon mosaic virus* (WMV) as the dominant virus species in zucchini in the region. Non-persistent aphid transmission and the abundance of host crops frequently results in very high disease levels by early flowering. Affected crops have reduced fruit set and high numbers of deformed unmarketable fruit.

Previous work some five years ago demonstrated the value of tolerant zucchini varieties in reducing the impact of virus disease in the crop. In project VG 16086, the work has been expanded and new generation varieties compared with those previously available. Three trials were completed in 2019, at Gatton (January, DAF QLD), Bundaberg (April-June, Agreco Australia) and Bowen (August-September, Prospect Agriculture). The aim was to assess varieties for tolerance in the presence of PRSV. In 2020 in WA, field trials were completed in the Carnarvon and Kununurra districts using natural aphid inoculations and glasshouse trials done in south Perth using artificial mechanical inoculation. Glasshouse plants were evaluated for virus titre in addition to symptom development.

Conclusion: At least six zucchini varieties were identified as having excellent tolerance to PRSV in QLD. All are worth evaluation by growers on their own properties to decide which varieties are best suited to the local environment and market requirements. The varieties highly tolerant to PRSV also performed well against ZYMV in trials in WA. Four of the six varieties were confirmed to have high tolerance to ZYMV as well. These were Baily, Desert, Apollonia, and Alessandra. Not all varieties evaluated in QLD were evaluated in WA and vice versa. WA glasshouse trials showed the level of virus detected in the zucchini correlated highly with leaf and fruit symptoms and was greatly reduced in the highly tolerant lines versus susceptible controls (up-to 10,000-fold less). This would suggest that these lines have potential to reduce virus spread as the ability for aphids to acquire virus from, and transmit to, other susceptible cucurbit plants are much lower than the susceptible varieties.

Further work included a side-by-side comparison of selected resistant varieties to the four main cucurbit potyviruses affecting Australian zucchini. Screening of zucchini varieties for potyvirus resistance was completed as a replicated trial with 12 varieties evaluated for their resistance to the four major Potyviruses which infect them in Australia (PRSV, WMV and 2 strains of ZYMV). Results showed durable resistance to all four viruses was present in varieties available to growers in Australia. This included a reduced number of plants which became infected, and reduced symptom (leaf and fruit) severity in plants which did become infected. The replication of the virus in these plants was greatly diminished which would also reduce the chances of aphids spreading the virus to other plants in the field. No single variety was the best performing for all four viruses, so growers need to choose from those most suitable for their district.

These varieties were also evaluated in the field for ZYMV resistance in Perth (2020), Carnarvon (2020, 2021) and Kununurra (2021). Consistent results were obtained in Carnarvon in both years, however a lack of virus in Kununurra and Perth occurred when the trials were held. Few plants of resistant varieties developed symptoms, and in those that did they were milder. Fruit from infected plants were often marketable, particularly in times of high disease pressure. Industry standard varieties by comparison were almost completely infected and no marketable fruit developed in the trial.

Management of potyvirus in zucchini without genetic tolerance

Potyvirus infection is a major limiting factor to zucchini production in many Australian production areas. *Papaya ringspot virus* (PRSV) type W is the main virus species found in Qld while *zucchini yellow mosaic virus* (ZYMV) dominates in WA. Surveys in the Swan Hill/Mildura area (Victoria) several years ago identified *watermelon mosaic virus* (WMV) as the dominant virus species in zucchini in the region. Non-persistent aphid transmission and the abundance of host crops frequently results in very high disease levels by early flowering. Affected crops have reduced fruit set and high numbers of deformed unmarketable fruit. Insecticides are seldom effective in reducing virus spread and crop hygiene to reduce inoculum levels is often poorly implemented. Options for virus management without crop genetic tolerance are limited to

insecticides, barrier crops, biological control agents and crop management. Multiple field trials were completed to evaluate this.

Conclusions:

- Barrier crops are useful to reduce virus spread into crops
- Establishing barrier crops as banker plants for biological control agents is difficult to do and requires further work to determine a good method for this, particularly at commercial farm-scale
- Secondary spread within crops is high and mostly due to mechanical spread from handling plants during crop monitoring
- Insecticides alone do not significantly reduce virus impact – reduction in the amount of rejected fruit was at best 17% compared to the tolerant variety which reduced this to 86.3% when managed with a traditional spray program
- Reducing adult aphid populations on old crops prior to their destruction will reduce spread to new crops

The recommended integrated management strategy for potyviruses in zucchini is:

The critical management point for control of potyviruses in commercial crops is firstly to prevent primary introduction into the crop, which is from weeds, older affected crops, infected seedlings, or volunteer plants from the previous crop. To reduce risk of transfer from external sources, remove these plants from nearby or treat them to reduce insect numbers. This should be done routinely.

Alternatively, using barrier non-virus host plants or fallow areas between and around crops will reduce spread of the viruses. Barrier plants are effective to both intercept aphids to reduce numbers entering crops and to cleanse aphid mouthparts of the viruses if they are a non-host for the virus (e.g sorghum, millet, corn). Aphids entering crops after feeding on the barrier plants will no longer spread the viruses. The barrier plants can also be used to multiply and maintain biological control agents to assist in controlling aphid populations.

Crop resistance is available in pumpkin, cucumber and particularly zucchini. It is generally partial resistance so some plants may still develop disease, but economic impacts are largely reduced, particularly in high disease pressure situations. Virus levels in resistant zucchini is greatly reduced which decreases spread by aphids to other non-resistant crops for which resistant varieties are not available. The level of resistance to each of the three viruses (PRSV, WMV and ZYMV) does vary so it is important to determine which varieties will work best in each region.

If virus is detected in crops, additional actions are:

- Diagnostic testing of symptomatic plants to confirm it is a potyvirus that requires management
- Rouging of infected plants, plus at least two additional plants either side which are not showing symptoms – recommended for virus incidences of <1%
- Reduce physical damage of plants where possible as the viruses are spread in sap between plants
- Regularly clean harvest knives to reduce risk of transfer in sap
- Normal insecticides applications within crops to control aphid populations and prevent colonisation
- Mineral oil foliar sprays can help reduce spread of virus in crops as they disrupt the aphid feeding – apply additional sprays as needed to protect new plant growth
- Biological control agents, insecticides, pest oil or some combination of these products applied to external sources could also be useful to control aphid populations multiplying on those plants (seek advice from APVMA on registration for non-crop usage of products)

Cover crop and biofumigant brassicas as hosts of potyviruses affecting commercial brassica crops

The brassica family includes the widely grown vegetables cauliflower, cabbage and broccoli, the oil seed crop canola and a diverse range of radish, turnip and swedes. Several members of the latter group are used as biofumigant cover crops in vegetable crop rotations as part of disease management to combat soil borne fungal pathogens. Forage brassicas provide high quality animal feed when pasture quality may be low and are also used in pasture improvement and rotation programs. Almost all brassica types, including species used as biofumigant and forage crops, are susceptible to virus

diseases. The most common and damaging of these is *turnip mosaic virus* (TuMV), which is spread by aphids and has a wide range of host plants across cultivated and weed species of the brassica family.

The susceptibility of biofumigant and forage brassica species and varieties were assessed to gauge their likely virus tolerance or resistance. This information is also useful as an evaluation of direct impact of TuMV on the biofumigant/forage crop performance. Details of the trial are provided in Appendix 3.

Conclusions:

- a wide range of susceptibilities to TuMV was detected across a range of genera, species and varieties of brassica - this indicates possible genetic segregation of the plant lines
- mustards ranged from no symptoms to very severe
- turnips, radish and rape plants were susceptible, from moderate to very severe

Recommendations: As several of the brassicas used for biofumigation, forage or as cover crops are susceptible to TuMV, care is needed when planting subsequent brassica vegetable crops to ensure any reservoirs of virus and/or aphids are destroyed prior to planting. This will prevent transfer of the virus from those crops to the vegetable crops.

Management of viruses in brassicas with insecticides

Virus diseases are prevalent in brassica crops in south Queensland, with Chinese cabbage (wombok) and daikon being particularly affected. The most prevalent virus in crops is turnip mosaic (TuMV) which is spread by aphids, in a non-persistent manner. This virus infects most cultivated brassicas and several weed species in the family. Aphids can spread the virus very quickly with the insect needing to feed for less than a minute to obtain the virus from an infected plant or introduce it into another plant as it feeds using a needle -like stylet. There are limited management options for the virus because of the potential for rapid spread, very few resistant or tolerant varieties and the prevalence of alternative weed and crop hosts. A field trial was completed at the Gatton Research Facility, to assess three insecticide treatments for efficacy against TuMV, in wombok (cv Matilda). This included a new chemistry insecticide and two pest oils. Details of the trial are provided in Appendix 3.

Conclusions:

- None of the products compared in the trial reduced TuMV spread effectively.
- Use of these products in other ways may provide useful options for control of these viruses, for example, use on old crops to decrease adult aphid levels before crop destruction. This would reduce risk of virus transfer into new crops. However, this would require evaluation before recommendation.

Recommendations: The critical management point for viruses spread by aphids in brassica crops is to prevent primary introduction. This will be from weeds, volunteer plants or older affected crops. To reduce this risk, remove these plants from nearby or treat them to reduce insect numbers. This should be done routinely.

Alternatively, using barrier non-virus host plants or fallow areas between and around crops will reduce spread of the non-persistently transmitted TuMV. Barrier plants are effective to both intercept aphids to reduce numbers entering crops and to cleanse aphid mouthparts of the viruses if they are a non-host for the virus (e.g sorghum, millet, corn). Aphids entering crops after feeding on the barrier plants will no longer spread the viruses. The barrier plants can also be used to multiply and maintain biological control agents to assist in controlling aphid populations.

If virus is detected in crops, additional actions are:

- Diagnostic testing of symptomatic plants to confirm it is TuMV, TuYV and/or CaMV that requires management
- Rouging of infected plants, plus at least two additional plants either side which are not showing symptoms – recommended for virus incidences of <1%
- Normal insecticides applications within crops to control aphid populations and prevent colonisation
- Biological control agents, insecticides, pest oil or some combination of these products applied to external sources could also be useful to control aphid populations multiplying on those plants (seek advice from APVMA on registration for non-crop usage of products)

Some anti-feeding insecticides may slow secondary spread of TuYV but will not be effective against TuMV or CaMV. Given secondary spread contributes little to virus disease outbreaks and these products have a very limited target range they are not recommended for use specifically as a virus disease control strategy. Usage should be limited to control insect populations as part of a routine spray program.

Further work is needed on aphid-transmitted viruses affecting brassica and lettuce crops to develop better management strategies. Research from this project on management of potyviruses affecting zucchini and the orthortospoviruses affecting capsicum/chili would provide a very high baseline for delivering similar management options for brassica and lettuce growers.

Orthotospovirus management in Solanaceae (QLD)

Tomato spotted wilt virus (TSWV) and *capsicum chlorosis virus* (CaCV) are both transmitted by thrips and cause disease in capsicum and chilli. Although resistance genes are available for TSWV in capsicum, their durability is questionable and evidence of resistance-breaking strains of the virus are reported nationally. CaCV was previously restricted in its distribution but is now emerging as an economically impacting disease in more areas. In the dry tropics of QLD during the 2018 season both viruses were detected in commercial crops at high levels and in Bundaberg, although both are present, CaCV is the dominant virus. There is no known resistance to either virus in chilli. The reported vectors of CaCV are *Thrips palmi* (melon thrips)*, Microcephalothrips abdominalis* (composite thrips) and *Frankliniella schultzei* (tomato thrips) (Persley et al. 2006).

Disease outbreaks are a function of virus incidence in weed hosts, thrips population levels and timing of planting. Worst case scenarios are where there is a significant overlap with weed populations with first plantings of crops. As the weeds dry off the thrips move into these crops carrying virus. If not managed appropriately, these crops then become a significant source of virus for subsequent crops. The management trial was aimed at assessing the importance of secondary virus spread within crops and to evaluate a range of management options to reduce orthotospovirus spread within chilli crops. Details of the trial are provided in Appendix 3.

The recommended integrated management strategy for Orthotospovirus management in Solanaceae is:

The critical management point for CaCV and TSWV in crops is to prevent primary introduction. This will be from weeds or older affected crops. To reduce this risk, remove these plants from nearby or treat them to reduce insect numbers. This should be done routinely. Consider resistant or tolerant varieties. Commercially available TSWV tomato varieties are generally robust, however, the TSWV resistance gene in capsicums can be overcome by resistance-breaking strains of the virus. There are no CaCV resistant crop varieties currently available. Chili and eggplant do not have resistance to either virus. If virus is detected in crops, additional actions are:

- Diagnostic testing of symptomatic plants to confirm it is TSWV and/or CaCV that requires management
- Identification of thrips to confirm there is a virus vector present and which species.
- Rouging of symptomatic plants – recommended for virus incidences of <1%
- Normal insecticides applications within crops to control thrips populations and prevent colonisation
- Thrips pupae (the dormant life stage between larvae and adults) do not feed on plants, and so they are not killed by systemic insecticides. Successive sprays often needed to eliminate newly emerged adult thrips. This should be done in crops only if larval stages are detected.
- Biological control agents, insecticides, pest oil or some combination of these products applied to external sources could also be useful to control thrips populations multiplying on those plants (seek advice from APVMA on registration for non-crop usage of products)

Reference: Persley DM, Thomas JE, Sharman M (2006) Tospoviruses—an Australian perspective. Austral Plant Pathol 35:161–180. https://doi.org/10.1071/AP06015

Glasshouse trial of *Pectobacterium* and *Xanthomonas campestris* pv. *campestris* in Wombok

Diseases caused by *Pectobacterium* spp., particularly in zucchini and brassicas, are identified as an emerging national concern. The diseases were detected in multiple growing districts nationally, with varying impact. Additionally, *Xanthomonas campestris* pv. *campestris* (Xcc) is known to cause disease in brassicas nationally. Both pathogens were

detected in a severe disease outbreak in Wombok in the Granite Belt, QLD in 2017. Investigation of bacterial survival between cropping seasons was evaluated with pot trials established to determine if either of the pathogens associated with the disease outbreak in Wombok could persist in soil in the absence of host material. Field soil was collected from a block where the badly affected crop (>70% disease incidence) had been incorporated into the soil. The soil was left for about three months and then wombok seedlings planted into the soil in a replicated trial with commercial potting mix used as the control. Details of the trials are provided in Appendix 3.

The trial showed:

- Seedlings were not infected by either *Pectobacterium* or Xcc direct from the stored field soil
- Seedlings were not infected by *Pectobacterium* from the field soil or potting mix when bacterial inoculum was added
- Seedlings were commonly infected with Xcc when the bacteria are added to potting mix (21/24 seedlings)
- Almost half the seedlings are infected with Xcc when the bacteria are added to the field soil (10/24 seedlings)

Conclusion: These results indicate that although newly planted seedlings can become infected by Xcc from soil infestation, the field soil is not a significant risk as a reservoir for this to happen. Only seedlings planted into pots of soil (either field or potting mix) deliberately infested with Xcc became infected. Those planted into either soil type without addition of the bacterial inoculum remained disease free. No plants became infected by *Pectobacterium* spp. in either trial.

Recommendations: Consider hot water treatment of seed to reduce risk of primary introduction into blocks. Allowing complete degradation of old crop debris and/or rotational cropping will mitigate risk of infection via soil from either of these pathogens. General control of insects is recommended, particularly chewing insects. Rouging infected plants at low disease incidence is likely to reduce disease spread, provided the crop debris is removed from the block and disposed of by deep burial or incineration. There are no known resistance genes for this bacterium in brassica crops.

Further work is needed, however, on managing disease outbreaks once they occur. This is through research to better understand the infection and spread processes of these pathogens which will ultimately lead to better management options.

***Pectobacterium spp.* causing crown rot in zucchini**

Diseases caused by *Pectobacterium* spp., particularly in zucchini and brassicas, are identified as an emerging national concern. The diseases were detected in multiple growing districts nationally, with varying impact. The source of disease outbreaks in zucchini were evaluated in separate studies in WA and QLD, with similar outcomes seen. Details of the trials are provided in Appendix 3.

Conclusions from the QLD trials:

- infections of zucchini seedlings are low risk from bacteria present in the soil
- transfer of bacteria on harvest knives from affected plants was not sufficient to initiate new infections, however, this may require further clarification

Conclusions from the WA trials:

- All varieties showed initial symptoms post-inoculation with *P. brasiliense* and in most varieties, this progressed to a brown soft rot of the stem, with necrotic streaks occurring down the petiole. In variety Regal Black this continued to cause complete plant collapse.
- Browning and some soft rots were seen with *P. carotovorum*, however, while this appeared milder externally, necrosis and browning occurred along the interior of the plant. No varieties displayed sufficient resistance which would likely be commercially useful.
- Other crop species common in Carnarvon where the zucchini soft rot disease was detected were evaluated for susceptibility to *P. brasiliense*. Some developed initial symptoms but no systemic wilt or collapse were seen
    - Pumpkins (including Jap, QLD blue and Butternut) and other cucurbits (watermelon, rockmelon, Luffa and cucumber)

- Capsicum, tomato and carrot

Recommendations: Consider hot water treatment of seed to reduce risk of primary introduction into blocks. Allowing complete degradation of old crop debris and/or rotational cropping will mitigate risk of infection via soil from these pathogens. The potential spread by insects also warrants further study before recommendations for insect control can be made. Rouging infected plants at low disease incidence is likely to have positive reductions in disease spread, provided the crop debris is removed from the block and disposed of by deep burial or incineration. There are no known resistance genes for this bacterium in cucurbit crops.

Further work is needed, however, on managing disease outbreaks once they occur. This is through research to better understand the infection and spread processes which will ultimately lead to better management options.

Data on pathogen and vector diversity

Significant advances in knowledge of the molecular and biological diversity were made for all targets selected.

Orthotospoviruses:

Investigation of resistance-breaking strains of TSWV (led by WA)

The *tsw* gene confers immunity resistance for capsicum against TSWV. Unfortunately, this resistance gene is readily overcome through genetic changes within the virus genome. The exact nature of these changes is unclear. Some work is published from overseas, however, the work was mostly completed in tomato using the *Sw-5b* resistance gene which has a different mode of action. As such, further work is required in this area.

In WA, a panel of TSWV isolates found breaking resistance in capsicum and other hosts, made during collections made in the project has been submitted for full genome sequencing, along with a selection of resistance breaking isolates from tomato, capsicum and snakeweed that were retrieved from the QLD virus collection, and collected from either Queensland or South Australia. 63 whole genome sequences were obtained altogether, with 51 known to cause resistance breaking.

Of the known or possible amino acid changes linked to TSWV resistance breaking, none of the sequenced isolates contained the *Sw-5* T-N mutation in the NSm protein position 120, and only one from WA contained the *Sw-5* C-Y mutation in the NSm protein at position 118. Mutations in the NSs protein at positions 74, 104 and 272 with potential links to resistance breaking the *tsw* gene in capsicum were also not found in any of these sequences. Manual inspection of the amino acid alignments of all sequences did not show any consistent amino acid changes that consistently correspond to either *Sw-5* or *tsw* resistance breaking in the isolates sequence here.

There is one amino acid change from E-G at position 694 in the RdRp gene which is found in 41 out of 51 resistance breaking strains we sequenced here. Importantly no other sequences from Genbank appear to show the same mutation, and this is observational only, further research would be required to determine if this is of any real significance.

A neighbor-joining phylogenetic tree of all coding complete genome sequences generated here, along with those available in Genbank was made, and showed no grouping of RB strains. This was as expected given the results of the comparison with the known RB sequences generated here.

This work links very closely with the activity listed below on host range and genetic diversity of TSWV. Phylogenetic trees made from all the new sequences generated from both WA and QLD did not yield different results to the trees produced from each data set separately.

Investigation of potential strains of TSWV affecting different vegetable commodities (led by QLD)

TSWV has an extremely wide host range, and this observed host range is not easily explained by only a single virus with low sequence diversity. Furthermore, the diagnostics used to detect TSWV are often done using assays designed to only one or two sites within the virus genome. The virus genome, however, is complex and comprised of three separate RNA components. The aim of this work is to investigate if there is re-assortment of these genome components between TSWV and another tospovirus which influences host range. It will also investigate if host range is influenced by sequence

variation that might be positively or negatively selected for by individual plant host species.

Full coding sequences of 31 isolates of TSWV were obtained through HTS sequencing, with a combination of RNA sequencing and HTS of overlapping PCR fragments for each genome segment. The isolates were from crop plants, weeds and ornamentals. Phylogenetic analysis of the complete coding regions yields no specific clustering based on host, location or year of collection with all isolates between 94.7 to 99.9% similarity. The most diverse was an isolate from capsicum from Perth in 2010 with a maximum similarity of 95.9% to the other isolates. There are three isolates that had quite a different glycoprotein coding region (93.6% to 96.3% similarity) two from Virginia, South Australia from basil and lettuce, and one from spider lily from south-east Queensland. No reassortment of TSWV isolates with other tospoviruses was detected. Like WA's results, no known resistance breaking amino acid changes were detected in any isolate sequenced.

Genetic diversity of CaCV (led by QLD)

CaCV is known to cause impacts to capsicum crops, particularly in QLD. Outside of this area there are only a few records across northern Australia and a recent detection during surveys for VG16086 in Mildura, Victoria. To date, no full genome sequences are available of this virus and genetic diversity studies are very limited. The virus is an emerging threat in new districts in QLD and possibly elsewhere.

Virus isolates from QLD, NT, WA, and VIC were sourced and include a range of older isolates from when the virus was first detected, to recent ones collected as part of VG16086 disease surveys. Twenty-four isolates were selected from the from the DAF QLD isolate collection spanning from 1992 through to 2019. These are from both crop and weed hosts and spread geographically from Northern NSW to the Atherton tablelands, QLD plus a single isolate from WA. Single primer sets have currently been designed and tested to amplify the complete S (approximately 4 kb) and M (approximately 5 kb) segments of CaCV, and both primer sets appear to work well, though the M segment does not amplify as highly due to its larger size. Further primers were designed to split the M segment into two ~3kb segments for better amplification, as well as three primer sets to amplify the L segment into three overlapping ~3 kb amplicons. Only eight isolates yielded enough HTS sequencing data to assemble complete coding regions. The isolates were 97.3% to 99.5% similar across the complete coding regions. The coding regions of the M segments of the more recently collected isolates, capsicum from Gumlu (2019), and pineapple from Yandina (2016) had more mutations than the rest of the sequenced isolates with 96.3% – 97.6% similarity versus 98.7% – 99.4% for the other isolates. It is unknown if the amino acid mutations in the glycoproteins would affect thrips transmissibility.

Genetic diversity of INSV, a recently introduced tospovirus for Australia (led by VIC)

Impatiens necrotic spot virus (INSV) is transmitted by western flower thrips (*Frankliniella occidentalis*) and is associated with necrotic spot disease mainly in ornamental plants. INSV has been important virus in the ornamental industry overseas and was first detected in Australia in 2010 infecting several ornamental species in a commercial nursery in New South Wales. Subsequent INSV outbreaks were detected in 2018-2020 in commercial lettuce production in NSW associated with lettuce necrosis and collapsed diseases. In 2020 and 2021 INSV was detected in ornamental plants in Victoria, but it has not been detected in lettuce or other vegetable crops in this state. The full genome of 14 lettuce isolates from the 2018-2020 outbreak in NSW and one begonia isolate from NSW collected in 2010 were sequenced and the lettuce isolates shared >99% nucleotide identity, regardless of the year they were collected, suggesting spread after a single introduction. The lettuce isolates share less than 97% nucleotide identity across the L, M and S genome segments with the NSW begonia isolate and with other published isolates from ornamental plants from other countries, indicating that spread from the original outbreak is not the cause of its occurrence in lettuce.

Separate phylogenetic analysis of each of the full nucleotide sequences of the three genome segments (L, M and S) showed that the lettuce isolates with published isolates clustered with isolates from China and Korea sharing 99% nucleotide identity, indicating a possible introduction from Asia (Figure 1). The Australian begonia isolate was 99% identical and clustered with other INSV ornamental isolates from USA and away from other Australian lettuce isolates.

Sequence analysis identified some unique amino acid changes across all the three genome segments within the lettuce isolates in comparison to all available published isolates and the Australian begonia isolate. In particular, four amino acid changes on the RNA-dependent RNA polymerase (RdRp) encoded by genome segment L, occurred only in the Australian lettuce isolates and their biological significance is not known.

Three amino acid changes were observed in protein encoded by the M segment of the Australian lettuce isolates, one in the non-structural movement protein (Nsm) and two in the glycoprotein (GnGc), and these changes also occurred on three isolates of ornamental species from China and Korea. These results provide some further evidence for a common evolutionary history and origin between the Australian and Asian isolates.

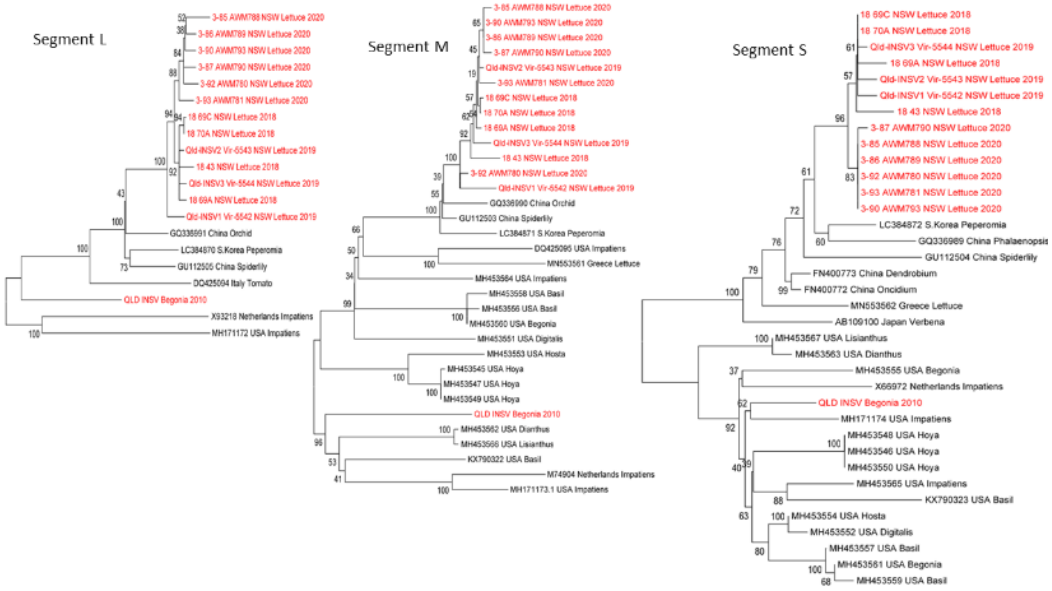

Figure 1 Phylogenetic analysis of the complete nucleotide sequences of *Impatiens necrotic spot virus* (INSV) genome segments L, M and S from Australian lettuce and begonia isolates (in red font) and published isolates from GenBank

Amino acid changes were also observed in Australian lettuce isolates nucleocaspid (N-gene) and non-structural protein (NSs) encoded by the INSV segment S. A single amino acid change was observed in the N-gene of Australian isolate which was similar in a Greek lettuce isolate and this change unique to lettuce only could be important in INSV ability to infect lettuce. Two changes were also observed in the NSs of some Australian lettuce isolates whereby one amino acid change was only observed in 2018 and 2020 isolates and the other change was only observed in the 2020 isolates. These varied changes observed amongst the NSs of Australian isolates through successive years could indicate ongoing INSV evolution or presence of two genetic strains of INSV in NSW.

Further monitoring of these evolutionary changes in subsequent years and their biological impact in the epidemiology of INSV in lettuce is required. Additionally, further sequence comparison of INSV lettuce isolates and other Australian ornamentals INSV isolates is required to better inform incursion pathways and genetic diversity of INSV in Australia. The Victorian ornamental INSV isolates will be sequenced and compared to the lettuce and Begonia isolates sequenced in this project, as part of the Hort Innovation funded project “NY19007 Improving surveillance strategies for orthotospoviruses and thrips to enhance the biosecurity of the nursery industry”, this information will be sued to inform the number of introductions and the regions from which they may have been introduced, which could assist in identifying risk pathways. The sequence information will also be used in NY19007 to develop in-field molecular assays from INSV detection

Genetic diversity of iris yellow spot virus (IYSV) (led by VIC)

Iris yellow spot virus (IYSV) is vectored by onion thrips *(Thrips tabaci)* and associated with lesion and necrotic spot disease in *Allium* species. IYSV was first detected in Australia in 2002 in commercial *Allium* crops in WA, NSW and VIC. The full genome of one isolate from a Victorian detection in 2019 and another sourced from Qld have been sequenced. The full genome sequence of the three genome segments of these two IYSV isolates share 98% nucleotide identity. Anecdotally, IYSV continues to impact yield in onion crops in South-Australia and further studies are recommended to better understand IYSV genetic diversity and epidemiology in Australia.

Genetic diversity of Beet pseudo yellows virus (BPYV) (led by VIC)

Beet pseudo-yellows virus (BPYV) is associated with yellowing disease in in a wide range of vegetable crops such has cucurbits, capsicum, lettuce, endive, carrot, spinach, and beet. The virus also infects ornamental crops and several weed species. BPYV is transmitted in a semi-persistent manner by greenhouse whiteflies, and it is an important virus for vegetable glass-house production across Australia.

The full genome of ten BPYV isolates sourced collected by the QLD team and by the VIC team from cucumber (6) in SA and QLD and various weeds (4) from QLD have been sequenced. Sequence analysis of RNA1 from Australian and published international isolates showed a wide diversity range of 80-99% nucleotide identity. The RNA1 of eight Australian BPYV isolates shared >98% nucleotide identity with each other and to other isolates available on GenBank. Two nettle isolates collected in QLD, which had 89% nucleotide identity with each other and were most closely related to Qld cucumber isolate 2841 with an identity of 97 and 90% than any other isolates. The nettle isolates and cucumber isolate 2841 had <89% nucleotide identity to the RNA1 of other Australian and published isolates (Figure 2).

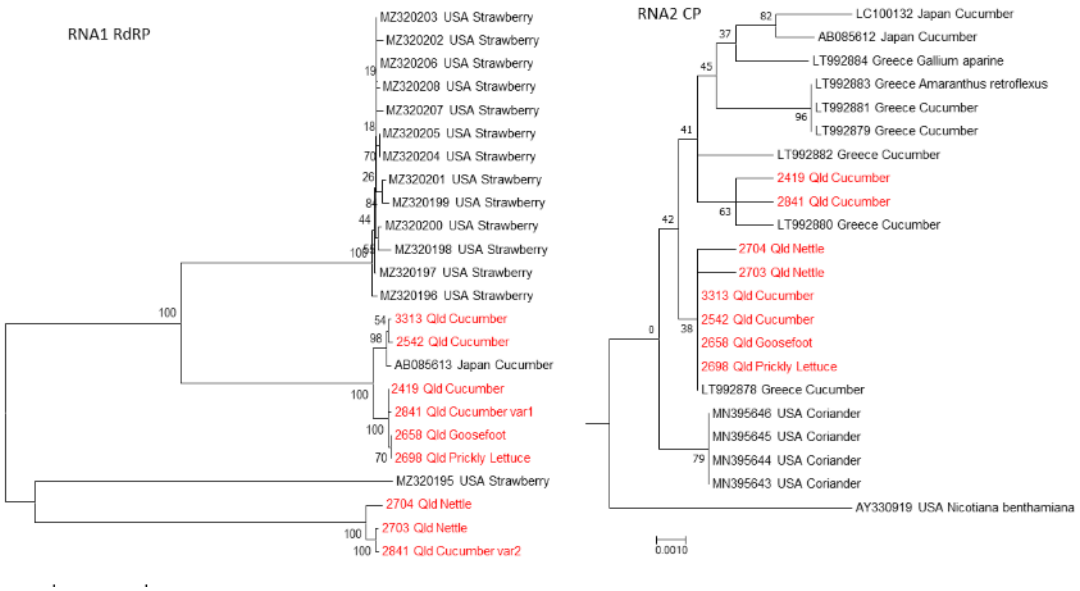

Figure 2 Phylogenetic analysis of *beet pseudo yellows virus* (BPYV) RNA1 RNA dependant RNA polymerase (RdRp) and RNA2 coat protein (CP) nucleotide sequences from Australian isolates (in red font) and published isolates from GenBan

Less diversity was observed amongst Australian and published BPYV isolates when RNA2 was compared, and all isolates shared >98% nucleotide identity. Phylogenetic analysis of BPYV coat protein (CP) nucleotide sequences (RNA2) showed one cluster containing the two nettle isolates and a second cluster containing the Australian isolates including cucumber isolate 2841 (Figure 2). Based on the phylogenetic analysis of BPYV RNA1 and 2, RNA dependent RNA polymerase (RdRp) gene on RNA1 was found to be more informative regarding diversity of Australian isolates sequenced. The RdRp phylogeny indicated occurrence of two BPYV populations, however, few isolates only from Queensland were analyzed. Further sequencing of isolates from other plant species, such as strawberry, which can act as an alternative host and isolates from other states is required to better understand the genetic diversity of BPYV and its epidemiology in Australia.

Genetic diversity of phytoplasma (PhD student Bianca Jardim)

Australia-wide survey of phytoplasma species diversity and host range were carried out as part of the phytoplasma PhD attached to this project. A total of 340 samples that include vegetables and weeds were tested for phytoplasma and 190 samples tested positive. The 16SrII phytoplasmas remain the most frequently detected group of phytoplasma. A total of

240 contemporary and historic phytoplasma-positive samples have been analysed using high throughput sequencing (HTS). The genomes of isolates within the 16SrII phytoplasma taxonomic group have been assembled using a metagenomic pipeline developed in the project and are currently being analyzed as part of a review of 16SrII group phytoplasma taxonomy. Preliminary results suggest at least four distinct phytoplasma species occur within the 16SrII specimens collected in Australia. A potential host range expansion has been identified for Vigna little leaf phytoplasma (ViLL), an undescribed 16SrII taxon that has been detected in bitter melon.

Genetic diversity of *Pseudomonas syringae* pv. *syringae* affecting zucchini (PhD student Noel Djitro)

A disease outbreak in zucchini caused by Pseudomonas syringae clade 2b was identified in Bundaberg, Australia for the first-time during autumn 2016 with symptoms including twisted petioles, necrotic leaves, crown-rot and internal fruit rot. To investigate the genetic diversity of 11 Australian isolates obtained from the outbreak, the genomes were compared to the publicly available *P. syringae* strains in phylogroup 2.

A phylogenetic tree classified the Australian isolates into clade 2b-a and refined clade 2b-a into four clusters. Pathogenicity assay showed all three isolates can infect pumpkin, squash, watermelon and zucchini var. Eva with different severity. Isolate 77-4C was not able to infect zucchini var. Rosa and only isolate KFR003-1 can infect rockmelon. This study supports the association between four type 3 effectors (avrRpt2, hopZ5, hopC1 and hopH1) and the susceptibility of some cucurbit hosts. The Biolog (biochemical utilization) profiles were similar for the isolates, with some differences in borderline usage observed between isolates 77-4C, KL004-k1 and KFR003-1. Identical phytotoxin and siderophore profiles were observed amongst all isolates in clade 2b-a, however, a difference in fluorescent phenotype was observed in one genetic clade. This difference is thought to be caused by a premature stop codon in one of the gene of pyoverdine biosynthesis pathway. Carbohydrate active enzyme profile was similar between clusters apart from the GH19, GH24, GT23 and GT4 families. Pan-genome analysis revealed a total of 22 orthologous groups of genes that are unique to clade 2b-a and six genes associated with *P. syringae* phylogroup 2 isolated from *Cucurbitaceae*. Most of clade 2b-a unique orthologous group of genes, some of carbohydrate active enzyme, type 3 effectors, syringolin biosynthesis pathway and type 6 secretion system were identified in region of genome plasticity.

The study clarified the taxonomy and provided improved virulence and genome-wide association study data of *P. syringae* clade 2b-a. This genetic analysis underpins the biological data of host range and informs industry on likely risk for rotation of cucurbit crops, particularly, high value melon crops following affected zucchini crops would be ill-advised.

Genetic diversity of viruses in weeds (PhD student Joanne Mackie)

As part of the PhD entitled 'Targeted surveillance strategies to support area wide management of viruses in vegetable crops'. *Cucumber green mottle mosaic virus* (CGMMV) isolates from vegetable, pollen and weed samples from NSW, NT, Qld, SA, WA and several contaminated imported seed lots, intercepted during routine seed testing, were characterised using HTS. HTS analysis of 100 hive-collected pollen samples received from NT detected the presence of CGMMV in 26 samples and papaya ringspot virus (PRSV) in two samples. A further 26 CGMMV samples have been sequenced using HTS. A total of 35 full genomes of Australian CGMMV isolates have been successfully assembled and analysis of the diversity, which is very low (<0.6% nucleotide differences) suggests that there has been a single introduction of CGMMV or a few introductions from single source into Australia. Nanopore sequencing using a MinION instrument and chemistry (Oxford nanopore technology) for targeted multiplex RT-PCR tiled amplicon sequencing is being developed in this PhD for targeted CGMMV detection. The method has been used to successfully detect and confirm or complete the genome arrangement of CGMMV in pollen, seed, and leaves. Validation using seed and leaf sample sets and comparison with short read (Illumina) HTS, qRT-PCR and ELISA will be carried out and form the basis of a methods paper for detection of CGMMV using.

Genetic diversity of of TuYV (PhD student Muhammad Umar)

Over 100 isolates of *turnip yellows virus* (TuYV) were obtained from pea and weed samples. Two distinct parts of the genome were examined to determine the genetic variability of TuYV isolates with sequences for both obtained from c. 75 isolates. There was a significant amount of diversity present among the Tasmanian TuYV isolates when compared with some evidence of recombination. Phylogenetic analysis of TuYV revealed that the isolates obtained from weed plants were closely related to the isolates reported from the pea crops.

Diversity of leafhoppers in the Granite Belt, QLD

Collection of leafhoppers in the Granite belt in tomato and capsicum crops affected by phytoplasma and from weed reservoirs commenced in summer 2019/2020. At the start of the project there was no information on the relative abundance of phytoplasma insect vectors within the district, nor of their identity. Monitoring during the project has significantly improved knowledge of both, and additionally in the incidence of phytoplasma in crop and weed reservoirs of the pathogen and its vector. Five different morphospecies were collected during the surveys. These are five groups based on different morphology and include potential phytoplasma vectors.

The two potential vector species, *Orosius argentatus* and *O. orientalis* were detected in the district. These are morphologically very difficult to differentiate, molecular bar coding was completed on 62 individual insects from the November 2020 collection to determine the relative abundance of them both. The analysis used the COI barcode gene and identified 59 of the 62 individuals as *O. argentatus*, two as *O. orientalis* and one as *O. canberrensis*. The last species, *O. canberrensis*, is morphologically very close to *O. argentatus* and its status as a vector for phytoplasma is unknown.

Diversity of thrips vectors in the Dry Tropics, QLD

Samples of thrips collected from sticky traps and flower samples from the dry tropics in QLD were evaluated morphologically and genetically. The samples were collected over several seasons. The species of virus vector thrips detected included tomato thrips (*Frankliniella schultzei*) and onion thrips (*Thrips tabaci*). No evidence for the presence of Western flower thrips (*Frankliniella occidentalis*) was found, however, the samples were not comprehensively evaluated.

Thrips identification morphologically from sticky traps is very difficult. Pale thrips which were Frankliniella-like were molecularly identified as onion thrips from at least two traps. Additionally, the literature reports evidence that the current *F. schultzei* species should be divided into two species, the morphologically pale form to be reclassified as *F. sulphurea*. There is molecular and biological evidence to support this with reports of different virus transmission efficiencies by the proposed two different species. Gene sequences derived from public databases and generated through the project from specimens collected in the Dry Tropics and elsewhere is assisting in further clarifying this. Generating good quality DNA from thrips specimens and amplifying bar-code genes was more difficult than expected. As such further work is needed to develop molecular assays to detect the different species, especially to allow more accurate identifications to be made from sticky traps. Some if this work will be done in “NY19007 Improving surveillance strategies for orthotospoviruses and thrips to enhance the biosecurity of the nursery industry”, by the PhD student based with Agriculture Victoria at La Trobe University, although the focus of the PhD is on thrips in temperate horticulture.

Recommendations: Further work is needed on thrips vector species in Australia and their relative efficiencies to transmit orthotospoviruses. The diversity of likely vector species found, and the complexity of this topic revealed within this project was completely unexpected and thus warrants further work.

Contingency plans on key exotic pathogens

The following contingency plans on six key exotic pathogens are prepared for upload onto to the Plant Health Australia portal:

1. Exotic Begomoviruses prepared by Paul Campbell and Cherie Gambley
2. Exotic Orthotospoviruses prepared by Denis Persley, Paul Campbell and Cherie Gambley
3. Exotic Tobamoviruses prepared by John Fletcher, Cliff Kinoti and Fiona Constable
4. Bacterial wilt of cucumber (*Erwinia tracheiphila*) prepared by Rebecca Roach, Nandita Pathania and Cherie Gambley
5. Bacterial blight of onion (*Xanthomonas axonopodis* pv. *allii*) prepared by Rebecca Roach and Cherie Gambley
6. Stewart’s wilt of corn (*Pantoea stewartii*) prepared by Rachel Mann, Cliff Kinoti and Fiona Constable

Diagnostic protocols field tested, and Ring-testing of diagnostic assays completed

A proposed diagnostic protocol validation and ring-test system was developed. *Cucumber green mottle mosaic virus* (CGMMV) in leaf/fruit tissues was used as the model and up two ELISAs, four endpoint RT-PCR assays and three RT-qPCR assays were tested by seven laboratories. The information was also used to assess the sensitivity of the tests. Each lab was sent seventeen samples, including ten CGMMV positive samples of varying concentrations, five negative samples and

one each of a known CGMMV negative and positive control and tested the samples using at least one of the molecular assays and an ELISA, if possible. Preliminary results of this CGMMV proficiency test indicate some variability within each laboratory which may be due to several factors such as one or a combination of differing extraction kits, molecular kits, equipment and primer quality. The results indicated that while the ELISA test was least sensitive compared to endpoint and RT-qPCR, it could detect virus in a dilution of 1/10000 of positive leaf homogenate in uninfected leaf homogenate. ELISA was more robust compared to endpoint RT-PCR and RT-qPCR methods as all labs that participated in using this method achieved similar results.

A second proficiency/ring-test was developed, in collaboration with the project *CMI C02897/C02755: Reducing constraints on the timely implementation of PCR tests for imported vegetable seeds for sowing,* to assess the detection of CGMMV, kyuri green mottle mosaic virus (KGMMV), zucchini green mottle mosaic virus (ZGMMV), cucumber fruit mottle mosaic virus (CFMMV) and melon necrotic spot virus (MNSV) in seed. A process for generating artificially pathogen contaminated seed was developed, because seed naturally contaminated by each of the viruses can be difficult to obtain. PCR evaluation of the artificially pathogen contaminated showed that CGMMV, KGMMV, ZGMMV and MNSV could be detected in the equivalent of one contaminated seed in a sample of 1000 seed using RT-qPCR and some endpoint RT-PCR assays, although the sensitivity is reduced compared to lower dilutions. This seed testing proficiency using the artificially pathogen contaminated seed produced by this project team members, is currently underway and scheduled for completion in May 2022. Results of this proficiency test will enable further validation of the detection protocols, and inform participating labs (EAMI, NSW) on their capability to accurately detect exotic seedborne pathogens and enable technical transfer of the protocols to the participating laboratories.

A standard template used to inform laboratories of test requirements was developed, along with a form that allowed each laboratory to provide sufficient detail about kits used, test start and finish times, etc. and the results of testing were developed. This latter form provides sufficient information to allow analysis and identify potential differences that can lead to different results between laboratories. The template and form can be modified for different pathogens and tests. A standard operating procedure was produced for for lyophilizing infected plant tissues and making serial dilutions for proficiency and ring testing and this was provided to members of the Diagnostic protocol working group (DPWG) of the Subcommittee on Plant Health Diagnostics (SPHD). A process for generating artificially pathogen contaminated seed has also been developed, because seed naturally contaminated by each of the viruses can be difficult to obtain. This has been used to aid ring testing of seed for several exotic pathogens. It was also used by Joanne Mackie (PhD student) to establish seed test panels to enable development of a novel tiled high throughput sequencing approach to detect and assemble full genomes of CGMMV in a single positive seed in different seed sample sizes.

In addition, at least 23 protocols were circulated for evaluation and feedback provided to the participating laboratories. This included seven PCR diagnostic protocols for bacteria (*P. syringae*, *Xanthomonas* spp., *Acidovorax* spp., *Pectobacterium* spp., *Ralstonia solanacearum* biovars and a generic 16S PCR for sequence identification of any bacterial species) and 10 PCR or qPCR assays and six LAMP assays were circulated for viruses and pospiviroids. This included generic endpoint PCRs for begomoviruses, orthotospoviruses, potexviruses, tobamoviruses and pospiviroids plus multiplex endpoint RT-PCRs for TYLCV and PLRV, specific endpoint RT-PCRs the brassica infecting viruses TuMV, TuYV and CaMV and as a triplex qRT-PCR assay (recently developed in this project). The LAMP assays included many that are in routine use (some of which were developed in this project) such as those for CaCV, CarVY, PMMoV, TSWV, LNYV, PRSV, ZYMV and CGMMV.

Available molecular tests for detecting and diagnosing *Agrobacterium* spp. and biovars were ring-tested. Unfortunately, the consensus for these assays was not favorable, reliable detection is not possible. The assay evaluated was that published by Puławska et al., (2006). The PCR and sequencing validation for this assay was not successful due to none-specificity of the assay, resulting in off-target amplifications. Further work is needed to develop a robust and accurate assay for *Agrobacterium* spp. as in addition to a need to accurately test samples from vegetable growers, some Horticulture businesses require phytosanitary clearance for this pathogen to export planting material (e.g blueberries).

Several other diagnostic protocols are under development and/or undergoing proficiency testing by Agriculture Victoria before circulating more widely. These include:

- Plant barcode ID - Maturase K gene for Caryophyllales PCR assay (Cuenoud et al., 2002) - PCR validated and confirmed by sequencing
- Plant barcode ID - Ribulose-1,5-Biophosphate Carboxylase/Oxygenase Large Subunit PCR assay (Levin et al., 2003; Kress & Erickson, 2007) - PCR validated and confirmed by sequencing

- ToBRFV RT-PCR assay (ISHI-Veg, 2019; Alkowni et al., 2019) - PCR validated and confirmed by sequencing
- KGMMV RT-PCR assay (Unpublished) – RT-PCR validated and confirmed by sequencing
- CGMMV (seed, fruit and leaves) ELISA and RT-PCR assay (Reingold et al., 2013; Ling et al., 2014; Berendsen, S. & Oosterhof, 2015) – ELISA and RT-PCR validated and confirmed by sequencing
- ZGMMV RT-PCR assay (Unpublished) – RT-PCR validated and confirmed by sequencing
- MNSV RT-PCR assay (Herrera-VÃ¡squez et al., 2009) – RT-PCR validated and confirmed by sequencing

In Western Australia, expanding the MALDI-TOF and BIOLOG databases has improved bacterial diagnostics. Due to recent changes in nomenclature from more frequent sequencing of bacteria, the identity of typed cultures needs verifying. For example, *Pectobacterium carotovorum* subsp. *carotovorum* was separated new species including *Pectobacterium carotovorum* and *Pectobacterium brasiliense*. So, we now need to determine if the MALDI-TOF and BIOLOG can separate these out enough to allow us to continue using these methods for identification.

A panel of 31 isolates of *Pseudomonas* species from three culture collections (NSW, Victoria and WA) have been sequenced to accurately determine identity including pathovar using the new nomenclature standards and once the analysis has been completed, these isolates will be added as standard isolates to the MALDI and BIOLOG databases.

Up to 117 typed bacterial cultures from the NSW culture collection were processed using MALDI-TOF and BIOLOG. In some cases, the results have come back matching by both methods. Where the results have been different isolates have been sequenced to confirm their identity. This type of analyses needs to continue as the national bacterial collections are large and these reference cultures underpin biosecurity decision making. The demonstration of using MALDI-TOF for the identification of bacteria through this project has encouraged other jurisdictions to explore the use of this equipment. The Commonwealth have now invested in the technology and will be creating a database of exotic bacteria that can be shared between jurisdictions that have access to this technology. This work was presented at the APPS meeting in November 2021 as an oral presentation.

The work in this project also enabled a comparison on the different methods used to identify plant pathogenic bacteria (Table 1).

Table 1 A comparison of different identification methods for bacteria including a cost estimate

	BIOLOG	MALDI	qPCR	Sequencing
Pros	• Provides biochemical reaction results • Easy to use • Now compatible with using LIMS and ISO 7025 requirements • Add to the database	• Results are very quick • Allows for a larger screener of bacterial isolates at once. • Can put up to 48 isolates on one plate • Can add to the database	• Quick turn-around time. • High-throughput and automation possible	• Provides most detailed info. • Additional analysis e.g. Phylogeny • Can use results to improve databases or update/develop new primers
Cons	• Can only identify bacteria isolates one at a time (ie 1 isolate per plate) • Database • Pipetting • Interpretation of results	• Database • Pipetting • Interpretation of results	• Interpretation of results • Only as good as the assay design • Current assays need sequence informed improvement	• Time to result • Interpretation is critical • Additional analysis can be time consuming
Time	3 days	1 day	1 day	Up to 1 week (or more)
Cost	$50 per plate/isolate	< $5 per target plate	$20.00 per assay*	$54 per gene region**

Reference: Puławska J, Willems A, Sobiczewski P (2006) Rapid and specific identification of four *Agrobacterium* species and biovars using multiplex PCR. Systematic and applied microbiology 29:470–9. https://doi.org/10.1016/j.syapm.2005.11.002

Recommendations for seed disinfestation and indexing

The investigation of seed disinfestation and indexing is largely completed, and an update was provided to members of the Imported Seeds Regulation Working Group, industry, and biosecurity agencies at the June 2021 Hort Connections Conference. This was via the two-hour workshop, 'Seed-how risky is it?' which was aimed at evaluating seed as a risk for entry of endemic and exotic pathogens. The workshop was organized in collaboration with Callum Fletcher, AusVeg.

The major outcome from the workshop was to continue the discussions on both disinfestation and indexing through a second workshop. This workshop is planned for early 2022 and will involve similar participants (about 90 participated in the first workshop). A summary of the workshop discussions and outcomes was circulated to all participants including Hort Innovation shortly following the workshop.

Additionally, discussion with Greg Chandler, Hort Innovation and David Dall, DAWE has progressed the topic through potential Hort Frontiers funding. A concept developed by Fiona Constable, Agriculture Victoria and Cherie Gambley, DAF QLD was tabled at the Vegetable Industry SIAP meeting in September 2021 and endorsed for further activity. The RFP AS1007 was discussed at a second workshop, 'Seed to production: improving efficiencies & reducing risk' being held in conjunction with the 2022 Hort Connections Conference. This RFP closed on the 29th July.

Extension activities: Grower forums and industry engagement; Factsheets; Benchmarking and case studies

Grower forums and industry engagement

Multiple industry engagements were held nationally during the project. The types of engagements included informal discussions on-farm during visits and phone calls, workshops on specific topics and webinars. There was significant impact on many events by COVID-19, particularly in Victoria and WA. The hands-on disease identification workshop was particularly successful with participants providing very positive feedback. Unfortunately, this was significantly impacted and instead of holding these nationally we were limited to QLD and one in Mildura, Victoria.

A list of the workshops and webinars held during the project is provided in Appendix 4.

Factsheets on key pathogens

The following factsheets were completed and available on the Hort Innovation Website:

- Area wide management of viral and bacterial disease of vegetables
- Virus disease of lettuce in Australia
- Lettuce necrotic yellows virus in temperate cropping areas of Australia
- Lettuce necrotic yellows virus in the Lockyer Valley
- Cucumber mosaic virus in vegetable crops
- Virus diseases of cucurbits in Australia
- Viruses infecting brassicas
- Managing virus diseases on zucchini
- Aphids spreading virus in brassicas and lettuce in the Lockyer Valley

Also available are the following resources:

- Understanding and identification of plant diseases: workshop presentation
- Understanding and identification of plant diseases: workshop slideshow
- A guide to understanding and management of bacterial diseases of vegetables
- A guide to understanding and management of viral diseases of vegetables

Factsheets on exotic viruses and bacteria were also prepared and submitted to Plant Health Australia for publication on their website. These were:

- Exotic vegetable begomoviruses : Cucurbitaceae prepared by Paul Campbell and Cherie Gambley
- Exotic vegetable begomoviruses : solanaceae prepared by Paul Campbell and Cherie Gambley
- Exotic vegetable tobamoviruses prepared by Cliff Kinoti, John Fletcher, Fiona Constable and Cherie Gambley
- Exotic vegetable tospoviruses prepared by Denis Persley and Cherie Gambley
- Stewart's wilt of corn (*Pantoea stewartii* subsp. *stewartii*) prepared by Rachel Mann
- *Erwinia tracheiphila* bacterial wilt of cucurbits prepared by Rebecca Roach, Nandita Pathania and Cherie Gambley

Regional case studies documented, and benchmarking completed

At the commencement of the project six case study topics were proposed for extension activities. The only case study where there was no real progress was the final one on non-native English-speaking growers. This proved to be very difficult and not delivering strongly for the overall outcomes of the project in relation to disease management development and as such was not further pursued. For the first three case studies listed below the information is incorporated directly into the two disease management guides published from the project. These re:

- A guide to understanding and managing bacterial diseases of vegetable crops
- A guide to understanding and managing virus diseases of vegetable crops

The case studies were:

1. Thrips transmitted viruses affecting capsicum crops
2. Bacterial leaf spot of capsicum and chilli
3. Mosaic disease of cucurbits
4. Area wide management (Completed using viruses spread by whitefly as the model – Appendix 5)
5. Investigate management strategies for insecticide resistance in Green Peach Aphid (GPA) (Completed – Appendix 5)
6. Adoption of management strategies by non-native English-speaking growers (no progress)

Outputs

A summary of project outputs is provided below, the details for each output is captured within the report body, appendices, on the Hort Innovation website as electronic copies and in the M&E plan in the below section. Specific details of extension activities are provided on pages 27-28 of this report.

Table 1. Output summary

Output	Description	Detail
Project commencement administration	agreement signed, project team meetings held, project reference committee formed, and annual meetings held, and the program logic, risk register, M&E and communication plans submitted and cross-checked annually	milestone reports 101, 102, 104, 106 and 108
A prioritised list of endemic and exotic viral and bacterial pathogens	completed by end of year 1, list reviewed and updated by project end if needed	milestone report 103, published on the Hort Innovation website and provided to Soil Wealth/ICP for publication on their website
A factsheet on the principles of area wide management	completed and disseminated by end of year 1	milestone report 103, published on the Hort Innovation website, handed out at various grower engagement activities
Electronic newsletter updating project activities	prepared and delivered annually	milestone reports 102, 104, 106 and 108, published through Vegetables Australia Magazine (four total)
Post-doc and PhD students	employed by end of years 1 and 2	milestone reports 103 and 105, all thesis due for submission by end of September 2022.
Review on exotics	review on exotic bacterial and viral pathogens affecting vegetables and development of contingency plans (3 for each group) – plans sent to industry and PHA for review by year 4	milestone report 109, contingency plans available through PHA portal, published on PHA website
Factsheets on the six exotic pathogens	prepared in collaboration with AusVeg and PHA, completed by year 3	milestone report 107, published on PHA website
Data on the survival and spread	of key viruses, insect vectors, phytoplasma and foliar bacteria in vegetable crops, native habitats and weeds during and between seasons (obtained through monitoring activities) – published annually in milestone reports and key information as factsheets by year 4	milestone reports 103, 105, 107 and 109, factsheets published on Hort Innovation website and provided to Soil Wealth/ICP for publication on their website, data used to provide advice in the two disease management guides
Data on the genetic and biological diversity	of bacterial and viral pathogens (obtained through monitoring and diversity activities) affecting vegetable crops to assist resistance breeding and deployment of resistant varieties and the development of new diagnostic methods - published annually in milestone reports and key results in journal papers by end of project	milestone reports 103, 105, 107, 109 and 190, diagnostics developed detailed on pages 27-29 of this report, journal papers listed on page 38-39 of this report.
A booklet on virus diseases of cucurbits	A booklet on virus diseases of cucurbits in Australia will be produced by year 2	published on Hort Innovation website and provided to Soil Wealth/ICP for publication on their

		website
Data on genetic and biological diversity of key insect vectors	obtained through monitoring and diversity activities - published annually in milestone reports and key points in refereed journal articles by end of project	milestone reports 103, 105, 107, 109 and 190, journal papers listed on pages 38-39 of this report.
Information on seasonal activity and migration patterns	of insect vectors (obtained through monitoring activities) - published annually in milestone reports and key points in refereed journal articles by end of project	milestone reports 103, 105, 107, 109 and 190, data used to provide advice in the two disease management guides, journal papers listed on page 38-39 of this report.
New diagnostic methods	for viruses and bacteria affecting nominated crops - available for field testing and to collaborating partners and the national diagnostic network by year 3	milestone reports 107 and 109, diagnostics developed detailed on pages 27-29 of this report
Formation of a diagnostic ring test system	by year 3 and at least two new diagnostic methods validated/proficiency tested through this system by year 4	milestone reports 106 and 109, diagnostics developed detailed on pages 27-29 of this report
Review of management strategies	to provide effective and sustainable disease management (e.g. biocontrol agents, chemicals, varietal selection, alternative host control, best crop management practices etc) including results from project trial work on potential management strategies- published in at least 3 factsheets by year 4 and key points in refereed journal articles by end of project	milestone reports 107 and 109, factsheets published on Hort Innovation website and provided to Soil Wealth/ICP for publication on their website, data used to provide advice in the two disease management guides
Risk mitigation strategy for seed treatment and/or testing	to reduce likelihood of introduction of bacterial and viral pathogens through this entry pathway – published in year 4 milestone report (109) and provided to the Imported Seeds Regulation Working Group by year	milestone reports 108 and 109, results of this output used to develop RFP AS1007 'Seed to production: improving efficiencies & reducing risk'. This RFP closed on the 29th July
Extension of regionally based management	to multiple districts including regional case studies documenting adoption of recommended management strategies and cost benefit analysis. Benchmarking of grower awareness, management strategies and losses from diseases done at the beginning, during, and at end of project	milestone reports 103, 107 and 109, multiple grower engagements held (refer Appendix 4). Extension also through the two disease management guides.
Grower forums	held in conjunction with disease surveys, at least two per state per year	milestone reports 103, 105, 107 and 109, multiple delivered each year per state, restricted at times by COVID, refer to Appendix 4
Conference presentations	At least 3 presentations at national and/or international conferences (industry and scientific) and submission of two manuscripts to peer-reviewed journals for publication by end of project	milestone report 190, multiple presentations delivered, restricted at times by COVID, multiple papers submitted, refer to pages 38-39 of this report
Six monthly reports	Six monthly milestone status reports	Milestones 103-109

Outcomes.

A summary of the project outcomes is listed below and aligned to the Hort Innovation Vegetable SIAP 2017-2021 strategic investment plan.

Table 1. Outcome summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Improved productivity and profitability through improved knowledge of disease management	Aligned to Outcome 3 'Improved farm productivity' subsection 4 – Pests and diseases This is through improved disease management and better prediction of disease outbreaks.	Improved productivity and profitability through lowered input costs, greater predictability of season yields and less downgraded produce resulting in improved marketable yields	This is a long-term outcome and very producer specific. Lowered input costs identified as not being a driver for adoption of new management, quality and quantity of product are drivers for adoption as was improved predictability for disease management
A more secured supply chain through better disease management	Aligned to Outcome 3 'Improved farm productivity' subsection 4 – Pests and diseases This is through improved disease management and better prediction of disease outbreaks.	A more secured supply chain by greater predictability of season yields and product quality (reduction in fluctuations in supply caused by these diseases)	This is a long-term outcome and very producer specific. Improved predictability for disease management identified as a driver for adoption of new practices.
Improved exotic pathogen interception	Aligned to Outcome 3 'Improved farm productivity' subsection 4 – Pests and diseases This is through biosecurity preparedness (6 contingency plans) and improved diagnostics	Increased potential for interception of infected seed lots through improvements in diagnostics	Hort Innovation commission a new project for this. RFP AS1007 'Seed to production: improving efficiencies & reducing risk'. This is a long-term outcome and project AS1007 will inform on this once it is finished.
Reduction in pesticide usage through improved knowledge of disease management	Aligned to Outcome 3 'Improved farm productivity' subsection 6 – environmental sustainability. This is through improved disease management leading to a reduction of chemical use.	Reduction in pesticide usage by more strategic use of chemicals, and/or identification of novel chemistries or biocontrol agents to assist in disease management	This is a medium-term outcome and very producer specific. Lowered input costs identified as not being a driver for adoption of new management, quality and quantity of product are drivers for adoption as was improved predictability for disease management. Also awareness of overuse of chemicals leading to reduced efficacy is acknowledged within the industry and helps drive adoption of new practices.

Sustainable management through improved knowledge of disease management	Aligned to Outcome 3 'Improved farm productivity' subsection 6 – environmental sustainability. This is through improved disease management leading to a reduction of chemical use.	Sustainable management of pests and diseases through protection of key chemistries	This is a medium-term outcome and very producer specific. Awareness of overuse of chemicals leading to reduced efficacy is acknowledged within the industry and helps drive adoption of new practices.
Lowered environmental impacts through improved knowledge of disease management	Aligned to Outcome 3 'Improved farm productivity' subsection 6 – environmental sustainability. This is through improved disease management leading to a reduction of chemical use. Also, through promotion of better weed control.	Lowered environmental impacts through strategic use of chemicals and better weed control	This is a medium-term outcome and very producer specific. Awareness of weeds as an important source for disease outbreaks has improved and will assist in driving adoption for better weed management, similarly awareness of overuse of insecticides and how this relates to virus disease control will drive reduction of use.
Improved preparedness to exotic bacteria and insect-vectored viruses	Aligned to Outcome 3 'Improved farm productivity' subsection 4 – Pests and diseases This is through biosecurity preparedness (6 contingency plans).	Improved preparedness to exotic bacteria and insect-vectored viruses resulting in swifter responses to incursions, improved chances of eradication and potential to reduce the quantum of funds via cost sharing required to address	Six contingency plans available.
Greater longevity of plant host resistance genes	Aligned to Outcome 3 'Improved farm productivity' subsection 4 – Pests and diseases This is through protection of elite resistant varieties through better management of disease sources and insect vectors of the diseases.	Greater longevity of plant host resistance genes through integrated approaches to disease management and protection of this valuable resource	This is a long-term outcome and district specific. Awareness of the effectiveness of host genetics for disease control is high and sought after. The potential to lose this will drive better management of disease sources.
Increased capacity of the plant protection discipline	Aligned to Outcome 5 'Improved industry capabilities for innovation and adoption' subsections 2 – Innovation support and 3 – Professional development This is through development of new plant protection specialists and	Increased capacity of the plant protection discipline to service the vegetable industry through investment into PhD students, post-graduates and early career scientists, and through improved mentoring of existing pathologists and	Four PhD students to graduate within the next few months. At least three mid-career scientist supported to develop knowledge for the vegetable industry within this project. Multiple extension activities delivered to

	improved networking of plant protection specialists available to industry to respond to need. This was through qualified research networks (government and private) and industry stakeholders.	entomologists. Improved knowledge of plant protection by industry stakeholders through.	improve knowledge of plant protection to industry stakeholders including hands-on workshops.

Monitoring and evaluation

A summary of the monitoring and evaluation activities throughout the project are detailed below. Details on specific project performance KPIs are provided in the body of this report.

Table 1. Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
Effectiveness		
1. To what extent has the project achieved its expected outcomes?	Number of reports and articles describing new information - nine factsheets published and disseminated (available on the Hort Innovation website and provided to SoilWealth/ICP for publication on their website) - four articles published by AusVeg (2018, 2019, 2020 & 2021) - articles published by various VegNet groups - multiple presentations on various topics nationally and internationally - two disease guides (available on the Hort Innovation website and provided to SoilWealth/ICP for publication on their website) Number of new diagnostic methods - assays for detection of at least 16 key viruses and 7 bacteria Number of case studies completed - 5 completed Number of new contingency plans - 6 completed	Publication of assays in peer reviewed journals, distribution of assays further through national diagnostic services. Further peer reviewed articles in preparation to be published within the next few months.
Relevance		

2. How relevant was the project to the needs of intended beneficiaries?	Number of growers adopting new practices - at least half of the brassica and tomato growers in Granite belt and some brassica growers in the Lockyer valley, QLD adopted hot water treatment of seed to mitigate risk of bacterial disease introduction - large south-east QLD nursery adopting and promoting hot water treatment of seed - WA growers adopting tolerant zucchini lines - Large QLD grower attempting barrier crops to control potyvirus in cucurbits Number of contingency plans endorsed by PHA and AusVeg - Six completed Number of factsheets and peer-reviewed papers published - Nine factsheets published (https://www.horticulture.com.au/search/?search=VG16086) - factsheets on disease issues in WA distributed in that state - factsheets on AWM developed for NT growers and translated into Vietnamese - multiple publications in preparation and planning, including 4 PhD thesis	Further education on the benefits of hot water treatment, further research to reduce practical barriers for this to happen, further publications of research outcomes anticipated. Further work on new management practices for viruses affecting brassicas and lettuce, new age bactericides, pectobaterium in zucchini and thrips diversity are areas for further development.
Process appropriateness		

3. How well have intended beneficiaries been engaged in the project?	Number of attendees at forums - between 5 and 30, depending on the district Number of project update articles supplied to AusVeg - initial project commencement article supplied, 2018 - update article submitted at end of May 2019, 2020 and 2021 - additional articles provided to jurisdiction agricultural journals and distribution networks Number of contingency plans endorsed by PHA - six completed - multiple presentations at discipline workshops	Further extension would be beneficial through a formalized training program for agronomists or continuation of the informal hands-on disease identification and management workshops developed within the project.
4. To what extent were engagement processes appropriate to the target audience/s of the project?	How well were extension events advertised - grower forums and farm visits well-advertised through VegNet, AusVeg and local grower groups - webinars done in 2020 due to COIVd-29 restrictions, at least 2 in QLD, both attended well (>50 participants) - Vegetable webinar series for DAF, QLD launched, 3 held to date with >50 participants at each - Webinar delivered for RMCG in July 2022 How often was extension delivered on-farm - extension of information is provided during all on-farm visits when the grower/consultant is available - For NT this was always on farm in collaboration with NTFA - For others it's a mix of on-farm and formal events How many extension activities were completed - multiple per state - new format of a disease identification workshop delivered in QLD (Gatton, in Sept 2019, Bundaberg in Dec 2019 and Ayr, Nov 2021), very positive feedback from all, was to be rolled out nationally but not done because of COVID-19, did hold one in Mildura, VIC in March 2022 - two on-farm field trials in QLD, 2021 Did contingency plans meet PHA criteria - yes, all six met criteria	New formalized training program for agronomists for disease identification and management would allow further extension of these outcomes, mini-series webinar organized by RMCG to be delivered August to October for further extension of key outcomes
Efficiency		

5. What efforts did the project make to improve efficiency?	How often were extension events done in collaboration with other industry activities (e.g. other projects, AusVeg, VegNet etc.) - multiple times per state and wherever practical How often were extension activities combined with survey trips - almost always	This should continue with both AusVeg and VegNet for all future crop protection projects.

Recommendations

Recommendations for relevant stakeholders such as growers, researchers, Hort Innovation and other audiences:

- Practical application of the project findings is through improved knowledge on understanding and management of virus and bacterial diseases of vegetables. This is delivered through the two comprehensive disease guides, distributed as printed copies and downloadable versions from websites such as Hort Innovation, jurisdictions involved in the project and Soil Wealth/ICP.
- Future RD&E ideas to extend and value-add to findings from VG16086:
    - Virus diseases affecting lettuce and brassica crops: extends research from mosaic disease of cucurbits and orthotospoviruses affecting capsicum (aphid and thrips transmitted viruses), key target areas would be Lockyer Valley QLD and Victoria, however other areas in WA and NSW are also impacted
    - New age bactericides: protectants aren't effective under high disease pressure which is evident from evaluating disease outbreaks in VG16086 and the trial work done in VG16086. There is a need for products to reduce bacterial numbers in infected plants, several products were identified in VG16086 which have the potential to do this
    - Pectobacterium disease management: zucchini and brassica. This is an emerging problem seen through surveys in VG16086, there is a need for research into infection processes and bacterial spread in crop so that effective management strategies can be developed.
    - Thrips diversity: little is known about vector species in the different vegetable districts where the viruses they spread cause disease. Different species vector different viruses.
- A formalized training program for agronomists would drive development and adoption of crop protection recommendations and ensure full value from the project's findings for industry. A carefully constructed curriculum to train agronomists in pest and disease identification plus management. This could include:
    - A small group of participants each year, capped at about 10
    - Three intensive hands-on workshops spread over a 12-month period, with assessment tasks to be completed between workshops
    - Participants to complete assessment tasks to gain a formal accreditation for the course
    - Requires expert plant pathologists and entomologists to develop curriculum and deliver workshop and specialist extensionist to develop and deliver the course

Refereed scientific publications

Journal article

Rodrigues-Jardim B, Kinoti W.M, Tran-Nguyen, L.T.T, Gambley, C., Rodoni, B and Constable, FE. (2019). The first steps towards investigating the molecular epidemiology of *Stylosanthes* little leaf phytoplasma in Australia. Phytopathogenic Mollicutes, 9 (1), 17-18

Kinoti, W.M., Moran, J.R., Gambley, C., Rodoni, B., and Constable, F.E. (2020). Genome characterization of two carrot virus Y isolates from Australia. *Microbiology Resource Announcements*, 9 (15).

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Mackie, J., Tran-Nguyen, L., Kinoti, C.W., Kehoe, M., Campbell, P., Rodoni, B., Constable, F. in preparation. Genome characterisation of the CGMMV virus population in Australia.

Mackie, J., Kinoti, C.W., Chahal, S., Lovelock, D., Tran-Nguyen, L., Campbell, P., Rodoni, B., Constable, F. in preparation. Assessment of a targeted multiplex PCR and Nanopore sequencing method for the detection of CGMMV.

Rodrigues Jardim, B., Tran-Nguyen, L., Gambley, C., Rodoni, B., Constable, F. (2021) Improved Phytoplasma Genome Assembly using a Novel Density Gradient Centrifugation Method. Accepted.

Djitro, N., Roach, R., Mann, R., Rodoni, B., Gambley, C. (2021) Characterisation of Pseudomonas syringae isolated from systemic infection of zucchini in Australia. Plant Disease PDIS-05-21-1039-RE. https://doi.org/10.1094/PDIS-05-21-1039-RE

Kinoti, C.W., Tesoriero L., Gambley, C., Constable, F., in preparation. Biological and molecular characterisation of a novel potyvirus infecting *Lactuca* species in Australia. In preparation

Chapter in a book or paper in conference proceedings

Kinoti, C (2018). Area-wide survey of virus and bacterial diseases of vegetables in Victoria. Presented at the Annual AgrBio conference.

Constable, F. (2019). The first steps towards investigating the molecular epidemiology of Stylosanthes little leaf phytoplasma in Australia. Presented at the 4th International Phytoplasma Working Group Conference.

Gambley, C., Nimmo, P., Persley, D., Campbell P. (2021) Carlavirus: is it a risk for beans in Australia? Presented at APPS and APS conferences.

Rodrigues Jardim, B., Tran-Nguyen, L., Gambley, C., Rodoni, B., Constable, F. (2021) Improved Phytoplasma Genome Assembly using a Novel Density Gradient Centrifugation Method. Presented at the IOM congress.

Djitro, N, Roach, R., Mann, R., Rodoni, B., Gambley, C. (2021) Internal fruit-rot and crown-rot disease on zucchini by Pseudomonas syringae in Australia. Presented at the APPS conference.

Mackie, J., Tran-Nguyen, L., Campbell, P., Edwards, J., Rodoni, B., Constable F. (2021) Molecular diversity of Cucumber green mottle mosaic virus in Australia. Presented at the APPS conference.

Rodrigues Jardim, B., Tran-Nguyen, L., Gambley, C., Rodoni, B., Constable, F. (2021) Investigating species delimitations within the 16SrII phytoplasmas using genome data. Presented at the APPS conference.

Webster, C., Wright, D., Kehoe, M., Constable, F., Persley, D., Gambley, C. (2021) Reduced Potyvirus symptom development and virus accumulation in new zucchini varieties. Presented at the APPS conference.

Wright, D., Bwye, A., Banovic, M., Webster, C., Kehoe, M., Wang, C. (2021) Plant bacteria to identify? Which method should I use? Presented at the APPS conference.

Umar, M., Tegg, R., Thangavel, T., Wilson, C. (2021) Diversity of Poleroviruses in Tasmanian pea crops. Presented at the APPS conference.

Constable, F. (2021). Seed Molecular Testing 101. Presented at the Australian seed federation (ASF) Seed Industry Education and Training Webinar Series.

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Presented at the Agriculture Victoria Research science webinar series.

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Constable, F. (2022). Pests and Seeds: Australia's #1 Intercepted Biosecurity Risk Item: Travelling Seeds. Presented at the Australian Biosecurity Webinar Series on 30 March 2022.

Thesis submissions

Bianca, R.J. For submission September 2022. Thesis title: A genomics approach to understanding the diversity and biology of phytoplasmas threatening vegetable production in Australia. Supervisory team: Rodoni, B., Gambley, C., Tran-Nguyen, L., and Constable, F.

Joanne, M. For submission May 2023. Thesis title: Targeted surveillance strategies to support area wide management of viruses in vegetable crops. Supervisory team: Tran-Nguyen, L., Campbell, P., Rodoni, B. and Constable F.

Umar, M. For submission June 2022. Thesis title: Poleroviruses of Legume Vegetable Crops: Diversity, impact, and control. Supervisory team: Tegg, R.S., Wilson, C.R. and Thangavel T.

Djitro, N. For Submission September 2022. Thesis title. Elucidating the epidemiology of bacterial crown and fruit rot, an unusual Pseudomonas disease of zucchini. Supervisory team Rodoni, B., Gambley, C., Roach, R., Campbell, P. and Mann, R.

Intellectual property

No project IP or commercialisation to report

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Appendices

Appendix 1: Pathogen detection

A summary of survey and diagnostic testing results to date are presented in the below tables. Note these are records of pathogen detection only. It is not an indication of disease incidence or frequency. Multiple crops were surveyed each year in many districts and only single records of detections are provided.

Disease survey results for Queensland: virus and bacteria detections from various crops and production districts (DT = dry tropics, GB = Granite belt, B = Bundaberg and SE QLD = Lockyer valley, Fassifern valley, peaks crossing and eastern downs). Virus acronyms are: AMV = alfalfa mosaic virus, CaCV = capsicum chlorosis virus, CGMMV = cucumber green mild mottle virus, CPMMV = cow pea mild mottle virus, LNYV = lettuce necrotic yellows virus, LBVD = lettuce big vein disease, PRSV = papaya ringspot virus, PVY = potato virus Y, TSWV = tomato spotted wilt virus, TuMV = turnip mosaic virus, TuYV = turnip yellows virus and WMV = watermelon mosaic virus. Bacteria abbreviations and acronyms are: Agro = *Agrobacterium* spp., Cmm = *Clavibacter michiganensis* subsp. *michiganensis*, Pseudo = *Pseudomonas* spp and Xantho = *Xanthomonas* spp.

Crops	2018		2019				2020				2021			
	DT	GB	DT	GB	B	SE QLD	DT	GB	B	SE QLD	DT	GB	B	SE QLD
Brassica										Xcc				Pseudo
Broccoli						TuYV				Pseudo				
Cabbage						TuYV				Xcc				Pseudo; Xantho
Capsicum	TSWV, CaCV		TSWV, CaCV	CaCV			CaCV				Xantho		CaCV	
Chilli	TSWV, CaCV		CaCV	PVY			TSWV							
chinese raddish (Daikon)						TuMV								
Chinese cabbage (wombok)		Xcc, Pecto				TuMV, TuYV								TuMV; Xantho
Coriander						AMV				AMV		Pseudo		
Cauliflower						TuYV				Xantho, Agro, AMV		Xanto		Xantho
Cucumber					CGMMV									
French bean					CPMMV	CPMMV	CPMMV			Pseudo., Xantho				
Lettuce						TuMV, TuYV; LBVD				Pseudo; Pecto; LNYV, TSWV, TuMV, TuYV				TuMV; LBVD; Xantho
Radish										TuMV, TuYV				

Parsley														AMV
Pumpkin			PRSV			PRSV, WMV	PRSV				PRSV			
Tomato									TYLCV	Cmm, TSWV	Xantho	Cmm		Pseudo
Zucchini		PRSV, Pecto	PRSV		PRSV, WMV, Pecto		Pecto	Pecto	PRSV		PRSV; Pecto, Pseudo			

Disease survey results for Victoria: virus and bacteria detections from various crops and production districts (SW = south west Melbourne, SE = south east Melbourne, G = Gippsland, WMB= Werribee, Melbourne and Bacchus Marsh; V = Virginia). Virus acronyms are: AMV = alfalfa mosaic virus, BWYV = beet western yellows virus, CaCV = capsicum chlorosis virus, CarYV = carrot virus Y, CeMV = celery mosaic virus, CMV = cucumber mosaic virus , CtRLV = carrot red leaf virus, LNYV = lettuce necrotic yellows virus, LBVD = lettuce big vein disease, LBVaV = lettuce big-vein associated virus, MiLV = mirafiori lettuce virus, MiLBVV = mirafiori lettuce big vein virus, RWMV = ranunculus white mottle virus, TMGMV = tomato mild green mosaic virus, TSWV = tomato spotted wilt virus, TuMV = turnip mosaiv virus, and TuYV = turnip yellows virus. Bacteria abbreviations and acronyms are: Cmm = *Clavibacter michiganensis* subsp. *michiganensis*, Erw = *Erwinia* spp., Pseudo = *Pseudomonas* spp and Xantho = *Xanthomonas* spp.

Crops	2018		2019		2020			2021		
	SW	SE	WMB	V	WMB	Mi	V	B	G	Mi
Baby spinach			Pseudo							
Basil					Pseudo					
Beetroot			Pseudo							
Bok Choy							CMV	TuYV		
Brassica			BWYV, TuMV, CMV							
Broccoli/broccolini	Polerovirus; Pseudo,				TuYV, Pseudo, Erw, Xantho			TuYV, Pseudo		
Brussel sprouts			Pseudo				*Pantoea*			
Cabbage	Pseudo, Xantho							Xantho		
Capsicum				RWMV, TSWV, TMGMV		TSWV	RWMV, TSWV			CaCV
Carrot			CtRLV, CarYV							
Celery					CeMV					
Chard								Pseudo		
Chili						TSWV				
coriander		*Polerovirus*						Pseudo		
cauliflower	Pseudo	*Polerovirus*	Pseudo, xantho					Pseudo		
Celery		*Pseudo*	TSWV		CeMV, TSWV			TSWV		

Cucumber				TSWV						
Endive		potyvirus, polerovirus								
lettuce	LBVaV, Pseudo	Polerovirus	TSWV, LNYV, LBVaV, MiLBVV, LMV, MiLBVV, Pseudo	LBVaV	TSWV, LNYV, LBVaV, Pseudo	LNYV, CMV		AMV, CMV, TSWV, LBVaV, MiLV, Pecto, Pseudo	CMV, Xantho	LNYV
onion			IYSV							
Pak choi					Pseudo					
Parsley		potyvirus	CMV		CMV, Polerovirus			CMV		
Peas			TSWV						AMV	
Pumpkin						WMV				
Radicchio		LBVaV2, LNYV, TSWV, Polerovirus, Pseudo								
Rocket								Pseudo		
spinach					CMV				Pseudo	
tomato					Pseudo			*TYLCV, Cmm*		
wild rocket					Xantho			Xantho		

Disease survey results for Western Australia: virus and bacteria detections from various crops and production districts (A = Albany, bm = Bunbury/Manjimup, Ca = Carabooda, C = Carnarvon, G = Geraldton, K = Kununurra, M = Myalup, P = Perth, SW = south-west, Wa = Waraloo, WB = Wheat belt, WG = Wanneroo & Gingin). Virus acronyms are: BCMV = bean common mosaic virus, CarYV = carrot virus Y, CGMMV = cucumber green mottle mosaic virus, CMV = cucumber mosaic virus, LBVaV = lettuce big-vein associated virus, PMMoV = pepper mild mottle virus, TSWV = tomato spotted wilt virus, WMV = watermelon mosaic virus, ZYMV = zucchini yellow mosaic virus. Bacteria abbreviations and acronyms are: Cmm = *Clavibacter michiganensis* subsp. *michiganensis*, Pseudo = *Pseudomonas* spp, Phyto = phytoplasma, Pecto = *Pectobacterium* Rhiz = *Rhizobium rhizogenes* and Xantho = *Xanthomonas* spp.

Crops	2018		2019					2020						2021						
	C	P	K	C	G	WG	SW	A	BM	Ca	C	K	P	C	P	G	M	K	Wa	WB
Bean																		BCMV		
Broccoli							Pecto, Xantho		Pecto, Pseudo								Xantho			
Capsicum	CMV			PMMoV, TSWV, Phyto		CMV	TSWV				Phyto, TSWV			CMV	Rhiz					
Carrots						CarVY							CarVY		CarVY					
Celery							Pseudo, Pa													
Chickpea			Phyto															phyto		
Chilli	TSWV			CMV, PMMoV, TSWV							TSWV, PMMoV									
coriander																				Pseudo
Cucumber	ZYMV				CGMMV							Phyto				CGMMV, ZYMV				
eggplant							TSWV				Phyto									
Fennel							Pecto													
Gai Lan (chinese broccoli)						Pseudo														
Kale													*Pseudo*							
lettuce		LBVaV		LBVaV		LBVaV														

luffa											Phyto, CGMMV								
melon (rockmelon)				CGMMV, ZYMV														Pseudo, Pecto	
mungbean			Phyto																
okra			poty																
Paprika	CMV										TSWV								
Parsley													*Pseudo*						
potatoes								Pecto	Pecto				Pecto						
Pumpkin, butternut	ZYMV																		
Pumpkin, Kent	ZYMV																		
pumpkin				ZYMV								Phyto		ZYMV				phyto	
spinach						TRV, F													
spring onions									Pseudo										
snake bean			poty								Phyto								
soybean			phyto																
Squash - yellow	ZYMV																		
tomato	CMV, phyto			Phyto, Cmm, Pseudo			Cmm, TSWV				Phyto, TSWV		*Pseudo*	*CMV, Pseudo, Xantho*					
Zucchini	ZYMV			ZYMV, Pecto, Pseudo	Xantho, Pa?	WMV					ZYMV	Phyto		ZYMV					

Disease survey results for Northern Territory: virus and bacteria detections from various crops and production districts. Virus acronyms are: CMV = cucumber mosaic virus, PeVYV = pepper vein yellows virus, PRSV = papaya ringspot virus, SPFMV = sweet potato feathery mottle virus, SPLCV = sweet potato leaf curl virus, ToLCV = tomato leaf curl virus and ZYMV = zucchini yellow mosaic virus.

Crops	2018	2019		2020			2021
	Darwin	Darwin	Katherine	Darwin	Katherine	Alice springs	Darwin
Bitter melon	Bacillus sp.	PRSV					PRSV, Ca. Phytoplasma aurantifolia, Vigna little leaf phytoplasma
Chilli		Pantoea anthophila, Pseudomonas spp., Xanthomonas spp.		PeVYV			
Cucumber							ZYMV
Eggplant				Ca.Phytoplasma aurantifolia			
Hairy melon	Pseudomonas spp.	PRSV					
Long melon		PRSV		PRSV			PRSV
Luffa	Ca. Phytoplasma aurantifolia	ZYMV, Ca. Phytoplasma aurantifolia					
Okra							

Peanut		Ca. Phytoplasma aurantifolia					
Pumpkin		ZYMV. PRSV		ZYMV			ZYMV
Sinquar							
Smooth gourd							PRSV
Snake bean	Ca. Phytoplasma aurantifolia	Ca. Phytoplasma aurantifolia		CMV			
Snake gourd				PRSV, new Tobamovirus			New Tobamovirus, PRSV. Ca. Phytoplasma aurantifolia
Sweet potato		SPFMV		SPFMV, SPLCV		SPFMV	SPLCV, Ca. Phytoplasma aurantifolia
Tomato		ToLCV		Ralstonia solanacearum, ToLCV			ToLCV, R. solanacearum
Unidentified legume species		Ca. Phytoplasma aurantifolia					
Unknown cucurbit				ZYMV			
Vietnamese cucumber							PRSV
Watermelon		PRSV	CGMMV				
Wax gourd	Pseudomonas sp.						

Zucchini		ZYMV, PRSV		ZYMV			ZYMV

Appendix 2: Seasonal vector

Knowledge gained from insect monitoring activities assisted with field trial design for development of management recommendations. This knowledge is incorporated the guide:

'A guide to understanding and management of viral diseases of vegetables'

Queensland: Leafhopper and Phytoplasma monitoring

Tomato big-bud disease (*Candidatus* Phytoplasma aurantifolia) is a damaging disease of capsicums, tomatoes, and other vegetable crops. The regular detection of phytoplasma in Queensland's Granite Belt horticultural district made this an ideal area to establish monitoring sites for the project. High incidences of phytoplasma were detected in this region in the 2016-17 production season, and across most of east coast Australia prompting inclusion of this work in this current project.

Capsicums and tomatoes are produced in the Granite belt from November through May depending on the occurrence of frost at both ends of the growing season. The more temperate weather conditions allow continuous production over the hotter Christmas and New Year period. For the first season, crop surveys in the district commenced in October 2019 and continued through February and April 2020. Surveys recommenced for the new cropping season in October 2020, however, during this 2020-21 season, dry conditions, a shortage of labor and poor prices brought an end to production in the study blocks in early March. In addition to commercial crops, nearby riparian areas and headlands were monitored for potential alternative phytoplasma hosts and vectors. Surveys of weeds continued from March 2021 through the non-production period, with a focus on blue heliotrope as this was identified in the earlier surveys as regularly hosting leafhoppers. The role of this weed as a reservoir for both the leafhoppers and the phytoplasma requires intensive investigation.

Monitoring involved counts of leafhoppers plus collection of individuals for potential species identification. Five broad species groupings of leafhoppers were collected as Morphospecies 1 through 5. Morphospecies 1 potentially represents *Orosius argentatus* and *Orosius orientalis* that are morphologically difficult to distinguish, and both share the common name of 'common brown leafhopper'. These two species are known vectors of phytoplasma. Through a combination of genetic and morphological means accurate specific names will be assigned to all five morphospecies groups and other leafhoppers of interest. Morphospecies 4 possibly contains further potential vector species, such as *Austroagallia torrida* (spotted leafhopper) and *Batrachomorphus angustatus* (large green jassid). Crop and weed species were also surveyed for phytoplasma disease symptoms. Representative symptomatic samples were collected to determine the identity of the phytoplasma.

Crop surveys for the 2019-20 season initially involved vacuum sampling of four panels on each of the first two rows and inspection of four panels of 20 rows for incidence of phytoplasma. The method was modified mid-season and increased the vacuum samples to 10 rows instead of two rows. This was to increase the potential for finding leafhoppers as few leafhoppers were being detected. Data for this first season was useful as a preliminary study to guide monitoring methods for subsequent seasons. All representative symptomatic plants samples tested were confirmed as having phytoplasma. Phytoplasma symptoms were generally detected two to four weeks after detection of leafhoppers which is consistent with expectations for this pathogen-vector combination.

Results for the 2020-2021 season showed for capsicum the incidence of phytoplasma was very low, with a maximum average of only five plants per survey area detected in March 2022. Tomato by contrast was much higher with a peak average of almost 250 affected plants in December 2020 (Figure 1). The data was very variable for tomato and highly site specific, with the maximum detected at a single site 474 at site 071 (Table 1).

In addition to crops, weeds were also surveyed for phytoplasma and leafhoppers. The most found weed with leafhoppers was blue heliotrope and it was found to host colonizing populations. This was evident through detection of juvenile stages of the insect. Insects were detected during winter (non-crop period) on the weed at low numbers.

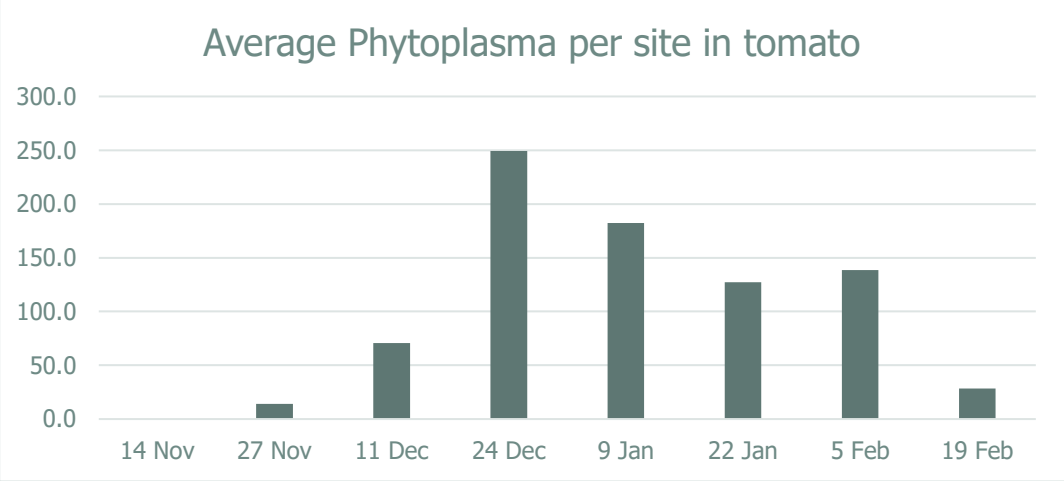

Figure 1. Graph of phytoplasma affected tomato plants during the 2020-2021 growing season.

Table 1. A list of phytoplasma and vector detections in capsicum and tomato crops during the 2020-2021 growing season.

Survey	Survey date	Host	Average Phytoplasma per survey site	Average leafhoppers detected per survey site
14/11/2020	14 Nov	Capsicum	0.0	10.0
27/11/2020	27 Nov	Capsicum	0.0	4.7
11/12/2020	11 Dec	Capsicum	0.7	3.0
24/12/2020	24 Dec	Capsicum	1.3	0.3
9/01/2021	9 Jan	Capsicum	4.0	0.0
22/01/2021	22 Jan	Capsicum	1.0	0.0
5/02/2021	5 Feb	Capsicum	3.7	0.0
19/02/2021	19 Feb	Capsicum	4.0	0.0
5/03/2021	5 March	Capsicum	4.7	0.0
14/11/2020	14 Nov	tomato	0.0	10.0
27/11/2020	27 Nov	tomato	0.0	2.3
11/12/2020	11 Dec	tomato	14.0	1.3
24/12/2020	24 Dec	tomato	70.7	0.0
9/01/2021	9 Jan	tomato	249.3	0.0
22/01/2021	22 Jan	tomato	182.4	0.0
5/02/2021	5 Feb	tomato	127.3	0.3
19/02/2021	19 Feb	tomato	138.7	0.0
5/03/2021	5 March	tomato	**28.5**	**0.0**

Conclusion: These results are important for industry as it shows phytoplasma diseases vary between different crops. This indicates a likely feeding preference by the insect vector. In the Granite Belt, presence of tomato crops is possibly attracting vectors away from the capsicum crops and thereby providing a level of protection to those crops from disease. The results also showed a peak in activity of the disease in December 2020 which was unrelated to the peak in leafhopper numbers. For industry, this means use of insecticides to manage phytoplasma disease would not be effective. Detection of blue heliotrope weed within the district that hosts both the leafhopper vector and the disease is of importance for management. Prior to this it was believed the leafhoppers bring the disease into crops from a longer distance, however, detection of a local weed hosts contradicts this idea. Management of this weed would assist in managing phytoplasma disease outbreaks.

Queensland: Thrips and Orthotospovirus monitoring

Tomato spotted wilt virus (TSWV) and *Capsicum chlorosis virus* (CaCV) are both known to occur in the dry tropics production area of QLD and cause disease in capsicum and chili. TSWV is normally the predominant orthotospovirus present in this district and caused significant impacts to tomato crops until resistant varieties were introduced. Since the commencement of this project in 2018, it's become evident that CaCV has also become an economically impacting disease in this area. Similarly, in the Bundaberg district, although both are present, CaCV is the dominant virus. There is no known crop-host resistance to CaCV. The reported vectors of CaCV are *Thrips palmi* (melon thrips)*, Frankliniella schultzei* (tomato thrips) and *Ceratothripoides claratris* (oriental tomato thrips) which is not known to occur in Australia (Persley et al. 2006). Both *F. occidentalis* (Western flower thrips, WFT) and *F. schultzei* vector TSWV. An understanding of the vector populations in relation to virus dominance is very important to manage disease, particularly as WFT requires more expensive chemicals for control. The two viruses also have very different host ranges, thus the environmental sources of the virus between cropping seasons are different. For TSWV it is well known that Jamaican snakeweed (*Stachytarpheta jamaicensis*) is a very important and widespread weed host for TSWV. At the start of the project the weed hosts of CaCV in this district were unknown. Blue billygoat weed (*Ageratum houstonianum*) is the known important weed host of CaCV in Bundaberg but it doesn't occur in the dry tropics.

Thrips monitoring in sticky traps was somewhat effective to generate broad data on potential vector flights, however, the lack of accurate identification complicates interpretation of the data. The trapping showed there was no significant difference in the use of blue versus white sticky traps (results not shown) and that there were big differences in seasonal variation of thrips during the study and this also varied between location (Figures 2 and 3). Note data collection started in week 1 (W1) which was the 15th April and concluded in week 33 (W33) which was the 4th of December and the numbers of thrips are for adult flying insects and calculated from a 36 cm^2 section of the sticky trap. These graphs show differences in when peak thrips activity occurs through the season, between seasons and between locations. For Bowen, the highest number of thrips recorded was in W8 in 2019, similarly the highest thrips recorded in Gumlu was also 2019, but it was at least two weeks later. For 2019, the numbers in Gumlu were significantly higher than Bowen, highest peak was 900 adult thrips per section of trap compared to just under 400 for Bowen, however, this trend doesn't hold for all season. Virus incidences at these locations was low during the project, except for 2018 where incidences were moderate, and the virus was mostly TSWV. This meant attempts to correlate thrips numbers with virus incidence was not possible from this monitoring activity.

During 2022, regular virus incidence counts, and thrips monitoring were done on a property in Gumlu and one in Bowen, in close collaboration with the agronomists from those businesses. This involved doing counts of symptomatic plants in a subsection of a production block, with multiple sections of 50 plants along rows inspected and a minimum of 300 in total inspected. From the same sub-area, 50 capsicum flowers were collected to detected adult thrips. In one survey trip, plants were beaten to attempt better detection of thrips, particularly larval stages. The sub-area was typically selected on the edge, preferable a corner of the block to maximize virus and thrips detections.

Virus incidences ranged from <1% to about 10% on these properties. Results from a single survey of a reported badly affected crop near Gumlu showed virus incidence of 50%. CaCV was detected in almost all samples collected from these crops, with TSWV only rarely found. Additionally, thrips monitoring in crops were completed but very low insect numbers were collected from any of the capsicum crops. The most was detected in an organic crop, but these were still quite low.

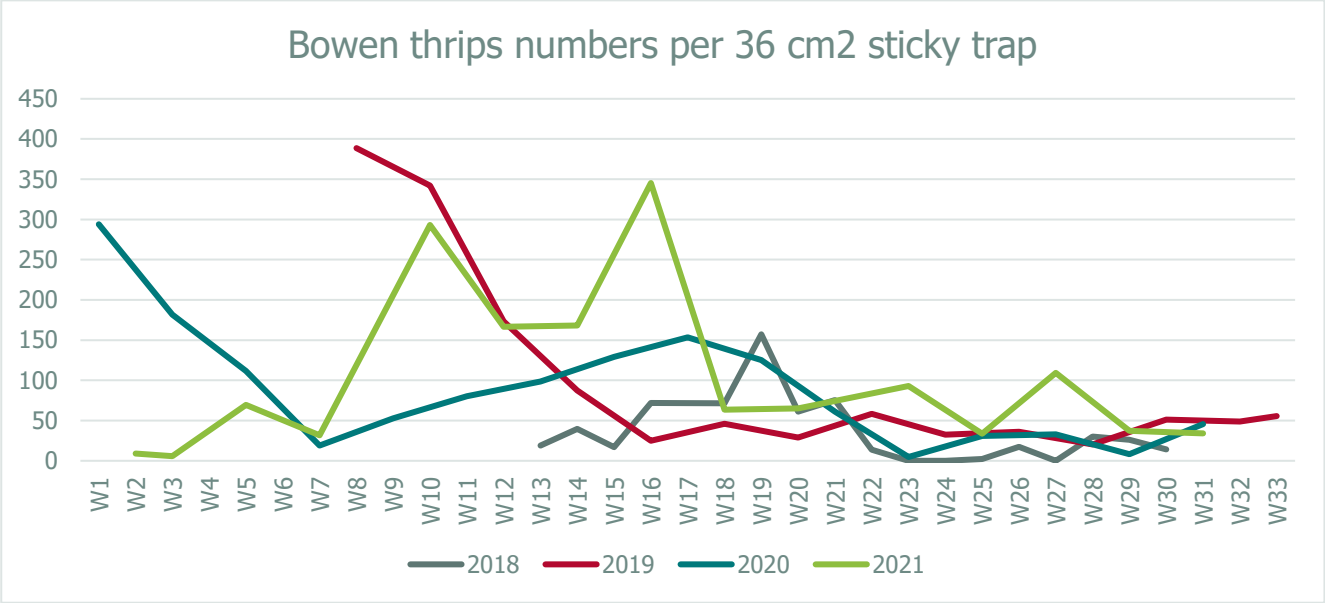

Figure 2. The number of adult flying thrips captured on sticky traps in Bowen for the four year study period. W1 = 15th April and W33 = 4th December.

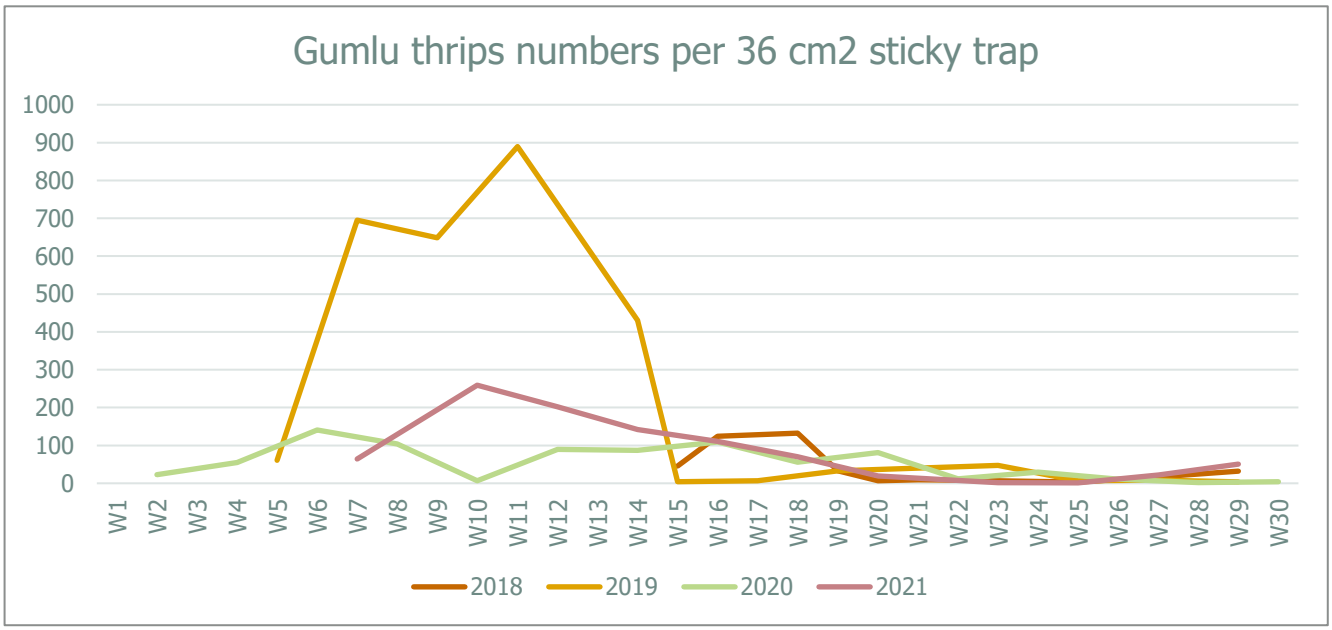

Figure 3 The number of adult flying thrips captured on sticky traps in Gumlu for the four year study period. W1 = 15th April and W33 = 4th December.

Weed surveys commenced in November 2021 and continued through to June 2022. New weed hosts for CaCV were detected and in some instances tomato thrips were present on the weeds. Molecular and morphological methods identified the key weed as *Praxelis clematidea*.

These results are very important for industry as they highlight a significant change in virus dominance in this district. Capsicum has resistance genes for TSWV, but none are commercially available for CaCV. It also shows that despite good thrips management in crop, virus diseases can still occur at economically damaging incidences. The 2022 growing season was unusual as there was continual and sporadic rainfall into winter, whereas normally the wet season is finished by early March. This continued moisture has resulted in ongoing weed presence well into the production window and thus its probable the virus incidence is from continual primary spread via ongoing thrips migration from the weeds. Similar

disease outbreaks occurred in this district in tomato with TSWV during the 2014 season due to late rainfall, economic impacts during that season were significant. A switch to TSWV-resistant tomato varieties has prevented further outbreaks.

The 2022 research was included in the disease management guide as a case study.

Vector trapping and virus monitoring in Carnarvon, WA

Vector populations for the major insect groups studied were variable each year of the monitoring (Figure 4). Thrips were the most caught vector in the district and were almost continually present on the traps. Peak catches of thrips were variable throughout the study period with the maximum number recorded in Sept (2019), Dec. (2020) and March (2021) with over 100 insects per trap. Only the tomato thrips (*Frankliniella schultzei*) and western flower thrips (*F. occidentalis*) were found on vegetable crops. A major outbreak of *tomato spotted wilt virus* (TSWV) in capsicum occurred in June to Sept. 2020, when thrips levels were high.

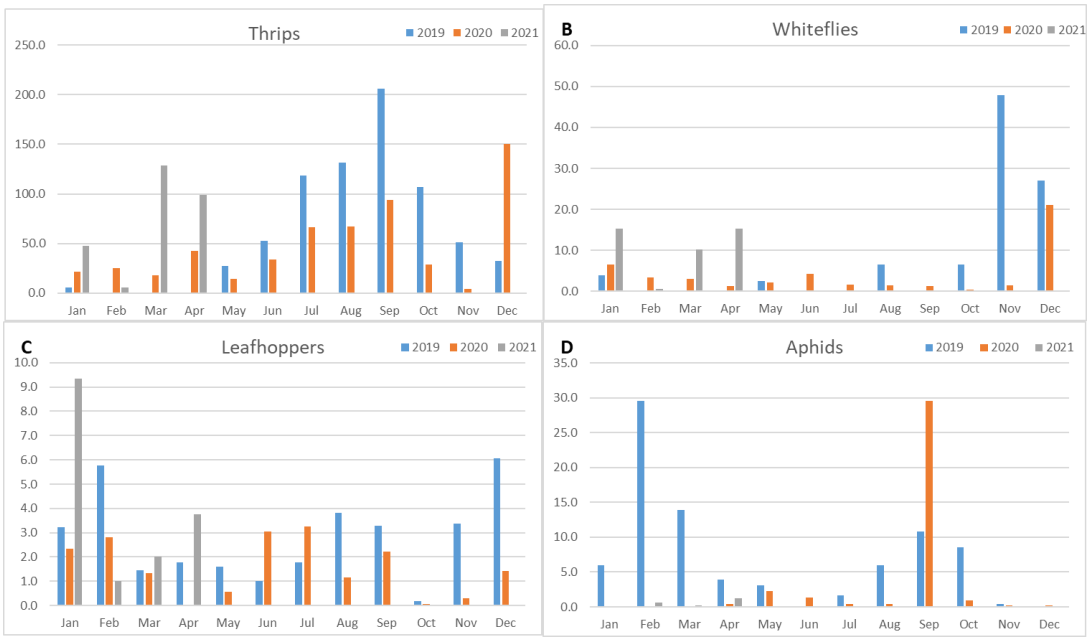

Figure 4 Numbers of the key virus disease vectors: thrips (A), whiteflies (B), leafhoppers (C) and aphids (D) caught in the Carnarvon horticultural area.

Trapping thrips using both blue and yellow colored sticky traps proved successful. High numbers of thrips were caught using both colored traps and no strong preference for thrips to either color was detected. Both types of traps could be useful for monitoring thrips populations in the region, although yellow traps are more appealing to the other key vectors so would provide more information and are recommended.

Whitefly were also commonly seen in the district; however, no whitefly transmitted diseases have been recorded so their presence is of low concern to the region. The whiteflies caught at several properties were identified as *Bemisia argentifolia* (formally MEAM-1 or B biotype of *B. tabaci*).

Leaf hopper of unknown species were detected throughout the project, these can spread phytoplasma diseases which were seen on several vegetable crops (capsicum, pumpkin, eggplant). While this disease was commonly found it rarely exceeded 1% incidence in the crop so was of low economic impact.

Of most concern were aphid numbers and the spread of virus diseases such as *zucchini yellow mosaic virus* (ZYMV; seen in all years of the project) and *cucumber mosaic virus* (CMV; seen in capsicums in 2021). Aphids were present in two major waves (Feb. 2019 and Sept. 2020) in events that were linked to rainfall in the district. Their numbers were also highly variable amongst traps on different farms, however during the periods of peak numbers there were present on nearly all traps. The identity of 192 aphids caught flying over vegetable crops were determined. Of these, the green peach aphid (*Myzus persicae*) was the most detected (>40%), while the majority of the remainder were turnip, oat or blue green aphids. All aphids detected (except the rice root and *Hyperomyzus carduellinus*) were able to transmit ZYMV, while the

melon aphid (the only aphid which regularly colonizes cucurbits) was only rarely detected (2.6%). This means that monitoring cucurbit crops via leaf inspections will miss the large majority of aphids which can spread the virus and trying to control virus infection by spraying systemic insecticides will largely fail (as the non-colonizing aphids transmit the virus before being killed by insecticides). Figure 5 shows the proportions of the various aphid species detected.

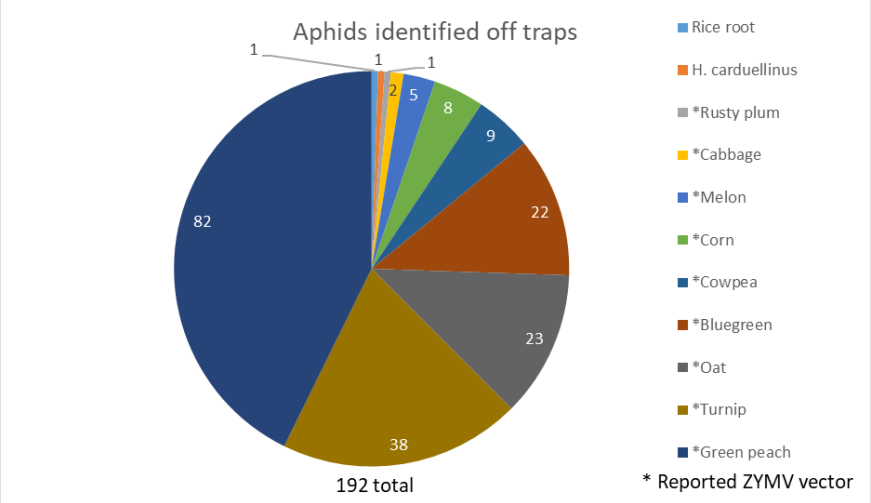

Figure 5 Aphids identified by sequencing of yellow sticky traps in proximity to vegetable crops.

Aphid and virus monitoring near Perth, WA

Aphids were first seen in April (2019 or 2021) of May (2020), following first rains in the area. Numbers increased rapidly and peaked in September of each year. They had dropped markedly by October and were not observed on traps for the remainder of the year (Figure 6). Young carrots planted during this window each year developed high incidences of virus (near 100%). Management of aphids in these crops was primarily through systemic insecticides.

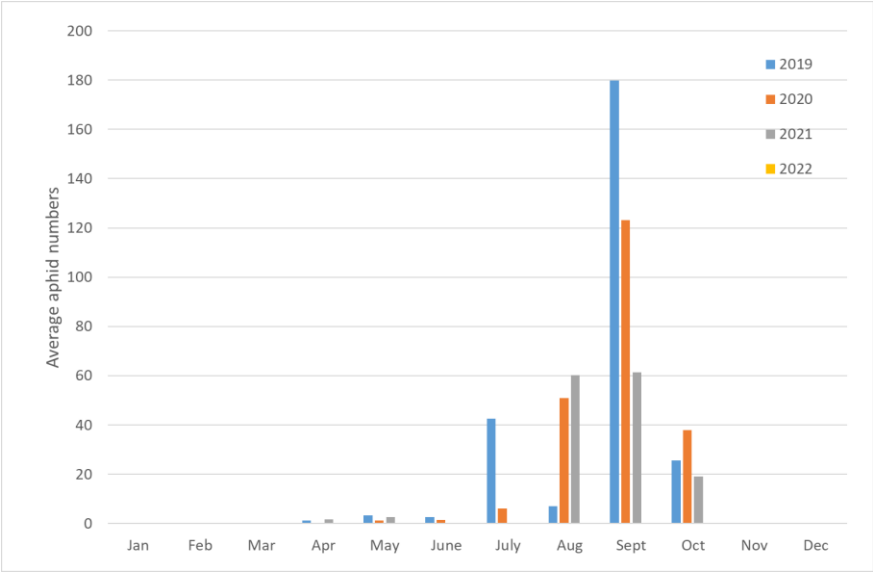

Figure 6 Average number of aphids on traps adjacent to carrots affected by annual epidemics of *Carrot virus Y*.

A total of 318 caught aphids were identified by sequencing (Table 2) the majority were the oat aphid (*Rhopalosiphum padi*) and green peach aphid (*Myzus persicae*) with 8 other species representing approximately 25% of the aphids caught. For several species of aphids, the efficiency of the aphid to spread *Carrot virus Y* was determined and this ranges from very poor (0.5% for *R. padi*) to very high (49% for *M. persicae*). From these numbers the relative role of each species to

contribute to virus epidemics was estimated and *M. persicae* was clearly contributing the most to the epidemics. This knowledge can help in targeting sprays to key areas, as the grass colonizing aphids (*R. padi* and *R. maidis*) do not contribute significantly, while the legume and brassicae colonizing aphids (*M. persicae*, *L. pseudobrassicae* and *A. kondoi*) together contributed >98% of virus spread. It was also noted the percentage of *M. persicae* changed on traps, generally increasing in both absolute number caught and percentage of the population over time.

Table 2 A list of aphid species detected on traps. Details on the total number caught, their relative percentage of total aphids caught, and their relative efficiency to transmit carrot virus Y are provided.

Species of aphid	Number caught	Percentage of total	Reported Efficiency	Relative role in transmission
Rhopalosiphum padi	152	47.8	0.50%	1.35%
Myzus persicae	91	28.6	49%	79.3%
Lipaphis pseudobrassicae	18	5.7	34%	10.9%
Acyrthoisphon kondoi	47	14.8	10%	8.4%
Rhopalosiphum maidis	1	0.31	2%	0.1%
Captiophorus elaeagni	1	0.31	N/A[1]	N/A
Therioaphis trifloii	1	0.31	N/A	N/A
Aphis craccivora	2	0.63	N/A	N/A
Rhopalosiphum rufiabdominale	5	1.57	N/A	N/A
Aphis lugentis	4	1.26	N/A	N/A

[1]N/A = the aphid species does not transmit carrot virus Y.

Appendix 3: Disease management trials

Knowledge gained from these field trials was distributed to industry at demonstration days where possible and is incorporated into management recommendations captured in the two guides published from the project. These are:

'A guide to understanding and management of bacterial diseases of vegetables'

'A guide to understanding and management of viral diseases of vegetables'

Genetic tolerance to potyviruses affecting zucchini (QLD and WA)

Potyvirus infection is a major limiting factor to zucchini production in many Australian production areas. *Papaya ringspot virus* (PRSV) type W is the main virus found in Qld while *zucchini yellow mosaic virus (*ZYMV) dominates in WA. Surveys in the Swan Hill/Mildura area (Victoria) several years ago identified *watermelon mosaic virus* (WMV) as the dominant virus in zucchini in the region. Non-persistent aphid transmission and the abundance of host crops frequently results in very high disease levels by early flowering. Affected crops have reduced fruit set and high numbers of deformed unmarketable fruit. Insecticides are seldom effective in reducing virus spread and crop hygiene to reduce inoculum levels is often poorly implemented.

Rapid aphid transmission and the abundance of host crops frequently results in very high disease levels by early flowering. Affected crops have reduced fruit set and high numbers of deformed unmarketable fruit. Due to aphids being able to spread these viruses in feeding times of less than one minute insecticides are seldom effective in reducing virus spread and crop hygiene to reduce inoculum levels is often poorly implemented.

Over the last decade or so considerable investment has been made by seed companies in developing *C. pepo* varieties with tolerance to the potyviruses and in some instances to Cucumber mosaic virus. Several genes such as *Prv* and *zym* have been used in various combinations. The varieties are tolerant not highly resistant in that plants do become infected following either aphid inoculation or sap inoculation in greenhouse tests. The value of the varieties is their capacity to produce good yields of saleable fruit under considerable virus pressure.

Previous work some five years ago had demonstrated the value of tolerant zucchini varieties in reducing the impact of virus disease in the crop. In project VG 16086, the work has been expanded and new generation varieties compared with those previously available.

PRSV resistance in zucchini (QLD)

Three field trials were completed in QLD in 2019 in collaboration with Agreco Australia and Prospect Agriculture. These were at Gatton, Bundaberg and Bowen. The aim was to assess varieties for tolerance in the presence of PRSV.

Trial design was four replicates with single row plots with 10 plants/ plot at 0.5m spacing. Squash plants inoculated with PRSV were used as virus reservoirs to allow aphid transfer to test plots. Test plants were not directly inoculated with virus. There were 16 varieties in the Gatton trial; 12 at Bundaberg and 27 at Bowen. Data were collected on virus incidence, symptom severity on plants and fruit and aphid populations. Virus severity was rated using a 0-7 scale with 1=very mild symptoms and 5 to 7 severe to very severe symptoms. Figure 1 shows an example of symptoms. Yield data were collected on at least three occasions in each trial. Total yields, yields of marketable fruit and severity of virus symptoms on fruit were measured. Selected symptomatic plants were sampled for molecular testing for PRSV, ZYMV and WMV to determine their virus status. The only virus detected in symptomatic plants tested by molecular assays was PRSV.

Results:

- Varieties ranked as **highly tolerant** over three trials were Desert, Apollonia, Alessandra, Ebano, 003-6, Baily. These varieties developed only very mild leaf symptoms and had few if any fruit symptoms. This tolerance was reflected in high yields of marketable fruit with little wastage.
- Varieties with **intermediate tolerance** were: Seduction, Rosa, Eva, Pascola. These varieties developed leaf symptoms of intermediate severity (2-3 on severity scale). Moderate virus symptoms were seen on a proportion of fruit.
- **Susceptible** varieties included Regal Black and Amanda. Varieties in this group had leaf and fruit symptoms 5 to 7 on the severity scale.

Figure 1 Field trial zucchini plants challenged with potyvirus. The photograph on the left is of a tolerant variety showing no symptoms of virus infection and on the right a susceptible variety showing very obvious potyvirus infection.

ZYMV resistance in zucchini (WA)

Infection of zucchini by *zucchini yellow mosaic virus* (ZYMV) remains a limiting factor to zucchini production in WA, in contrast to the *papaya ringspot virus* (PRSV) or *watermelon mosaic virus* (WMV), which affects areas on the east coast. This work built on the previous work done in QLD as part of this project to identify Potyvirus resistance in Cucurbita pepo varieties. Field trials were established in the Carnarvon and Kununurra districts and the same varieties were evaluated in the glasshouse in South Perth using mechanical inoculation. Virus affected plants were included in Carnarvon, but no aphid control was applied to the trial. In Kununurra local grower opposition to introducing virus to the environment after its absence for 3 seasons was respected and no virus or aphids were detected in the trial.

Varieties ranked as highly tolerant over the trials were 065-0, Luda, Nitro, Syros, Baily, HMX588615, Desert, Windsor, Apollonia, Brookton and Alessandra. These varieties became infected at a low rate (<50% in trials and greenhouse mechanical inoculations), and symptoms on the fruit and leaves were absent or mild (Figure 2). Where symptoms did appear, they were also delayed by up to 4 weeks, compared to susceptible controls.

Intermediate tolerance was seen in HMX586539, Rosa and Eva with leaf symptom scores of 2 to 3 and virus symptoms on fruit making them unmarketable, albeit at a lower rate than in susceptible controls.

Susceptible varieties included Naxos, Regal Black, Black Jack and Goldie in which severe levels of virus damage (4 to 5) were seen, which appeared within 2 weeks of inoculation and resulted in the large majority of fruit being unmarketable.

The amount of virus in each variety was also tested in glasshouse grown and mechanically inoculated plants. The level of virus detected in the zucchini correlated highly with leaf and fruit symptoms, and was greatly reduced versus susceptible controls (up-to 10,000 fold less). This would suggest that these lines would also be impaired in their ability for aphids to acquire virus from, and transmit to, other susceptible cucurbit fruit and vegetables.

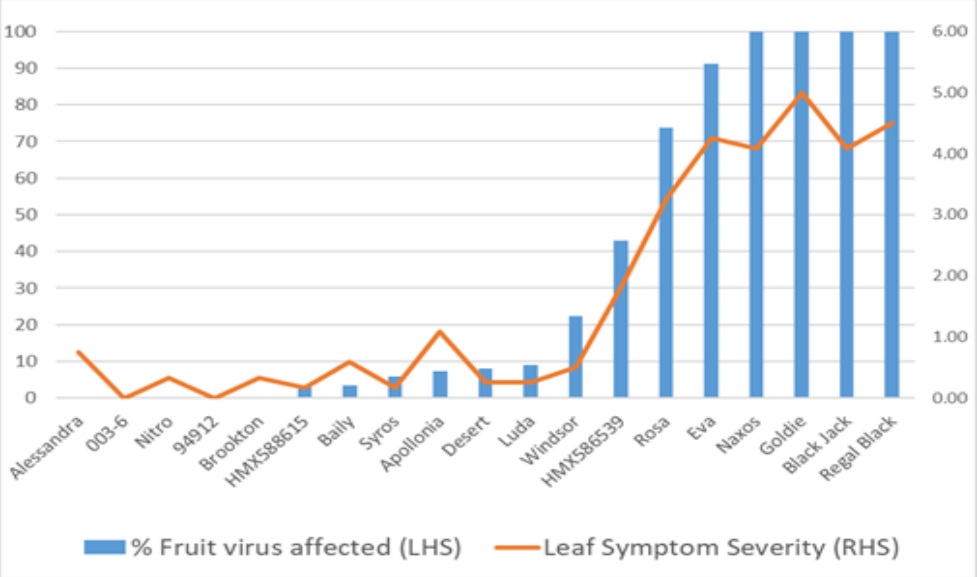

Figure 2 Zucchini with resistance to ZYMV following mechanical inoculation. Plants were rated for up to 8 weeks post inoculation for foliar symptoms (orange line, right hand Y-axis) on a 0 to 5 scale, with 0 being no symptoms and 5 being severe symptoms of stunting, shoestring and mosaic). The percentage of fruit showing symptoms of virus affection (blue bar, left hand Y-axis) was also recorded. Each zucchini variety evaluated is listed on the X axis.

Further work included a side-by-side comparison of selected resistant varieties to the four main cucurbit potyviruses affecting Australian zucchini. Screening of zucchini varieties for potyvirus resistance was completed as a replicated trial with 12 varieties evaluated for their resistance to the four major Potyviruses which infect them in Australia (PRSV, WMV and 2 strains of ZYMV). Results showed durable resistance to all four viruses was present in varieties available to growers in Australia. This included a reduced number of plants which became infected, and reduced symptom (leaf and fruit) severity in plants which did become infected. The replication of the virus in these plants was greatly diminished which would also reduce the chances of aphids spreading the virus to other plants in the field. No single variety was the best performing for all four viruses, so growers need to choose from those most suitable for their district.

These varieties were also evaluated in the field for ZYMV resistance in Perth (2020), Carnarvon (2020, 2021) and Kununurra (2021). Consistent results were obtained in Carnarvon in both years, however a lack of virus in Kununurra and Perth occurred when the trials were held. Few plants of resistant varieties developed symptoms, and in those that did they were milder. Fruit from infected plants were often marketable, particularly in times of high disease pressure. Industry standard varieties by comparison were almost completely infected and no marketable fruit developed in the trial.

Conclusion: Tolerant varieties perform very well to both PRSV and ZYMV.

Recommendation: To trial several lines of these tolerant varieties on-farm to choose those suitable to the local production climate.

Management of potyvirus in zucchini without genetic tolerance (QLD and WA)

Potyvirus infection is a major limiting factor to zucchini production in many Australian production areas. Papaya ringspot virus (PRSV) type W is the main virus found in Qld while zucchini yellow mosaic virus (ZYMV) dominates in WA. Surveys in the Swan Hill/Mildura area (Victoria) several years ago identified watermelon mosaic virus (WMV) as the dominant virus in zucchini in the region. Non-persistent aphid transmission and the abundance of host crops frequently results in very high disease levels by early flowering. Affected crops have reduced fruit set and high numbers of deformed unmarketable fruit. Insecticides are seldom effective in reducing virus spread and crop hygiene to reduce inoculum levels is often poorly implemented.

Trial work was conducted to review tolerant varieties compared to management of aphid vectors through banker plants

and barrier plantings, management of vectors through insecticides and management of disease through treatment of old crops to prevent migration of viruliferous aphids into new crops.

Conclusion: Banker plants are useful to maintain beneficial biocontrol agents, but it is difficult to establish this. Timing is critical and requires optimization in the local setting where it would be used. Reduction of adult aphids on old crops prior to their destruction is important to reduce risk of virus transfer to new crops.

Recommendations: Further research works is needed before banker plants can be adopted as a management strategy for virus control.

Queensland:

A glasshouse trial to evaluate a range of species in the *Brassicaceae* family for susceptibility to *turnip mosaic virus* (TuMV). This included a range of plants that are recommended for use as cover crops. Results are shown in Table 1 and highlight a wide range of susceptibilities to this virus across a range of genera, species and varieties. Of the commercial vegetable crops Pak choy, Chinese broccoli and rocket showed no symptoms of virus infection whereas Chinese cabbage was severely affected. For the cover, biofumigant or forage brassicas evaluated in this study, there was a lot of variation within species, for example, mustards ranged from no symptoms to very severe. Similarly, turnips, radish and rape plants were susceptible, from moderate to very severe. The three plant lines tested showed a big variation within the replicates, from only moderate to very severe (Table 1). This indicates possible genetic segregation of the plant lines. As several of the brassicas are used for biofumigation, forage or as cover crops are susceptible to the virus, care is needed when planting subsequent brassica vegetable crops to ensure any reservoirs of virus and/or aphids are destroyed prior to planting. This will prevent transfer of the virus from those crops to the vegetable crops.

Table 1 List of plant species and varieties tested for susceptibility to turnip mosaic virus in glasshouse trials. Crops listed as other include those used as cover, biofumigation or forage crops.

Species	Variety	Crop	Average rating[1]
B. juncea	Nemclear	Other	0
B. carinata cv. cappuccino	Ethiopian mustard	Other	0
Eruca sativa	*Rocket*	Vegetable	0
Brassica rapa subsp. *chinensis*	Pak Choy - Joi Choi	Vegetable	0
Brassica oleracea var *alboglabra*	Chinese broccoli	Vegetable	0
Brassica carinata	Ethiopian mustard	Other	1
B. napus	Invitation Swede	Other	1
B. juncea	Calinete Rojo[2]	Other	2
B. napus	Interval forage rape	Other	2
Brassica napus	Leafmore forage rape	Other	2
B. juncea	Mustard[2]	Other	3
B. juncea	Mustclean 666[2]	Other	3
R. sativus	Blackjack radish	Other	3
Brassica rapa var. *pekinensis*	Chinese cabbage - Tokyo Bekana	Vegetable	3
Brassica alba	White mustard	Other	4
Brassica nigra	Black mustard	Other	4
Raphanus sativus	Tillage radish P14148	Other	4
R. sativus	Oilseed radish Terranova	Other	4
B. rapa	Dynamo forage turnip	Other	4
B. rapa	Falcon leafy turnip	Other	4

[1] Rating system: 0=no symptoms; 1=mild; mosaic mottle on new growth but mild; 2=mod; mosaic mottle obvious, possible ringspots; 3=severe; prominent mottle mosaic symptoms; 4=very severe; severe stunting, severe mottle mosaic and distortion. [2] These plants had a big variation in responses amongst replicates.

Recommendation: As several of the brassicas used for biofumigation, forage or as cover crops are susceptible to TuMV, care is needed when planting subsequent brassica vegetable crops to ensure any reservoirs of virus and/or aphids are destroyed prior to planting. This will prevent transfer of the virus from those crops to the vegetable crops.

Queensland: Potyvirus in brassicas

Virus diseases are prevalent in brassica crops in south Queensland and elsewhere, with Chinese cabbage (wombok) and daikon being particularly affected. The most prevalent virus in crops is turnip mosaic (TuMV) which is spread by aphids, in a non-persistent manner. This virus infects most cultivated brassicas and several weed species in the family. Aphids can spread the virus very quickly with the insect needing to feed for less than a minute to obtain the virus from an infected plant or introduce it into another plant as it feeds using a needle -like stylet. There are limited management options for the virus because of the potential for rapid spread, very few resistant or tolerant varieties and the prevalence of alternative weed and crop hosts. A field trial was completed at the Gatton Research Facility, to assess three insecticide treatments for efficacy against TuMV, in wombok (cv Matilda). This included a new chemistry insecticide and two pest oils.

A field trial was completed and compared the insecticide treatments Versys (afidopyropen), Sero-X (extract of Citoria ternatea) and Biopest Oil (paraffinic oil). Versys and Sero-X are both claimed by the manufacturers to have rapid antifeedant effects, and Versys is also claimed to reduce virus transmission, although this is for persistently transmitted viruses. TuMV and other potyviruses are non-persistently transmitted by aphids. Mineral oils have previously demonstrated efficacy for reducing transmission of non-persistent viruses in field trials. Seedlings were drenched with Durivo prior to planting and then Sero-X and Biopest Oil were applied weekly, and Versys every second week, from one week post planting onwards. Insecticides were compared with an untreated control. The trial was set up in a randomised block design, with four replicates for the Sero-X and control treatments, and five replicates for the Versys and Biopest Oil treatments. TuMV introduced into the guard plants at the ends of the replicates. This was either by sap inoculation of plants or transplanting infected plants. Additionally, a TuMV infected seedling was planted in each bed through the center of the trial.

Aphid numbers remained low throughout the trial period. There was no effect of treatment on aphids and there were no significant differences between the treatments for virus control (Figure 3).

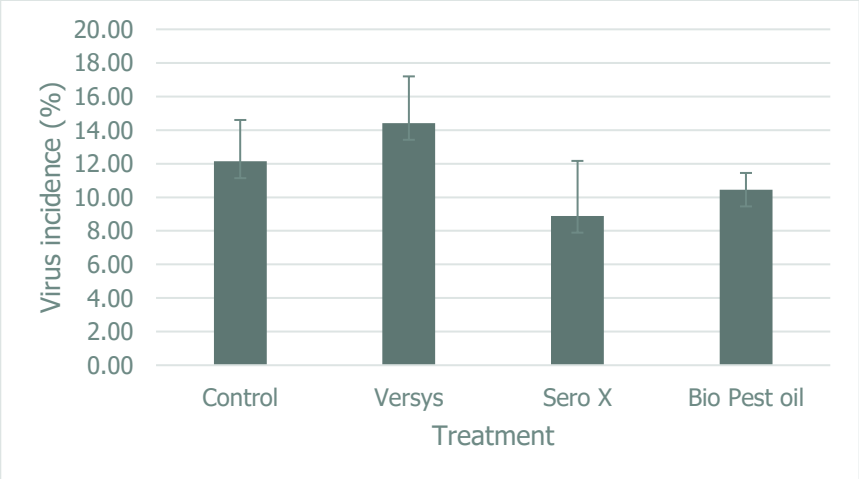

Figure 3 Graph of the average virus incidence for each of the four management treatments. Standard deviation between replicates is shown as vertical lines. No significant differences between treatments

Conclusions:

- None of the products compared in the trial reduced TuMV spread effectively.
- Use of these products in other ways may provide useful options for control of these viruses, for example, use on

old crops to decrease adult aphid levels before crop destruction. This would reduce risk of virus transfer into new crops. However, this would require evaluation before recommendation.

Recommendations: further work is needed on aphid-transmitted viruses affecting brassica and lettuce crops to develop better management strategies. Research from this project on management of potyviruses affecting zucchini and the orthortospoviruses affecting capsicum/chili would provide a very high base-line for delivering similar management options for brassica and lettuce growers.

Orthotospovirus management in Solanaceae (QLD)

Tomato spotted wilt virus (TSWV) and *Capsicum chlorosis virus* (CaCV) are both transmitted by thrips and cause disease in capsicum and chilli. Although resistance genes are available for TSWV in capsicum, their durability is questionable and evidence of resistance-breaking strains of the virus are reported nationally. CaCV was previously restricted in its distribution but is now emerging as an economically impacting disease in more areas. In the dry tropics of QLD during the 2018 season both viruses were detected in commercial crops at high levels and in Bundaberg, although both are present, CaCV is the dominant virus. There is no known resistance to either virus in chilli.

The reported vectors of CaCV are *Thrips palmi* (melon thrips)*, Ceratothripoides claratris* (oriental tomato thrips) and *Frankliniella schultzei* (tomato thrips) (Persley et al. 2006). There was no record of *Frankliniella occidentalis* (western flower thrips) as a vector of CaCV in that review paper and it is assumed it isn't a vector.

Disease outbreaks are a function of virus incidence in weed hosts, thrips population levels and timing of planting. Worst case scenarios are where there is a significant overlap with weed populations with first plantings of crops. As the weeds dry off the thrips move into these crops carrying virus. If not managed appropriately, these crops then become a significant source of virus for subsequent crops.

The management trial was aimed at assessing the importance of secondary virus spread within crops and to evaluate a range of management options to reduce tospovirus spread within chilli crops. The persistent transmission mode of orthotospoviruses by thrips allows potential to reduce secondary spread. Adult thrips are unable to transmit orthotospoviruses unless they acquired the virus as a larva. Control of thrips populations reproducing within a crop provides an opportunity to reduce secondary spread, particularly through the control of the juvenile stages.

This management trial was discontinued due to low plant establishment from on-going wet weather. Research results from field disease outbreaks in North QLD 2022 indicate that secondary spread of virus within crops is not significant. Existing pest control measures manage the thrips in capsicums to negligible levels and colonization is rare. Without colonization secondary spread of these viruses does not occur. This results were captured in the virus disease guide and provided directly to the growers in that district through a grower workshop in Gumlu, July 2022.

Glasshouse trial of *Pectobacterium* and *Xanthomonas campestris* pv. *campestris* in Wombok

Diseases caused by *Pectobacterium* spp., particularly in zucchini and brassicas, are identified as an emerging national concern. The diseases were detected in multiple growing districts nationally, with varying impact. Additionally, *Xanthomonas campestris* pv. *campestris* (Xcc) is known to cause disease in brassicas nationally. Both pathogens were detected in a severe disease outbreak in wombok in the Granite Belt, QLD in 2017. Investigation of bacterial survival between cropping seasons was evaluated with pot trials established to determine if either of the pathogens associated with the disease outbreak in wombok could persist in soil in the absence of host material. Field soil was collected from a block where the badly affected crop (>70% disease incidence) had been incorporated into the soil. The soil was left for about three months and then wombok seedlings planted into the soil in a replicated trial with commercial potting mix used as the control. The wombok seed were treated to remove potential contamination with bacterial pathogens using the protocol developed by Berg et al. (2004). Each soil type was replicated in three blocks of 10 plants. Plants were rated for disease using the following symptoms and scale:

- Systemic infection – lesion(s) extending down the leaf midrib
- Wound response – lesion(s) on leaf (not extending) and papery in appearance
- Local lesions – lesion(s) on leaf (not extending) and water-soaked in appearance, with or without a yellow halo

Scale of severity:

0. None
1. 1.Mild – one leaf only affected
2. 2.Moderate – two leaves affected
3. 3.Severe – multiple leaves affected
4. 4.Completely affected

A second trial was established which included additional treatments of soil plus a known concentration of the pathogen (Xcc at 1.5 x 10^8 CFU/ml or *Pectobacterium* spp at 3 x 10^8 CFU/ml). The treatments were:

1. 1.potting mix no pathogen added,
2. 2.field soil no pathogen added,
3. 3.potting mix plus Xcc,
4. 4.field soil plus Xcc,
5. 5.potting mix plus *Pectobacterium* spp.
6. 6.field soil plus *Pectobacterium* spp.

Each treatment was replicated in three blocks of eight plants and rated using the above system.

Putative Xcc infections were evaluated by testing lesions for bacterial ooze, isolation culturing from lesion fragments soaked in sterile water and by PCR using the method described by Berg et al. (2005). For PCR both the lesion fragments and pure bacterial cultures from isolation attempts were tested. Putative Pectobacterium spp. infections were evaluated by bacterial ooze testing and isolation culturing only as no PCR test is currently available.

From the first trial, eight plants were tested for putative bacterial infection, all from field soil. The symptoms, however, were not typical of either pathogen and none of the lesions showed evidence of bacterial ooze. All lesions were also negative by PCR for Xcc and no bacterial cultures were obtained of Xcc or Pectobacterium spp. The conclusion was there was no bacterial infections of wombok seedlings from either soil type for either pathogen.

In the second pot trial, two of the 24 seedlings planted in the field soil and four of the 24 seedlings planted in potting mix (i.e control treatments, without deliberate infestation with Xcc or Pectobacterium spp.) became infected with Xcc. Given this low rate of infection and that none of the 48 plants grown in soil infested with Pectobacterium spp. were infected with Xcc, the positive infections by Xcc in the control plants is therefore most likely due to cross-contamination from infested pots grown in close proximity. These results were therefore removed from the analyses. By contrast, of the 24 pots of potting mix infested with Xcc, 21 showed signs of disease and were subsequently confirmed positive for Xcc by PCR testing. Although infestation of the field soil also resulted in plants succumbing to disease caused by Xcc, there was only 10 of the 24 plants affected. Most of the plants affected by disease showed symptoms of systemic infections and most were rated as 2 or above. Plants were rated a second time, 10 days later. Further plants succumbed to infection and disease symptoms were rated higher (most 3 or more). Of the 48 plants in total exposed to Xcc (both soil types combined), only eight were recorded as uninfected at this second rating.

Conclusions:

- No plants were infected with Pectobacterium spp. in any of the treatments.
- These results indicate that although newly planted seedlings can become infected by Xcc from soil infestation, the field soil is not a significant risk as a reservoir for this to happen. Only seedlings planted into pots of soil (either field or potting mix) deliberately infested with Xcc became infected. Those planted into either soil type without addition of the bacterial inoculum remained disease free. No plants became infected by Pectobacterium spp. in either trial.

Recommendations: Further work is needed, however, on managing disease outbreaks once they occur. This is through research to better understand the infection and spread processes which will ultimately lead to better management options.

References:

Berg, T, L Tesoriero, and D Hailstones. 2004. “Quality Assurance for Improved Management of Black Rot of Brassicas: Improved Detection and Disinfestation in Seed; Management Protocols for Seedling and Field Production,” 66.

Berg, T., L. Tesoriero, and D. L. Hailstones. 2005. “PCR-Based Detection of Xanthomonas Campestris Pathovars in Brassica Seed.” Plant Pathology 54 (3): 416–27. https://doi.org/10.1111/j.1365-3059.2005.01186.x.

Pectobacterium spp. causing crown rot in zucchini (QLD and WA)

Diseases caused by *Pectobacterium* spp., particularly in zucchini and brassicas, are identified as an emerging national concern. The diseases were detected in multiple growing districts nationally, with varying impact. The source of disease outbreaks in zucchini were evaluated in separate studies in WA and QLD, with similar outcomes seen.

In QLD, a zucchini field was conducted at the Redlands Research Facility, Redland Bay to study a bacterial disease reported from the Granite belt field production in 2020-2021 season. The aim of this trial was to replicate the crown rot symptoms of the Pectobacterium seen in the field outbreak and to investigate different sources of the disease through use of different methods of plant inoculation. The feasibility of studying bacterial spread on harvesting equipment was also assessed. The timing and field conditions required for the trial were also monitored to determine the optimal sampling time to recover the causal bacterium of any observed symptoms. Using this pilot trial, we were able to determine disease expression only occurred with injection of bacteria into the stems, no disease was observed when bacteria was applied to the rhizosphere as a suspension. The possibility of in-field spread was noted as late-stage symptoms caused by Pectobacterium were also seen in control rows. There was no obvious spread of bacteria on harvest knives contaminated from cutting fruit off disease affected plants and then use on healthy plants. Photographs of the field trial and an enlarged view of an affected zucchini crown is shown in Figure 4. These results support those from experiments conducted in WA (described below).

Figure 3 Field trial site showing infected plants from injection inoculation. A close up of crown rot symptoms is also shown in the right-hand photo.

Conclusions:

- infections of zucchini seedlings are low risk from bacteria present in the soil
- transfer of bacteria on harvest knives from affected plants was not sufficient to initiate new infections, however, this may require further clarification

In WA, a series of glasshouse experiments were completed to evaluate infection and spread pathways.

A). A number of glasshouse experiments have been conducted, trying to determine how *Pectobacterium* enters the zucchini plants and whether it is retained in the soil between crops. All experiments used susceptible variety “Black Jack”. Two treatments were included:

1. Contaminated soil (collapsed zucchini that was previously infected with *Pectobacterium* added to the soil). Clean transplants were then planted into the contaminated soil. A healthy control of clean potting mix was included

2. Transplanted seedlings were watered with inoculum (inoculum concentration was approx. 10^6 cfu/mL). A healthy control of sterile water was included.

Six days after treatment, there was no collapse of the plants. Four plants had a leaf removed and dirt rubbed onto the wound, or a leaf removed and the wound sprayed with inoculum.

Two weeks after inoculation, plants were sampled, and roots were tested for presence of Pectobacterium. No Pectobacterium was detected in the plants.

B). A second experiment to test variety and host range susceptibility to Pectobacterium was also completed. Varieties Black jack, Nitro and Eva were tested for resistance to Pectobacterium infection in the greenhouse. Seedlings were inoculated at the first true leaf stage by infecting the stem with a 19 gauge needle with approx. 250 µL of bacterial suspension, and there were three dilutions used. Seedlings were injected with inoculum at 10^6 10^5 and 10^4 cfu on day 1. On day 2 no symptoms of collapse were seen, so they were re-inoculated using the same method. When an aliquot of bacteria was plated onto nutrient agar, the viability of the bacteria was confirmed.

The results (Table 2) showed all varieties were susceptible with browning and wilting occurring. The bacteria were re-isolated from all treatments. Black Jack and Nitro appeared the most susceptible varieties with the majority of plants collapsing, although a clear reduction in the number of plants, which collapsed or showed symptoms, occurred at higher inoculum levels. Eva was more tolerant with fewer plants developing browning at the stem at the site of injection and none developed the characteristic collapse seen in Nitro and Black Jack.

This trial was repeated on a wider selection of varieties, including several identified as having Potyvirus resistance, as these are increasingly being used in area's affected by the viruses. Several *Pectobacterium* cultures collected from vegetable crops were used. These included *P. brasiliense* and *P. carotovorum* subsp *carotovorum* cultures, which were identified by MALDI-TOF, BIOLOG® and qPCR.

All varieties injected with *P. brasiliense* had extensive browning of the stem with bacterial ooze occurring at the injection site (Table 3). In most varieties this progressed to a brown soft rot of the stem, with necrotic streaks occurring down the petiole. In variety Regal Black this continued to cause complete plant collapse. Browning and some soft rot was seen in plants injected with *P. carotovorum*, however, while this appeared milder externally, necrosis and browning occurred along the interior of the plant. No varieties displayed sufficient resistance which would likely be commercially useful.

Table 2 Effect of dilution of *Pectobacterium* on three varieties of zucchini.

Variety	Pectobacterium concentration	Symptoms
Black Jack	10^6 cfu/mL	All brown and collapsed
	10^5 cfu/mL	5/5 brown
	10^4 cfu/mL	2/5 brown with wilting
	Control	0 brown and collapsed
Nitro	10^6 cfu/mL	5/5 brown
	10^5 cfu/mL	2/5 brown, 1 soft and 1 slightly brown
	10^4 cfu/mL	0/5 brown
	Control	0 brown and collapsed
Eva	10^6 cfu/mL	2/5 quite brown, 2/5 bit brown and 1 okay
	10^5 cfu/mL	1/5 brown
	10^4 cfu/mL	0/5 brown
	Control	0 brown and collapsed

Table 3 Response of zucchini varieties to a range of *Pectobacterium* isolates with (B = Browning), (W = Wilt) and (O = Ooze).

Variety	*P. brasiliense (zucchini-WA)*	*P. brasiliense (zucchini Qld)*	*P. brasiliense (capsicum-WA)*	*P. carotovorum subsp. carotovorum (zucchini-WA)*

Apollonia	B,O	B,O	B,O	B
Black Jack	B,O	B,O	B,O	B
Brookton	B,O	B,O	B,O	B
Desert	B,O	B,O	B,O	B
Eva	B,O	B,O	B,O	B
HMX586615	B,O	B,O	B,O	B
Luda	B,O	B,O	B,O	B
Nitro	B,O	B,O	B,O	B
Regal Black	B,O,W	B,O,W	B,O,W	B
Rosa	B,O	B,O	B,O	B
Syros	B,O	B,O	B,O	B

Finally, preliminary experiments have identified that other vegetables may be susceptible to this bacterium. Ten varieties were injected, as above, with a 10^6 cfu/mL suspension of *Pectobacterium brasiliense* (Table 4). Several of the vegetables developed stem browning, although the number of plants was lower than in the zucchini controls. In addition, no plants developed the wilt and collapse often seen in zucchini and *P. brasiliense* could be re-isolated from representative plants of each species. Although this work indicated several other crops which are grown in the Carnarvon area are susceptible the lack of systemic wilt indicates they may not be of economic concern in these crops, but future surveys will continue to check if there are any indications of disease caused by this bacterium.

Table 4 Symptoms of soft rot observed in vegetables injected with *Pectobacterium brasiliense*

Species	Variety (crop)	Symptoms
Cucurbita pepo	Black Jack (zucchini)	Stem browning, collapse, wilt (5/5)
Cucurbita pepo	Jap Pumpkin	No symptoms
Cucurbita pepo	Queensland Blue	Stem browning, ooze (3/5)
Cucurbita moschata	Butternut	No symptoms
Citrullus lanatus	Afghan melon (weed)	Stem browning (1/5)
Citrullus lanatus	Candy Red (watermelon)	Stem browning (2/5)
Cucumis sativus	Reko (cucumber)	No symptoms
Cucumis melo	Claudia (rockmelon)	Stem browning (2/5)
Luffa acutangular	(Luffa)	No symptoms
Darcus carota	Stefano (Carrot)	No symptoms
Capsicum annum	California wonderer (Capsicum)	Stem browning (2/5)
Solanum lycopersicum	Grosse Lisse (Tomato)	No symptoms

In Qld, several small scale trials were conducted to investigate initial questions around symptom replication and bacterial spread in the field. A pot trial using soil from a field infected with Pectobacterium investigated the potential for this soil to infect new plantings. This trial was conducted on zucchini var. Alessandra seedlings that were inoculated using the stab inoculation method. Potting mix and water inoculation was used as controls. This trial showed that while the symptoms could be replicated with the stab inoculation method, the field soil resulted in less disease expression compared to the potting mix (Figure 5). This indicates re-infection from soil contamination is unlikely.

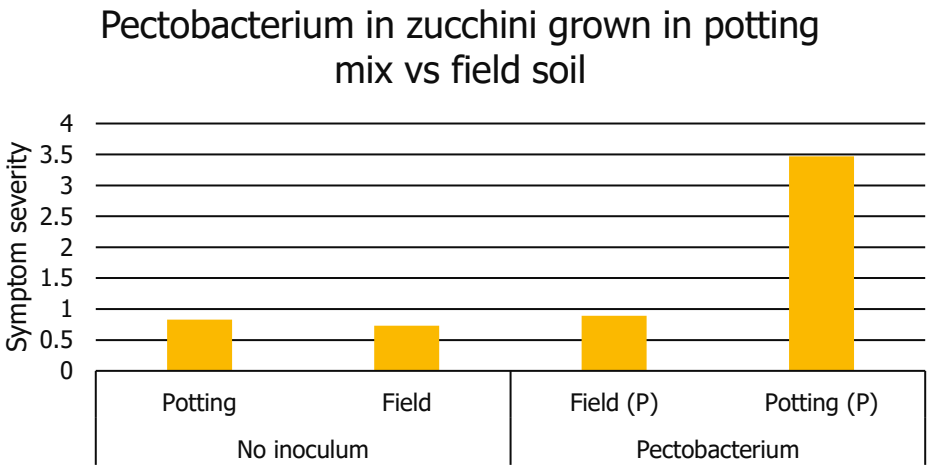

Figure 5. symptom severity of Pectobacterium inoculated in zucchini planted in field soil and potting mix.

Two field trials were then conducted to further explore inoculation methods and in field spread. Both field trials used zucchini var. Rosa and aimed to test to the following questions:

- Do tools transfer the bacteria between plants?
- Does drenching seedling roots with inoculum result in disease?
- Does the stab inoculation method reliably replicate the disease symptoms in the field?
- Can we track the inoculated bacteria spreading through the field?

The first trial concluded that bacterial transfer on tools did not result in increased disease spread. Drenching with inoculum did not reliably result in symptom replication either. The stab inoculation method did reproduce symptoms in the field. As some plants in the control rows showed symptoms of infection (wilt and crown rot with Pectobacterium detected), the next trial aimed to track the spread of the inoculated bacteria through the field.

A rifampicin mutant of the Pectobacterium isolate was generated (using the method of bacterial growth on rifampicin media) and inoculated to track bacterial spread. These isolates were injected and drenched (along with controls). Indicator plants were uninoculated zucchini at the end of the rows. This trial resulted in rapid death (wilting and rot) of the entire field regardless of inoculation, possibly because of an increased rain period. Where the rifampicin was inoculated, it was also able to be recovered as it grew within one day on the rifampicin media. Where disease was noted on uninoculated or indicator plants, no rifampicin mutant was recovered (table 5). Isolations were also carried out on control media to isolate non rifampicin resistant bacteria. This shows that while the inoculated bacteria did persist in zucchini plants, there is also evidence of naturally occurring Pectobacterium inoculating other plants.

These trials addressed our questions in the following way:

- Can we track the inoculated bacteria through the field? - yes
- Tracking with rifampicin mutant seems to work well
- No evidence of the inoculated mutant bacteria moving through the field
- Evidence of naturally occurring disease in control plants

Table 5. growth of isolated bacteria on rifampicin and control media. Column 1 describes the method of sample inoculation and column 2 shows if the plant was inoculated or not.

Method	**Rif mutant inoculum**	**Rif media growth 1d**	**growth on control media**
drench pect-Qld	yes	yes	yes
drench ctrl	no	no	yes

stab pect-WA	yes	yes	yes
stab ctrl	no	no	yes
stab pect-Qld	yes	yes	yes
stab pect-WA	yes	yes	yes
drench ctrl	no	no	yes
stab pect-WA	yes	no	yes
stab ctrl	no	no	yes
stab pect-WA	yes	yes	yes
indicator (ctrl)	no	no	yes
indicator (ctrl)	no	no	yes

Recommendations: Further work is needed, however, on managing disease outbreaks once they occur. This is through research to better understand the infection and spread processes which will ultimately lead to better management options.

Appendix 4: Grower forums and industry engagement

For all states and NT, a lot of engagement was conducted on individual properties with growers and agronomists, particularly in 2019 and 2019. This was very limited post-COVID and very few in-person activities were held. By 2021, delivery via webinars was becoming easier. Engagement during 2020 was very challenging.

Queensland

- 2018: forums held in Gatton (May), North QLD (August) and Granite Belt (September), these were in collaboration with VegNet; a lot of on-farm engagement
- 2019: forums held in Bundaberg (April), Ayr and Bowen (September), mostly in collaboration with AusVeg; first disease identification workshop held (Gatton, September) and second in Bundaberg (December) in collaboration with VegNet; a lot of on-farm engagement
- 2020: webinar for Lockyer Valley growers in response to a significant disease outbreak (July); webinar in collaboration with AusVeg and GrowCom (November)
- 2021: two field trial demonstrations (Bundaberg and Gatton); launch of bimonthly webinar series for vegetables and presentation of VG16086 results at two webinars; presentation at a PCA event in Gatton and presentation in Brisbane at Hort Connections; delivery of seed workshop with Agriculture Victoria in June (Brisbane); delivery of a disease identification workshops in North QLD with AusVeg (November); some on-farm engagement
- 2022: delivery of a disease identification workshops in North QLD (March); delivery of a disease identification workshop with Agriculture Victoria, VegNet and AusVeg in Mildura (April); monitoring of disease outbreaks in North QLD with close involvement by local agronomists; presentation at the DAF, QLD webinar series in June and the RMCG webinar series in July; delivery of seed workshop with Agriculture Victoria in June (Brisbane); delivery of a virus inoculation technique workshop in Bowen (June); delivery of a specific disease management presentation in North QLD (July); some on-farm engagement
- Ongoing mini-series outlining key findings from the project to be delivered by RMCG from August -October. This includes presentations from Queensland, Victorian and Western Australia project team members.

Victoria and South Australia

- 2018: forums and/or farm surveys held in SW Melbourne (June), Werribee (June, December), East Gippsland (October) and in Virginia (SA; October)
- 2019: forums and/or farm surveys held in SE Melbourne (May, November), Bacchus Marsh (November), Werribee (February, May, October, November), Gippsland (February, October, December), Mildura (March) and in Virginia (SA; September)
- 2020: forums and/or farm surveys held in Gippsland (December) and Mildura (February); COVID impacts severe in Victoria during 2021
- 2021: forums and/or farm surveys held in SE Melbourne (February, October), Bacchus Marsh (February), Werribee (February, April), Mildura (March) and webinar in collaboration with AusVeg (October); COVID impacts severe in Victoria during 2021
- 2022: forums and/or farm surveys held in Melbourne (February), Gippsland (April), Mildura (April) and delivery of a disease identification workshop with DAF, QLD and AusVeg in Mildura (April);

Western Australia

- 2018: workshops and on-farm surveys were held in Geraldton (August), Perth metro (August), Carnarvon (August) and the south-west (August). These were held in conjunction with AusVeg and Vegetables WA (August). Spoke with cucurbit growers on disease management at the Melon's Australia meeting (Townsville, September). An article on the project was included in the August issue of the WA Grower magazine.
- 2019: workshops and on-farm surveys were held in the south-west (April, May), Perth (April), Carnarvon (May, September) and Kununurra (August, October). Workshops in May were held in

conjunction with AusVeg and Vegetables WA. Workshops in October were in conjunction with QDAF project partners. Articles on the project were included in the February, May and August issues of the WA Grower magazine.

- 2020: on-farm surveys were held in the south-west (February), Perth (February, May) and Carnarvon (September, December). Impacts from COVID prevented holding workshops in person and travelling regionally to visit grower properties. Articles on the project were included in the February, May, August and November issues of the WA Grower Magazine.
- 2021: on-farm surveys and workshops held in March in both Perth and Carnarvon with Vegetables WA. COVID impacted again on ability to travel and hold in person meetings. Articles on the project were included in the February and May issues of the WA Grower magazine.
- 2022: Participation in the RCMG webinar series on cucurbit virus management (August).

Northern Territory

- March 2019: "Pre-season Meeting for vegetables, melons and all row crops" Held by NT Farmers Association, factsheets were handed out to attendees and an overview of the project and its current findings was presented.
- August 2021: "Financial fitness bootcamp" Held by NT Farmers Association targeting Vietnamese vegetable growers. The NT project team attended and provided information on the project, vector control and the detection of the Novel Tobamovirus.
- On site surveillance training for industry representatives; in particular NT Farmers Association Biosecurity officers. Conducted regularly throughout the project.

Appendix 5: Case studies

Case study: Area wide management of viruses spread by whitefly

Background

The benefits of implementing area wide management (AWM) to insect-transmitted viruses was evaluated using case studies of two viruses spread by whitefly. In Australia, there are two viruses which impact vegetable production and are spread by silverleaf whitefly (SLW; *Bemisia argentifolii*). The first is *tomato yellow leaf curl virus* (TYLCV) and the second is *cowpea mild mottle virus* (CPMMV). TYLCV has significantly impacted field tomato production in most regions of QLD since its introduction in 2006. CPMMV occasionally impacts French bean production and although present in many districts in QLD, the economic impacts to date are limited mostly to one growing region, the Fassifern Valley.

Detailed studies of both viruses were conducted through previous Hort Innovation funding, projects VT13003-TYLCV and VG15073-CPMMV. The detail studies were conducted in the dry tropics district near to Bowen, dry tropics QLD for TYLCV and the Fassifern Valley, south-east QLD for CPMMV. Both studies highlighted the influence of weather on SLW population levels, risk of virus transfer from the environment and provide a guide on spatial distances required for an AWM approach.

The same type of analyses can be applied to other insect vectors and the viruses they spread. This would include in particular aphids and thrips which spread a multitude of viruses to a range of different vegetable crops. Additionally, there are a high number of viruses spread by SLW which are exotic to Australia. Applying AWM to manage these viruses if introduced will be important as genetic resistance will not be available for many of the virus/crop combinations and existing ones may work less effectively over time.

TYLCV in the Dry Tropics

Tomato crops in three sub-areas within the Bowen growing district were surveyed three times per season, at approximately 2-month intervals for four years, 2013-2016. The incidence of TYLCV, the population of SLW and proportion of SLW which carried the virus was recorded for each survey. Additionally, the weather data from local Bureau of Meteorology sites was obtained and rainfall events evaluated for the survey period.

Figure 1 shows the spatial separation of the three sub-areas within the district. Each sub-area was separated by approximately 5-15 km and represented very different landscapes in relation to adjoining land usage.

The major learnings from the study were:

1. There is a complex interaction between SLW adult population levels, the proportion of the SLW individuals which carry the virus and the level of virus disease within crops

Figure 2 shows the relationship that exists between SLW population levels and virus incidence within crop is not straightforward and there is no real correlation between them. This highlights the complexity of managing virus diseases spread by insects, simply setting a threshold value based on adult insect numbers will not be effective. The impact from virus is a function of the SLW population present and where the SLW have migrated from. Low levels of SLW can be very damaging if they migrate from an infected crop or weed plants. For example, late 2013 had low SLW populations but they had a very high proportion of individuals carrying TYLCV and subsequently, this resulted in high levels of TYLCV in crop. By contrast, high levels of SLW may not cause virus disease outbreaks if the virus sources in the environment are rare. For example, most of 2015 and early 2016, had high SLW populations but very little virus disease in crops. Data collected during the study also showed that most virus spread was from SLW migrating into the crop from external sources and not from spread within the crop. Several weed species were identified as hosts for TYLCV and are the source for spread of virus into early crops. Once established in crops, these crops become external sources of TYLCV for later planted crops and can exponentially increase disease in these later crops.

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Figure 1 Map of the Bowen district production area showing the three sub-areas, Euri Creek Rd, the Delta area and the Collinsville Rd area. A scale bar of 5 km is included for reference.

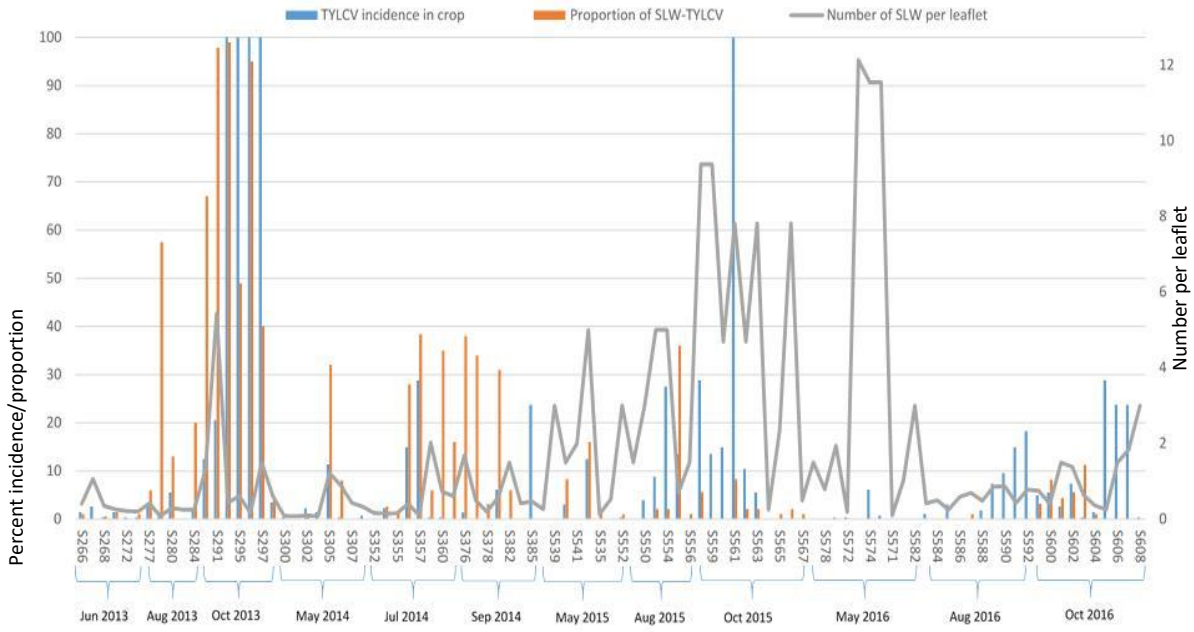

Figure 2 The incidence of TYLCV, SLW population levels and the proportion of SLW carrying TYLV during the study period.

2. Rainfall volume and timing influences SLW adult populations levels.

The dry tropics region of Bowen has a distinct wet and dry season which influences SLW population development. Figure 3 shows the differences in rainfall over the survey period and the subsequent SLW population levels. Typically, high levels of rainfall either reduce SLW adult numbers through physical damage or delay their increase. For example, SLW populations in 2013 and 2014 were quite low compared to 2015. This coincided with high rainfall which extended into late autumn. By contrast, in 2015 there was a relatively normal wet season with high rainfall over summer that finished in early autumn. This year saw the highest populations of SLW during the study period. Similarly, in 2016 there were high SLW numbers in late autumn due to a combination of a drier than normal wet season and very high SLW numbers towards the end of 2015. The SLW numbers subsequently fell significantly due to a late rain event in June.

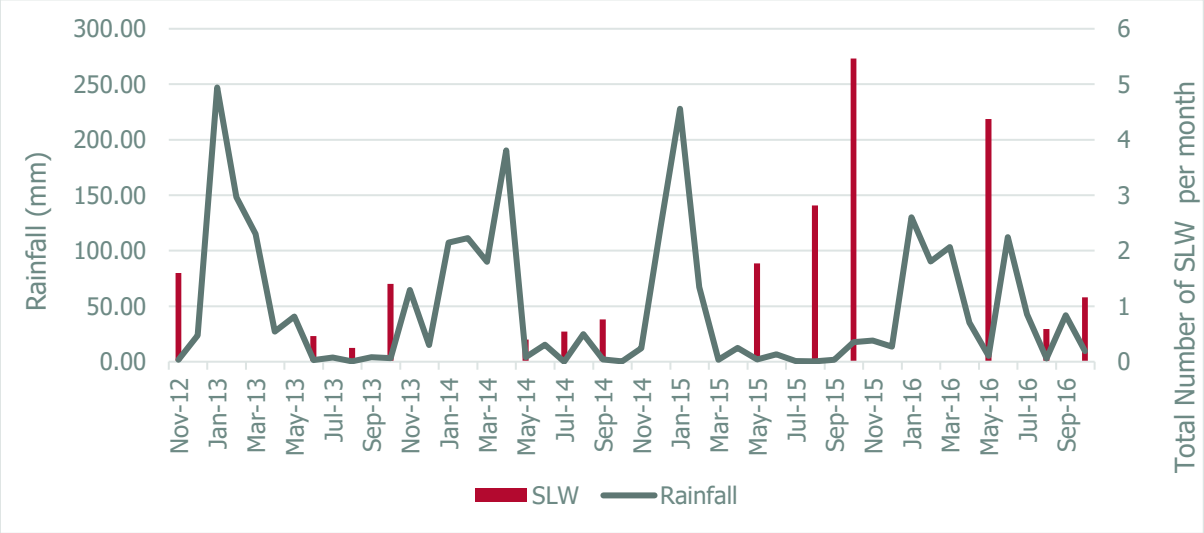

Figure 3 Total amount of rainfall and SLW numbers for each month during the study period.

3. Location can influence TYLCV disease but not always

The three subareas within the district differed in adjacent land use. Horticulture production and large areas of non-cultivated land extend along Euri Creek Rd, the Delta area has pockets of tomato production amongst rural residential and residential zones and Collinsville Rd is a mix of horticulture production and non-cultivated areas but with less tomato production. Table 1 shows the differences in TYLCV incidence across these three subareas which also differed between the years. The Delta and Collinsville Rd had TYLCV detected at less sites than Euri Creek Rd for two of the years (2014 and 2015), whereas the other two years TYLCV was detected at almost all sites across the whole area. This highlights the need for monitoring on farm as well as district wide.

Table 1 The distribution of TYLCV across the district and over study period.

Survey area	Number of sites TYLCV detected per sites inspected each year				
	2013	2014	2015	2016	Total
Collinsville Rd	3/4	3/8	1/7	6/9	13/28 (46.4%)
Euri Creek Rd	10/10	9/11	12/16	7/10	38/47 (80.8%)
Delta	4/4	4/7	0/6	8/11	16/28 (57.1%)

CPMMV in the Fassifern Valley

A severe outbreak of CPMMV occurred in the Fassifern Valley, QLD during the autumn of 2016. Crop surveys in the Fassifern Valley commenced in early November 2016 and continued until June 2019. The crops surveyed included French bean, soybean and an occasional mungbean crop. The surveys were done every two to three weeks during legume production periods. The incidence of CPMMV and SLW were recorded for each survey site. Additionally, the weather data from local Bureau of Meteorology sites was obtained and rainfall events evaluated for the survey period.

In addition to survey of commercial crops, riparian areas and field edges were inspected for potential alterative hosts for CPMMV. This was done multiple times during the study. Whiteflies were also collected at various times indicating these areas were a reservoir for the vector.

Figure 1 shows the spatial separation of the survey sites within the valley. The French bean production was fairly uniformly spread across the valley with very short distances between production blocks. Similarly, the landscapes in relation to adjoining land usage was fairly similar across the valley.

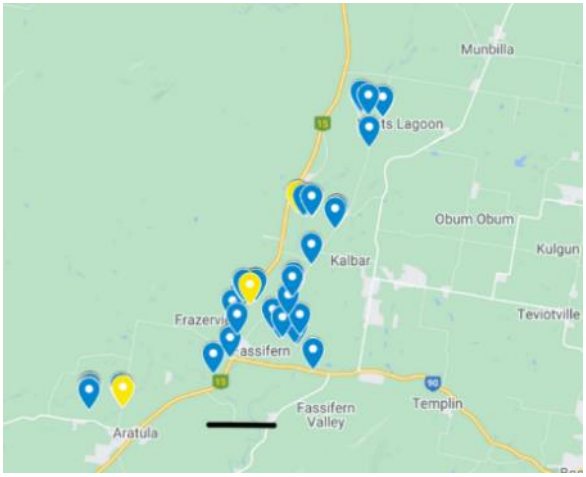

Figure 1 Map of the Fassifern valley production area showing the locations of positive CPMMV detections in French bean crops (blue markers) and weed hosts (yellow markers). A scale bar of 5 km is included for reference.

The major learnings from the data were:

1. CPMMV affects French bean crops during autumn planting windows and is influenced by SLW numbers

French beans are produced in the Fassifern valley from spring through to late December with a break over the hot, humid summer months and then planting resumed in February. Final harvest is in May-June. Intensive surveys for the virus and vector in this valley was completed from spring 2016 to June 2019. SLW numbers are typically low in bean crops in late summer and spring plantings and CPMMV was not found in those crops. CPMMV was regularly detected during the autumn planting period and disease incidences reflected SLW population levels. In 2015 and 2017, both SLW and CPMMV levels were low and caused negligible impact to production. In 2019, however, SLW levels were very high in March and coincided with high incidences of CPMMV in bean crops, resulting in considerable economic losses, similar to those reported by growers from the 2016 autumn outbreak. Surveys of two blocks with 99% virus incidences in mid-March 2019 showed virus incidence within the crops was linked to SLW coming into the crop, rather than SLW spreading the virus within the crop. These blocks had very high numbers of SLW and within a week CPMMV had increased from about 10% to 99%. Virus symptoms typically take a week from infection to be seen in crops, thus the disease incidence of 99% was a result of the SLW seen the week prior. Secondary spread within a crop is likely to occur but probably contributes less to large disease outbreaks than high numbers of SLW arriving already laden with virus acquired from external sources.

2. Major virus reservoirs were legume weeds within the riparian areas

Surveys of riparian areas and field edges identified at least two epidemiologically significant alternative hosts for CPMMV. Virus infected siratro and glycine vines were detected in close proximity to badly affected French bean crops (Figure 1). Conversely, the virus incidence in bean crops grown towards the northern end of the district, near Kents Lagoon, was consistently lower than other areas and no large areas of siratro or glycine were obvious in this area. Other weed hosts such as Phasey bean were present in the valley and confirmed as infected with CPMMV but the distribution and abundance of this weed was much lower than siratro and glycine.

3. The influence of other crops on SLW populations and CPMMV incidences

No CPMMV was detected in other legume crops grown within the valley. Pumpkin is not considered a host of CPMMV but is a preferred host for SLW and is grown within the valley. Whitefly numbers were monitored on these crops and a summary is provided in Table 1. These results indicate that pumpkin is a very good breeding host for SLW. Soybean and French bean were also identified as a breeding host for SLW, however, the numbers of pupae observed was much lower than pumpkin, particularly on the French bean. Releases of the parasitic wasp, *Eretmocerus hayati*, commenced in 2017 and its distribution monitored. The greatest parasitism of SLW eggs was observed on soybean and in some instances at high levels.

4. Severe disease outbreaks were influenced by climate

Over the period 2016-2019 there were two years of severe disease outbreak and two of negligible disease in the bean crops. The original severe outbreak was in 2016 and one of similar severity occurred again in 2019. Evaluation of the weather data showed temperature was unlikely to influence SLW populations as this was relatively uniform across all

years. Rainfall amount and timing, however, varied considerably (Figure 2). In particular, the summers for 2015/2016 and 2018/2019 were dry compared to the other two years. By comparison, for 2016/2017 and 2017/2018, there was significant rainfall in either February or March which influenced adult SLW numbers.

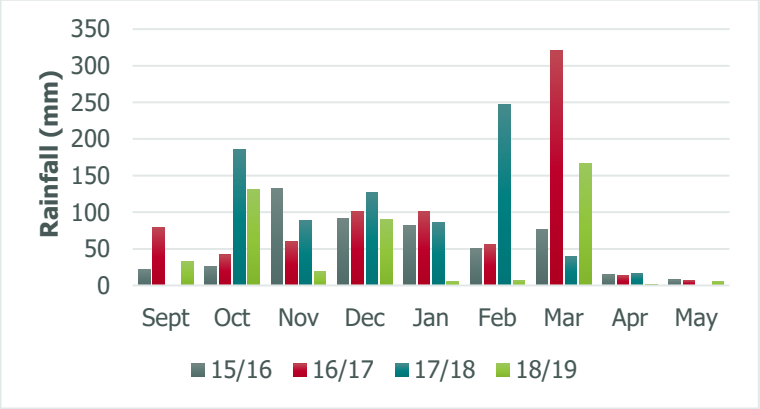

Figure 2 Average monthly rainfall in mm for the September to May period of years 2015/2016 to 2018/2019.

Table 2 Silverleaf whitefly (SLW) populations observed over time in the Fassifern Valley from late 2016 to mid-2019. The counts were done on three different crops and are an average of the SLW counted per site for that month. For each site the adult SLW and pupae were counted on a leaf from 150 and 50 individual plants, respectively. The percent parasitism by the wasp Eretmocerus hayati was also recorded.

Year	Month	SLW pupae per site with % parasitism			Average adult SLW per site		
		French bean	Soybean	Pumpkin	French bean	Soybean	Pumpkin
2016	Dec				0.8		
2017	Jan		193 (14%)		34.8		23.8
	Feb		211 (18%)			42.5	
	March		167 (69%)		11.2	10.3	
	April				3.8	0.0	
	May				2.5		
	Sept			2095 (1.4%)			73.0
	Oct			1573 (1.3%)	12.0		105.2
	Nov			1694 (2%)	71.5		95.8
	Dec		8 (0%)	1789 (2%)	4.9	31.3	85.4
2018	Jan		218 (20%)			27.4	
	Feb		158 (19%)			27.9	
	March		147 (32%)		56.9	21.6	
	April				9.3	20.8	
	May				2.3		
	Oct				0.3		600.0[A]
	Nov				0.0		
	Dec				0.5		
2019	Jan				0.7		
	Feb				219.3		
	March	71 (0%)[B]			39.6		

	April	95 (2.4%)[B]			19.4		
	May				33.3		

[A]The population of adult SLW was estimated at 600. A total of only 20 plants at the two sites were monitored, instead of the normal 150 and 40 SLW adults observed from each site. [B]SLW pupae and parasitism counted from only 15 leaves at these sites.

Figure 3 shows the interaction between SLW population levels and CPMMV incidence during a severe disease outbreak. The rapid decrease in SLW numbers at the end of February 2019 was due to a significant rain event which affected the whole valley. This figure also shows that once there is a very high incidence of CPMMV going into the autumn season, incidences will remain relatively high for most of that season, even with only low levels of SLW detected in crop.

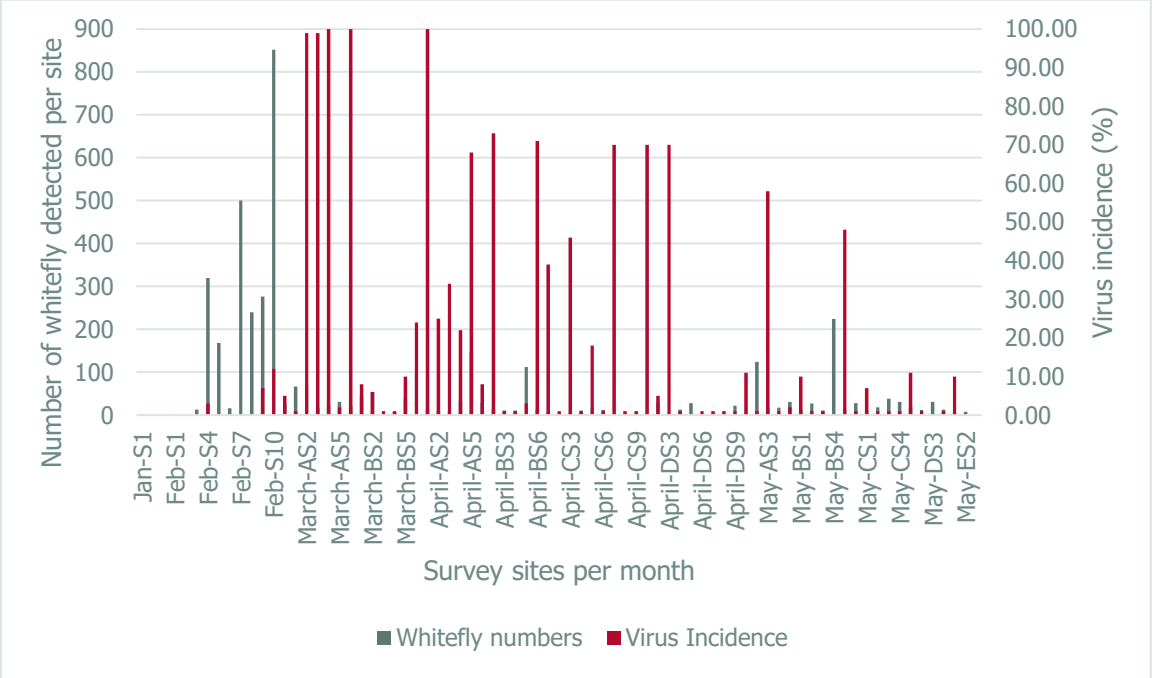

Figure 3 Graphical comparison of the level of silverleaf whitefly observed and the in-crop incidence of CPMMV for each site surveyed in 2019 from January to the end of May.

How are these learnings applied to Area Wide Management

- The data shows that the importance of AWM to manage SLW-transmitted viruses such as TYLCV and CPMMV will depend on a whole range of factors including rainfall, host diversity for both the viruses and SLW across the area and insect life-cycle rates.
- Rainfall influences SLW adult populations. For TYLCV in the dry tropics, years where there is a drier than normal wet season SLW will be high and if virus sources are present then disease outbreaks are likely to be significant. For CPMMV landscape host diversity is also a factor and in years where there is a predicted dry summer SLW will be high going into autumn plantings, particularly if this coincides with presence of pumpkin crops over summer.
- For these and other SLW-transmitted viruses, area wide control of the adult insects early in these high-risk seasons through biological control agents and/or insecticides will lower the risk of severe disease outbreaks later in the season. These control strategies if applied to sources of SLW other than crops, prior to crop planting would further reduce likelihoods of both SLW populations increasing rapidly and the spread of virus from environmental sources to early crops.
- Monitoring across the district will assist in determining the baseline SLW population early in the season and how it is increasing through the season. Further interventions to control this population can then be

implemented as needed.

- District wide management of virus sources within the environment, particularly prior to early crops being planted will reduce the likelihood of disease outbreaks. Once established in crops it's important to manage these crops as a virus source for subsequent crops. In particular, when the crop is finished, adult SLW populations should be reduced prior to natural senescence of the crop or its destruction. Failure to do this will drive migration of SLW carrying virus into new crops.

Acknowledgments

Detailed studies of both viruses were conducted through Hort Innovation funding with support from industry and the QLD Department of Agriculture and Fisheries (VT13003-TYLCV and VG15073-CPMMV)

Case study: Investigate management strategies for insecticide resistance in Green Peach Aphid (GPA)

Background

Green peach aphid (GPA; *Myzus persicae*) is a widespread and economically impacting pest of Australian horticulture production areas. GPA is highly polyphagous and feeds on plant species in over 40 different families, including *Brassicaceae*, *Solanaceae*, *Poaceae*, *Leguminosae*, *Cyperaceae*, *Convolvulaceae*, *Chenopodiaceae*, *Compositae*, *Cucurbitaceae* and *Umbelliferae*. This includes a broad range of vegetable crops such as capsicum, cucurbits, eggplants, tomatoes, brassicas, carrots, spinach, pea, parsley, asparagus and lettuce (https://www.cabi.org/isc/datasheet/35642).

In addition to the feeding damage and economic impacts due to contamination of product the aphid also spreads over 100 species of plant viruses (https://www.cabi.org/isc/datasheet/35642). These viruses cause additional economic impacts. Those which affect vegetable crops in Australia include *alfalfa mosaic virus* (AMV), *bean common mosaic virus* (BCMV), *beet western yellows virus* (BWYV), *cauliflower mosaic virus* (CaMV), *cucumber mosaic virus* (CMV), *lettuce mosaic virus* (LMV), *onion yellow dwarf virus* (OYDV), *papaya ringspot virus* (PRSV), *pea enation mosaic virus -1* (PeMV-1), *potato leafroll virus* (PLRV), *turnip mosaic virus* (TuMV), *turnip yellows virus* (TuYV), *watermelon mosaic virus* (WMV), *and zucchini yellow mosaic virus (*ZYMV). GPA and many of these viruses are also hosted by a range of weed species common in production districts.

Insecticides are generally not useful for prevention of in-crop spread of viruses by aphids. Insecticides can agitate the aphids making them more mobile, so that they spread the virus further and more rapidly. This is particularly problematic for non-persistently spread viruses, which is most of the viruses which affect vegetable crops. This is where aphids need to feed for less than a minute to pick up the virus and to deposit it into another plant. Management of virus diseases can incorporate strategic use of insecticides if needed, however, the most effective control is through preventing the introduction of virus into crops by invading insects.

GPA was reported to have wide-spread resistance to many insecticides, including organophosphates, carbamates and synthetic pyrethroids and neonicotinoids (Umina, P., 2016). This research project identified low levels of resistance to neonicotinoids in many areas as well. The project developed methods for testing new chemistries and recommendations for regional resistance management strategies. The project focus was on the insect pest and not as a vector, although it provided some general recommendations for virus control. Population thresholds for insect pests as virus vectors are much lower and thus other strategies are essential to limit impact.

Umina, P. (2016) also published a factsheet on GPA resistance management for the grains industry in 2015. Similarly, it is also of concern for the cotton industry. Many horticulture production districts are in close proximity with grains and cotton production districts thus management of GPA resistance needs to be considered as a cross-industry concern.

Proposed resistance management strategy - 2016

A resistance management strategy (RMS) was developed for the vegetable industry and launched in 2016 (Umina, P., 2016). The key recommendations to minimize resistance were:

1. Rotate chemical compounds from different mode of action (MoA) groups
2. Implement non-chemical control tactics and consider beneficial insects when managing GPA populations
3. Other integrated pest management (IPM) recommendations to consider:
    - Monitor aphids and beneficials over time to determine if insecticide is needed
    - Use economic spray thresholds where available and do not spray if pest pressure is low

- Avoid use of pyrethroids and organophosphates – already national resistance in GPA
- Comply with label directions and calibrate spray rigs to ensure good coverage
- No not re-spray a paddock in the same season where a known spray failure has occurred with that product or the same chemical group

Recommendations for adoption of GPA resistance management as an area wide strategy

The extent to which these above recommendations were implemented needs to be evaluated. A follow-up survey of production districts using the same methodology as reported by (Umina, P., 2016) would provide data for direct comparison. Strategies to manage insecticide resistance in other pests such as diamond back moth have also been developed. Combining these into an integrated pest management strategy would provide the most cost-effective and robust system for pest control. Additionally, an area wide management approach is needed for resistance management to work.

Actions require:

1. Evaluate current adoption rates of GPA and other insect pest resistance management
2. Repeat testing of districts to obtain a base-line level of GPA insecticide resistance (this is important as it will improve adoption through managing existing resistance or through prevention of it occurring)
3. Compare methodologies and insecticides used to manage a broad-range of insect pests
4. Provide management strategies by a regional basis – landscape diversity of crops, weeds and insect pests will vary considerably, adoption is likely to be greater with specific rather than general information
5. Develop cost-effective ways to monitor pest levels within crops and between cropping windows – minimise the cost for monitoring which will provide the best advice on when intervention is required and what chemistry and/or biological agents are needed to reduce pest population build-up
6. Collaborate with the cotton and grain industries to develop systems

References

Umina, P., 2016. Management of insecticide resistance in the green peach aphid. Horticulture Innovation Australia.