Horticulture Innovation Australia

Final Report

Development of molecular diagnostic tools to detect endemic and exotic pathogens of Prunus species for Australia

Dr Fiona Constable
The Dept of Economic Development Jobs, Transport & Resources

Project Number: MT12005

MT12005

This project has been funded by Horticulture Innovation Australia Limited using funds from the Australian Government and the following industry levies:
the almond industry
the summerfruit industry

ISBN 0 7341 3757 5

Published and distributed by:
Horticulture Innovation Australia Limited
Level 8, 1 Chifley Square
Sydney NSW 2000
Tel: (02) 8295 2300
Fax: (02) 8295 2399

Contents

Summary

This project has developed world's best practice, diagnostic capability for the detection of economically significant pathogens of almonds and summerfruit.

Molecular diagnostic tools were developed for the detection of exotic and endemic viruses, viroids and bacteria for almonds and summerfruits to support the biosecurity continuum of the Australian almond and summerfruit industries at the border during post entry quarantine (PEQ) facilities, during an incursion event and internally through schemes that supply high-health planting material.

The primary molecular diagnostic tools that have been developed use polymerase chain reaction (PCR) techniques. For a few pathogens, (e.g. *Xylella fastidiosa*, almond leaf scorch) some simpler tests, such as Loop Mediated Isothermal Amplification, have been developed, which are potentially adaptable to field-based diagnostics for smart surveillance.

To validate the molecular diagnostic tests 101 samples, comprising of 33 almonds, 55 summerfruit species and 13 cherries, were tested for the presence of five bacteria, 10 phytoplasmas or phytoplasma groups and 3 viroids. They were also tested using specific assays for 34 viruses. Each sample was also tested using generic PCR tests to detect viruses in the family *Closteroviridae* and in the genera *Ampelovirus, Capillovirus, Foveavirus, Ilarivirus and Trichovirus*, including some viruses for which no specific test is available.

Synthetic positive controls, incorporating pathogen primers and/or probes sequences of the assays developed in this project, were custom-made for the viruses and bacteria where nucleic acid was unavailable. This strategy ensures that there is a reliable, cheap, long lasting supply of positive controls that are essential for quality control of molecular tests to ensure preparedness for incursion response.

The following viruses and viroids which are of quarantine concern to almonds and/or summerfruit species in Australia were not detected during the survey: *American plum line pattern Ilarvirus* (APLPV), *Apricot latent virus foveavirus* (ApLV), *Asian Prunus virus 1 foveavirus (*APruV-1), APruV-2, APruV-3, *Arabis mosaic nepovirus* (ArMV), *Cherry leaf roll nepovirus* (CLRV), *Cherry mottle leaf trichovirus* (CMLV), *Cherry rasp leaf cheravirus* (CRLV), *Cherry rusty mottle associated virus* (CRMaV), *Cherry twisted leaf foveavirus* (CTLaV), *Little cherry virus 1* (LCHV1), *Peach chlorotic mottle foveavirus* (PCMV), *Peach mosaic virus trichovirus* (PcMV), *Peach rosette mosaic nepovirus* (PRMV), *Plum pox potyvirus* (PPV), *Raspberry ringspot nepovirus* (RpRSV), *Strawberry latent ringspot virus* (SLRSV), *Tobacco ringspot nepovirus* (TRSV), *Tomato black ring nepovirus* (TBRV), *Tomato ringspot nepovirus* (ToRSV), *Tomato bushy stunt tombusvirus* (TBSV) and *Apple scar skin viroid* (ASSVd). *X. fastidosa*, *Erwinia amylovora*, *Xanthomonas arboricola pv. pruni* and Phytoplasmas were also not detected.

The pathogens that were detected during the survey include *Apple chlorotic leaf spot trichovirus* (ACLSV), *Apple mosaic virus Ilarvirus* (ApMV), *Apricot pseudochlorotic leaf spot trichovirus* (APCLSV), *Apple stem grooving virus capillovirus* (ASGV), *Cherry green ring mottle virus* (CGRMV), *Cherry necrotic rusty mottle foveavirus* (CNRMV) , *Cherry virus A* (CVA), *Little*

cherry virus 2 ampelovirus (LChV2), Plum bark necrosis stem pitting associated ampelovirus (PBNSPaV), *Prune dwarf Ilarvirus* (PDV) and *Prunus necrotic ringspot Ilarvirus* (PNRSV), an uncharacterised *Ilarvirus*, *Hop stunt viroid* (HSVd), *Peach latent mosaic viroid* (PLMVd), *Pseudomonas syringae* and *Agrobacterium tumefaciens*. This is the first report of CGRMV, CNRMV, CVA and PBNSPaV in Australia. ASGV is endemic in pome and citrus fruit species and this survey provides the first evidence that it can also infect *Prunus* species in Australia. ASGV, CGRMV, CNRMV, CVA and PBNSPaV should be included in routine pathogen-testing for production of high health planting material for summerfruit and/or almonds.

Significant diversity was observed within each of the *Ilarvirus* species (PNRSV, PDV and ApMV), APCLSV and ACLSV infecting *Prunus* trees in Australia. This can affect the reliability of molecular tests for these viruses. Therefore the use of both species and genus specific assays was recommended to ensure their detection.

A next generation sequencing pipeline has been developed and will be used to further investigate *Ilarvirus* diversity and a putative and previously uncharacterised *Ilarvirus* detected in almonds and summerfruit.

The diagnostic tools developed within the project will be used by the Australian almond and summerfruit industries for the production and maintenance of high health planning material and by the Department of Agriculture during Post Entry Quarantine (PEQ). An important output from this research is a draft diagnostic manual that incorporates the diagnostic protocols for endemic and exotic pathogens. These protocols will form the basis of a national certification standard to support the production of pathogen tested almonds and summerfruit propagation material in Australia.

Best practice protocols for the day–to-day management of almond and summerfruit high-health budwood repositories were developed based on the information gathered in this project. The protocols include information about the endemic pest or pathogens that occur in Australia, surveillance, preferred location of the repository block, farm hygiene including movement of people, plant material and equipment, signage, product management, staff training and pest and pathogen awareness. These protocols, in hand with the diagnostic manual, will ensure that high-health budwood repository blocks remain sustainable and productive for many years and will reduce the risk of incursion of a quarantine pest or pathogen.

Keywords

Pathogen-testing, diagnostics, polymerase chain reaction, PCR, next generation sequencing, biosecurity, biosecurity plan, certification, post entry quarantine, virus, bacteria, viroid, phytoplasma, *Prunus,* almond, summerfruit, stone fruit

Introduction

The biosecurity of the Australian Summerfruit and Almond industries are maintained at the border by Department of Agriculture (DA) Biosecurity (formerly Australian Quarantine Inspection Service, AQIS) in Post Entry Quarantine (PEQ) facilities and internally through schemes that supply high-health planting material throughout Australia.

Prunus germplasm imported into Australia requires a minimum of two years post entry quarantine testing using a range of diagnostic techniques for detection of diseases and pathogens of quarantine significance.

Within the recently completed HIA Limited funded project entitled "Review of the post entry quarantine conditions for imports of almond germplasm" (AL10001) Victorian DEDJTR scientists reviewed the bacteria, phytoplasmas, viruses, viroids and fungi that are currently known to infect almond and other *Prunus* species and identified which of these pathogens are significant to Australian PEQ for almonds. The review identified several pathogens that do not appear on the current *Prunus* species PEQ lists for Australia. The most appropriate diagnostic tests for detection of the listed quarantinable pathogens were also identified. The review identified the following:

1. *Prunus* species can be infected by five bacteria that are significant at the quarantine level and three are important for almond and require testing during PEQ. Another four bacteria are significant at the certification level within Australia. Eleven phytoplasmas or phytoplasma groups that are known to infect *Prunus* and all require testing during PEQ.
2. There are 47 viruses that are known to infect *Prunus* species, of which 34 are significant at the PEQ level. Ten of the 34 quarantine viruses infect almonds and are significant at the PEQ level for Australia.
3. Three viroids infect *Prunus* species; two are reported in Australia and infect *Prunus* species including almond.

The Almond Board of Australia (ABA) manage an industry based certification programs for the production of high health almond budwood. In Australia high-health pathogen-tested almond trees are routinely tested for *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *and Apple chlorotic leafspot virus (ACLSV) and Apple mosaic virus (ApMV).* Production of pathogen tested material for the Summerfruit industry is conducted by individual nurseries with their own specified protocols for pathogen testing. Summerfruit species are usually tested for PNRSV, PDV and ACLSV.

In recent years there have been significant advances in molecular and diagnostic technology that have allowed characterisation of new and emerging pathogens, new strains of known pathogens of *Prunus* species and clarification of pathogens associated with diseases of *Prunus* species which had an unknown aetiology, especially for viruses, viroids and phytoplasmas. As a consequence it is likely the list of quarantine pathogens of *Prunus* species for Australia will require further updating to reflect this knowledge. It is also likely these advances have resulted in new and/or improved molecular tools for the rapid and sensitive detection of these pathogens. These diagnostic tools can be used to better support

pathogen detection during PEQ, for stone fruit certification programs and to improve stone fruit biosecurity in Australia. The development of these molecular protocols will support the industry biosecurity plans that have been developed for both almonds and summerfruit which identify, as a priority, the need for adequate diagnostic tools for the detection of exotic pathogens at the border and post border during incursions and to support market access.

Viruses, including PNRSV, PDV, ACLSV and ApMV, can have a significant impact on fruit and nut quality and yield. Consequently these routine testing for these viruses is undertaken within Australian almond and summerfruit certification and high health programs to minimize the risk of their introduction and spread in propagation materials. Genetic variation in natural populations of viruses may influence the reliability of molecular diagnostic tests, such as RT-PCR. There is a requirement to determine genetic diversity of PNRSV, PDV, ACLSV and ApMV populations in Australia for development of rapid and reliable detection assays to support the production of high health almond and summerfruit propagation material.

The aim of certification schemes is to reduce the threat of spread of endemic (primarily) pathogens and pests, to provide traceability, and to ensure provision of high quality planting material that is true to type. Budwood repository blocks established with such material, if properly maintained, should remain sustainable and productive for many years. An additional benefit of a well-managed repository is the reduced risk of incursion of a quarantine pest or pathogen.

Methodology

This project focuses on the priority area of biosecurity for the Summerfruit and Almond industries. Research activities of this three year project will centre around five objectives:

1. Update the PEQ list for stone fruit to include recently reported pathogens and update the existing information on the known pathogens of stone fruit.
2. Identify, develop and validate molecular diagnostic tools for the detection of endemic and exotic pathogens of stone fruit under Australian conditions.
3. Develop a post entry quarantine diagnostic manual for *Prunus* species including almonds.
4. Investigate strain variation of major endemic viruses of *Prunus* species in Australia.
5. Identify optimal pest and disease management strategies for maintaining stone fruit budwood repository blocks.

1. **Update the PEQ list for *Prunus* species with recently reported pathogens and updated information of known pathogens.**

During the HIA Limited funded project entitled "Review of the post entry quarantine conditions for imports of almond germplasm" (AL10001) Victorian DEDJTR scientists reviewed the bacteria, phytoplasmas, viruses, viroids and fungi that are currently known to infect almond and other *Prunus* species. The review identified several pathogens that do not appear on the current *Prunus* species PEQ lists in Australia. Within AL10001 a list of quarantine pathogens that are specific to almond was also produced. Using the information that was gathered in this review and a further review of the current literature, specific lists of pathogens that are significant for quarantine in Australia and require testing during PEQ will be created for apricots, cherries, plums and peaches/nectarines. These lists will be supplemented by information about the biology and epidemiology of the pathogen and the tests that are required to identify and detect them.

2. **Identify, develop and validate molecular diagnostic tools for the detection of endemic and exotic pathogens of stone fruit under Australian conditions.**

A literature search will be conducted to identify PCR primers for detection of endemic and exotic bacteria, viruses and viroids of *Prunus* species. Detailed bioinformatic analysis will be done on the primer sequences to determine their specificity and chance of success in detecting all known strains of each pathogen. Positive controls for each pathogen will be obtained from international researchers and/or international pathogen collections to assist in the establishment of the most appropriate PCR based test for each pathogen. This work is a fundamental requirement for understanding the capability of a given primer set/PCR test for detection of a pathogen. Strains of these pathogens are being sequenced at an increasing rate and it is important to continue the bioinformatic analysis on an ongoing basis. Two or three primer pairs for each pathogen will be selected, or designed if necessary, and where possible qPCR techniques will be developed. The new molecular diagnostic tools for exotic pathogens will be assessed for inclusion in the nationally endorsed SPHDS protocols for quarantine pathogens of almonds and other stone fruit species.

To complete the validation of the diagnostic test, an Australia-wide survey will be conducted in the final two years of the project. Approximately 200 trees of different commercial and ornamental *Prunus* species, and collected from major *Prunus* sp. growing districts throughout Australia, will be sampled and the tested using the protocols selected and developed in this project. The purpose of the survey is two-fold: (i) to update the disease status for each pathogen in Australia and (ii) to test protocols under "local" conditions and identify any potential "false positives" or organisms that can make interpretation of results difficult. It is vitally important that these surveys are conducted prior to the use of these protocols for certification in a quarantine or commercial situation. Isolates of pathogens will be further characterised by sequencing. The validation of molecular assays will assist in the endorsement of the SPHDS protocols for quarantine pathogens of almonds and other stone fruit species.

Some virus pathogens of *Prunus* species, such as nepoviruses and ilarviruses, are transmitted in pollen. Pollen imported into Australia therefore represents a risk for introduction of exotic viruses at the border as well as endemic viruses that are controlled in certification programs. As a part of this project strategies for the detection of viruses in pollen will be assessed, including appropriate nucleic acid extraction techniques and PCR technology to ensure the most reliable and sensitive detection of pollen borne viruses. Pollen will be collected from trees that are known to be infected with an Ilarvirus and this will be used to develop nucleic acid extraction techniques. Nucleic acid extraction procedures will be assessed for the quantity and quality of RNA using spectrophotometry. A dilution series of infected and uninfected pollen will be used to assess the sensitivity of conventional and qRT-PCR assays that are developed as a part of this project for virus detection in pollen.

3. **Development of a post entry quarantine diagnostic manual that is specific for *Prunus* species including almonds**

Some specific guidelines for almond pathogen testing exist in the current PEQ manual. However this manual does not incorporate specific tests for some recently identified virus, viroid and bacterial pathogens. As a part of this project a manual will be developed for *Prunus* species including almonds, which incorporates the most up to date list of *Prunus* pathogens, and the biological, serological and molecular diagnostics tests that can be used for their detection. This manual will be submitted to Department of Agriculture Biosecurity for consideration.

4. **Investigate variation of major endemic viruses in Australia.**

Significant genetic variation can exist within a virus species. This variation can affect the reliability of biological, serological and molecular diagnostic tests and may also affect the biology and epidemiology of the pathogen. This analysis also enables industry and quarantine agencies to determine the strains, particularly aggressive strains, of pathogens that are not present in Australia and which should be screened for at the border. Up to 10 isolates each of PNRSV, PDV, ACLSV and ApMV, some of which are currently held by DEDJTR Victoria and some to be collected from different hosts and different regions during the project, will be characterised by sequencing at the molecular level and uploaded to GenBank if appropriate. This information will be compared to the information that is published on

GenBank and will be used to improve our understanding of the biology of the viruses and improve the reliability of the tests used for their detection.

5. **Identify optimal pest and disease management strategies for maintaining stone fruit and almond budwood repository blocks.**

Many of the endemic viruses and some of the quarantine viruses, and bacteria are transmitted in the field by insect vectors. Most of the pathogens are also transmitted in budwood. Some of the viruses may be transmitted via pollen. A literature review and discussions with relevant pathologists and entomologists will be conducted to determine the epidemiology of each pathogen. This information will be used to identify effective strategies to minimize the risk of an incursion. Best practice protocols for the day–to-day management of the budwood repository will be developed. The plan will include information about each pest or pathogen, surveillance, preferred location of the repository block, farm hygiene including movement of people, plant material and equipment, signage, product management, staff training and pest and pathogen awareness.

Outputs

The outputs of this project are:

1. Updated post entry quarantine pathogen lists that are specific for the Australian almond and summerfruit industries (see Appendix 2).
2. A summary of endemic and exotic pathogens of Australian almonds and summerfruit that were identified in the project through a review of the literature and the results of the survey which was published in the industry journals.
3. The development of validated molecular diagnostic tools for the detection of endemic and exotic pathogens, including viruses, viroids and bacteria, of almonds and summerfruit under Australian conditions (see Appendix 3).
4. A diagnostic manual that can be used during post entry quarantine and by certification and high health almonds and summerfruit in Australia (see Appendix 5).
5. Improved understanding of the genetic variation of PNRSV, PDV, ApMV and ACLSV and the impact of the variation on detection and disease (see Appendix 4).
6. An update on the pest free status of almonds and summerfruit in Australia (see Appendix 3 and via industry-focussed articles see Appendix 8)
7. Recommendations for optimal pest and disease management strategies for maintaining and supporting the biosecurity of summerfruit and almond budwood repository blocks (see Appendix 6).

Outcomes

1. Enhanced biosecurity of the Australian Summerfruit and Almond industries at the border and post border in stone fruit growing districts, through the availability of best available, validated and internationally accepted protocols for the detection of endemic and exotic virus, viroid and bacterial pathogens of *Prunus* in Australia.
2. The efficient and sustainable production of summerfruit and almonds through the availability of high health planting material which will be screened in PEQ and potentially certified locally as a result of development of reliable diagnostic protocols for pathogens that affect orchard sustainability and productivity. A direct outcome of this project is a world's best practice diagnostic capability that will support the *Prunus* PEQ and industry-based certification schemes.
3. Reduced risk of pest and disease incursion into budwood repositories through the development of optimal pest and disease management strategies.

Evaluation and Discussion

This project focused on the priority area of biosecurity for the Summerfruit and Almond industries. Research activities centered around five objectives:

1. Update the PEQ list for summerfruit to include recently reported pathogens and update the existing information on the known pathogens of summerfruit.
2. Identify, develop and validate molecular diagnostic tools for the detection of endemic and exotic pathogens of summerfruit under Australian conditions.
3. Develop a post entry quarantine diagnostic manual for *Prunus* species including almonds.
4. Investigate strain variation of major endemic viruses of *Prunus* species in Australia.
5. Identify optimal pest and disease management strategies for maintaining summerfruit budwood repository blocks.

The PEQ and certification lists for almonds and summerfruit were updated based on a review of the literature and discussions with Australian and International experts.

There are three bacteria, eight phytoplasma or phytoplasma groups, eight viruses, one viroid and 17 fungi that are of quarantine significance to the almond industry of Australia. However new pathogens and updates in pathogen biology and disease aetiology occur and it is important that there is continual surveillance of the literature to ensure these pathogen lists remain current. There are 29 fungal pathogens of almonds that may be significant to quarantine. Twelve of these 25 fungi have been reported in Australia on *Prunus* species and/or other hosts and their presence in Australian *Prunus* orchards and alternative hosts requires clarification to determine if they should remain on the PHA and PEQ pathogen list or be considered for certification programs.

There are four bacteria, ten phytoplasma or phytoplasma groups, 31 viruses, two viroids and 50 fungi that are of quarantine significance to the summerfruit industry of Australia.

Four bacteria, 13 viruses, two viroids and three fungi that are known to infect summerfruit species in other countries have also been recorded in Australia. Three of the four bacteria, 7/13 viruses and both viroids have also been reported to infect almond overseas. Almonds and summerfruit will require testing for ApMV, ACLSV, PNRSV, PDV and PBNSPaV during certification as each of these viruses are known to spread naturally via a vector and pollen. Summerfruit species will require additional testing for APCLSV, ASGV, CGRMV and CNRMV. Plums may require additional testing for CVA and LChV2. Molecular diagnostic tools for important quarantine pathogens and pathogens important to production of high health planting material for almonds and summerfruit species were validated under Australian conditions. The primary molecular diagnostic tools that have been developed use polymerase chain reaction (PCR) techniques. For a few pathogens, (e.g. *Xylella fastidiosa*, almond leaf scorch) some simpler tests, such as Loop Mediated Isothermal Amplification, have been developed, which are potentially adaptable to field-based diagnostics for smart surveillance.

Synthetic positive controls were developed to support many of the tests that were validated. These are a reliable, cheap, long lasting supply of positive controls that are essential for quality control of molecular tests to ensure preparedness for incursion response.

A total of 101 samples, comprising of 33 almonds, 55 summerfruit species and 13 cherries, were tested for the presence of five bacteria, 10 phytoplasmas or phytoplasma groups, 3 viroids. They were also tested using specific assays for 34 viruses. Each sample was also tested using generic PCR tests to detect viruses in the family *Closteroviridae* and in the genera *Ampelovirus, Capillovirus,* Foveavirus*, Ilarivirus and Trichovirus*, including some viruses for which no specific test is available.

The following viruses and viroids which are of quarantine concern to almonds and/or summerfruit species in Australia were not detected during the survey: *American plum line pattern Ilarvirus* (APLPV), *Apricot latent virus foveavirus* (ApLV), *Asian Prunus virus 1 foveavirus (*APruV-1), APruV-2, APruV-3, *Arabis mosaic nepovirus* (ArMV), *Cherry leaf roll nepovirus* (CLRV), *Cherry mottle leaf trichovirus* (CMLV), *Cherry rasp leaf cheravirus* (CRLV), *Cherry rusty mottle associated virus* (CRMaV), *Cherry twisted leaf foveavirus* (CTLaV), *Little cherry virus 1* (LCHV1), *Peach chlorotic mottle foveavirus* (PCMV), *Peach mosaic virus trichovirus* (PcMV), *Peach rosette mosaic nepovirus* (PRMV), *Plum pox potyvirus* (PPV), *Raspberry ringspot nepovirus* (RpRSV), *Strawberry latent ringspot virus* (SLRSV), *Tobacco ringspot nepovirus* (TRSV), *Tomato black ring nepovirus* (TBRV), *Tomato ringspot nepovirus* (ToRSV), *Tomato bushy stunt tombusvirus* (TBSV) and *Apple scar skin viroid* (ASSVd)*. X. fastidosa*, *Erwinia amylovora*, *Xanthomonas arboricola pv. pruni* and Phytoplasmas were also not detected.

The pathogens that were detected during the survey include *Apple chlorotic leaf spot trichovirus* (ACLSV), *Apple mosaic virus Ilarvirus* (ApMV), *Apricot pseudochlorotic leaf spot trichovirus* (APCLSV), *Apple stem grooving virus capillovirus* (ASGV), *Cherry green ring mottle virus* (CGRMV), *Cherry necrotic rusty mottle foveavirus* (CNRMV) , *Cherry virus A* (CVA), *Little cherry virus 2 ampelovirus (LChV2, Plum bark necrosis stem pitting associated ampelovirus* (PBNSPaV), *Prune dwarf Ilarvirus* (PDV) and *Prunus necrotic ringspot Ilarvirus* (PNRSV), an uncharacterised *Ilarvirus*, *Hop stunt viroid* (HSVd), *Peach latent mosaic viroid* (PLMVd), *Pseudomonas syringae* and *Agrobacterium tumefaciens*. This is the first report of CGRMV, CNRMV, CVA and PBNSPaV in Australia. ASGV is endemic in pome and citrus fruit species and this survey provides the first evidence that it can also infect *Prunus* species in Australia. ASGV, CGRMV, CNRMV, CVA and PBNSPaV should be included in routine pathogen-testing for production of high health planting material for summerfruit and/or almonds.

Significant diversity was observed within each of the *Ilarvirus* species (PNRSV, PDV and ApMV), APCLSV and ACLSV infecting *Prunus* trees in Australia. This can affect the reliability of molecular tests for these viruses. Therefore the use of both species and genus specific assays was recommended to ensure their detection.

A next generation sequencing pipeline has been developed and will be used to further investigate *Ilarvirus* diversity and to a putative and previously uncharacterised *Ilarvirus* with detected in almonds and summerfruit. Next generation sequencing and bioinformatics analyses should be considered for further development as a diagnostic tool to support PEQ and certification.

These molecular diagnostic protocols have been used to develop a specific PEQ diagnostic manual for almonds and summerfruit.

Many of the endemic viruses and viroids and some of the quarantine viruses, and bacteria are transmitted in the field by insect vectors. Most of the pathogens are also transmitted in budwood. Some of the viruses may be transmitted via pollen.. The aim of certification schemes is to reduce the threat of spread of endemic (primarily) pathogens and pests, to provide traceability, and to ensure provision of high quality planting material that is true to type. Budwood repository blocks established with such material, if properly maintained, should remain sustainable and productive for many years. An additional benefit of a well-

managed repository is the reduced risk of incursion of a quarantine pest or pathogen. As a part of this project optimal pest and disease management strategies for maintaining summerfruit budwood repository blocks were identified and recommendations for the management were made.

Recommendations

The following recommendations have been made based on the findings of this project:

1. Department of Agriculture adopt the updated PEQ list of pathogens for active testing in almonds and summerfruit at the border.
2. Almond and summerfruit industries adopt the updated list of pathogens for testing during the production of high health planting material.
3. Almond and Summerfruit industries and DA Biosecurity should decide if some of the "minor" viruses require active testing.
4. Adoption of the diagnostic manual that was developed in this project between the almond and summerfruit industries and DA Biosecurity (post entry quarantine) for routine pathogen testing at the border and in certification programs.
5. Adoption of the biosecurity plan that has been developed in this project by the almond and summerfruit industries
6. Continual surveillance of scientific literature for information about new pathogens and updated information about known pathogens of almonds and summerfruit. This information should be used to update the lists of pathogens significant to quarantine protocols, certification of high health planting material and industry biosecurity plans.
7. Development of next generation sequencing and bioinformatics analysis as a tool to assist Australian PEQ and certification of pathogen tested almond and summerfruit planting material for vegetatively transmitted pathogens.
8. Develop and validate molecular diagnostic assays and determine the presence or absence in Australia of *Apricot vein clearing associated virus*, Caucasus *Prunus* virus (CPrV) and *Prunus virus T.*
9. Determine the pathogenicity of Australian HSVd isolates in *Prunus* sp. to hops and use this information to inform the quarantine status of HSVd for Australia.
10. Development of in-field diagnostic tests, such as Loop mediated isothermal amplification (LAMP) for field based diagnostics to support the biosecurity of the almond and summerfruit industries
11. Survey for the following fungi (and their alternate anamorph or telomorph states) to determine if they are present and widely distributed in Australian *Prunus* orchards and alternative hosts: *R. necatrix, N. coryli L. persoonii, A. mellea, M. marginata, B. jaapii, D. sarmentorum, G. lucidum, N. dimidiatum*, *L. sulphureus M. cerasella* and *P. guttata.*
12. Further work is required to characterise an *Ilarvirus* most closely related to *Parietaria mottle virus* that was detected in almond and summerfruit species and to determine its impact on Australian *Prunus* species.
13. Utilise qiaextractor as a reliable high throughput nucleic acid extraction procedure for the isolation of DNA and RNA from *Prunus* species.
14. The RT-PCR and PCR assays for most pathogens, except ACLSV, APCLSV, APLPV, CNRMV and HSVd, were reliable and are recommended to be used for the detection of viruses, viroids, phytoplasmas and bacteria of *Prunus* species.
15. Further work is required to gain knowledge of the genetic diversity of ACLSV and APCLV so that better molecular tests can be developed.

16. The assays for APLPV, CNRMV and HSVd generate non-specific PCR products that could result in a false positive result and another assay for each pathogen requires development.
17. To overcome the risk of false negative results use of both virus species specific and genus or family specific molecular diagnostics tests is required to ensure detection of viruses in the genus *Ilarvirus, Foveavirus, Trichovirus, Capillovirus and Ampelovirus* and in the family *Closteroviridae.*
18. A divergent strain of CVA was detected and further work is required to determine if this is in fact CVA or another *Capillovirus* species.

Intellectual Property/Commercialisation

The intellectual property arising from this project aligns with the project outputs.

1. Updated post entry quarantine pathogen lists that are specific for the Australian almond and summerfruit industries
2. A summary of endemic and exotic pathogens of Australian almonds and summerfruit that were identified in the project through a review of the literature and the results of the survey which was published in the industry journals.
3. The development of validated molecular diagnostic tools for the detection of endemic and exotic pathogens, including viruses, viroids and bacteria, of almonds and summerfruit under Australian conditions.
4. A diagnostic manual that can be used during post entry quarantine and by certification and high health almonds and summerfruit in Australia.
5. Improved understanding of the genetic variation of PNRSV, PDV, ApMV and ACLSV and the impact of the variation on detection and disease
6. An update on the pest free status of almonds and summerfruit in Australia
7. Recommendations for optimal pest and disease management strategies for maintaining and supporting the biosecurity of summerfruit and almond budwood repository blocks.

This information is being shared directly with and for the benefit of the almond and summerfruit industry and Australian biosecurity agencies and as such no commercialisable IP is envisaged to arise from this project.

Scientific Refereed Publications

See Appendix 8 for the full list of conference abstracts, papers and industry articles.

Intellectual Property/Commercialisation

No commercial IP generated

References

A detailed list of references is provided in Appendix 7.

Acknowledgements

This project was funded by Horticulture Innovation Australia Limited using the almond and summerfruit levies with co-investment from Department of Economic Development, Jobs, Transport and Resources and funds from the Australian Government. We would like to acknowledge and thank the Almond Board Australia and Summerfruit Australia for their support. Many thanks also to Bruce Tomkins (DEDJTR), Mark Whattam (Department of Agriculture - Biosecurity), Ben Brown, Project and Technical Manager at Select Harvests Ltd (Formerly Almond Board Australia, ABA), Brett Rozenzweig (ABA), Ross Skinner (ABA) and John Moore (Summerfruits Australia) for their input and helpful discussions. We would also like to thank the following Australian and International researchers who provided helpful discussions and information for the tests that were developed: Dr. Alison Dann (Department of Primary Industries, Parks, Water and Environment, Tasmania), Dr. Benedict Lebas (Ministry for Primary Industries, New Zealand), Dr. Ken Eastwell (Washington State University, USA), Dr. Thierry Candresse (Institut National de la Recherche Agronomique, France) and Dr. Xavier Foissac (Institut National de la Recherche Agronomique, France).

Appendix 1: Background

Introduction

Industry biosecurity is the protection from risks posed by exotic pests through actions at the pre-border (identifying exotic pest threats and managing quarantine risks offshore), border (implementing effective quarantine for people, plants and goods) and post-border (minimising risk of regional and property entry establishment e.g. planting pathogen tested material).Australia's national quarantine system helps to prevent the introduction of harmful exotic threats to plant industries.

Rapid increases in overseas tourism, imports and exports, mail and changing transport procedures, as well as the potential for pests to enter via natural routes, has increased industry reliance on effective quarantine measures. This is particularly the case for viruses and bacteria, including phytoplasmas, as these pathogens are generally not eradicated by fumigation or heat treatments of plant material on arrival at the border. Consequently imported material requires monitoring for such pathogens in post entry quarantine facilities.

1.1 Post entry quarantine

The biosecurity of the Australian Summerfruit and Almond industries are maintained at the border by Department of Agriculture (DA), Biosecurity, (formerly Australian Quarantine Inspection Service, AQIS) in Post Entry Quarantine (PEQ) facilities and internally through schemes that supply high-health planting material throughout Australia. Over the last 3 years 261 accessions of summerfruit, eight accessions of almonds and 75 cherry accessions have been screened and released from the PEQ station based in Victoria (Mark Whattam and Zoee Maddox, pers. Comm). Additional accessions may have been screened in New South Wales.

On arrival into Australian PEQ, imported *Prunus* propagation material is inspected for insect pests and disease symptoms. If there are no obvious disease symptoms, the budwood is fumigated with methyl bromide (32gms/m^2 for 2.5hrs) and then dipped in 1% sodium hypochlorite for 2 minutes to kill any epiphytic organisms. If disease symptoms are detected, the budwood is stored in the fridge until the causal agent is identified.

The fumigation and dipping procedures kill most fungal pathogens on the propagation material. However, all summerfruit viruses, viroids, phytoplasmas and bacteria and some fungi can be transmitted internally in propagation material and fumigation and dipping will not kill these pathogens. Many *Prunus* pathogens do not cause symptoms on the stems of their host and may be missed during visual inspection of the propagation material. Consequently all imported summerfruit material budwood is propagated and grown for a minimum of 16 months in PEQ to identify these diseases and pathogens.

Department of Agriculture suggests budwood of *Prunus* germplasm should be imported during October – February to ensure the material can be established and tested for pathogens in a timely manner. Post entry quarantine testing using a range of diagnostic techniques for detection of diseases and pathogens of quarantine significance (Table 1). Currently the DA Biosecurity recognises two bacteria, six phytoplasmas or phytoplasma-like diseases, 13 viruses, one viroid and 70 fungi, to be of quarantine significance for *Prunus*

species. DA Biosecurity also recognises that there are many diseases of *Prunus* of unknown aetiology.

Table 1. Testing methods for detection of bacteria, fungi, phytoplasma and viruses in imported Prunus germplasm during post entry quarantine (Source: http://apps.daff.gov.au/icon32/asp/ex_querycontent.asp)

Pathogen type	Mandatory screening[1]				
	Visual screening	Woody Indexing[4]	Herbaceous indexing	PCR tests[2]	ELISA
Bacteria	✓			2	
Fungi	✓				
Phytoplasma	✓	✓		1	
Viruses	✓	✓	1 test[3]	13	1

[1]Additional testing of symptomatic plants may include culturing, microscopy and molecular testing.
[2]The plants must be indexed for any quarantinable pathogenic organisms that have not been indexed for by the supplier.
[3]Herbaceous indexing is performed using *Chenopodium quinoa.*
[4]Woody indexing is performed using Bing (cherries) and GF305 (all *Prunus* spp.).

Although not mandatory, importers may also request AQIS to test their imported *Prunus* germplasm for the pollen borne viruses, *Prunus necrotic ringspot virus* and *Prune dwarf virus* by ELISA, PCR or grafting to shirofugen (PDV only). This benefits importers who wish to place their germplasm into certification programs as they may be able to request destruction of material if it is infected or they may wish to begin a process of virus elimination while the material undergoes PEQ testing.

1.2 Quarantine pathogens of *Prunus sp.*

Within the recently completed HIA Limited funded project entitled "Review of the post entry quarantine conditions for imports of almond germplasm" (AL10001) Victorian DEDJTR scientists reviewed the bacteria, phytoplasmas, viruses, viroids and fungi that are currently known to infect almond and other *Prunus* species and identified which of these pathogens are significant to Australian PEQ for almonds. The review identified several pathogens that do not appear on the current *Prunus* species PEQ lists for Australia. The most appropriate diagnostic tests for detection of the listed quarantinable pathogens were also identified. The review identified the following:

Prunus species can be infected by many bacteria and five of these are significant at the quarantine level, including *Xylella fastidiosa* which is a high priority pest threat and another four are significant at the certification level within Australia. Of these bacteria three are important for almond and require testing during PEQ. Two bacteria of quarantine significance to other *Prunus* species (not almonds) were also identified that are not currently on the PEQ list for summerfruit. Phytoplasmas are not known to infect *Prunus* species within Australia. In other countries there are 11 phytoplasmas or phytoplasma groups that are

known to infect *Prunus* species including European stone fruit yellows and X disease, both of which are high priority pest threats.

There are 47 viruses that are known to infect *Prunus* species, of which 34 are reported from other countries and are significant at the PEQ level. This list includes *Plum pox virus* (PPV) which is a Formal Category 2 pest threat to the Australian summerfruit industry and the overall pest risk threat is rated as "Extreme". Ten of the 34 quarantine viruses infect almonds and are significant at the PEQ level for Australia.

Three viroids infect *Prunus* species; *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd) are reported in Australia and infect *Prunus* species including almond. However, hop strains of HSVd are quarantinable as they are not known to occur in Australian hops or other hosts including *Prunus* species and may still require testing in PEQ. The third viroid, *Apple scar skin viroid* which does not occur in Australia, infects other *Prunus* species but not almond

Based on the findings of the HIA Limited project AL10001, the following recommendations were made:

1. Update the PEQ list for almonds and/or summerfruit with recently reported pathogens and updated information of known pathogens.
2. Determine if some of the "minor" pathogens require active testing.
3. Adopt, develop and validate molecular diagnostic tools under Australian conditions for the important quarantine pathogens of almonds.
4. Develop a post entry quarantine diagnostic manual that is specific for almonds.

1.3 High Health and certification

The production of high health *Prunus* material during the 1970's and 1980's, which primarily included *Prunus* species other than almonds, was done through the Fruit Variety Foundation (FVF) and was managed by the Victoria Department of Agriculture (now DEDJTR). The FVF is still in existence and is managed by NSW DPI, but the scheme only maintains citrus. The only industry based certification programs for *Prunus* species that currently exist are for the production of high health almond budwood, and are run by the Almond Board of Australia. In Australia high-health pathogen-tested almond trees are routinely tested for several viruses that can significantly affect quality and yield, including *Prunus necrotic ringspot virus*, *Prune dwarf virus*, *Apple chlorotic leafspot virus* and *Apple mosaic virus*. *Cucumber mosaic virus* (CMV) and PLMVd which occur and HSVd are also known to infect *Prunus* species including almond in other countries. PLMVd is not known to infect Australian *Prunus* species while CMV and HSVd occur in Australia in other crops. The effect of CMV on almonds is unknown but it is associated with diseases in other *Prunus* species, and might also have significant impact on quality and yield. PLMVd and HSVd can also be associated with loss in quality and yield in some *Prunus* species. The incidence of CMV, PLMVd, and HSVd in Australian *Prunus* species including almond is not known and they are not tested for within the certification program.

Production of pathogen tested material does occur for the Summerfruit industry but is conducted by individual nurseries with their own specified protocols for pathogen testing,

rather than an industry based program. When these species are tested they are usually tested for PNRSV, PDV and ACLSV. However there are also other viruses and viroids that are known to infect *Prunus* species and which also occur in other crops in Australia and these include: CMV, Apple stem pitting associated virus, Apricot pseudochlorotic leaf spot virus, Apple stem grooving virus, Sowbane mosaic virus, Tobacco necrosis virus, Tobacco mosaic virus, Carnation ringspot virus, HSVd and PLMVd. The incidence of these pathogens in Australian *Prunus* species is unknown and for many, except APCLSV, CMV, PLMVd and HSVd, their economic impact in *Prunus* species is uncertain.

1.4 Diagnostic methods

A combination of diagnostic methods can be used by quarantine and certification programs to identify diseases and pathogens of significance:

- Visual inspection of the imported germplasm, which is propagated and grown in PEQ, is mandatory for observation of symptoms associated with fungi, bacteria, viruses, viroids and phytoplasmas. Visual inspection is currently the only active method used for detection of fungal pathogens and *Pseudomonas syringae pv. amygdali*.
- Biological indexing onto herbaceous and woody indicators is mandatory and is specifically used for the detection of viruses, viroids and graft transmissible diseases of unknown aetiology. If symptoms are observed various techniques may be used to determine which virus is present such as electron microscopy, ELISA or PCR.
- Electron microscopy is optional and may be used to detect virus particles in disease plants or biological indicators; the symptomatology and virus particle morphology may be indicative of a specific virus.
- Light microscopy and culturing are used for confirmation if fungal or bacterial pathogens are suspected.
- Enzyme linked immunosorbent assay (ELISA) is only mandatory for *Plum pox virus* detection. ELISA may be used for confirmation of the presence of other viruses.
- Molecular (polymerase chain reaction, PCR) techniques are mandatory for detection of *Xylella fastidiosa* on plums, peaches, nectarines and almonds originating from countries where the pathogen has been reported. PCR is also mandatory for phytoplasma detection. For all other pathogens this method is optional and dependent on the availability of a validated diagnostic test. Molecular techniques, such as polymerase chain reaction (PCR), may be used to identify viroids, viruses or phytoplasma that might also be associated with some symptoms.

1.4.1 Visual inspection

Many summerfruit pathogens are associated with specific symptoms on sensitive varieties and visual inspection can assist in identifying these infected varieties (Diekmann and Putter 1996, Di Terlizzi 1998). Conversely, symptomless infections can occur in some varieties; these infected trees may act as a reservoir for other sensitive varieties (Diekmann and Putter 1996, Di Terlizzi 1998). Also, other factors can induce similar symptoms and result in a misdiagnosis of disease. Consequently visual inspection alone cannot be relied upon for disease diagnosis and pathogen detection. Active diagnostic testing is required to confirm the presence of viruses and bacteria.

1.4.2 Biological indexing

Biological indexing takes advantage of a sensitive plant response to the presence of pathogens. Indicator plants are inoculated with material of another source and are observed for characteristic symptoms. Two biological indexing methods are used for the detection of summerfruit viruses: herbaceous indexing by sap inoculation and chip bud inoculation of woody indicators (Diekmann and Putter 1996, Di Terlizzi 1998).

Herbaceous indicators are used in Australian post entry quarantine for detection of nepoviruses and *Strawberry latent ringspot virus* (SLRSV). Herbaceous indicator plant species that are sensitive to nepoviruses include *Chenopodium* spp., in particular *C. quinoa* and *C. amaranticolor* (Martelli 1993). Plant tissue is ground in an appropriate buffer and rubbed onto the leaves of the indicator plants that have been dusted with an abrasive powder. Symptoms can develop within seven days of inoculation, however plants are often observed for up to six weeks post inoculation. Symptoms are dependent on the virus species but can include: chlorotic local lesions, systemic mottling and leaf malformation, tip necrosis and plant death. Some similar symptoms might also be associated with other virus species that have been reported to infect summerfruit including *Sowbane mosaic virus* (SoMV) and *Cucumber mosaic virus* (CMV).

Woody indictors are currently used for diagnosis of virus associated diseases in Australian PEQ and certification schemes. Chip buds of a candidate plant are grafted onto sensitive summerfruit indicators. The indicators used in PEQ are listed in Table 1. This method is time consuming and symptoms may take several years to appear.

Biological indexing onto woody indicators is dependent on the successful transmission of associated viruses from the chip bud via the graft union to the indicator plant. This virus inoculation process is affected by graft take and presence of viruses in the grafted chip buds. Disease expression in the indicators is also affected by strain variation amongst the associated virus species, and some viruses may not induce obvious symptoms on the selected biological indictors. It is possible that environmental factors, such as temperature, light and nutrition, will affect symptom expression. Some indicators develop symptoms that cannot be readily attributed to a known causal agent, or combination of causal agents.

1.4.3 Enzyme-Linked ImmunoSorbent Assay

The Enzyme-Linked ImmunoSorbent Assay (ELISA) is a serological technique using antibodies to detect the presence of an antigen, such as a virus, in a plant sample (Clark and Adams 1977). The technique is simple and is useful for testing large numbers of samples and results can be gained within 24 hours. ELISA assays have been prepared for many summerfruit viruses and are commercially available. However there are many summerfruit viruses where ELISA tests have not been developed (Boscia and Myrta 1997).

The reliability of ELISA tests may be affected the degree of amino acid sequence conservation within the coat protein of the virus. False negative results can occur where there is high variation of the antigenic coat protein within a species and the antibodies fail to detect all strains of a defined viral species. False positive results can occur where there is a high degree of conservation of the coat protein between viral species and antibodies react with a number of virus species. Where polyclonal antisera is prepared against poorly purified

viruses that contain co-purified viruses and proteins, cross reactions with antibodies may occur also leading to false positive results (Boscia and Myrta 1997, James et al 1994, James et al 1996).

ELISA reliability is affected by virus concentration within a plant and low virus concentration may lead to false negative results. In addition viruses can be unevenly distributed and in uneven titre within a plant and appropriate tissue sampling is required to ensure reliability of detction. Virus concentration is also affected by environmental factors and timing of sampling may also be critical to reduce the risk of false negative results (Fuchs, 1980, Fuchs, 1982; Boscia and Myrta 1997).

1.4.4 Molecular testing

In recent years there have been some significant advances in molecular diagnostics for summerfruit pathogens using reverse transcription polymerase chain reaction (RT-PCR) and PCR methods. Most summerfruit viruses have an RNA genome and reverse transcription (RT) is required to make a DNA copy (cDNA) of the viral RNA before the PCR can proceed. During PCR a thermostable enzyme (e.g. *Taq* DNA polymerase) is used to generate multiple copies of a specific nucleotide sequence from DNA or cDNA. Once the PCR reaction is complete the products are separated on an agarose gel by electrophoresis, stained with a fluorescent dye and viewed under UV illumination. A positive result is recorded when a product of the correct size is observed. More recently real-time (quantitative) PCR (qPCR) techniques have been developed that simultaneously amplify and measure the presence of the target in a PCR reaction (VanGuilder et al 2008). Real-time PCR technology is being adapted for pathogen detection in summerfruit.

Regardless of the chemistry used, PCR creates multiple copies of the original target nucleic acid and thus is considered much more sensitive than ELISA, which can be limited by the number of virus particles present in a plant sample (Wetzel et al 1992, Lopez et al 1999, Levy et al 2000). However, PCR can also be affected by genetic variability of strains causing false negative results. False negative results can also be obtained due to inhibition of the reverse transcriptase or DNA polymerase activity by compounds co-extracted with the nucleic acids. False positive results can also occur when the primers have a high degree of similarity to other genetic material, including that of the host plant or other organisms and it is important that PCR tests are thoroughly validated in their hosts and in the regions where they will be used before they are adopted for routine diagnostic testing.

Next generation sequencing (NGS) methods are currently and primarily being used in research settings for pathogen detection, discovery and genomic analysis. However they are being developed as a “one-stop-shop” diagnostic test for plant pathogen detection to support plant biosecurity and certification programs (Barba et al 2014, Kehoe et al 2014, and Massart et al 2014). The positive benefits of this approach are the ability to detect multiple pathogens in one sample in one test and the identification of novel pathogens that may be associated to a disease. However, NGS will require further development to overcome some weaknesses.

The impact on pathogen detection by NGS such as timing for sampling, tissue types and sample preparation is not yet understood. Different sequencing platforms and the impact on

the NGS output and dataset quality is also not fully understood. An appropriate bioinformatics data analysis pipeline addressing limits of detection is required, i.e. how much of the pathogen genome is required to call a positive result? Should this pipeline be backed up by biological information or an alternative detection method for validation to give confidence in application of NGS in a diagnostic setting? The bioinformatics analysis of volumes of data generated by NGS may be laborious and time consuming in a diagnostic setting, increasing the cost of testing. Consequently a suitable bioinformatics analysis pipeline also needs to be assembled. This pipeline could be similar to the approach taken by Stobbe et al (2014) in the development of the E-probe Diagnostic Nucleic acid Analysis (EDNA) process which essentially mines the NGS data set with electronic probes, removes and eliminates the need for assembly of NGS data. The limitation of this approach is that it only may detect pathogens for which genetic information is known and novel pathogens may be missed. The NGS procedure is highly sensitive and prone to laboratory contamination occurring during sample preparation and from contaminated sequencing equipment, possibly leading to false positive results and an overload of contaminant might mask low abundance targets leading to a false negative result (Lawrence et al 2014, Hadfield and Eldridge 2014). Finally, if a novel virus is discovered there may be implications for biosecurity and market access, therefore any detection of a novel pathogen must be supported by biology (McDiarmid et al 2013). As NGS technology and knowledge develops, these weaknesses will be dispelled or managed and it is highly likely that this will be the method of choice for pathogen detection and routine diagnostics in the near future.

1.5 Project aims

In recent years there have been significant advances in molecular and diagnostic technology that have allowed characterisation of new and emerging pathogens, new strains of known pathogens of *Prunus* species and clarification of pathogens associated with diseases of *Prunus* species which have an unknown aetiology, especially for viruses, viroids and phytoplasmas. As a consequence it is likely the list of quarantine pathogens of *Prunus* species for Australia will require updating to reflect this knowledge. It is also likely these advances have resulted in new and/or improved molecular tools for the rapid and sensitive detection of these pathogens. These diagnostic tools can be used to better support pathogen detection during PEQ, for summerfruit certification programs and to improve summerfruit biosecurity in Australia. The development of these molecular protocols will support the industry biosecurity plans that have been developed for both almonds and summerfruit which identify, as a priority, the need for adequate diagnostic tools for the detection of exotic pathogens at the border and post border during incursions and to support market access.

This project focuses on the priority area of biosecurity for the Summerfruit and Almond industries. Research activities of this three year project will center around five objectives:

1. Update the PEQ list for summerfruit to include recently reported pathogens and update the existing information on the known pathogens of summerfruit.
2. Identify, develop and validate molecular diagnostic tools for the detection of endemic and exotic pathogens of summerfruit under Australian conditions.

3. Develop a post entry quarantine diagnostic manual for *Prunus* species including almonds.
4. Investigate strain variation of major endemic viruses of *Prunus* species in Australia.
5. Identify optimal pest and disease management strategies for maintaining summerfruit budwood repository blocks.

Appendix 2: An updated list of pathogens of almonds and summerfruit significant to quarantine and certification.

Introduction

Prunus germplasm, including summerfruit and almonds, imported into Australia requires a minimum of 16 months post entry quarantine testing and during this period is tested for a prescribed list of diseases and pathogens of quarantine significance using a range of diagnostic techniques (Appendix 1). In recent years there have been significant advances in molecular and diagnostic technology that have allowed characterisation of new and emerging pathogens, new strains of known pathogens and clarification of pathogens associated with diseases of unknown aetiology, especially for viruses, viroids and phytoplasmas, As a consequence it is likely the list of quarantine pathogens for summerfruit will require updating to reflect this knowledge.

Department of Agriculture may not differentiate between the pathogens that infect almonds and summerfruit species and the specific pathogen testing protocols for almond germplasm in post entry quarantine (PEQ) have not been identified. Additionally, the quarantine status of almond diseases may have changed over the past decade with new reports of various pathogens (e.g. *Apricot pseudochlorotic leafspot virus*) in Australia that infect summerfruit. It is unclear if many of these pathogens can infect almond. Indeed it is unclear if *Plum pox virus* (PPV) can infect almond in the field. As a result, Australian quarantine agencies would consider almonds to be a host of PPV and would include almonds in any survey and eradication program for this serious disease of summerfruit. Clarity on the "field" host-range of PPV and other viruses and viroids could prove to be of significant benefit to the almond industry.

As a part of this project a review of the actionable diseases and pathogens of summerfruit has been generated and will include a list of diagnostic tools that are available for each pathogen. Analysis of this data may identify a strategy for reducing the period of time imported germplasm spends in post entry quarantine. At a minimum this review will ensure the Australian almond industry has rapid access to overseas germplasm whilst minimizing the risk of introducing new exotic diseases.

The key diseases and pathogens of almonds and summerfruit are reviewed. Information on their biology, distribution, host range, economic importance and entry pathway is provided. The focus will primarily be on bacteria, phytoplasmas, viruses and viroids as these pathogens are of higher risk of transmission in propagation material entering PEQ when compared to the fungi. However several high risk fungi are also included.

Based on the information gathered in the review this project aims to:

- identify the diseases and pathogens of quarantine importance to almonds and summerfruit;
- identify diagnostic techniques available to detect these diseases and pathogens; and
- prepare a report with recommendations for AQIS to consider changes in the post entry quarantine testing of imported almond germplasm.

2.1 Review of significant pathogens

2.1.1 Bacteria

Quarantine

Organism: *Erwinia amylovora*

Disease: Associated with fire blight disease in plants species of *Rosaceae* including apple and pear. Associated with shoot blight on European plum, *Prunus domestica* L. 'd'Agen' and apricot .

Distribution: The Americas, Europe, Asia (Armenia, Azerbajhan, India Israel, Iran, Jordon, Lebanon, Turkey), Africa (Egypt and Morocco) and New Zealand.

Host range: *E. amylovora* mainly infects members of the sub-family Pomoideae of the family *Rosaceae*: Primary hosts include*: Malus* (apple), *Pyrus* (pears), *Crataegus* (hawthorns), *Cotoneaster*, *Cydonia*, *Eriobotrya* and *Pyracantha* species. Other hosts include *Amelanchier*, *Mespilus*, *Chaenomeles*, *Rubus* (blackberry, raspberry), chokeberry (*Aronia melanocarpa*), strawberry and *Sorbus.* It has been reported to infect almond under experimental conditions. References to infections in almond in general non-refereed publications may be associated with flowering almond (*Prunus triloba*). Natural infections are reported for other *Prunus* sp. in the field, including apricot, peach, Japanese and European plum, sour cherry and cherry plum. Other research has shown that all *Prunus* sp., except *P. domestica*, can support epiphytic growth of the bacterium on flowers.

Economic impact: The disease can cause severe economic impact in *Maloideae* host species such as pear and apple, although the effect is dependent on environment and cultivar. Although disease is observed the impact on summerfruit production is not known, yet these hosts could represent a source of inoculum for other more susceptible species such as pome fruits.

Pathway: Contaminated plant material

Diagnostic tests:

- Isolation and culturing onto general and selective media
- Morphological methods: ooze test and moist incubation
- Hypersensitivity assay in *Nicotiana tabacum.*
- Pathogenicity tests onto pear fruitlets and apple and pear seedlings
- Fatty acid analysis
- Molecular diagnostics: Many conventional and real time PCR tests are available, however some tests may not detect all strains and other tests may detect *Erwinia* sp. other than *E. amylovora.* The currently accepted SPHDS protocol utilises primers that are known to cross react with other bacterial sp. and which have not been validated for summerfruit or almonds. The EPPO protocol recommends to use two conventional PCR tests to detect plasmid and chromosomal DNA (EPPO 2013) especially as some studies show that not all PCR tests will detect all strains (Powney et al 2007). qPCR assays have been developed that may be more sensitive than conventional assays (EPPO 2013, Gottsberger 2010 and Pirc et al 2009). A LAMP based assay has also been developed (Buhlmann et al 2013).

Notes: The bacterium enters plants through blossoms and the open stomata of new shoots and leaves in spring and then systemically invades the hosts, where it can persist in parenchyma and xylem tissue. It can also enter though wounds. It is transmitted by wind, rain and water splash, insects and birds. It can also be transmitted in plants and propagation material.

Symptoms include: flower or shoot tip necrosis, blight of fruitlets, shepherd's crook shape of affected shoots, red or reddish brown streaks of the vascular tissue, cankers on larger branches, bacterial ooze may be present on shoot tips, blossoms and at the margins of cankers and blighted blossoms and leaves may remain attached after leaf fall.

References: Atanasova et al 2005, Berger et al 2000, EPPO 2013, Johnson et al 2006, Korba and Sillerova 2010, Mohan and Thomson 1996, Mohan and Bijman 1999, Mohan et al 2001, Mohan 2007, Moltmann and Viehrig 2008, Pirc et al 2009, Snow, 1922, Vanneste et al 2002, Végh et al 2012, Végh and Palkovic 2013, Vojinovic 2010.

Organism: *Pseudomonas amygdali*

This bacterium is listed on the PHA Almond Industry biosecurity plan.

Disease: Bacterial (Hyperplastic) canker of almond.

Distribution: Greece, Afghanistan and Turkey.

Host range: *S*pecific to almond. Some almond varieties are more susceptible to *P. amygdali* than others.

Economic impact: Infection by *P. amygdali* can result in yield loss and decline and death of trees, therefore having a significant negative economic impact to industry.

Pathway: Propagation material

Diagnostic tests: No specific diagnostic tool exists.

- Symptom expression, bacterial culturing onto selective media and biochemical tests together should suggest the presence of this pathogen.
- It is specific to almond; therefore pathogenicity onto other hosts may assist diagnosis. An HR response is induced on inoculated *Nicotiana tabacum*.
- A combination of culturing and the use of PCR and sequencing of the *rpoD* locus should distinguish *P. amygdali* from other Pseudomonads that may be isolated from *Prunus* species. This should allow isolates to be assigned to a specific assign phylogroup or genomospecies. Further confirmation could be done using PCR and sequencing of the *acn, pgi* and *cts* loci.

Notes: *P. amygdali* overwinters in cankers and cankers are perennial. It is spread by wind and rain/water splash from active cankers to other parts of a tree and to other trees. It enters the host though leaf scars on twigs and shoots or through wounds. Infection of the wounds results in cankers that consist of swollen bark that cracks open longitudinally around infection points on shoots, twigs and branches. Cankers forming around buds can result in no bud burst. Cankers can girdle shoots, twigs and branches resulting in dieback. Affected

trees decline. The canker symptoms are distinct to *P.* amygdali and differ significantly from cankers associated with *P. syringae pv. syringae* infection.

References: Ercolani and Ghaffer 1985, Gundogdu and Demir 1990, Janse 2010, Psallidas 1997, Sarkar & Guttman 2004, Parkinson et al 2011.

Organism: *Pseudomonas syringae pv. persicae*

Disease: Bacterial dieback of peach and nectarine. It also infects plum in New Zealand.

Distribution: New Zealand, France, Germany and Portugal Possibly Yugoslavia, but not confirmed. It was isolated once in the UK from *P. cerasifera*.

Host range: Peach, nectarine and Japanese plum. It is not known to infect almond.

Economic impact: The bacterium may have serious economic impacts as it can reduce quality and yield of fruit and quickly cause decline and death of trees

Pathway: Infected propagation and planting material. Mechanically transmitted by pruning.

Diagnostic tests:

- Characteristic symptoms on affected plants should indicate possible infection.
- Isolation and culturing onto semi-selective media
- Biochemical analyses
- PCR tests for the detection of genes associated with toxin production.
- *P. s. pv. persicae* can be distinguished from *P. s. pv. syringae* and *P. s. pv. mors-prunorum* which occur in Australia, by biochemical tests: GATTa (gelatine liquefaction, aesculin hydrolysis, tyrosinase activity, Na-tartrate utlization)test; lack of acid production from sorbitol, erythritol, inositol; non-utilization of lactate, D(-) and L(+) tartrate.); slow growth and non-fluorescence on Kings B media
- A combination of culturing and the use of PCR and sequencing of the *rpoD* locus should distinguish *P. s. pv. persicae* from other Pseudomonads that may be isolated from *Prunus* species. Sequencing of the *rpoD* locus PCR product may not distinguish this species from *P. s. pv. avii.* This should allow isolates to be assigned to a specific assign phylogroup or genomospecies. Further confirmation could be done using PCR and sequencing of the *acn, pgi* and *cts* loci.
- Repetitive PCR, multi-locus sequence typing and melting profile PCR may also be used to distinguish between some *P. syringae* pathovars.

Notes: *P. s. pv. persicae* is spread from epiphytic populations on leaves and enters through wounds such as leaf scars, pruning wounds and water soaked areas on bark that occur due to freeze- thaw cycles. It is spread by pruning, wind and rain.

Symptoms develop through winter as the bacteria spreads systemically within the plant. In spring infection can lead to wilting and dieback of shoots, death of larger branches and in severe infections death of the entire tree. The bacterium can cause necrotic spots on leaves which fall out causing a shot-hole appearance. Necrotic spots can also form on fruit, especially nectarine, which can be covered in a transparent gum that browns quickly.

References: EFSA 2014, Eppo 2005, Janse 2010, Kaluzna et al 2012, Parkinson et al 2011, Prunier et al 1970, Young 1988.

Organism: *Pseudomonas syringae pv. avii*

Disease: Bacterial canker

Distribution: France

Host range: Wild and cultivated cherries. It is not known to occur on other *Prunus* sp. including almond

Economic impact: *P.s. pv. avii* can cause severe dieback and death of affected trees and therefore can have a significant negative economic impact on Cherry production.

Pathway: Propagation material.

Diagnostic tests:

- Characteristic cankers on affected plants should indicate possible infection.
- Isolation and culturing onto semi-selective media
- Biochemical analyses
- PCR tests for the detection of genes associated with toxin production.
- *P. s. pv. avii* can be distinguished from other pathovars, including those which occur in Australia, though biochemical tests: GATTa test and using the Toxin PCR tests
- A combination of culturing and the use of PCR and sequencing of the *rpoD* locus should distinguish *P. s. pv. avii* from other Pseudomonads that may be isolated from *Prunus* species. Sequencing of the rpoD locus PCR product alone may not distinguish the species from *P. s. pv. persicae.* This should allow isolates to be assigned to a specific assign phylogroup or genomospecies. Further confirmation could be done using PCR and sequencing of the *acn, pgi* and *cts* loci.
- Repetitive PCR, multi-locus sequence typing and melting profile PCR may also be used to distinguish between some *P. syringae* pathovars.

Notes: It is spread by pruning, wind and rain. It invades plants through leaf scars and wounds from pruning, feeding etc. and can spread systemically within the plant. It can also spread in propagation material and plants as it can over-winter in cankers and buds

References: Janse et al 2010, Kaluzna et al 2012, Menrad et al 2003, Parkinson et al 2011, Santi et al 2004.

Organism: *Xylella fastidiosa*

This bacterium is listed on the PHA Industry biosecurity plan for almonds

Disease: *X. fastidiosa* causes almond leaf scorch disease (ALSD), phony peach disease, plum leaf scald and leaf scorch of the ornamental Purple leafed plum (*Prunus cerasifera*). Schipka laurel (*P. laurocerasus* 'Schipkaensis'), and Japanese flowering cherry (*P. serrulata* 'Kwanzan') are also reported as hosts.

Distribution: Asia (Taiwan, Turkey), Italy, North America (Canada, Mexico, USA), Central America and Caribbean (Costa Rica), and South America (Argentina, Brazil, Paraguay, Venezuela). There are unconfirmed reports of its occurrence in Kosovo and France in imported grapevine propagation material from the USA. Almond leaf scorch disease has been observed in India and the presence of *X. fastidiosa* was presumed based on the use of a chemical (acid based) test; its presence has not been confirmed by other methods.

Host range: Broad host range (more than 150 plant species in 30 families) that includes both woody and herbaceous plants such as: alfalfa, almond, blueberry, citrus, coffee, elm, grape, maple, mulberry, oak, oleander, peach, pear, sycamore. Some strains are specific to their hosts e.g. almond strains do not infect grapevine but grapevine strains can infect almond.

Economic impact: Infection by *X. fastidiosa* can result in yield loss and decline and death of trees, therefore having a significant negative economic impact to industry.

Pathway: Propagation material. Infectious vectors

Diagnostics tests:

- Isolation and culturing.
- Dark field microscopy.
- A bio-assay on Nicotiana tabacum cv SR-1 has been developed.
- ELISA is available however this technique is not as sensitive as molecular methods.
- Many PCR tests are available, including conventional and real-time techniques. Many PCR tests are generic and should pick up all strains of *X. fastidiosa*. A specific conventional PCR test is available for strains associated with almond leaf scorch disease. A real-time PCR and a loop mediated isothermal amplification (LAMP) assay have been developed and are used by Ministry for Primary Industries in New Zealand; these assays could be used for routine detection and during an incursion. The LAMP based assay is colourmetric and could be used in the field.
- The current SPHD accepted PCR test for detection of *X. fastidiosa* in Australian grapevines requires validation for use in summerfruit and almonds under Australian conditions. Conventional PCR tests using the primer pairs RST31/RST33 (Minesavage et al 1994) and XF1-F/XF6-R (Firrao and Bazzi 1994) are included in this protocol and the former primer pair still recommended for detection of *X. fastidiosa* in Almonds and summer fruit. This protocol should be updated with the most currently used PCR tests available:
    - Conventional tests could use: HL5/HL6 (Francis et al 2006).
    - qPCR tests could use: HL5/HL6 and probe (Francis et al 2006); ITS and 16S primers and probes (Schaad et al 2002); LAMP and Real-time PCR assays targeted to the *rim*M gene (Harper et al 2010)

Notes: *X. fastidiosa* is a xylem limited, gram-negative bacterium.

There are several subspecies: *X. f.*subsp *fastidiosa* (infects grape, almond, alfalfa and maple), *X. f. multiplex* (infects peach, plum, almond, sycamore, elm and pigeon grape) and *X. f. pauca* (infects Citrus spp. and possibly coffee) *X. f.* subsp. *Sandyi* (infects *Nerium oleander)* and *X. f.* subsp. *taske (*infects *Chitalpa tashkentensis*, this subspecies needs confirmation)

Susceptible hosts may exhibit symptoms such as wilting, leaf scorching, premature leaf fall, chlorosis, stunting, early shooting and flowering, decline and dieback. *X. fastidiosa* has a serious impact on production in almonds, peach and plum as well as other host species such as citrus, coffee and grape due to loss in quality and yield of the crop and decline and death of infected host plants.

Symptom expression is dependent on the bacterial strain and plant host species and cultivar. Differences in susceptibility are observed amongst almond cultivars. Not all infected plants host species exhibit symptoms, but these may serve as a source of inoculum.

X. fastidiosa is transmitted by xylem feeding insects. It has been suggested that most sucking insects which feed predominantly on xylem fluid are potential vectors. The most important vectors are species of *Cicadellidae* and *Cercopidae* (Insecta: Hemiptera: Homoptera) and include. *Homalodisca vitripennis* (glassy winged sharpshooter), *Xyphon* (formerly *Carneocephala*) *fulgida* (red-headed sharpshooter), *Draeculacephala minerva* (green sharpshooter) and *Graphocephala atropunctata* (blue-green sharpshooter).

X. fastidiosa is also spread via infected planting material.

References: Cabrera-la *Rosa* et al 2008, Chang et al 2009, Connell et al 2011, Davis et al 1981,Doddapaneni et al 2007, EFSA 2015 , Firrao and Bazzi 1994, Francis et al 2008, Harper et al 2010, Hernandez-Martinez et al 2006a, 2006b, Janse 2010, Janse and Obradovic 2010, Janse et al 2012, Jindal and Sharma 1987, Luck et al 2002, Mircetich et al 1976, Purcell, 1989, Qi 2007, Rodrigues et al 2003, Schaad et al 2002 , Schaad et al 2004.

Present in Australia

Organism: *Agrobacterium tumefaciens and A. rhizogenes*

Disease: Crown gall, root knot and hairy root.

Distribution: Worldwide.

Host range: Many hosts. *Prunus* spp including almond.

Economic impact: *A. tumefaciens* is not considered to be a significant pathogen of *Prunus* sp. in Australia if effective management practices are used.

Pathway: Propagation material, sSoil.

Diagnostic tests:

- Characteristic symptoms of galls on affected plants should indicate possible infection.
- Isolation and culturing on selective media.
- Pathogenicity tests.
- Biochemical tests: these can assist in discriminating between *Agrobacterium* sp.
- Several specific PCR tests are available (Lopez et al 2010) including a multiplex PCR that allows detection and differentiation between 4 different agrobacterium species including *A. tumefaciens* and *A. rhizogenes* (Pulawska et al 2006). These have not been validated for *Prunus* species. Several primers were developed using an in silico

analysis (Albuquerque et al for the specific detection of *A. tumefaciens* but these have not been validated in vivo 2012)

Notes: The bacteria invade the tissue of roots through wounds and move systemically.

A. tumefaciens causes large tumour-like growths of affected *Prunus* sp. on roots and at the collar just above the soil. It may be more problematic in young plants and rootstocks, where it can cause significant losses at the nursery level. Affected plants may be stunted, decline and die due to disruption to the vascular tissue and girdling of the trunk. Fissures within the galls can lead to secondary infections by other pathogens. Older trees are often less affected by the disease and may have no economic loss. However the occurrence of the bacterium in trees used for nursery stock production could represent a risk for contamination of propagation material.

A. rhizogenes causes proliferation of the roots and root hairs. It can be used to promote root production in almonds and other plant species undergoing micropropagation. An avirulent strain is used to cross protect against other *Agrobacterium sp.* in many plant hosts but is not reported on almond.

Both species have a broad host range. They are soil-borne, where they can last for several years in the absence of a host. They can be transmitted through planting material and may be transmitted from plant to plant on pruning equipment.

References: Cubero et al 1999, Cubero et al 2006, Escobar and DanLopes et al 1997, Janse et al 2010, Lopez et al 2010, Pulawska et al 2006, Albuquerque et al 2012.

Organism: *Pseudomonas syringae pv. syringae* and *P. s. pv. mors-prunorum*

Disease: Bacterial canker and blast

Distribution: Worldwide.

Host range: *P.s. pv. syringae* infects many *Prunus* sp. and causes bacterial canker. It is associated with bacterial canker and blast of almond in Italy, Iran, Algeria and USA. *P. s. pv. mors-prunorum* infects several *Prunus* spp but is not reported on almond.

Economic impact: Both pathovars can cause yield loss due to blast and death of buds and decline and death of *Prunus* trees and therefore can have negative impact on production of summerfruits.

Pathway: Transmitted on infected propagation material and plants.

Mechanical transmission during pruning.

Diagnostic tests:

- Characteristic symptoms on affected plants should indicate possible infection.
- Isolation and culturing onto semi-selective media.
- Biochemical analyses.
- PCR tests for the detection of genes associated with toxin production.

- *P. s. pv. syringae and P. s. pv. mors-prunorum* can be distinguished from one another and from *P. s. pv. avii* and *P. s. pv. persica*e, which do not occur in Australia, using biochemical and PCR based tests.
- A combination of culturing and the use of PCR and sequencing of the *rpoD* locus should distinguish *P. s. pv. avii* from other Pseudomonads that may be isolated from *Prunus* species. Sequencing of the rpoD locus PCR product alone may not distinguish the species from *P. s. pv. persicae*. This should allow isolates to be assigned to a specific assign phylogroup or genomospecies. Further confirmation could be done using PCR and sequencing of the *acn, pgi* and *cts* loci.
- Repetitive PCR, multi-locus sequence typing and melting profile PCR may also be used to distinguish between some *P. syringae* pathovars.

Notes: These bacteria are spread by pruning, wind and rain. They can also spread in propagation material and plants as it can overwinter in cankers and buds, survive epiphytically on leaf and bud surfaces in spring and it can invade the vascular system.

Both pathovars prefer wet, cool conditions for growth and disease development. In less favourable conditions they may live as saprotrophs. They overwinter in cankers and on infected leaves and buds.

Both pathovars are distributed worldwide but are often managed through production of high health planting material in certification programs.

References: Harzallah et al 2004, Janse 2010, Kaluzna et al 2012, Parkinson et al 2012, Samavatian, 2006, Scortichini 2010, Vavaro 1983

Organism: *Xanthomonas arboricola pv. pruni*

Disease: Bacterial leaf spot, shot-hole and black spot of almond, apricot, cherries, nectarine, peach, plum and *P. salicina*.

Distribution: Occurs in Australia, Europe, the Americas, New Zealand, Africa and Asia

Host range: It infects only *Prunus* species including *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry) *P. cerasus* (sour cherry) *P. persica* (nectarine, peach) and *P. domestica* (plum). It also infects the ornamental *species P. davidiana, P. japonica* and *P. laurocerasus* and *P. salicina*

Economic impact: This bacterium affects yield and quality of fruit and therefore can have negative impact on production of summerfruits.

Pathway: Disseminated through rain and wind from cankers. Entry is through wounds. Transmitted mechanically on pruning equipment. Transmitted in plants and propagation material and infected fruit.

Diagnostic tests:

- Characteristic symptoms on affected plants should indicate possible infection.
- Pathogenicity tests on young shoots or leaves and fruit.
- Isolation/culturing onto general and specific media.
- Fatty acid and protein profiling.

- Specific conventional and real time PCR diagnostic tests are available for detection. Most recently a Bio-PCR has been developed in Australian conditions that can detect the pathogen in symptomless and symptomatic tissues in plum (Ballard et al 2011). This test could be used to support Australian certification programs for almond and summerfruit.
- A conventional PCR (Pagani et al 2004) was further modified to improve detection of this bacterium (Lopez et al 2012).
- Several other PCR tests have been developed including conventional and real-time assays but all require further evaluation for specificity on different *Prunus* species and in with Xap isolates world-wide (Palacio-Bielsa et al 2011, Ballard et al 2011, Pothier et al 2011a, Pothier at al 2011b, Palacio-Bielsa et al 2012).

Notes: Severe infections can result in defoliation of trees. Severely affected trees may decline and die back. Fruit may be small and unmarketable. Infected fruit may develop spotting, cracks and lesions resulting in poor quality. The bacterium affects production in warm and moist environments.

X. arboricola pv. pruni can overwinter on peach in the intercellular spaces of the cortex, phloem and xylem parenchyma towards the tips of twigs. It can overwinter in cankers formed in summer on plum and apricot providing a source of inoculum in the following spring. It can also overwinter in buds of plum and fallen leaves.

References: Ballard, 2008, Ballard et al 2011, EFSA 2014, EPPO Bulletin 2006, Hetherington et al 2005, Janse 2010, Lopez et al 2011, Pagani, 2004, Palacio-Bielsa et al 2010, Park et al 2010, Palacio-Bielsa et al 2011, Pothier et al 2011a, Pothier et al 2011b, Palacio-Bielsa et al 2012

2.1.2 Phytoplasmas

Quarantine

Organism: *Candidatus* Phytoplasma prunorum

Synonyms: European stone fruit yellows phytoplasma

This phytoplasma is listed on the PHA Industry biosecurity plan for almonds

Disease: European stone fruit yellows and decline in almond**,** Apricot chlorotic leaf roll, Apricot witches broom, Plum leptonecrosis, Molieres disease of cherry, Peach yellows, Plum chlorotic leaf roll

Distribution: Europe, Azerbaijan and Turkey

Host range: Natural Hosts*:* apricot (*Prunus armeniaca)*, Japanese (flowering) cherry (*P. serrulata)*, black cherry *(P. mahaleb),* peach *(P. persica),* Japanese plum *(P. salicina),* European plum (*P.domestica),* cherry (myrobalan) plum (*P. cerasifera*) and almond (*P. dulcis* syn. *P. amygdalus* Batsch). Rootstocks can be infected: including *P. marianna, P. domestica*, *P. cerasifera, P. domestica* x *P. cerasifera* hybrid*, P. salicina* x *P. Spinosa* hybrid*, and P. persica* x *P. cerasifera* hybrid.

Natural alternative hosts include Hackberry *(Celtis australis),* Ash *(Fraxinus excelsior*), Dog rose *(Rosa canina),* Wild cherry *(Prunus avium) and* Blackthorn *(Prunus spinosa*). Non-*Prunus*

species may be symptomless. These hosts are important in the epidemiology of the phytoplasma as they act as a source of inoculum for orchards.

Ca. P. prunorum has been experimentally inoculated to several other *Prunus* sp.

Economic impact: Causes significant reduction in yield and quality of fruit and decline and death of trees. Therefore this phytoplasma has negative economic impact in *Prunus* sp. including almond.

Pathway: Propagation material, infection vectors

Diagnostic tests: Universal and specific PCR. A national diagnostic protocol for Australia has been developed but the PCR tests require validation (Constable et al 2009a). A real time PCR using the *Ca.* P. prunorum specific primers ECA1/ECA2 has been developed (Jarausch et al 2010); these primers can be used in conventional PCR for detection of this phytoplasma. A specific probe based assay has been developed for detection of this phytoplasma (Nikolic et al 2010). Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). The Christensen and Hodgetts qPCR assays have been validated by ring testing in several European laboratories (Euphesco 2011). LAMP based assay for generic detection of phytoplasmas have also been developed (Tomlinson et al 2010, Hodgetts et al 2011).

Notes: Bacteria; Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16SrX (Apple proliferation group). *Candidatus* Phytoplasma prunorum

Many strains exist and may be associated with biological differences such as host and symptom expression.

The severity of symptom expression in *Prunus* sp. is dependent on the species and the variety and phytoplasma strain.

Cacopsylla pruni (Scopoli) is the vector of *Ca. P.* prunorum. It is persistent and propagative in these insects. There is some indication of transovarial transmission but this needs to be confirmed. Blackthorn is a preferred host of the vector.

References: Berges et al 2000, Carraro et al 1998, 2001, 2004a, 2004b, Castelain et al 1997, Christensen et al 2004, Conci et al 1992, Constable et al 2009a, Deng and Hiruki 1991, Domenichini, 1967, Ermacora etal., 2009, Green et al 1999, Jarausch et al 1998, Jarausch et al 1999, Jarausch , et al 2000, Jarausch et al 2009, Kirkpatrick, 1991 , Lee et al 1993, Loi et al 2008, Lorenz et al 1995, Lorenz et al 1994, Marcone et al 1996, Marcone et al 2010, Marzachì et al 2004, McCoy, 1984, Morvan et al 1986,1991, Necas and Krska, 2006, Necas et al 2008, Nemeth, 1986. Ossiannilsson, 1992, Pignatta et al 2008, Poggi Pollini et al 2002, Schaub and Monneron, 2003, Schneider et al 1995, Seemuller and Schneider, 2004, Seemüller and Foster 1995, Seemuller et al 1998, Seemüller et al 1998c, Sertkaya et al 2005, Tedeschi et al 2002, Tedeschi et al 2006, Thebaud 2005, Thebaud et al 2009, Varga et al 2001, Yvon et al 2009

Organism: X-Disease phytoplasma

Suggested scientific name of X-disease phytoplasma is *Candidatus* Phytoplasma pruni, Synonyms:Western X (WX**)** mycoplasma-like organism (MLO), Western X phytoplasma**,** Peach –X phytoplasma, Cherry-X phytoplasma**,** Cherry buckskin MLO, Eastern peach X disease phytoplasma, Western peach-X phytoplasma, Green Valley X (GVX) phytoplasma, Napa Valley X (NVX) phytoplasma, Peach yellow leafroll 1 (WX/PYLR1) phytoplasma.

This phytoplasma is listed on the PHA Industry biosecurity plan for almonds.

Disease: Cherry-X disease, Decline of almond, Cherry blossom anomaly, Cherry albino, Cherry buckskin , Cherry Western X, Peach little peach, Peach yellows , Peach yellow leafroll, Peach rosette, Peach red suture

Distribution: North America.

There are reports of this phytoplasma in declining cherry in Italy. Related stains in subgroup IIIB of the 16SrIII group of phytoplasmas have been detected in declining sweet and sour cherry trees in Lithuania and Italy.

Host range: *Prunus* hosts: *Prunus avium* (sweet cherry), *P. cerasus* (sour cherry), *P. persica* (peach), *P. dulcis* (almond), *P. virginiana* (wild chokecherry), *P. emarginata* (bitter cherry), Japanese plum (*Prunus salicina), Prunus angustifolia, P.injucunda, P. Mexicana, P. munsoniana*, *P. glandulosa* (flowering almond), *P. armeniaca* (apricot), *P. instititia* (Damson plum), *P. domesticca* (European plum), *P. mahaleb* (Mahaleb cherry), *P. tomentosa* (Manchu cherry), *P. avium* (Mazzard cherry), *P. pumila* (sand cherry), *P. besseyi* (western sand cherry).

Alternative non-*Prunus* hosts: red maple, burclover (*Medicago polymorpha*), clovers (Trifolium sp.), and dandelion (*Taraxacum officinale*). The last three alternative host plants may also act as hosts for the vectors

Economic impact: Causes significant reduction in yield and quality of fruit and decline and death of trees. Therefore this phytoplasma has negative economic impact in *Prunus* sp. including almond.

Pathway: Infected propagation material, infectious vectors.

Diagnostic tests: Universal and specific PCR. A national diagnostic protocol for Australia has been developed but the PCR tests require validation (Constable et al 2009b). Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009).

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16Sr III (X-disease group).

Four X-disease phytoplasma strains are reported: Green Valley (GVX), Napa Valley (NVX), Peach yellow leafroll 1 (WX/PYLR1) and the Seibe strain The GVX strain is most common. GVX and NVX strains are associated with slightly different symptoms in cherry.

Transmitted by leafhoppers *Colladonus montanus, C. clitellarius*, *C.geminatus, Fieberiella florii, Graphocephala confluens, Gyponana lamina*, *Macropsis trimaculata, Norvellina seminuda, Paraphlepsius irroratus* and *Scaphytopius acutus.* It is persistent and propagative in these insects.

Risk of transmission through propagation material is considered low by USA researchers

References: Berges et al 2000, Christensen et al 2004, Constable et al 2009b, Deng and Hiruki 1991, Gilmer and Blodgett, 1976, Gilmer et al 1966, Green et al 1999, Guerra and Eastwell, 2006, IRPCM 2004, Kirkpatrick et al 1995, Landi et al 2007, Lee et al 1993, Lee et al 1994, Lukens et al 1971, McClure 1980, McCoy, 1984, Necas and Krska, 2006 , Nemeth, 1986, Paltrinieri et al 2001, Paltrinieri et al 2007, Rosenburger and Jones 1978, Schneider 1946, Schneider et al 1995, Scott and Zimmerman 2001, Seemuller et al 1998 , Suslow and Purcell 1982, Uyemoto, 1989, Valiunas et al 2009, Wolfe 1955.

Organism: *Candidatus* Phytoplasma pyri

This phytoplasma is listed on the PHA Industry biosecurity plan for almonds

Disease: Almond brown line – symptomatic trees die, almond shrivelled kernal disease, yellow canopy of almond, Peach yellow leaf roll, Decline of Cherry

Distribution: Europe, North America and Libya

Host range: *Pyrus communis, P. pyrifiolia and P. usseriensis, Cydonia oblonga, Prunus dulcis and P. persiCa.*

Economic impact: This phytoplasma has a serious economic impact on all host species due to loss of yield and quality of fruit and decline and death of trees.

Pathway: Propagation material, infectious vectors.

Diagnostic tests: Universal and specific PCR. A 16Sr X group conventional PCR test has been developed for detection of all phytoplasma in this group (Lorenz et al 1995). A specific probe based assay has been developed for detection of this phytoplasma (Nikolic et al 2010). Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). The Christensen and Hodgetts qPCR assays have been validated by ring testing in several European laboratories (Euphesco 2011).

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16Sr X (Apple proliferation group) strains of *Candidatus* Phytoplasma pyri.

Vectored by psyllids: *Cacopsylla pyri* and *C. pyricola*. *C. pyricola* vectors PYLR associated strains. The phytoplasma is persistent and propagative in its vectors.

References: Blomquist and Kirkpatrick, 2002, Carraro et al 1998a, Carraro et al 2001, Cieslinka and Morgas 2010, Del Serrone et al 1998, Guerra, 1997, Jensen et al 1964, Kison & Seemuller, 2001, Kison et al 1997, Lee et al 1995, Lorenz et al 1995, Marcone et al 1996a Mehle et al 2007, Navratil et al 2001, Paltineri et al 2001, Seemuller, 1992, Seemuller and Schneider 2004, Seemuller et al 1998a Topchiiska et al 2001, Uyemoto 1997, Uyemoto 1998, Uyemoto et al 2000.

Organism: *Candidatus* Phytoplasma phoenicium'

Disease: Almond witches' broom, Shoot proliferation diseases of Nectarine and Peach (trees decline and die), Apricot chlorotic leafroll.

Distribution: Iran and Lebanon.

Host range: *P. dulcis* (Almond), *P. persica* (peach and nectarine).

Economic impact: High economic impact due to loss of yield and decline and death of trees.

Pathway: Propagation material, infectious vectors.

Diagnostic tests: Universal PCR. A probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Christensen et al 2004).

Notes: Bacteria; Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16SrIX (Pigeon pea witches'-broom group). *Candidatus* Phytoplasma phoenicium.

A vector is unknown but there is evidence for spread. The phytoplasma was detected in the following leafhopper sp. *Fieberiella macchiae, Euscelidius mundus, Asymmetrasca decedens, Thamnottetix seclusis, Balclutha sp., Lylatina inexpectata, Allygus sp., Annoplotettix danutae,* and Empoasca *decipiens* indicating their ability to acquire the phytoplasma. Further work is required to determine their transmission ability.

Transmitted in propagation material.

References: Abou-Jawdah et al 2002, Abou-Jawdah et al 2003, Abou-Jawdah et al 2009a, 2009b, Bove et al 1999, Choueiri et al 2001, Dahkil et l 2011, Salehi et al 2005, Salehi et al 2006, Verdin et al 2003, Zirak et al 2009b.

Organism: Peanut witches' broom group phytoplasmas *(*16SrII - *Candidatus* Phytoplasma aurantifolia related strains)

Disease: Almond little leaf, Shoot proliferation of almond, yellowing of almond, Peach rosetting, peach yellowing.

Distribution: Group II Phytoplasma species and strains occur worldwide, including in Australia; however they have only been reported from Iran on summerfruit.

Host range: *P. dulcis,* (almond) *P. persica* (peach).

Economic impact: May have some economic impact in almond and peach in Iran.

Pathway: Propagation material, infectious vectors.

Diagnostic tests: Universal nested PCR. Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). A group II specific qPCR has also been developed (Hodgetts et al 2009).

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16SrII (Peanut witches' broom group) *Candidatus* Phytoplasma aurantifolia related strains. Phytoplasma strains and species in this group can have a broad host range (e.g. Tomato big bud [TBBp] in Australia is found Australia wide in many plant host species). However Australian Group II phytoplasmas have not been reported in summerfruit, including almond. It is likely the group II phytoplasmas reported elsewhere differ genetically and biologically to the Australian phytoplasmas and should be considered of quarantine significance.

Although several vectors are reported for various species and strains in some plants hosts (e.g. *Orosius orientalis* tansmits TBBp in Australia) none are reported to transmit this phytoplasma to summerfruit.

References: Bagheri et al 2009, Perez et al 2010, Zirak et al 2009a, Zirak et al 2009b

Organism: Clover proliferation group phytoplasmas (16SrVI - *Ca. P.* trifolii related strains)

Disease: Shoot proliferation in almond, peach rosetting, yellowing.

Distribution: Species and strains of 16SrVI group phytoplasmas are found in North America, Europe and Asia. However they have only been found in almonds and peaches in Iran.

Host range: *P. dulcis* and *P. persica.*

Economic impact: May have some economic impact in almond and peach in Iran.

Pathway: Propagation material.

Diagnostic tests: Universal PCR. Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma group (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009).

Notes: Bacteria; Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16SrVI (Clover proliferation group)

The leafhoppers *Macrosteles fascifrons* and *Cirulifer tenellus* transmitted *Ca. P.* trifolli strains in some hosts. A vector is not reported for peach and almond in Iran.

References: Hiruki and Wang 2004, Shaw et al 1993, Zirak et al 2009a, Zirak et al 2009b.

Organism: Stolbur (16SrXII-A) group phytoplasmas

Disease: Strains associated with yellowing or little leaf in almond, Moliere's disease of cherry in Europe and decline in Cherry in Italy, Peach leaf rolling, bronzing, tattering and shot hole in Iran, Peach reddening, yellowing decline witches' broom in Serbia, and Japanese Plum leaf rolling, rosetting yellowing and shoot proliferation.

Distribution: Europe and Asia (Lebanon, Iran, Armenia, Azerbaijan, Cyprus, Israel, Kyrgyzstan, Tajikistan, Turkey, Uzbekistan).

Host range: Broad host range. Strains of this phytoplasma are reported from *P. dulcis* (almond) in Iran and from *P. avium* (sweet cherry), *P. cerausus* (sour cherry), *P. persica* (peach) and *P. salacina* (Japanese plum) in Europe.

Economic impact: Strains of phytoplasmas in this group have a serious economic impact on their hosts.

Pathway: Propagation material, infectious vectors

Diagnostic tests: Universal and specific PCR. Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). A group XII specific qPCR has also been developed (Hodgetts et al 2009).

Notes: Bacteria; Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16SrXII-A (Stolbur group).

Vectors include planthopper species, including *Hyalesthes obsoletus, Oncopsis alni, Reptalus panzeri* and *Pentastiridius* leporinus and the leafhopper *Macrosteles quadripuntulatas.*

References: Batle et al 2008, Bressan et al 2009, Duduk et al 2008, Gattineau et al 2001, Jovic et al 2009, Marcone et al1999, Maixner 1994, Paltrinieri et al 2001, Paltrinieri et al 2008, Schneider et al 1993, Sforza et al 1998, Valiunas et al 2009a, Zirak et al 2009a, Zirak et al 2009b, Zirak et al 2009c.

Organism: *Candidatus* Phytoplasma mali

Disease: Cherry: decline, floral and phloem necrosis, and wilting. Apricot trees: stem necrosis and leaf wilting; Plum tree: blooming.

Distribution: Europe.

Host range: Primary hosts are *Malus* sp.(apple) but other plant host species can be infected including *P. armeniaca* (apricot), *P. domestica* (plum) and *P. avium* (cherry). Not known in almond.

Economic impact: Strains of this phytoplasma have a serious economic impact in their hosts.

Pathway: Propagation material, infectious vectors.

Diagnostic tests: Universal and specific PCR. A specific probe based assay has been developed for detection of this phytoplasma (Nikolic et al 2010). Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). The Christensen and Hodgetts qPCR assays have been validated by ring testing in several European laboratories (Euphesco 2011).

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; *Candidatus* Phytoplasma; 16Sr X (Apple proliferation group) strains of *Candidatus* Phytoplasma mali.

Cacopsylla picta and *C. melanoneura* are confirmed vectors and *Fieberiella florii*, is a likely vector.

References: Bliefernicht and Krczal, 1995; Cieslinka and Moras 2011, Carraro et al 2008, Krczal et al 1988, Mehle et al 2006, Mattedi et al 2008, Mayer et al 2009, Schaper and Seemuller 1982, Seemuller et al 1984, Tedeschi and Alma 2004, Tedeschi and Alma 2006

Organism: Elm yellows (16SrV) group phytoplasmas

Disease: Cherry lethal yellows (16SrV-B phytoplasma); Plum: Leafroll, proliferation and little leaf in Plum (16SrV-B phytoplasma); Peach decline. Not known in almond.

Distribution: Phytoplasmas in the 16SrV group occur in North America, Europe and Asia.

Host range: The host range for this phytoplasma group is diverse, including *P. avium* (cherry), *P. domestica* (plum) and *P. persica* (peach).

Economic impact: Strains of this phytoplasma have a serious economic impact in their hosts.

Pathway: Propagation material, infectious vectors.

Diagnostic tests: Universal PCR. Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma group (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). A group V specific qPCR test has also been developed (Hren et al 2007).

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; *Candidatus* Phytoplasma; 16Sr V (Elm yellows group)

Various leafhopper species transmit phytoplasma in the group but none are reported from summerfruit. *Hishimonoides chinesis* is a vector of *Ca.* Phytoplasma ziziphi, a phytoplasma very closely related to those detected in plum and cherry in Asia.

References: Cieslinka et al 2004, Jovic et al 2011, Jung te al 2003, Hong et al 2010, Lee et al1995, Lee et al 2004, Paltinieri et al 2004, Paltinieri et al 2006, Thakur et al 1998, Zhung et al 2003.

Organism: Aster yellows group (I-B, I-F, I-Q) phytoplasmas

Disease: Apricot chlorotic leafroll, Decline of Sweet and Sour cherry with decline in Lithuania.

Distribution: North America, Africa, Europe and Asia

Host range: Strains and species of Aster yellows group phytoplasmas infect a broad host range. AY group phytoplasma in the subgroups I-B, I-F and I-Q have been detected in *Prunus* sp. in Europe including *P. armeniaca* (apricot) *P. avium* (sweet cherry), *P. cerasus* (sour cherry), *P domestica* (plum) and *P. persica* (peach). Not known in almond.

Economic impact: Strains of this phytoplasma have a serious economic impact in their hosts.

Pathway: Propagation material.

Diagnostic tests: Universal PCR. Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma group. A group 16SrI specific qPCR test has also been developed.

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; *Candidatus* Phytoplasma; 16Sr I (Aster yellows group).

This is the largest group of phytoplasmas and strains and species infect a broad range of hosts. Leafhopper vectors are reported for various AY group phytoplasma but none were reported in summerfruit. Aster yellows group phytoplasmas occur in North America, South America, Africa, Europe and Asia.

References: Lee et al 1998, Navratil et al 2001, Paltrinieri et al 2001, Valiunas et al 2009b, Varga et al 2001. Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). (Hodgetts et al 2009).

2.1.3 Viruses

Quarantine

Organism: *American plum line pattern Ilarvirus* (APLPV)

Disease: Plum line pattern, peach line pattern.

Distribution: Canada, USA, Palestine, Albania, Tunisia, Lebanon and Italy.

Because this virus is associated with symptoms that are also caused by PNRSV and ApMV its distribution may be wider than has been reported.

Host range: APLPV is thought to occur naturally on many *Prunus* sp. It is known to occur in *P. domestica* (Plum), *P. persica* (peach) *P. salicina* (Japanese Plum) and *P. serrulata* (Japanese flowering cherry). Not formally reported on almond but pathogen testing schemes in the USA actively test for this virus in Almond. It has been experimentally transmitted to at least 85 plant species.

Economic impact: It is not considered a serious pathogen on its own but it may have greater impact when found in combination with other viruses.

Pathway: Planting material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR

Notes: APLPV is an *Ilarvirus* in the family *Bromoviridae*.

APLPV causes similar symptoms to strains of *Prunus necrotic ringspot virus* (PNRSV) or *Apple mosaic virus* (ApMV) in plum. It is possible that previous reports of viruses associated with diseases in *Prunus* sp. for which PNRSV and ApMV could not be detected might be associated with APLPV

No vector is known. Transmitted in propagation material.

A dot blot hybridisation approach has also been developed using a polyprobe for APLPV in combination with PNRSV, PDV, ApMV, PPV and ACLSV. This approach may be useful for screening in PEQ or certification programs.

References: Alayasa et al 2003, Al Rwahnih et al 2004, Fulton 1984, Herranz et al 2008, Herranz et al 2005, Kirkpatrick and Fulton 1976, Sanchez-Navarro et al 2005, Scott and Zimmerman 2001.

Organism: *Apricot latent virus foveavirus* (ApLV)

(syn. = Peach sooty ringspot virus)

Disease: Chlorosis and leaf deformation in apricot cultivars Tirynthos and Haward, sooty ringspot in experimentally inoculated peach.

Distribution: France, Turkey, Palestine, Lebanon, Egypt, Italy and Spain.

Host range: Natural host is *P. armeniaca* (apricot). Experimentally transmitted to *Prunus persica* (peach), *P. domestica* European (plum), *P. salicina* (Japanese plum), *P. avium* (sweet cherry) and *P. cerasifera.*

Economic impact: Little information is available on the economic impact of this virus. As it is latent in many apricot cultivars ApLV may not be a significant pest.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- PCR. This virus can be detected by specific conventional RT-PCR (Nemchinov and Hadidi, 1998, Ghanem-Sabanadzovic et al 2005, Garcia-Ibarra et al 2010). A polyprobe has also been designed for simultaneous detection of ApLV, seven additional viruses and two viroids of *Prunus* species (Peiro et al 2012).

Notes: ApLV is a member of the genus *Foveavirus* in the family *Betaflexiviridae*.

It is known to naturally infect apricot and is symptomless in many cultivars except cvs Tirynthos and Haward.

Experimental transmission indicated that cherry and plums were symptomless but the virus was associated with asteroid or sooty ringspot symptoms on the leaves and therefore could be a causal agent of Peach sooty ringspot and peach asteroid spot diseases. The peach sooty ringspot strain of ApLV causes sooty ringpsot symptoms on inoculated GF305 indicators

Reference: Ghanem-Sabanadzovic et al 2005, Barone et al 2008, El-Maghraby et al 2007, Garcia-Ibarra et al 2010, Gentit et al 2001, Grimova et al 2010, Gümüs et al 2004, Jarrar et al 2006, Jarrar et al 2007, Nemchinov and Hadidi, 1998, Nemchinov et al 2000, Zemtchik and Verdevskaya, 1993, Zemtchik et al 1998.

Organism: *Apricot latent ringspot nepovirus* (ALRSV)

Disease: Unclear.

Distribution: France: limited distribution.

Host range: *P. armeniaca* (apricot).

Economic impact: The economic impact of this virus is unknown, although it is likely to cause a significant reduction in yield in susceptible varieties.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: ALRSV is a member of subgroup C of the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* It is only reported to naturally infect apricot trees, which appeared bare due to reduced foliage and had reduced yield. The rootstock scion combination affected symptom expression in some varieties. Cultivar differences were observed. The virus was also experimentally transmitted to peach, cherry and plum, which also showed a range of foliar symptoms and stunting.

ALRSV is transmitted in propagation material and plants. No vector is reported.

Reference: Anon 2011, Gentit et al 2001.

Organism: *Apricot vein clearing associated virus* (AVCaV)

Disease: Vein clearing in apricot cv. Jameloppis

Distribution: Europe

Host range: *P. domestica (*plum), *Prunus armeniaca* (apricot)

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- PCR

Notes: Member of the genus in the family *Betflexiviridae.* No symptoms reported on plums or other infected apricot cultivars.

References: Elbeaino et al 2014, Abou-Kabaa et al 2014

Organism: *Arabis mosaic nepovirus* (ArMV)

Disease: European rasp leaf of cherry.

Distribution: Europe, North America, Asia, South Africa, Chile, New Zealand and Australia (Victoria and Tasmania). Australian reports are from hops and likely to have been eradicated.

Host range: Broad host range. In Europe it is reported on *P. persica* (peach), *P. armeniaca* (apricot) and *P. avium* (cherry).

Economic impact: Unknown, however ArMV has significant economic impact in other crops such as grapevine.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR: Probe based real time PCR assays have been developed (Bertolini et al 2010, Wei et al 2012). A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: ArMV is a member of the genus subgroup A in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.*

In cherry ArMV causes "European rasp leaf" in combination with *Prunus* necrotic ringspot or *Prune dwarf virus*es. It has also been associated with decline in cherry. The association with disease in peach and apricot is unknown.

It is transmitted by *Xiphinema divericaudatum*. It is transmitted in propagation material and plants. Seed transmission is reported in other hosts.

Reference: Cropely 1964, Cropely 1961, Digiaro et al 2007, Gambino and Gibraudo et al 2006, Jenser et al 1984, Maligoka et al 2004, Munro 1987, Vuittenez and Kuszala 1971, Wei and Clover 2008, Wetzel et al 2002. Anon 2011, Bertolini et al 2010, Wei et al 2012

Organism: *Asian Prunus virus 1 Foveavirus (*APruV-1), *Asian Prunus virus 2 Foveavirus (*APruV-2) and *Asian Prunus virus 3 Foveavirus* (APruV -3)

Disease: Unknown.

Distribution: USA and Europe.

Host range: *Prunus* sp. of Asian origin: *Prunus mume* and *Prunus persica* (Ta Tao 24).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing

- ELISA
- PCR. Degenerate primers have been designed to amplify the coat protein gene of these viruses (Marais et al 2006). Degenerate primers that amplify member of the genera (*Trichovirus*, *Capillovirus* and *Foveavirus* may detect strains of these viruses but are not reliable for routine detection. A generic RT-PCR test might be used to detect these viruses but needs development and validation (Dovas and Katis 2003)

Notes: APruV-1, -2 and -3 are members of the genus *Foveavirus* in the family *Betaflexiviridae*.

APruV 1 was detected in both *Prunus mume* and *Prunus persica* (Ta Tao 24). APruV 2 was detected in *P. mume*, and APruV 3 was detected in *P. persiCa.*

Little is known about the association between these viruses and disease. However, it is possible that APruV-3 is associated with blossom delay in peach cultivars. This effect is used to reduce the risk of damage and reduced yield associated with frost, which will improve production in environments where flowering trees are at risk of frost damage.

They can be transmitted in propagation material. No vector is reported.

These viruses have the potential to cross react with some PPV antisera.

Reference: Foissac et al 2005, Gibson et al 2001, Gibson et al 2008, Hari et al 1995, James et al 1994, James et al 1996, Marais et al 2006, Marini et al 2009. Dovas and Katis 2003.

Organism: *Cherry leaf roll Nepovirus* (CLRV)

Disease: Leafroll disease in cherry

Distribution: Widespread in Europe and North America. It also occurs in Asia in Turkey, China and Japan. It occurs with limited distribution in Peru, Chile and infects *Rubus idaeus* in New Zealand but is not reported on summerfruit in these countries. It has been reported to occur in Australia but specific details of host and location are unknown.

Host range: CLRV has a wide host range. In *Prunus* it is known to infect *P. myrobalan* (myrobalan plum), *P. avium* (sweet cherry), *P. serotina* (black cherry), *P. persica* (peach) and *P. armeniaca* (apricot).

Economic impact: CLRV is of economic importance in cherry as it causes reductions in yield and decline of trees.

Pathway: Seed, pollen, propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR. A National Diagnostic Manual for Australia has been prepared (Rodoni and Thomas 2011, SPHDS) but the methods require validation in Australia. A specific probe based real time RT-PCR has been developed in NZ (Anon 2012, Tang unpublished) for detection of this virus in grapevine in quarantine. Specific primers

were developed for detection of CLRV in the North America (Osman et al 2012). A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: CLRV is a member of subgroup C in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* There are many strains, which have apparently diversified according to plant host species. Strain variation could impact on detection by ELISA and PCR.

In *Prunus* spp it is transmitted in propagation material, seed and pollen.

References: Anon 2011, Anon 2012, Cropley, 1961, Crosslin et al 2010, Eastwell and Howell 2010, Herrera and Madariaga, 2001, Jones, 1985 , Keglar 1972, Kurcman 1977, Kumari 2009, Murant, 1983, Osman et al 2012, Posnette and Cropley 1955, Rodoni and Thomas 2011, Schimanski et al 1975, Schimanski et al 1976, Sipahioglu et al 2011, Rebenstorf et al 2006, Walker 2004, Werner et al 1997.

Organism: *Cherry mottle leaf trichovirus* (CMLV)

This virus is listed on the PHA Industry biosecurity plan for almonds, although it is not formally reported on almond in the literature.

Disease: Mottle leaf of cherry, Peach wart disease on peach fruit.

Distribution: USA, Europe, China and South Afri*Ca.*

Host range: *P. avium* (sweet cherry)*, P. cerasus (*sour cherry), some cherry hybrids, *P. persica* (peach), *P. emarginata* (bitter cherry)*, P. armeniaca (apricot), P. serrulata* (Japanese flowering cherry) and *P. yedoensis.*

CMLV has been experimentally transmitted to *P. dulcis* (almond) but natural infections are not known to occur (D. James Pers. comm.).

Economic impact: The virus is of economic importance in cherry as it reduces quality and yield.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA - not commercially available
- PCR. Conventional RT-PCR tests are available (James and Upton 2001, Rott and Jelkmann 2001). Generic RT-PCR tests for detection of Foveaviruses and Tricohviruses may detect PcMV (Dovas and Katis 2003, Foissac et al 2005).

Notes: CMLV is a *Trichovirus* in the family *Betaflexiviridae*. It causes mottle leaf symptoms in sweet cherry. It is symptomless on sour cherry, some cherry hybrids, peach, bitter cherry, apricot, flowering cherry and *P. yedoensis.* It is associated with peach wart disease of peach fruit

It is transmitted in propagation material and by the bud mite, *Eriophyes inaequalis.*

The listing of CMLV in the PHA biosecurity plan for almonds may be due to confusion with reports regarding the occurrence of the closely related *Peach mosaic trichovirus* which is reported in almond and may cause significant disease.

References: James et al 2000, James and Upton 1999, James et al 1999, James and Mukerji 1993, James and Mukerji 1996, Nemeth 1986 , Rott and Jelkmann 2001, Mekuria et al 2013, Ma et al 2014.

Organism: *Cherry rasp leaf cheravirus* (CRLV)

Disease: Rasp leaf of cherry.

Distribution: North America and China. There are unconfirmed reports from South Africa and New Zealand. These reports may be associated with a description of disease rather than detection of a specific virus. Rasp leaf symptoms in cherry may be caused by other viruses such as *Strawberry latent ringspot virus*.

Host range: *P. avium (*sweet cherry) *P. cerasus* (sour cherry), *P. mahaleb* (rootstock) and *P. persica* (peach). It has been detected in other plant species in infected orchards. It also causes flat apple disease and is known to infect potato.

Economic impact: In sweet and sour cherries and peach it causes loss in yield and quality of fruit and stunting and decline of trees. Therefore it may have significant economic impact.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR. A specific conventional RT-PCR has been developed (James and Upton 2005). A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: CRLV is a member of the family *Secoviridae*, subfamily *Comovirinae*, genus *Cheravirus*.

CRLV is named for the enations it causes on the underside of cherry leaves.

CRLV is seed and pollen borne in some herbaceous hosts. It has been detected in cherry pollen but transmission was not confirmed. Seed collected from the infected branches of cherry trees did not grow. It is also transmitted by nematodes in the complex *Xiphinemea Americanum, sensu lato*.

References: Anon 2011, Bobine et al 1942, Fry and wood 1973, Hansen et al 1974, James et al 2001, James and Upton 2002, James and Upton 2005, Ma et al 2014b, Nyland 1974, Nyland et al 1969, Thomson et al 2004, Wagnon et al 1968, Wood and Fry 1972

Organism: *Cherry rosette nepovirus* (ChRV)

Disease: Rosetting disease of cherry.

Distribution: Switzerland.

Host range: *P. avium* (sweet cherry).

Economic impact: The economic impact of the virus is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011)

Notes: ChRV is a tentative member of the genus *Nepovirus.*

ChRV is reported from Switzerland Europe and associated with a rosetting disease of cherry. The disease in the USA could be a mix of RRSV + CLRV.

ChRV is transmitted by *Longidorus arthensis.* It is likely to be transmitted in propagation material and plants.

Reference: Brown et al 1994, Brown et al 1998, Kunz and Bertschinger 1998, Kunz 1998.

Organism: Cherry rusty mottle asssociated virus

Disease: Rusty mottle disease.

Distribution: North America.

Host range: *Prunus avium* (sweet cherry), *Prunus* lusitanica (Portugese laurel)

Economic impact: This virus may have a negative economic impact in sweet cherry

Pathway: Propagation material.

Diagnostic tests:

- Woody indexing.
- PCR. A specific conventional RT-PCR has been developed (Villamor and Eastwell 2013).

Notes:

References: Villamor and Eastwell 2013, Villamor et al 2015. Villamor et al 2014, Villamor et al 2013.

Organism: Cherry twisted leaf associated virus

(= Apricot ring pox virus).

Disease: Cherry twisted leaf and Apricot ring pox diseases

Distribution: Cherry twisted leaf disease is reported in North America, Denmark and Romania, although its distribution is limited. Apricot ring pox has been reported in North America, Europe (Italy) and there is one report of this disease in NSW, Australia.

Host range: Natural hosts are *P. avium* (Sweet cherry) *and P. armeniaca* (apricot). The associated virus has been experimentally transmitted to *P. mahaleb (*rootstock), *P. serotina* (black cherry), *P. cerasus* (sour cherry), *P. persica* (peach), *P. dulcis* (almond) and *P. salacina* (Japanese plum) and *P.* besseyi.

Economic impact: It is not considered to be of economic importance due to its limited distribution. However it may have an impact on quality and yield in sensitive cherry and apricot varieties.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- PCR.

Notes: CTLaV is a distinct virus forming part of a proposed new genus (Robigovirus) in the family *Betaflexiviridae*. Variation in sweet cherry and apricot cultivar susceptibility is observed and some maybe symptomless. The associated virus has been experimentally transmitted to mahaleb, black and sour cherry, apricot, peach, almond and Japanese plum, which were symptomless hosts. Previous work has suggested that the agent(s) associated with Apricot ring pox and Cherry twisted leaf diseases in the USA naturally infect western chokecherry and hybrid plums (*P. salicina* x *P. simomi*). The virus is transmitted in propagation material and plants. It may be transmitted by root grafting between trees. The distribution patterns within an orchard are suggestive of a vector, although none is known.

References: Fidlund 1964, Foissac et al 2005, Hansen 1976, Hansen and Cheney 1976, James 2011, James et al 1995, Keane and May 1963, Liberti et al 2003, Nemeth 1986, Zhang et al 1992, Villamor and Eastwell 2013, Villamor et al 2014, Villamor et al 2013, Villamor et al 2015.

Organism: *Little cherry Velarivirus 1* (LCHV1)

Disease: Little cherry disease

Distribution: Europe and Asia and with limited distribution in North America. The disease is reported in New Zealand and there is an unconfirmed report of the disease occurring in Australia. The occurrence of associated viruses such as LCHV1 in Australia and New Zealand is unknown.

Host range: Naturally infects *P. serrulata* (flowering cherry), *P. avium* (sweet cherry), *P. cerasus* (sour cherry), *P. dulcis* (almond), *P. persica* (peach), and *P. domestica* (plum). The disease was transmitted from symptomless *Prunus emarginata* var. mollis to cherry but the associated virus is unknown.

Economic impact: The disease may have significant economic impact in sweet and sour cherry.

Pathway: Propagation material.

Diagnostic tests:

- Woody indexing
- PCR. A generic *Closteroviridae* RT-PCR has been developed for detection of viruses in the family *Closteroviridae* and can be used to detect this virus (Dovas and Katis 2003). Specific conventional tests are available (Rott and Jelkmann 2001, Osman et al 2012, Matic et al 2010),

Notes: LCHV1 is a member of the genus *Velarivirus*, family *Closteroviridae*. A virus described as LChV3 may be a variant of this species. LCHV1 is one of two viruses associated with little cherry disease. The other virus is *Little cherry virus 2*. The disease is associated with a reduction in yield and quality of fruit in sweet and sour cherry and may be associated with stunting in some flowering cherry varieties. A divergent strain of LChV1 is associated with shirofugen stunt disease. It is not associated with symptoms in almond, peach and plum. Symptomless hosts could represent a source of infection for susceptible cherry varieties.

Transmitted in propagation material. There is no known vector of LCHV1.

References: Candresse et al 2013, EPPO 1992, Jelkmann 2010, Martelli et al 2012, Matic 2007, Matic 2010, Matic et al 2009 a, Matic et al 2009b, Rott and Jelkmann 2001, Rott and Jelkmann 2005, Theilmann et al 2001, Vitushkina et al 1997, Wilks and Welsh 1961, Wilkes and Reeves 1960, Wood and Fry 1970.

Organism: *Myrobalan latent ringspot nepovirus* (MLRSV)

Disease: Peach rosetting. Leaf enation in cherry.

Distribution: France.

Host range: *Prunus cerasifera* (myrobalan plum), *Prunus persica* (peach), *P. avium* (sweet cherry).

Economic impact: MLRSV is not considered to be economically important.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: MLRSV is a member of subgroup C in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* MLRSV is latent in myrobalan (*P.s cerasifera*). It causes short

internodes and rosetting in peach (*P. persica*) and enations on the leaves of sweet cherry (*P. avium*).

The mode of transmission is unknown but a nematode vector is suspected.

References: Anon 2011, Dunez and Delbos 1976, Dunez et al 1971, Diekmann and Putter 1996, Gallitelli et al 1981, Polak 2008.

Organism: *Peach enation nepovirus* (PEV)

Disease: Enation on peach leaves.

Distribution: Japan.

Host range: *P. persica* (Peach).

Economic impact: The economic importance of this virus is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau.

Notes: PEV is a member of the genus *Nepovirus* in the family *Comoviridae*, subfamily *Comovirinae.* Spread is observed but a vector is unknown. A nematode vector is presumed.

References: Anon 2011, Kishi et al 1973.

Organism: *Peach rosette mosaic nepovirus* (PRMV)

Disease: Rosetting, stunting, chlorosis, mottling.

Distribution: PRMV infects peach in Turkey, Egypt, Canada and the USA. It is reported on almond from Turkey.

Host range: Peach, almond *Vitis labrusca, V. vinifera*, French-American *Vitis* sp. Hybrids, highbush blueberry (*Vaccinium corymbosum) Rumex crispus, Solanum carolinense* and *Taraxacum officinale*

Economic impact: PRMV can cause serious economic impact in peach and grapevines. The full economic impact of this virus in almonds is not known. Introduction in any host may pose a risk to all three horticultural crops, especially in areas where vectors occur. The ability of other Australian *Xiphinema* and *Longidorus* species to transmit this virus is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA

- PCR. Specific primers have been developed in New Zealand for detection of grapevine in quarantine. A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau.

Notes: PRMV is member of subgroup C in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.*

Almond trees infected with PRMV showed one or a combination of the following symptoms: chlorotic spots, mosaic and narrowing of the leaves. Leaves on affected peach trees may be delayed or develop slowly. Symptoms on peach leaves include chlorotic mottling, wavy leaf margins and severe distortion of leaves. Shoots on affected peach trees have severe shortening of the internodes resulting into rosette type of growth. Infected peach trees can die. PRMV is also a serious pathogen of grapevines in which it causes degeneration and decline.

PRMV is transmitted in planting material. PRMV is transmitted by two nematode species: *Xiphinema Americanum* and *Longidorus diadecturus.* Although not reported, transmission is likely to be in a semi-persistent manner. *X. Americanum is* reported in Australia in Victoria and South Australia although the extent of its distribution is not known. The occurrence of *L. diadecturus* is not reported. Several other *Xiphinema* and *Longidorus* species have been shown to transmit the virus experimentally. PRMV is seed transmitted in *V. labrusca* cv. Concord. It is not known to be transmitted by seed in summerfruit or almonds.

References: Anon 2011, Anon 2012; Allen et al 1984, Allen, et al 1999, Azery and Cycek 1997, Brown et al 1994, Brown et al 1995, Digiaro et al 2007, Kheder et al 2004, Nicol et al 1999 , Quader et al 2003, Ramsdell and Meyers 1974, Ramsdell and Gillett 1981, Sanafaçon et al 2009.

Organism: *Peach chlorotic mottle foveavirus* (PCMV)

Disease: Chlorotic mottle on GF305.

Distribution: USA.

Host range: *P. persica* (peach).

Economic impact: The economic impact of this virus is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- PCR - Generic *Foveavirus* PCR tests may detect this virus (Dovas and Katis 2003, Foissac et al 2005).

Notes: PCMV is a member if the genus *Foveavirus* in the family *Betaflexiviridae.*

It occurs in the USA on a peach cultivar originally imported from Mexico. PCMV causes chlorotic mottle symptoms in the indicator GF305. It can cross react with PPV antisera.

It is likely to be transmitted in propagation material. No vector is reported.

Reference: Foisssac et al 2005, James et al 2007, James et al 1996, James et al 1994.

Organism: *Peach mosaic trichovirus* (PcMV)

Disease: Peach mosaic disease.

Distribution: North America, Europe (Greece, Italy) and Asia (India).

Host range: *P. persica.* The disease also affects *P. dulcis* (almond), *P. armeniaca* (apricot), nectarine and *P. domestica* (plum). Further elucidation of the host range for PcMV is required.

Economic impact: PcMV has a negative economic impact in susceptible peach varieties. Some varieties of almond are symptomless hosts of the disease.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- ELISA - but not commercially available.
- PCR. Generic RT-PCR tests for detection of Foveaviruses and Tricohviruses may detect PcMV (Dovas and Katis 2003, Foissac et al 2005). A specific conventional RT-PCR test is also available (James et al 2006).

Notes: PcMV is a member of the genus *Trichovirus* in the family *Betaflexiviridae*

PcMV is associated with peach mosaic disease, which can also affect almond, apricot, nectarine and plum. A graft transmissible agent from almond can cause mosaic symptoms in peach, indicating that almond is a likely host of the virus.

PcMV is transmitted by the eriophyid mite *Eriophyes insidiosus* in peach. The virus is transmitted in propagation material.

References: Bodine and Durell 1941, Cochran and Hutchins 1938, , Gispert et al 1998a, Gispert et al 1998b, James and Howell 1998, James and Upton 1999, James et al 2006, Larsen et al 1998, Oldfield et al 1994, Pine 1965.

Organism: *Plum pox potyvirus* (PPV)

This virus is listed on the PHA Industry biosecurity plan for almonds.

Disease Sharka, Plum pox.

Distribution: Europe, Turkey, Syria, Egypt, India, the United Kingdom, Africa, the former USSR and parts of the United States, Canada and South America.

Host range: Primary hosts of PPV include apricots (*P. armeniaca*), peaches (*P. persica*), nectarines (*P. persica var. nucipersica*), plums (*P. domestica* and *P. salicina*) and sweet (*P.*

avium) and sour cherry (*P. cerasus*). Almond (*P. dulcis*) can be infected and express mild symptoms. Other wild and ornamental *Prunus* species are also hosts of PPV. Cultivated or weedy annual plant species have been reported as hosts for PPV but they are unlikely to act as a reservoir of the virus.

Economic impact: In susceptible commercial *Prunus* species and cultivars fruit quality and yield are severely affected. PPV is considered to be one of the most economically important pathogens of summerfruit worldwide.

Pathway: Propagation material**.** Viruliferous aphids.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA: The latest draft ISPM protocol for PPV detection recommends the DASI-ELISA kit based on the 5B-IVIA monoclonal antibody which is available from AC Diagnostics, Inc. (Fayetteville, USA), Agritest (Valenzano, Italy), AMR Lab (Barcelona, Spain) and Real/Durviz (Valencia, Spain) as the most reliable ELISA method for PPV detection as it detects all known strains.
- PCR: The ISPM protocol (Cambra et al 2012) recommends several molecular tests for PPV detection and conventional and real-time assays are available for the universal detection of PPV strains. Some assays can also differentiate between strains. Several molecular tests have also been identified in the National Diagnostic Protocol for Australia. A LAMP assay has also been designed and may be useful for detection in the field during an incursion (Hadersdorfer et al 2011, Hadersdorfer et al 2012). A polyprobe has also been designed for simultaneous detection of PPV, seven additional viruses and two viroids of *Prunus* species (Peiro et al 2012). Melt Peak analysis may also be useful for detection of this virus by RT-PCR without the need for traditional gel based technology (Winder et al 2011)

Notes: PPV is a member of the genus *Potyvirus* in the family *Potyviridae*. There are nine strains of PPV: An (Albania), D (Dideron), M (Marcus), C (Cherry), CR (Cherry Russian), EA (El Amar), W (Winona), Rec (Recombinant) and T (Turkish).

PPV causes plum pox disease in several *Prunus* sp. The disease is also called Sharka. Symptoms appear on leaves, fruits, flowers, and seeds. Symptom expression varies depending on the PPV strain, host species and cultivar. Other wild or ornamental *Prunus* sp. such as *P. cerasifera, P. insititia, P. spinosa, P. salicina* may be symptomless.

It is transmitted in a non-persistent manner by aphids. There are more than 20 aphid species that can transmit PPV. Some of the most important vectors are *Aphis spiraecola, Brachycaudus cardui, B. helichrysi, Myzus persicae* and *Phorodon humuli.* The virus is transmitted in propagation material.

References: Cambra et al 2012 (ISPM 27;2012), Candresse and Cambra, 2006, Damsteegt et al 2007, Dosba et al 1987, EPPO 2004, Festic,1978, Garcia et al 2014, James and Glasa, 2006; Kunze and Krczal, 1971, Leclant, 1973, Levy et al 2000, Minoiu 1975, Olmos et al 2002, Olmos et al 2005, Schneider et al 2004, Ulubaş Serçe et al 2009, Van Oosten 1970, Varga and James 2006, Wetzel et al 1991, Wetzel et al 1992, Hadersdorfer et al 2011, Hadersdorfer et al 2012, Peiro et al 2012, Winder et al 2011.

Organism: *Prunus* Tepovirus T (PrVT)

Disease: Unknown as has PrVT has only been reported in mixed infections with other viruses.

Distribution: Europe.

Host range: *P. domestica (*plum), *P. avium* (sweet cherry), *P. cerasifera* (cherry plum and myrobalan plum).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- PCR.

Notes: Member of the genus in the family *Betflexiviridae.* Reported with low prevalence (1%) in Italy and Azberjain and may be a minor pest.

References: Marais et al 2015.

Organism: *Raspberry ringspot nepovirus* (RpRSV)

This virus is listed on the PHA Industry biosecurity plan for almonds.

Disease: Chlorosis, mosaic, decline death in *Prunus* sp.

Distribution: Europe and Asia: (Kazakhstan, Turkey).

Host range: It is known to infect *P.avium* (sweet cherry*)* and *P. domestica (*Plum*)* and has been reported in almond in Turkey. RRSV also infects many other plant species, including grapevine, currant, raspberry and strawberry.

Economic impact: The economic impact of this virus in almonds is unknown however it can cause yield loss in cherry due to decline and dieback. Plum trees infected by RpRSV also showed stunting and decline and necrosis was observed at the graft union.

Pathways: Propagation material. Seed transmission in plum.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR. Specific primers have been developed in New Zealand (Tang unpublished) for detection of this virus in grapevine in quarantine. A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau. An RT-LAMP assay has also been designed for detection of this virus and may be useful for laboratory and field based applications.

Notes: RpRSV is a member of subgroup A in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* Serologically two strains can be differentiated: Scottish strain RRSV-S

and English strain RRSV-E. Strain variation can impact on detection by ELISA and PCR detection.

Almond trees infected with RRSV showed one or a combination of the following symptoms: chlorotic spots, mosaic and narrowing of the leaves.

RpRSV is transmitted in propagation material and plants. There is evidence for seed transmission in plum. RpRSV is seed transmitted in other host species. Nematode vectors include *Longidorus macrosoma, L. elongata* and *L. arthensis*. There are also reports of transmission by *Xiphinema sp.* and *Paralongidorus maximus*

References: Anon 2011, Anon 2012; Azery and Cycek 1997, EPPO 1995, Hubschen et al 2004, Jones et al 1984, Ochoa-Corona 2006, Trudgill et al 1983, Wei et al 2008, Zawadzka 1985, Morimoto et al 2011.

Organism: *Stocky prune cheravirus* (StPV)

Disease: Stunting.

Distribution: France: limited distribution.

Host range: *P. domestica* (plum and prune cultivars). Experimentally inoculated to other *Prunus* sp.

Economic impact: It is not considered to be of economic importance due to its limited distribution. It can cause yield loss in infected trees.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: StPV. is a member of genus *Cheravirus* in the family *Secoviridae.*

StPV causes stunting in prune (plum). In transmission experiments it caused rosetting on peach.

There is evidence for field transmission and a nematode vector is suspected.

Reference: Candresse et al 1998, Candresse et al 2006, Desvinges 1990, LeGall et al 2007, Sanafacon et al 2009.

Organism: *Strawberry latent ringspot virus* (SLRSV)

Disease: Peach rosetting, decline, poor growth and graft union failure in combination with other viruses on peach, bare twig and unfruitfulness in apricot.

Distribution: Europe, North America, Israel, New Zealand and Turkey. There is one report of the virus occurring in Australia – no details were available about the host species.

Host range: *Broad host range.* It has been detected in flowering *P. avium* (sweet cherry), *P. cerasus* (sour cherry), *P. persica* (peach), *P. armeniaca* (apricot), *P. domestica* (plum) and *P. lusitaniCa. P. dulcis* (almond) and *P. laurocerasus* (cherry laurel) are also reported.

Economic impact: This virus may be associated with reduced yield and quality in peach and apricot and therefore may have an economic impact to industry.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- ELISA.
- PCR. Conventional assays exist (Postman et al 2004, Faggioli et al 2005, Martin et al 2004, Tzanetakis et la 2006) and could be converted to a Sybr based realt time RT-PCR assay.

Notes: SLRSV is a tentative member of the genus *Sadwavirus* in the family *Secoviridae*.

SLRSV is associated with rosetting disease in peach, bare twig and unfruitfulness in apricot, poor growth and graft union failure in combination with other viruses on peach. Associated with decline of peach in combination with PDV.

Experimentally inoculated to *P. dulcis* (almond) but these appeared tolerant. There is little other information to support the report in almond therefore SLRSV is likely to be a minor pest.

It is transmitted by the nematode vector *Xiphinema diversicaudatum*. It is transmitted in propagation material and plants.

Reference: Belli et al 1980, Blattny and Janeckova 1980, Brown 1985, Faggioli et la 2005, Elbeaino et al 2007, Everett et al 1994, Fry and wood 1973, Huguet et al 1977, Lamberti et al 1986, Lamberti et al 1993, Lister 1964, Nemeth 1980, Polak et al 2004, Postman et al 2004, Richter and Kegler 1967, Saric and Velagic 1980, Scotto la Massese et al 1973, Sweet 1980.

Organism: *Tobacco ringspot nepovirus* (TRSV)

Disease: Associated with Eola rasp leaf of cherry, stem pitting of peach

Distribution: Europe, North America, Central America (Cuba), South America (Brazil, Uruguay), Africa, Australia, Papua New Guinesa and New Zealand.

Host range: Broad host range. *P. avium* (sweet cherry), *P. serrulata, P. incisa* and *P. serrula* (ornamental cherries) and *P.persica* only in the USA.

Economic impact: It is not considered to have an economic impact in summerfruit species.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- ELISA.
- PCR. A Probe based real time RT-PCR assays has been developed (Yang et la 2007). A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: TRSV is a member of the genus *Nepovirus* subgroup A in the family *Secoviridae*, subfamily *Comovirinae*.

Reports of an association with disease in *Prunus* species are mixed and TRSV may be symptomless in some sweet cherry varieties. Transmitted by *Xiphinema Americanum sensu lato* in some hosts; seed in some hosts; in propagation and planting material.

Reference: Anon 2011, Digiaro et a 2007, Fuchs et al 2010, Liu and Allen 1965, MacNish 1963, Martin et al 2009, Randles and Franco 1965, Reynolds and Teakle,1976, Shiller et al 2010, Stace-Smith 1985, Stace-Smith and Hansen 1974, Uyemoto et al 1977, Watson 1949, Wilkinson 1952, Yang et al 2007.

Organism: *Tomato black ring nepovirus (TBRV)*

This virus is listed on the PHA Industry biosecurity plan for almonds.

Disease: Yellow peach bud and peach shoot stunting.

Distribution: Europe, Japan, India and Chile.

Host range: TBRV has many horticultural, wild and weed hosts. *Prunus* sp. include *P. dulcis* (almond), *P. avium* (sweet cherry) and *P. persica* (peach).

Economic impact: TBRV is considered an economically important pathogen of peach. Its economic impact on almond is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- ELISA - Multiple strains mean that more than one test may be required.
- PCR. A specific probe based real time RT-PCR has been developed in New Zealand for detection of this virus. A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau.
-

Notes: TBRV is a member of subgroup B in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* There are many TBRV strains.

In Peach TBRV has been associated with enations, yellow peach bud and peach shoot stunting and it was associated with yield loss. In almond it has been associated with leaf enations, but its impact on quality and yield of fruit is unknown. It has also been isolated from sweet cherry with ringspot symptoms, although its association with these symptoms was inconsistent.

TBRV is transmitted by nematode vectors including: *Longidorus attenuatus* and *L. elongatus* (infrequently). Transmissibility may be affected by virus strain. TBRV is seed transmitted in some plant host species (e.g. lettuce) but its seed transmission in *Prunus* sp. is unknown. It is transmitted in propagation material and plants.

References: Anon 2011, Bercks and Mishcke 1964, Digiaro et al 2007, Jacob 1974, Harrison and Murant 1977, Harper et al 2010, Harper et al 2011, Le Gall et al 1995, Martelli and Savino 1997, Mischke and Bercks 1963, Mischke and Bercks 1965, Wei and Clover 2008

Organism: *Tomato ringspot nepovirus (ToRSV)*

This virus is listed on the PHA Industry biosecurity plan for almonds.

Disease: Yellow bud mosaic of almond and peach stem pitting and decline in peach, cherry and apricot, brown line disease in plum.

Distribution: USA. It also occurs with limited distribution in other hosts in Europe, Asia, South and Central America, Australia and New Zealand. ToRSV is not reported on *Prunus* sp. in the EPPO regions in Europe, Central America, Australia and New Zealand.

Host range: ToRSV has a broad host range. ToRSV infects various *Prunus* sp., including *P. dulcis* (almond) in North America. ToRSV is associated with diseases of almond in Turkey.

Economic impact: This virus is economically important in summerfruit species including peach and almond as it reduces production in peach and seriously affects quality and yield in almond**.**

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA - Multiple strains mean that more than one test may be required
- PCR - Some evidence to suggest that current D1/U1 primers recommended for use in many countries may not detect all isolates. A specific probe based real time RT-PCR has been developed in New Zealand (Tang unpublished) for detection of this virus in grapevine in quarantine. Specific primers were developed for detection of ToRSV in the North America. A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau.

Notes: ToRSV is a member of subgroup C in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae*. There are several ToRSV strains. Several species/strains within the nematode complex *Xiphinema Americanum sensu lato* are vectors.

Strain variation can affect detection by ELISA and the RT-PCR.

References: Auger et al 2009, Azery and Cycek 1997, EPPO 2005, Griesbach 1995, Kommineni and Ramsdell 1997, Mircetich and Moller 1977, Li et al 2011, Moini et al 2009,, Anon 2012, Li et al 2011, Osman et al 2012, Anon, 2011.

***Tomato bushy stunt tombusvirus* (TBSV) and *Petunia asteroid mosaic tombusvirus* (PeAMV).**

Disease: Twig necrosis in sweet and sour cherry and plum.

Distribution: Europe, North America Argentina, Morocco and Tunisia.

Host range: Various plants species including *P. persica* (peach) and *P. domestica* (plum) PeAMV and TBSV have not been reported in almond.

Economic impact: Has a negative economic impact due to reduction in yield and quality of fruit.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- ELISA.
- PCR.

Notes: TBSV and PeAMV are members of the genus *Tombusvirus* in the family *Tombusviridae*. A *Tombusvirus* reported from sour and sweet Cherry in the USA and Europe and Plum in Europe where it was associated twig necrosis and fruit pitting. Although initial studies suggested TBSV was associated with this disease in cherry and plum subsequent studies showed that the virus was PeAMV. The occurrence of TBSV in cherries cannot be dismissed; however it is likely this virus occurs with lower frequency than PeAMV.

Tombusvirus species are transmitted in propagation material and may be transmitted in seed. TBSV (= PeAMV) was detected in cherry pollen. *Tombusvirus* species have been isolated from soil and water.

References: Albechtova et al 1980, Allen and Davidson 1966, Hansen and Yorsten 1975, Hollings and Stone 1975, Jelkmann 2011, Kegler et al 1983, Kegler and Kegler 1980, Keldysh et al 2005, Koenig and Kunze 1982, Koenig and Lesemann 1985, Koenig et al 2004, Novak and Lanzova 1977, Novak and Lanzova 1980, Pfeilstetter et al 1996, Tomlinson and Faithful 1984, Tremaine 1969.

Minor quarantine viruses

Organism: *Carnation Italian ringspot tombusvirus* (CIRV)

Disease: Viral twig necrosis of cherry.

Distribution: Europe and North America.

Host range: *P. avium* (cherry). Carnation and some forest trees.

Economic impact: The economic impact of this virus is minor.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- ELISA.
- PCR - PCR primers used to amplify part of the genome for dot blot probe development.

Notes: CIRV is a member of the genus *Tombusvirus* in the family *Tombusviridae*.

Soil-borne and has been isolated from surface water.

Reference: Allen and Davidson 1967, Buttner et al 1987, Jelkmann 2011, Koenig et al 2004, Lesemann et al 1989, Pfeilstetter et al 1992, Rubino et al 1995, Sanchez Navarro et al 1999, Tremaine 1970.

Organism: *Epirus cherry ourmiavirus* (EpCV)

Disease: Rasp leaf of cherry.

Distribution: Greece.

Host range: *P. avium* (sweet cherry).

Economic impact: There are no reports of economic impact.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.

Notes: EpCV is a member of the genus *Ourmiavirus* for which there is no assigned family.

It is transmitted in seed. There is no evidence for spread in affected orchards.

Reference: Accotto et al 1997, Avgelis et al 1989, Rastgou et al 2009.

Viruses in the genus *Marafivirus*

Disease: Unknown.

Distribution: Europe.

Host range: *P. persica* (peach).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- None described.

Notes: Uncharacterised viruses of the genus Marafivirus, family *Tymoviridae*. Preliminary report based on an analysis RNA transcriptomic data of gf305 deliberately inoculated with PPV. Possible multiple novel species present.

References: Candresse et al 2015, Rubio et al 2015.

Present in Australia

Organism: ***Apple chlorotic leaf spot trichovirus*** **(ACLSV)**

Disease: Pseudopox disease of plum and apricot, apricot viruela disease and plum bark split.

Distribution: Worldwide.

Host range: *P. dulcis, P persica (*peach), *P. domestica* (plum), *P. avium* (sweet cherry), *P. cerasus* (sour cherry), *P. armeniaca* (apricot), *Prunus cerasoides, Prunus glandulosa. P orientalis, P korschinskii, Malus* spp and *Pyrus* spp.

Economic impact: ACLSV may be of economic significance in apricot and plum. May not be important in almond as a single infection.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- ELISA.
- PCR. Several specific conventional RT-PCR tests are available (Osman et al 2012, Constable et al 2007, Nakahara et al 2011). A probe based real-time RT-PCR assay has also been developed (Salmon et al 2002).

Notes: ACLSV is a member of the genus Trichovirus in the family *Betaflexiviridae*.

It has been associated with pseudopox disease of plum and apricot, apricot viruela disease and plum bark split. Possibly associated with chlorotic leafroll of almond in combination with PDV

Transmitted in propagation material.

References: Al Rwahnih et al 2004, Candresse et al 1995, Canizares et al 2001, Constable et al 2007, Dunez and Marenaud 1969, Foissac et al 2001, Garcia_Ibarra 2010, German et al 1990; German et al 1997, Jelkmann 1996, Kanaan-Atallah et al 2000, LLacer et al 1985, Marini et al 2008, Martelii and Savino 1997, Menzel 2002, Nemeth, 1986, Rana et al 2007, Rana et al 2008, Rana et al; 2009, Salmon et al 2002, Sato *etal.* 1993, Spiegel et al 2005, Sutic et al 1999, Ulubas and Ertunc 2005, Youseff and Shalaby 2009.

Organism: *Apricot pseudochlorotic leaf spot trichovirus* (APCLSV)

Disease: decline, stem-grooving, butteratura (pockmark) in apricot.

Distribution: Europe and Australia.

Host range: *P. ameniaca (*Apricot) *P. domestica* (plum), *P. salicina* (Japanese plum) and *P. persica* (peach).

Economic impact: APCLSV could have serious economic impact in plum and apricot if it is the cause of the diseases with which it has been associated. It is not reported in almond.

Pathway: Propagation material.

Diagnostic tests:

- PCR specific RT-PCR tests are available . A generic RT-PCR test can be used to detect this virus but needs development and validation .

Notes: APCLSV is a member of the genus *Trichovirus* in the family *Betaflexiviridae.*

It has been associated with various symptoms including decline of plum, stem-grooving symptoms in plum, butteratura symptoms on apricot fruit. It is also associated with symptoms similar to those caused by ACLSV and symptoms of apricot ringpox disease.

No vector is reported. Transmitted in propagation material.

References: Barone et al 2006, Barone et al 2008, Dovas and Katis 2003, Foissac et al 2005, Liberti et al 2004, Liberti et al 2005, , Niu et al 2012, Sarec et al 2003.

Organism: *Apple mosaic virus Ilarvirus* (ApMV)

Disease: European plum line pattern. Line pattern in various other *Prunus* sp.

Distribution: Worldwide.

Host range: Broad host range, including *Prunus* spp.: *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry) *P. cerasus* (sour cherry), *P. persica* (peach) and *P. domestica* (plum).

Economic impact: In some instance this virus, alone or in combination with other viruses, can impact on quality and yield of fruit, therefore this virus can have a negative economic impact.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- ELISA.
- RT-PCR.

Notes: ApMV is a member of the genus *Ilarvirus* in the family *Bromoviridae*. Genetic variants exist that may be associated with plant host species.

In almond it is associated with mosaic symptoms. It is associated with line pattern in plum, almond and other *Prunus* sp. ApMV may be symptomless in some varieties of various *Prunus* sp.

ApMV is transmitted in propagation material. It is seed and pollen borne in some plant host species. It has been detected in the ovules of one cultivar of almond but not in pollen nor in seedlings.

References: Barba et al 1985, Ciccarone, 1958, Digiaro et al 1992, Digiaro and Savino, 1992, Digiaro et al 1992, Fulton 1972, Fulton 1983, Garau et al1989, Gilmer 1956, Hamilton, 1985, Imed et al 1997, Llacer et al 1997, Maliogka et al 2010, Marenaud and Lansac, 1977, Martelli and Savino 1997, Menzel et al 2002, Petrzik and Svoboda 1997, Petzrik and Lenz 2002, Saade et al 2000, Savino et al 1989a, Tirro and Catara, 1982, Uyemoto and Scott 1992, Youseff and Shalaby 2009.

Organism: *Cherry necrotic rusty mottle foveavirus* (CNRMV)

Disease: Rusty mottle of cherry, Lambert mottle.

Distribution: Australia, North America, Europe and Japan. The associated disease is also reported in Chile, Israel and New Zealand.

Host range: *P. avium* (sweet cherry), *P. serrulata* (flowering cherry), *P. cerasus* (sour cherry, *P. armeniaca* (apricot), *P. persica* (peach) and *P. domestica* (plum). Experimental hosts include peach, apricot and almond.

Economic impact: This virus is of economic importance on cherry where it is associated with a reduction in fruit quality and yield.

Pathway: Propagation material.

Diagnostic tests:

- Woody indexing.
- ELISA.
- PCR. Various conventional RT-PCR tests are available. A generic RT-PCR test can be used to detect this virus.

Notes: CNRMV is a member of the genus *Foveavirus* in the family *Betaflexiviridae*. Strains infect sweet cherry, flowering cherry, sour cherry and apricot. The flowering cherry strain has been experimentally transmitted to peach, apricot and almond and all three experimental hosts did not show symptoms. In 1960, a cherry necrotic rusty mottle strain of Lambert mottle virus was transmitted to sour cherry, peach and, *P. mahaleb*; these experimental hosts were symptomless.

Surveys of symptomless host are not reported so their natural occurrence in these hosts is unknown.

No vector is reported. It is likely to be spread in propagation material.

References: Fry and Wood 1973, Dovas and Katis 2003, Isogai et al 2004, Li and Mock 2005, Li and Mock 2008, Mandic et al 2005, Osman et al 2012, Rott and Jelkmann 2001a, Rott and Jlekmann 2001b, Sabandsovic et al 2005, Zhou et al 2013.

Organism: *Cherry green ring mottle foveavirus* (syn. Sour cherry green ring mottle virus, CGRMV)

Disease: Green ring mottle disease.

Distribution: Australia, North America North America, Europe, New Zealand, Africa, and Asia.

Host range: *Prunus cerasus* (sour cherry), *P. avium* (sweet cherry), *P. serrulata* (flowering cherry), *P. persica* (peach), *P. domestica* (plum) and *P. armeniaca* (apricot). Symptom expression is dependent of the virus strain and *Prunus* species and cultivar.

Economic impact: This virus may have a negative economic impact in sweet and sour cherry.

Pathway: Propagation material.

Diagnostic tests:

- Woody indexing.
- PCR. A specific conventional RT-PCR has been developed. A generic RT-PCR test might be used to detect these viruses but needs development and validation .

Notes: CGRMV is a member of the genus *Foveavirus* in the family *Betaflexiviridae.*

This virus is associated with green ring mottle disease of flowering and sour cherry.

Sweet cherry and peach may be symptomless hosts.

It is transmitted in propagation material. A vector is not reported.

Reference: Desvignes, 1999, Dovas and Katis 2003, Li and Mock 2005, Liberti et al 2005, Nemeth, 1986, Parker et al 1976, Rott and Jelkmann 2001, Sipahioglu et al 2007, Wang et al 2009, Zhang et al 1998, Zhang et al 2000, Zhou et al 2011, Villamor et al 2015.

Organism: *Cherry A capillovirus* (CVA)

Disease: CVA is considered a latent virus in most infected hosts. In *Prunus domestica* subsp. Insititia it may be associated with Krikon necrosis disease in which symptoms include chlorotic mottling of leaves and stem necrosis**.**

Distribution: Australia, Asia, Europe and North America.

Host range: It infects several *Prunus* sp. including *P. avium* (sweet cherry) and *P. cerasus* (sour cherries), *P. mume* (Japanese apricot), *P. armeniaca* (apricot), and *P. domestica* (plum).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- PCR. Specific RT-PCR tests are available. Generic RT-PCR tests for detection of Foveaviruses and Tricohviruses may detect PcMV.

Notes: CVA is a member of the genus *Capillovirus*, family *Betaflexiviridae.*

It has been detected in symptomless plants.

It is graft transmissible and may be transmitted in propagation material.

It is possible that this virus is present in regions where it has not been reported as it could be transmitted in symptomless material.

Cherry virus A has been associated with Krikon necrosis disease in *Prunus domestica* subsp. Insititia (http://agris.fao.org/aos/records/US201302277539 and https://www.bordeaux.inra.fr/cherry/docs/dossiers/Activities/Meetings/02%2009%202013_WG3%20Small%20Group%20Meeting_Olomouc/Candresse_Olomouc.pdf)

References: Barrone et al 2008, Dovas and Katis 2003, Foissac et al 2005, Jelkmann 1995, Marais et al 2008, Marais et al 2012, Noorani et al 2010, Osman et al 2012, Rao et al 2009, Sabanadzovic et al 2005.

Organism: *Little cherry virus 2 ampelovirus* (LChV2)

Disease: Little cherry disease.

Distribution: Australia, North America and Europe.

Host range: *P. avium* (cherry)*, P. serrulata, P emarginata, P. domestica* (plum)*.*

Economic impact: LChV2 has affects the quality of fruit and may have a negative economic impact.

Pathway: Propagation material. Infectious vectors.

Diagnostic tests:

- Woody indexing.
- PCR A generic *Closteroviridae* RT-PCR has been developed for detection of viruses in the family *Closteroviridae* and can be used to detect this virus. Specific conventional tests are available.

Notes: LChV2 is a member of the genus *Ampelovirus* in the family *Closteroviridae.*

It is associated with little cherry disease in sweet cherry. It may be symptomless in flowering cherry. *Prunus emarginata* may be a natural host.

LChV2 is vectored by the apple mealybug (*Phenacoccus aceris*) and transmitted in propagation material and plants.

Reference: Bajet et al 2008, Dovas and Katis 2003, Eastwell and Bernardy 2001, Isogai et al 2004, Jelkmann et al 1997, Kiem-Konrad and Jelkmann 1996, Matic et al 2010, Osman et al 2012, Raine et al 1986, Rott and Jelkmann 2001, Rott and Jelkmann 2005, Yorston et al 1981.

Organism: *Plum bark necrosis stem pitting-associated ampelovirus* (PBNSPaV)

Disease: Plum bark necrosis, stem pitting

Distribution: Australia, North America, Europe, Jordan, Turkey, Morocco and Egypt.

Host range: *P. dulcis (*almond), *P. domestica* (plum), *P. mume* (Japanese plum) *P. persica* (peach), *P. avium* (sweet cherry) *P. cerasus* (sour cherry) and *P. armeniaca* (apricot). It was also experimentally transmitted to *P. serrulata* and colt cherry (*P. avium* x *P. pseudocerasus*).

Economic impact: Its economic importance is unclear however it has been associated with decline.

Pathway: Propagation material.

Diagnostic tests:

- Woody indexing.
- PCR. A generic *Closteroviridae* RT-PCR has been developed for detection of viruses in the family *Closteroviridae* and might be used to detect this virus although this needs validation. Specific conventional tests are available. This virus can also be detected along with eight other viruses and two viroids using a polyprobe.

Notes: PBNSPaV is a member of the genus *Ampelovirus* in the family *Closteroviridae.*

It is associated bark necrosis and stem pitting disease in susceptible almond, plum, prune, peach, cherry, and apricot varieties. In sensitive cultivars can also cause gummosis and graft union failure. Symptomless infections occur. It was also experimentally transmitted to. *P. serrulata* and colt cherry (*P. avium* x *P.* pseudo*cerasus*).

It is transmitted in propagation material. No vector is reported although natural spread occurs

References: Abou Ghanem-Sabanadzovic et al 2001, Al Rwahnih et al 2007, Amenduni et al 2005, Amenduni et al 2004a, Amenduni et al 2004b, Bouani et al 2004, Di Terlizzi and Savino 1994, Dovas and Katis 2003, El Maghraby et al 2006, Ghanem-Sabanadzovic et al 2001, Garcia-Ibarra et al 2010, Gumus et al 2007, Mandic et al 2005, Marais et al 2009, Marini et al 2002, Matic et al 2010, Peiro et al 2012, Sánchez-Navarro et al 2005, Usta et al 2007, Uyemoto and Teviotdale, 1996, Dovas and Katis 2003, Peiro et al 2012.

Organism: *Prune dwarf Ilarvirus* (PDV)

Disease: Prune dwarf, almond mosaic.

Distribution: Worldwide.

Host range: Several *Prunus* species, including *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry) *P. cerasus* (sour cherry), *P. persica* (peach) and *P. domestica* (plum).

Economic impact: PDV alone or in combination with other viruses can have a serious economic impact in many *Prunus* sp., including almond.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.

- Woody indexing.
- ELISA.
- PCR. Several conventional RT-PCR tests exist that could be assessed in this project in comparison to those already used. A real time RT-PCR assay has been developed. A polyprobe assay has been developed to detect PNRSV as well as seven other viruses and two viroids. Two generic *Ilarvirus* tests are also available but may not detect all strains of PDV. Further work is being undertaken to develop RT-PCR assays for detection of Australian isolates of PDV.

Notes: PDV is a member of the genus *Ilarvirus* in the family *Bromoviridae*. Genetic variants exist that may be associated with plant host species and region.

Associated with almond mosaic disease. It causes stunting in peach and plum, sour cherry yellows, chlorotic spots, shot hole of leaves and defoliation fruit cracking in sweet cherry.

Symptom expression in some *Prunus* sp. can vary from year to year depending on climate. Some cultivars may be symptomless.

Transmission occurs through seed and pollen and in propagation material, including almond. Some evidence for spread by vectors including mite (*Vasates fockeui*).

References: Al-Chaabi and Darwesh 2008, Bertozzi et al 2002, Boulila 2009, Boulila and Marrakchi, 2001, Boulila, 2002, Brunt et al 1996, Di Terlizzi et al 1994, Digiaro and Savino 1992, Fridlund, 1965; Foneseca et al 2005, Greber et al 1992, Helguera et al 2002, Jarasova and Kundu 2010, Johnstone et al 1995, Kelly and Cameron 1986, Maliogka et al 2007, Martelli and Savino 1997, Massart et al 2008, Mekuria et al 2005, Mekuria et al 2003, Osman et al 2012, Nemeth 1986, Parakh et al 1995, Peiro et al 2012, Raquel et al 1998, Saade et al 2000, Savino et al 1994, Spiegel et al 1996, Spiegel et al 1998, Ulubas Serce et al 2009, Untiveros et al 2010, Uyemoto et al 1992, Vaskova et al 2000, Waterworth and Fulton, 1964, Youssef et al 2002, Youseff and Shalaby 2009.

Organism: *Prunus necrotic ringspot Ilarvirus* (PNRSV)

Disease: Almond calico, sweet cherry rugose mosaic, necrotic ringspot, European plum line pattern.

Distribution: Worldwide.

Host range: Many commercial, wild and ornamental *Prunus* species, including almond, apricot, cherry, peach and plum. Also many *Rosa* sp., and other plants species.

Economic impact: Alone and in combination with other viruses PNRSV can have a serious economic impact due to reduction in yield.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA

- PCR Several conventional RT-PCR tests exist. A real time RT-PCR assay has been developed. A polyprobe assay has been developed to detect PNRSV as well as seven other viruses and two viroids. Two generic *Ilarvirus* tests are also available but may not detect all strains of PDV.

Notes: PNRSV is a member of the genus *Ilarvirus* in the family *Bromoviridae*. Genetic variants exist that may be associated with plant host species and region.

In almond it has been associated with necrotic shock, bud failure, calico and chlorotic mottling. It may be symptomless in some almond cultivars. In other *Prunus* spp. PNRSV may be associated with more serious disease and especially when occurring in mixed infection with other viruses.

Spread in pollen and seed and propagation material. Some evidence for spread by vectors including mite (*Vasates fockeui*), nematode (*Longidorus macrosoma*) and thrips (*Frankliniella occidentailis*).

References: Amari et al 2009, Aparicio et al 1999, Barba 1986, Bertozzi et al 2002, Boulila 2002, Boulila and Marrakchi et al 2001, Cole et al 1992, Crosslin and Mink 1992, Digiaro and Savino 1992, Greber et al 1992, Heleguera et al 2001, Howell and Mink 1988, Jarasova and Kundu 2010, Lansac et al 1980, Maliogka et al 2007, Martelli and Savino 1997, Massart et al 2008, Mekuria et al 2003, Mink 1983, Mink et al 1987, Moury et al 2000, Nyland and Lowe 1964, Nyland et al 1976, Osman et al 2012, Peiro et al 2012, Saade et al 2000, Salem et al 2003, Savino et al 1994, Sanchez-Navarro et al 1998, Spiegel et al 1998, Sweet 1976, Untiveros et al 2010, Uyemoto, 1996, Uyemoto et al 1989, Varveri et al 1997, Williams et al 1970, Youseff and Shalaby 2009.

Minor viruses of Prunus sp. present in Australia

Organism: *Apple stem grooving virus capillovirus* (ASGV)

Disease: Unknown in *Prunus* sp.

Distribution: Worldwide.

Host range: Naturally infects citrus, lily and pome fruits. Natural infections are also reported from *P. persica* (nectarine), *P. domestica* (plum), *P. armeniaca* (apricot), Japanese apricot (*Prunus mume*) and *P. avium* (cherry).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- ELISA.
- PCR. Several conventional RT-PCR assays have been developed for the detection of ASGV. The primers developed by Menzel et al (2002) were successfully used in a Sybr based RT-qPCR assay.

Notes: ASGV is the type species of the genus *Capillovirus* in the family *Betaflexiviridae*.

The fungus *Talaromyces flavus* is reported as a vector of ASGV in Korea. It is transmitted in propagation material.

References: Constable et al 2007, Fuchs and Grntzig 1994, Hassan et al 2006,Hilf 2008, Ito et al 2002, James 1999, James 2008, Kinard et al 1996, Marinho et al 1998, Massart et al 2008, Menzel et al 2002, Negi et al 2010, Nickel et al 2004, Roy et al 2005, Takahashi et al 1990, Yoshikawa et al 1992, Yoshikawa et al 1996.

Organism: *Apple stem pitting associated Foveavirus* (ASPV)

Disease: It is primarily a pathogen of pome fruit but has close relationships to viruses in summerfruit. Associated yellow vein disease in sweet and sour cherry in India needs confirmation.

Distribution: Worldwide.

Host range: Pome fruit species. Possibly *P. cerasus* (sour cherry) *and P. avium* (sweet cherry).

Economic impact: The economic impact of this virus in summerfruit is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- ELISA.
- PCR. Many conventional assays exist. A probe based real time RT-PCR assay has also been developed.

Notes: ASPV is the type member of the genus *Foveavirus* in the family *Betaflexiviridae*

There is a recent report of ASPV in cherry trees with vein yellows symptoms (P *avium* and *P. cerasus*) in India. Further work to confirm this result is required. It is possible that closely related summerfruit Foveaviruses, such as ApLV, have cross reacted with both antisera and RT-PCR primers for ASPV

References: Cameron 1989, Dhir et al 2009, Gugerli and Ramel 2004, Komorowska et al 2009, Mackenzie et al 1997, Malinowski et al 1998, Mathioudakis et al 2009, Menzel et al 2002, Schwarz and Jelkmann 1998, Stouffer 1989, Yousseff et al 2011, Komorowska et al 2010, Mathioudakis et al 2009, Salmon et al 2002.

Organism: *Carnation ringspot dianthovirus* (CRSV)

Disease: Unknown.

Distribution: Worldwide.

Host range: Naturally infects *Dianthus sp. P. domestica (* plum), *P. cerasus* (sour cherry) and *P. avium* (sweet cherry).

Economic impact: Is likely to be of little economic significance.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- ELISA.
- PCR.

Notes: CRSV is the type member of the genus *Dianthovirus* in the family *Tombusviridae.*

It has been detected in summerfruit orchards in plum, sour cherry, and sweet cherry in Germany: an associated disease is unknown.

Likely to be transmitted in propagation material, spreads in soil in the presence and absence of nematodes however transmission by *Longidorus elongatus, L. macrosoma and Xiphinema diversicaudatum* is reported and questioned.

References: Brown and Trudgill 1984, Fritzsche et al 1979, Kegler et al 1983, Kleinhempel et al 1980, Koenig et al 1988, Koenig et al 1989, Jelkmann 2011, Sanchez-Navarro et al 1999, Sit et al 2001.

Organism: Citrus enation - woody gall virus (CVEV)

Disease: Unknown

Distribution: Libya, Spain, Turkey, China, India, Iran, Japan, Kenya, Libya, Réunion, South Africa, Tanzania, USA Peru, Australia, Kiribati and New Zealand.

Host range: Primarily infects Citrus. One report on *P. domestica* (plum).

Economic impact: Unlikely to be of economic significance in *Prunus* sp.

Pathway: Propagation material.

Diagnostic tests: None.

Notes: CVEV is a graft transmissible agent in citrus. It may be a member of the genus *Luteovirus* but is not a recognised virus species.

It is primarily infects Citrus and is not considered of economic importance as it is symptomless on commercial cultivars. The occurrence on plum was reported from NSW, Australia and probably needs confirmation.

Aphid transmitted: *Toxoptera citricidus*, *Myzus persica*e and *Aphis gossypii* .Transmitted in propagation material.

References: EPPO 1997, Fraser and Broadbent 1979, Maharaj and da Graca, 1989, Mali et al 1976, Wallace and Drake, 1960.

Organism: *Cucumber mosaic cucumovirus* (CMV)

Disease: Associated with pseudopox disease of plum and chlorotic mottle of cherry.

Distribution: Worldwide.

Host range: Broad host range. *Prunus* sp. including *P. armeniaca* (apricot), *P. dulcis* (almond), *P. serrulata* (flowering cherry), *P. avium* (sweet cherry) *P. cerasus* (sour cherry), *P. mume* and *P.domestica* (plum).

Economic impact: May have a significant impact on susceptible *Prunus* sp. in combination with other viruses. The economic impact when CMV occurs on its own is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- ELISA.
- PCR.

Notes: CMV is the type member of the genus *Cucumovirus* in the family *Bromoviridae*.

In Japan it is associated with a severe disease of *P. mume* when found in combination with PNRV. In China it was detected in sweet cherry with deformed, chlorotic mottled leaves. Detected in *Prunus* sp. in Russia, almond, flowering cherry, sour cherry, and plum. CMV was associated with pseudopox disease of plum in Germany. It was detected in combination with PPV in apricot in Egypt.

It is transmitted by many aphid species. It is transmitted in propagation material.

References: Ahmed and Fath-Allah, 2012; Bashir et al 2006, Berniak et al 2010, Bertolini et al 2003, Casper 1977, Keldish et al 1998, Kishi et al 1973, Kurihara et al 1998, Tan et al 2010, Topchiiska and Topchiiski 1976, Tremain 1968, Waterworth and Kaper 1980, Zitikaite and Stanliulis 2006.

Organism: *Sowbane mosaic sobemovirus* (SoMV)

Disease: Unknown.

Distribution: Worldwide.

Host range: Many plant hosts including *P. cerausus* (sour cherry) and *P. domestica* (plum).

Economic impact: Economic significance is unknown. Likely to be low.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- ELISA.
- PCR.

Notes: SoMV is the type member of the genus *Sobemovirus*, which is not yet assigned to virus family.

SMoV is transmitted by insects (thrips, leafminer fly, beet leafhopper, fleahopper, aphid) and pollen and seed in some plant hosts.

References: Bennett and Costa, 1961, Eastwell et al 2010, Hull and Fargette 2005, Saric and Velagic 1980, Sutic and Juretic 1976.

Organism: *Tobacco mosaic tobamovirus* (TMV)

Disease: Detected in cherry with mottle leaf, and peach with red leaf.

Distribution: Worldwide.

Host range: Broad host range. Including *P. domestica* (plum), *P. persica* (peach) and *P. avium* (cherry).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- ELISA.
- PCR.

Notes: TMV is the type member of the genus *Tobamovirus* in the family *Virgaviridae.*

Transmitted mechanically and in propagation material.

References: Babovic et al 1980, Burgyan et al 1980, Gilmer 1967, Jacobi et al 1998, Letschert et al 2002, Niu et al 2009.

Organism: *Tobacco necrosis necrovirus* (TNV)

Disease: Considered symptomless in *Prunus* sp.

Distribution: Worldwide.

Host range: Broad host range including; *P. domestica (*plum), *P. armeniaca* (apricot), *P. persica* (peach), *P. cerasus* (sour cherry).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- ELISA.
- PCR.

Notes: TNV is a member of the genus *Necrovirus* in the family *Tombusviridae.*

Found in water. Transmitted by *Olipidium brassicae* and propagation material.

References: Albrechtova et al 1980, Kegler et al 1969, Paulechova 1983, Paulechova and Baumgartnerova 1980, Mitrofanova and Teslenko 1982, Uyemoto and Gilmer 1972, Zitikaite et al 2005, Zitikaite and Staniulis 2006.

2.1.4 Viroids

Organism: *Peach latent mosaic viroid* (PLMVd)

Disease: Plum spotted fruit, peach mosaic, peach yellow mosaic, peach calico

Distribution: Strains occur worldwide, including Australia.

Host range: Many hosts including *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry)*, P. domestica* (plum) and *P. persica* (peach).

Economic impact: Loss of quality and yield in affected crops.

Pathway: Propagation material. Pollen.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- PCR.

Notes: PLMVd is a member of the genus *Pelamoviroid* in the family *Avsunviroidae*. Many genetic variants exist. Latent infections occur that may be associated with strain variation and/or host. In peach infection may be latent for many years.

References: Barba et al 2007, Boubouakas et al 2009, Desvignes 1986, Di Serio et al 1999, Flores et al 2006, Hadid et al 1997. Hassen et al 2006, Hassen et al 2009, Hernandez and Flores 1992, Luigi and Faggioli 2011, Parisi et al 2011, Ragozzino et al 2004.

Organism: *Hop stunt viroid* (HSVd)

Disease: dapple fruit.

Distribution: Strains occur worldwide, including Australia in grapevine and citrus. Not known to occur in *Prunus* species in Australia.

Host range: Many hosts including *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry)*, P. domestica* (plum) and *P. persica* (peach).

Economic impact: Loss of quality and yield in affected crops.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing.
- Woody indexing.
- PCR.

Notes: HSVd is a member of the genus *Hostuviroid* in the family *Pospoviroidae*. Many genetic variants exist. Latent infections occur that may be associated with strain variation and/or host. HSVd is noted in the PEQ conditions for almonds as being present in crops in Australia and therefore not of quarantine significance. However hop strains are not reported on hops in Australia and are considered quarantine pathogens. Consequently it may be necessary to actively test imported almonds and other *Prunus* species for HSVd.

References: Amari et al 2007, Astruc et al 1996, Biosecurity Australia 2010, Cañizares et al 1999, Gillings et al 1988, Hadidi et al 1992, Hadidi et al 2003, Kofalvi et al 1997,Koltunow et al 1988, Pallas et al 2002, Pallas et al 2003, Pethybridge et al 2008, Ragozzino et al 2004, Sano et al 1989, Sano 2003, Zhou et al 2006

Organism: *Apple scar skin viroid* (APSVd)

Disease: Cherry mosaic and fruit spot. Epinasty and distortion in apricot.

Distribution: Europe, North America, Asia. In Cherry in Greece and peach and apricot in China.

Host range: This viroid is primarily a pathogen of *Malus sp.* and *Pyrus sp*. *Prunus* species that are are known hosts include *P. avium, P. cerasoides P. persica* and *P. armeniaCa.* Other Pomoideae hosts include *Cydonia oblonga*, *Pyracantha coccinea*, *Chaenomeles japonica, Sorbus aucuparia, S. domestica, S. mougeotii, S. prattii and × Pyronia veitchi*.

Economic impact: May have an economic impact in cherry due to a reduction in the quality of fruit. Its impact on apricot and peach is unknown.

Pathway: Propagation material.

Diagnostic tests:

- PCR.

Notes: ASSVd belongs to the genus *Apscaviroid* of the *Pospiviridae* family. It is seed borne in apples. Seed transmission in summerfruit species is unknown.

Reference: Behl et al 1998, Campbell and Sparks, 1976, Desvignes et al 1999 Hadidi et al 1991, Handa et al 1998, Kaponi et al 2010, Kyriakopoulou and Hadidi, 1998, Lee et al 2001, Osaki et al 1996, Tharkur et al 1995, Walia et al 2012, Zhao and Niu 2006, Zhao and Niu 2008, Zhu et al 1995.

2.2 Summary and conclusions

In this Appendix the lists of pathogens significant quarantine and Australian certification programs for almonds and summerfruit that were identified in the HIA Limited funded project entitled “Review of the post entry quarantine conditions for imports of almond germplasm” (AL10001) were updated (Tables 2.1-2.3). This information was used to identify the most appropriate diagnostic tests for detection of the pathogens and which were validated within this project (Appendix 3 and 4).

During the course of this project several *Prunus* viruses have been detected in Australia (Appendix 3 and 4) including *Cherry virus A* (CVA), *Cherry green ring mottle virus* (CGRMV),

Cherry necrotic rusty mottle virus (CNRMV), *Little cherry virus 2* (LChV2) and *Plum bark necrosis stem pitting associated virus* (PBNSPaV). These are incorporated into the lists of pathogens present in Australia that may be significant for certification (Table 2.3).

Hop stunt viroid (HSVd) is reported to infect grapevines and citrus in Australia and was detected in almonds and summerfruit during this project (Appendix 3). Specific HSVd strains are considered quarantine pathogens for the Australian hop industry and it is possible the severe hop strains of HSVd may also infect *Prunus* species consequently *Prunus* species including almonds may require testing for HSVd in PEQ. Further work to determine the pathogenicity of Australian HSVd strains to hops may also assist in understanding the importance of the detections in *Prunus* and to determine if *Prunus* sp. should be tested for HSVd in PEQ.

The review and the lists (Tables 2.1-2.3) represent the most recent research regarding known and characterised almond and summerfruit pathogens. However new technology continually aids the discovery of pathogens associated with known and emerging diseases. It is critical that this list is updated regularly and frequently to ensure it remains relevant and continues to protect the biosecurity of the Australian almond industry. For example in June 2015 a final review of the literature was undertaken and the 23rd meeting of the International Council for the Study of Virus and other Graft Transmissible Diseases of Fruit Crops (ICVF), Japan, was attended by Dr. Constable and several new *Prunus* infecting viruses have been reported and there was new information on existing viruses. There was an update on the detection of *Cherry virus A* (CVA) in new hosts which has been included in the description in 2.1.3. New viruses include *Prunus virus T* (PrVT) and *Apricot vein clearing associated prunevirus* (AVCaV) which have been incorporated into the list of pathogens (Tables 2.1 and 2.2) in this Appendix, a preliminary announcement of several peach infecting Marafiviruses which are mentioned above but will not be incorporated into the tables 2.1 and 2.2 due to the lack of information about their biology and detection, and a novel almond infecting *Prunevirus* from Azerbaijan for which information will soon be published and which is also not yet incorporated into tables 2.1 and 2.2 (Elbeaino et al 2014, Marais et al 2015a, Marais et al 2015b). The new almond infecting *Prunevirus* for and Marafiviruses will need to be included in the almond and summerfruit lists once information becomes available. Diagnostic tests for PrVT, AVCaV, the Marafiviruses and the almond infecting *Prunevirus* were not able to be adopted within this project and should be considered for future work. *Cherry rusty mottle associated virus* was recently described and associated with rusty mottle disease of cherry – it is not known to naturally infect almond or summerfruit species although peach and apricot were reported as experimental hosts of the disease (Zeller and Milbrath 1947, Villamor and Eastwell 2013, Villamor et al 2015). In describing CRMaV, updated information and diagnostic tests were for CNRMV, CTLaV and CGRMV were also published and were not able to be adopted in this project (Villamor and Eastwell 2013, Villamor et al 2015). The identification of these viruses are associated with advancements in molecular technologies, specifically next generation (deep) sequencing (NGS), which is increasingly being used for the detection and whole genome sequencing of known viruses and discovery of novel viruses. The increasing use of this tool is likely to lead to a rapid expansion in the number of known *Prunus* infecting viruses in the coming years.

Recommendation: Determine the pathogenicity of Australian HSVd isolates in *Prunus* sp. to hops and use this information to inform the quarantine status of HSVd for Australia.

Recommendation: Development of NGS and bioinformatics analysis as a tool to assist Australian PEQ and certification pathogen testing of almond and summerfruit for vegetatively transmitted pathogens.

Recommendation: Continual surveillance of scientific literature for information about new pathogens and updated information about known pathogens of almonds and summerfruit. This information should be used to update the lists of pathogens significant to quarantine and certification and industry biosecurity plans.

2.2.1 Pathogens of quarantine significance for almonds

There are three bacteria, eight phytoplasma or phytoplasma groups, eight viruses, one viroid and 17 fungi that are of quarantine significance to the almond industry of Australia (Table 2.1). Each of these pathogens may have a significant negative economic impact on almonds and their alternative host crops. Caucasus Prunus virus (CPrV) is recently described and proposed species in the proposed genus Prunevirus which has been incorporated into the table but for which further diagnostic development may be required (Marais et 2015).*Cherry mottle leaf trichovirus* occurs on the PHA biosecurity plan for almonds. However this virus is not known to naturally infect almond, it has only been transmitted experimentally. Therefore it has not been included in Table 2.1 and could be removed from the biosecurity plan.

As reported in AL10001, There are 29 fungal pathogens of almonds that may be significant to quarantine. Twelve of these 25 fungi have been reported in Australia on *Prunus* species and/or other hosts and their presence in Australian *Prunus* orchards and alternative hosts requires clarification to determine if they should remain on the PHA and PEQ pathogen list or be considered for certification programs. These fungi include, and *Armillaria mellea, Blumeriella jaapii, Dothiorella sarmentorum, Ganoderma lucidum, Maireina marginata, Laetiporus sulphureus, Leucostoma persoonii, Mycosphaerella cerasella, Nematospora coryli, Neoscytalidium dimidiatum*, *Phyllactinia guttata* and *Rosellinia necatrix* or their alternate (anamorph or telomorph). If these fungi are present management plans for their control in certification programs should be developed.

Several fungi including *Polystigma rubrum, P.ochraceum and P. amygdalinum,* (Cannon 1996) cause similar diseases in almonds and other summerfruit species and are not reported in Australia. All three *Polystigma* species could be added to the Almond and summerfruit biosecurity plans and the PEQ pathogen lists. *Collophora hispanica sp. nov., Diplodia olivarum, Phaeoacremonium amygdalinum, Pm. Iranianum* have been recently associated with wood declay diseas in almonds (Gramaje te al 2012) and should also be included on the PEQ list and biosecurity plan for almonds.

Fumigation and dipping will greatly reduce the risk of introducing these fungi through PEQ. Additionally, 8/25 fungi may be at low risk of transmission as they infect leaf or root tissues which are rarely imported and are less likely to infect budwood, which is imported as propagation material.

The lists compiled in this review will facilitate targeted testing for almond and summerfruit specific pathogens that may reduce the amount of testing required during PEQ. These lists will also aid in the development of an almond and summerfruit focused PEQ diagnostics manual. Based on the information gathered in this review a biosecurity plan specifically aimed at almonds and summerfruit can be updated.

Recommendation: Survey for the following fungi (and their alternate anamorph or telomorph states) to determine if they are present and widely distributed in Australian *Prunus* orchards and alternative hosts: *R. necatrix, N. coryli L. persoonii, A. mellea, M. marginata, B. jaapii, D. sarmentorum, G. lucidum, N. dimidiatum*, *L. sulphureus M. cerasella* and *P. guttata.*

Recommendation: Each of the pathogens listed in table 2.1 should be included in the PEQ list for active testing in almonds.

Recommendation: Development of a diagnostic assay for Caucasus *Prunus* virus (CPrV) is required.

Table 3.1 Bacteria phytoplasmas, viruses, viroids and fungi that are known to infect almonds and are of quarantine significance and should be included in a PEQ list of quarantine pathogens for almonds.

Bacteria	Phytoplasmas and phytoplasma groups	Viruses	Viroids	Fungi
Pseudomonas amygdali *Xylella fastidiosa* *Erwinia amylovora*	*Candidatus* Phytoplasma prunorum X-Disease phytoplasma *Candidatus* Phytoplasma pyri *Candidatus* Phytoplasma phoenicium' Peanut witches' broom group phytoplasmas *(*16SrII - *Candidatus* Phytoplasma aurantifolia related strains) Clover proliferation group phytoplasmas (16SrVI - *Ca.* P. trifolii related strains) Stolbur (16SrXII-A) group phytoplasmas	Caucasus *Prunus* Prunevirus (CPrV) *Little cherry virus 1* (unassigned genus, LCHV1) *Peach mosaic trichovirus* (PcMV) *Peach rosette mosaic nepovirus* (PRMV) *Plum pox potyvirus* (PPV) *Raspberry ringspot Nepovirus* (RpRSV) *Tomato black ring nepovirus* (TBRV) *Tomato ringspot nepovirus* (ToRSV)	*Hop stunt viroid*	*Apiosporina morbosa* *Armillaria cepistipes* *Armillaria mellea** *Armillaria tabescens* *Blumeriella jaapii*†* *Collophora hispanica sp. nov.* *Cytospora leucosperma* *Diplodia olivarum* *Dothiorella sarmentorum** *Fomes fomentarius* *Ganoderma lucidum*†* *Laetiporus sulphureus** *Leucostoma cincta* *Leucostoma persoonii** *Leucotelium pruni-persicae* *Maireina marginata** *Mycosphaerella cerasella*†* *Nematospora coryli** *Neoscytalidium dimidiatum** *Passalora rubrotincta* *Phaeoacremonium amygdalinum* *Phaeoacremonium iranianum*

		Phomopsis amygdali *Phyllactinia guttata** *Phymatotrichopsis omnivora*† *Polystigma rubrum*† *Polystigma ochraceum* † *Polystigma amygdalinum*† *Rosellinia necatrix**†

†Fungi at low risk of entry as they are less likely to infect imported propagation material.

*Fungi occur on the PHA Industry biosecurity plan for almonds and/or the PEQ list of *Prunus* pathogens but have been reported in Australia on *Prunus* species or other hosts and their current presence or absence needs to be determined.

2.2.2 Pathogens of summerfruit species which are of quarantine significance for Australia

There are four bacteria, ten phytoplasma or phytoplasma groups, 31 viruses, two viroids and 50 fungi that are of quarantine significance to the summerfruit industry of Australia (Table 2.2). All bacteria and phytoplasmas pathogens may have a significant negative economic impact on summerfruit. Of the 31 viruses ALRSV, ChRV, CIRV, EpCV, MLRSV, PCMV, PEV, and StPV are minor reports and may not require testing in quarantine. However it should also be noted that the interaction between viruses in mixed infections is not well understood and, although it is not reported, these viruses might contribute to a negative economic impact in some instances.

In addition to the 31 viruses there are several viruses, including several *Prunus* infecting Marafiviruses, which have been informally reported and are not included in table 2.2. These will need to be considered as a part of the quarantine lists when further information becomes available.

The fungus *Phellinus ignoramus* has been reported once in Australia on *Casuarina trulosa* and its presence needs to be determined.

This list will aid the summerfruit industry and PEQ by enabling improved and targeted testing for summerfruit pathogens. It will also aid in the development of an up-to-date PEQ diagnostics manual specifically for summerfruit.

Recommendation: Each of the pathogens listed in table 2.2 should be included in the PEQ list for active testing in summerfruit.

Recommendation: Further work is required to develop diagnostics test for *Prunus virus T* and *Apricot vein clearing associated virus*

Recommendation: The decision to include some of the "minor" (*) viruses for active testing needs to be made in consultation with the summerfruit industry and DA Biosecurity.

Table 3.3 The viruses, bacteria phytoplasmas and fungi that are known to infect summerfruit (*Prunus* species other than almonds) and are of quarantine significance and should be used to update the existing PEQ list of quarantine pathogens for summerfruit.

Bacteria	Phytoplasmas and phytoplasma groups	Viruses	Viroids	Fungi
Xylella fastidiosa	*Candidatus* Phytoplasma prunorum	*American plum line pattern Ilarvirus* (APLPV)	*Apple scar skin viroid*	*Apiognomonia erythrostoma*
Erwinia amylovora	X-Disease phytoplasma	*Apricot latent ringspot nepovirus* (ALRSV)*	*Hop stunt viroid*	*Auerswaldiella puccinioides*
Pseudomonas syringae pv. persicae	*Candidatus* Phytoplasma pyri	*Apricot latent virus* f*oveavirus* (ApLV)		*Catenophora pruni*
Pseudomonas syringae pv. avii	*Candidatus* Phytoplasma phoenicium	*Apricot vein clearing associated prunevirus* (ACVaV)		*Corilopsis gallica*
	Peanut witches' broom group phytoplasmas *(*16SrII - *Candidatus* Phytoplasma aurantifolia related strains)	*Asian Prunus virus 1 foveavirus (*APruV-1)		*Cristulariella pruni*
		*Asian Prunus virus 2 foveavirus (*APruV-2)		*Cylindrosporium nuttalli*
		Asian Prunus virus 3 foveavirus (APruV-3)		*Diaporthe decorticans*
		Arabis mosaic nepovirus (ArMV)		*Diaporthe pennsylvanica*
		Carnation Italian ringspot tombusvirus (CIRV)*		*Diaporthe pruni*
		Cherry leaf roll nepovirus (CLRV)		*Diaporthe prunicola*
		Cherry mottle leaf trichovirus (CMLV)		*Diplodia vulgaris*
		Cherry rasp leaf cheravirus (CRLV)		*Diplodina persicae*
		Cherry rosette nepovirus (ChRV)*		*Fomitopsis cajanderi*
		Cherry twisted leaf foveavirus (CTLaV)		*Fomitopsis pinicola*
		Epirus cherry ourmiavirus (EpCV)*		*Fomitopsis rosea*
		Little cherry virus 1 (unassigned genus, LCHV1)		*Fomitopsis spraguei*
		Myrobalan latent ringspot nepovirus (MLRSV)*		*Ganoderma zonatum*
		Peach chlorotic mottle foveavirus (PCMV)		*Gloeocystidiellum porosum*
		Peach enation nepovirus (PEV)*		*Gloephyllum sepiarium*
		Peach mosaic virus trichovirus (PcMV)		*Gloephyllum trabeum*
		Peach rosette mosaic nepovirus (PRMV)		*Helicobasidium mompa*
		Petunia asteroid mosaic tombusvirus (PeAMV)		*Irpex lacteus*
		Plum pox potyvirus (PPV)		
		Prunus virus T (PrVT)		
		Raspberry ringspot nepovirus (RpRSV)		
		Strawberry latent ringspot virus (SLRSV)		

Bacteria	Phytoplasmas and phytoplasma groups	Viruses	Viroids	Fungi
		Stocky prune cheravirus (StPV)* *Tobacco ringspot nepovirus* (TRSV) *Tomato black ring nepovirus* (TBRV) *Tomato ringspot nepovirus* (ToRSV) *Tomato bushy stunt tombusvirus* (TBSV)		*Laxitextum bicolor* *Melanconium cerasinum* *Meruliopsis corium* *Monilia angustior* *Monilia kusanoi* *Monilinia fructigena* *Monilinia seaveri* *Morrisographium persicae* *Mycocentrospora cladosporioides* *Mycosphaerella nigerristigma* *Mycosphaerella pruni-persicae* *Nectria galligena* *Pestalotia adusta* *Pestalotia psidii* *Phellinus prunicola* *Phialophora parasitica* *Phellinus igniarius* *Phyllosticta congesta* *Phyllosticta psidii* *Phyllosticta serotina* *Postia balsamea* *Rhodosticta quercina*

Bacteria	Phytoplasmas and phytoplasma groups	Viruses	Viroids	Fungi
				Septoria pruni *Steccherinum ochreceum* *Taphrina flectans* *Trichaptum biforme* *Xylaria longiana* *Xylaria mali*

2.2.3 Pathogens of almond and summerfruit species that occur in Australia and may be of significance at the certification level

Four bacteria, 13 viruses, two viroids and three fungi that are known to infect summerfruit species in other countries have also been recorded in Australia. Three of the four bacteria, 7/13 viruses and both viroids have also been reported to infect almond overseas.

Almonds and summerfruit will require testing for ApMV, ACLSV, PNRSV, PDV and PBNSPaV during certification as each of these viruses are known to spread naturally via a vector and pollen. Summerfruit species will require additional testing for APCLSV, ASGV, CGRMV and CNRMV. Plums may require additional testing for CVA and LChV2.

Almond is an experimental host of CNRMV and does not require testing for this virus. ASPV, CMV, CRSV, CVEV, SoMV, TNV and TMV viruses were not detected in almond or summerfruit during this project (Appendix 3). ASPV, CMV, CRSV, CVEV, SoMV, TNV and TMV are minor pathogens of summerfruit and/or almonds and should not require testing during certification.

HSVd and PLMVd are reported in Australia in grapevine, citrus and peach (Koltunow et al 1988, Gillings et al 1988, Di Serio et al 1999). Both are known to infect *Prunus* species including almond in other countries (Tables 2.1-2.3). HSVd was detected in almonds and summerfruit samples in this project (Appendix 3).

The two fungi are not reported on *Prunus* species in Australia but *A. citri* has localised distribution on Citrus in Australia (CABI 2005, Simmonds 1966) and *L. threobromae* is widespread in Australia (Qiu et al 2010; Pitt et al 2010). *A citri* may not be a significant pathogen of *Prunus* species.

Recommendation: Where possible molecular diagnostic tools for important quarantine pathogens of almonds and other *Prunus* species should be validated under Australian conditions and adopted by quarantine agencies. These protocols can then be used to develop a specific PEQ diagnostic manual for almonds and update the existing protocols for summerfruit.

Recommendation: Assess new molecular diagnostic tools for inclusion in the nationally endorsed SPHDS protocols for *X. fatsidiosa*, *E. amylovora* and PPV. If suitable these protocols should be validated under Australian conditions to determine their reliability prior to inclusion within the protocols.

Table 2.3 A list of pathogens that are known to infect almonds and/or summerfruit species that occur in Australia and may be significant at the certification level.

Pathogen group	Pathogens known to infect almond	Pathogens known to infect summerfruit species
Bacteria	*Agrobacterium tumefaciens* *Pseudomonas syringae pv. syringae* *Xanthomonas arboricola pv. pruni*	*Agrobacterium tumefaciens* *Pseudomonas syringae pv. mors-prunorum* *Pseudomonas syringae pv. syringae* *Xanthomonas arboricola pv. pruni*
Viruses	*Apple chlorotic leaf spot trichovirus* (ACLSV) *Apple mosaic virus Ilarvirus* (ApMV) *Plum bark necrosis stem pitting associated ampelovirus* (PBNSPaV) *Prune dwarf Ilarvirus* (PDV) *Prunus necrotic ringspot Ilarvirus* (PNRSV) *Cucumber mosaic cucumovirus* (CMV) – not known on summerfruit in Australia* *Cherry necrotic rusty mottle foveavirus* (CNRMV) - Almond is an experimental host*	*Apple stem pitting associated foveavirus** (ASPV) – not known on summerfruit in Australia *Apricot pseudochlorotic leaf spot trichovirus* (APCLSV) *Apple stem grooving virus capillovirus* (ASGV) *Apple chlorotic leaf spot trichovirus* (ACLSV) *Apple mosaic virus Ilarvirus* (ApMV) *Cherry green ring mottle virus* (CGRMV) *Cherry necrotic rusty mottle foveavirus* (CNRMV) *Cherry virus A* (CVA) *Little cherry virus 2 ampelovirus (LChV2)* *Plum bark necrosis stem pitting associated ampelovirus* (PBNSPaV) *Prune dwarf Ilarvirus* (PDV) *Prunus necrotic ringspot Ilarvirus* (PNRSV) *Cucumber mosaic cucumovirus* (CMV)* – not known on summerfruit in Australia *Carnation ringspot dianthovirus** (CRSV)– not known on summerfruit in Australia Citrus enation - woody gall virus (CVEV)* *Sowbane mosaic sobemovirus* (SoMV)* – not known on summerfruit in Australia *Tobacco necrosis necrovirus* (TNV)* – not known on summerfruit in Australia *Tobacco mosaic tobamovirus* (TMV)* – not known on summerfruit in Australia
Viroids	*Hop stunt viroid* (Australian strains) *Peach latent mosaic viroid*	*Hop stunt viroid* (HSVd, Australian strains) *Peach latent mosaic viroid* (PLMVd)
Fungi		*Alternaria citri* *Calsophaeria pulchella* *Lasiodiplodia theobromae*

*Pathogens marked with an asterisk are minor reports or not economically important and may not need to be included into pathogen testing programs.

2.4 Diagnostic testing

In this review we identified the types of tests that are available for detection of each of the pathogens that are significant to *Prunus* species. Although traditional methods for detection of these pathogens should not be discarded, molecular diagnostics are available that can assist in the rapid and specific detection of many quarantine pathogens of almonds and summerfruit species, especially for viruses, viroids and phytoplasmas which are uncultivable. The adoption of these rapid and specific tests could assist in reducing the amount of time almonds and summerfruit species spend in PEQ prior to release. All molecular tests that are developed for quarantine pathogens must be validated under Australian conditions by surveying their known hosts before they are adopted. Validation will determine their reliability by ensuring that the tests do not give false positive results due to detection of nucleic acids belonging to plant hosts or other organisms that occur in Australia. The survey would also update the disease status and areas of freedom for almonds and summerfruit species in Australia. Detection of a quarantine pathogen will simplify the suggested PEQ lists for almonds and summerfruit.

Bacteria can be detected using traditional methods of isolation and culturing and biochemical tests. All bacteria except *Pseudomonas amygdali* have molecular tests available. Nationally endorsed protocols exist for the detection and identification of *X. fastidiosa* and *E. amylovora*. The International Plant Protection Convention (IPPC) International Standards for Phytosanitary Measures (ISPMs) recommend the same tests for *E. amylovora*. However since their publication several new molecular protocols have become available for *X. fastidiosa* and *E. amylovora* (Harper et al 2010, Hernandez-Martinez 2006, Rodrigues et al 2003, Taylor et al 2001, Powney et al 2011) and these and the endorsed protocols should be validated for their reliability in Australian *Prunus* crops. Molecular tests for the remaining bacterial species should also be adopted and validated under Australian conditions.

PCR methods are used for phytoplasma detection. Two protocols for *Ca.* P. prunorum and X-disease phytoplasma have been endorsed by SPHD (Constable 2009a, Constable 2009b). Both protocols describe a universal test that can be used for detection of all phytoplasmas in *Prunus* and other plant host species. These protocols require validation for use in *Prunus* species under Australian conditions.

Viruses are detected by biological indexing onto herbaceous and/or woody indicators, ELISA and/or PCR. Many of the recently reported viruses have been discovered through molecular methods and PCR tests have been developed and reported but no commercial ELISA test is available. The reason for this is likely to be associated the use of molecular methods for virus discovery and that the PCR technique tends to be cheaper and easier to develop and are more sensitive that serological methods such as ELISA. The advantage of developing PCR detection methods is that they can be updated as more information becomes available about new strains and species. For example, since the nationally endorsed detection protocol for PPV was written, which uses the same tests recommended by the IPPC ISPMs, new PPV strains have been identified and real-time tests have been published (Cambra et al 2010, Olmos et al 2005, Schneider et al 2004). These new tests should be validated for reliability under Australian conditions and included in the protocol if successful. Similarly many new tests for PDV and PNRSV have been reported in recent years and these could be adopted and validated for use in almond and summerfruit pathogen testing schemes.

Some of the minor viruses, including ALRSV, ChRV, CIRV, EpCV, MLRSV, PCMV, PEV, SoMV and StPV do not have molecular tests available. The risk of introduction of these viruses is low and active testing may not be required. The use of a generic *Foveavirus* test, which is available (Foissac et al 2005), could aid detection of PCMV and other *Foveavirus* species, although they may not detect all *Foveavirus* species and strains (X. Foissac pers. comm.). Likewise a generic *Tombusvirus* test (Russo et al 2002) is available that may detect CIRV and can be used for detection of TBSV and PeAMV. Several generic *Nepovirus* tests have been published (Digiaro et al 2007; Maliogka et al 2004, Wei and Clover 2008) which might detect ALRSV, ChRV and MLRSV but those tests have not been validated for these species in other countries. ALRSV, ChRV and MLRSV should be detected using herbaceous indicators during PEQ.

Biological indexing on woody indicators for graft transmissible agents (GTA) such as viruses, viroids and phytoplasmas, requires time and space as the candidate plant must be grafted onto a sensitive indicator plants and observed for symptom development, which can take up to three years. PCR protocols for GTAs could assist in reducing the time newly imported varieties spend in PEQ by rapidly identifying *Prunus* GTAs of quarantine significance upon entry and for pathogen identification in symptomatic biological indicators. However a combination of PCR and biological indexing is ideal for PEQ screening for exotic pathogens as this should increase the chances of detection of all pathogen strains. Biological indexing is still required for those diseases and GTAs for which there are no other available diagnostic tests.

The risk of entry of many fungal pathogens via PEQ is low as they may not be carried in propagation material or the inoculum load will be knocked out during fumigation and dipping. The continued use of visual observations while imported material is growing in PEQ may suffice for detection. PCR is available for many of the almond fungal pathogens at risk of transmission in propagation material including *Apiosporina morbosa, Armillaria mellea, A. tabescens, A. cepistipes, Cytospora leucosperma Dothiorella sarmentorum, Laetiporus sulphureus, Leucostoma cincta, L. persoonii, Neoscytalidium dimidiatum, Phomopsis amygdali and Phyllactinia guttata*. PCR is also available for detection of *Rosellinia necatrix, Phymatotrichopsis omnivore, Blumeriella jaapii and Ganoderma lucidum* although these fungal pathogens pose a low risk of entry due to the tissue type that they infect (leaves or roots). PCR could be used for identification if one of these fungal pathogens is suspected based on symptom expression.

Recommendation: Where possible molecular diagnostic tools for important quarantine pathogens of almonds and other *Prunus* species should be validated under Australian conditions and adopted by quarantine agencies. These protocols can then be used to develop a specific PEQ diagnostic manual for almonds and update the existing protocols for summerfruit.

Recommendation: Assess new molecular diagnostic tools for inclusion in the nationally endorsed SPHDS protocols for *X. fastidiosa*, *E. amylovora* and PPV. If suitable these protocols should be validated under Australian conditions to determine their reliability prior to inclusion within the protocols.

2.5 Conclusions and recommendations:

Based on the information in this review we recommend the following:

1. Update the PEQ list for almonds and/or summerfruit with recently reported pathogens and updated information of known pathogens.

2. Determine if some of the “minor” (*) pathogens require active testing.
3. Adopt, develop and validate molecular diagnostic tools under Australian conditions for the important quarantine pathogens of almonds.
4. Development of a post entry quarantine diagnostic manuals that are specific for almonds and summerfruit.

Specific recommendations, based on the information gathered in the review include:

1. The PEQ list for almonds and summerfruit should be updated with information gathered in this review about the aetiology of diseases and the occurrence of newly reported and/or characterised bacterial, phytoplasma, viral, viroid and fungal pathogens that occur in almonds and summerfruit species. Continual surveillance of the literature is required into the future to ensure these remain current.
2. A separate PEQ list of quarantine pathogens for almonds and summerfruit should be created that includes the bacterial phytoplasma, viral, viroid and fungal pathogens listed Table 2.1 and 2.2.
3. Where possible molecular diagnostic tools for important quarantine pathogens of almonds and other *Prunus* species should be validated under Australian conditions and adopted by quarantine agencies. These protocols can then be used to develop a specific PEQ diagnostic manual for almonds and update the existing protocols for summerfruit. Next generation sequencing and bioinformatics analyses should be considered for further development as a diagnostic tool o support PEQ and certification.
4. The decision to include some of the “minor” (*) viruses for active testing needs to be made in consultation with the almond and summerfruit industries and the federal Dpeartment of Agriculture.
5. Assess new molecular diagnostic tools for inclusion in the nationally endorsed SPHDS protocols for quarantine pathogens of almonds and other summerfruit species. If suitable, these protocols should be validated under Australian conditions to determine their reliability prior to inclusion within the protocols.
6. The quarantine status of HSVd and several fungi including *R. necatrix, N. coryli L. persoonii, A. mellea, M. marginata, B. jaapii, D. sarmentorum, G. lucidum, N. dimidiatum*, *L. sulphureus M. cerasella* and *P. guttata* needs to be determined. This includes surveillance for the presence of the fungi in Ausralia and assessment of the pathogenicity of *Prunus* infecting HSVd isolates in hops.

Appendix 3: Identify, develop and validate molecular diagnostic tools for the detection of endemic and exotic pathogens of summerfruit under Australian conditions

3.1 Introduction

In recent years there have been significant advances in molecular and diagnostic technology that have allowed characterisation of new and emerging pathogens, new strains of known pathogens of *Prunus* species and clarification of pathogens associated with diseases of *Prunus* species which have an unknown aetiology, especially for viruses, viroids and phytoplasmas. These advances have resulted in new and/or improved molecular tools for the rapid and sensitive detection of these pathogens associated with diseases of almonds and summerfruit, which are quarantinable or controlled through certification programs in Australia.

A literature search was conducted to identify PCR primers for detection of endemic and exotic bacteria, viruses and viroids of *Prunus* species. Detailed bioinformatic analyses were done on the primer sequences to determine their specificity and chance of success in detecting all known strains of each pathogen. Based on this information tests for endemic and exotic viruses, viroids and bacteria that are important to the almond and summerfruit industries were established in the laboratory.

A national survey of summerfruit and almond growing regions of Australia was conducted to assess and validate the molecular tests developed in this project under Australian conditions. This validation process was required to identify the potential for 'false negatives', 'false positives' or banding that can make interpretation of results difficult.

The disease survey would also update the disease status for viruses, viroids and bacteria of almond and summerfruit industrues in Australia. It is possible that some of the exotic pathogens are present in Australia but remain symptomless within their hosts. However, it is also possible that, in combination with selection of healthy plant material from the country of origin, Australian post entry quarantine (PEQ) facilities have been successful in preventing entry of these pathogens.

There are seven viruses that are known to infect *Prunus* species which are pollen-borne and include: PNRSV, PDV, CLRV, RRSV, TRSV, TBRV and ToRSV (Card et al 2007). In addition SLRVS, ApMV and HSVd are suspected to be pollen transmitted, but the evidence is inconclusive (Card e al 2007). Pollen may be imported into Australia as a source of germplasm for breeding purposes and therefore is a risk of introducing quarantine viruses, whilst pollen produce in locally grow trees may be a source of endemic viruses during deliberate pollination for breeding and passive pollination in the field. Therefore a method to detect viruses in pollen was also trialled (Shiller et al 2010).

Adoption of these tests will improve the efficiency of the Australian post entry quarantine for almonds and summerfruit and may reduce the existing 18 month waiting period. This will also improve our ability to respond in case of an incursion by providing more accurate and sensitive identification of almond and summerfruit pathogens. The tests will also improve the ability of the Australian almond and summerfruit certification schemes to determine the pathogen status of selected varieties prior to inclusion and during regular maintenance and pathogen testing of the high health programs.

3.2 Materials and Methods

3.2.1 Samples

In total, samples were collected from 101 trees throughout Australia. The samples were received between August 2013 and March 2015. Samples were collected from major summerfruit and almond growing regions and some home gardens in New South Wales (34), Queensland (16), South Australia (5), Tasmania (17), Western Australia (3) and Victoria (26).

Trees that were sampled included almond (33), apricot (7), cherry (13), cherry plum (2), nectarine (8), peach (16), peach almond hybrid (2), plum (19) and rose (1). Older varieties were selected in preference to more recent importations. Diseased trees were selected in preference to healthy trees.

Each sample was tested for the presence of five bacteria (*Agrobacterium* sp., *Erwinia amylovora*, *Pseudomonas* sp., *Xanthomonas arbicola pv. pruni* and *Xylella fastidiosa*), phytoplasmas, 3 viroids (HSVd, ASSVd and PLMVd) and specifically for 34 viruses (ACLSV, APCLSV, ApLV, APLPV, ApMV, APruV1, APruV2, APruV3, ArMV, ASGV, ASPV, CGRMV, CLRV, CMLV, CMV, CNRMV, CRLV, CRMaV, CTLaV, CVA, LChV1, LChV2, PBNSPaV, PcMV, PDV, PNRSV, PPV, PRMV, RRSV, SLRSV, TBRV,TBSV, TRSV and ToRSV (See Appendix 2 for a list of full names and abbreviations). Each sample was also tested using generic PCR tests to detect viruses in the family *Closteroviridae* and in the genera *Ampelovirus, Capillovirus, Foveavirus, Ilarivirus* and *Trichovirus*, including some viruses for which no specific test is available *e.g Peach chlorotic mottle foveavirus* (PCMV). *Petunia asteroid mosaic tombusvirus* (PeAMV) may be detected using the TBSV RT-PCR assay.

3.2.2 Positive controls - viruses present in Australia

Positive controls for the following viruses were obtained from Crop Health Services diagnostic laboratories (CHS; Victorian DEDJTR): *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Apple stem grooving virus* (ASGV), *Apple stem pitting virus* (ASPV), *Cucumber mosaic virus, Prune dwarf virus* (PDV) and *Prunus necrotic ringspot virus* (PNRSV). *Cherry green ring mottle virus* (CGRMV), *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry virus A* (CVA), and *Plum bark necrosis stem pitting associated virus* (PBNSPaV) were detected during the project in material supplied by Dr. Alison Dann from Department of Primary Industries, Parks, Water and Environment (DPIPWE), Tasmania and the material subsequently used as positive controls for the validation and survey. *Little cherry virus 2* provided by Dr. Alison Dann. A positive control for *Peach latent mosaic viroid* (PLMVd) was not available.

3.2.3 Positive controls – exotic viruses, phytoplasmas and bacteria

Positive controls for some exotic pathogens were obtained from DSMZ via Ms Joanne Mackie (DEDJTR; Table 3.1). DNA of was provided by. Nucleic acid of *Cherry virus A* (CVA), *Erwinia amylovora, Hop stunt viroid* (HSVd), *Phytoplasma*, *Tomato bushy stunt virus* (TBSV), and *Xylella fastidiosa* were provided by Ms Joanne Mackie (DEDJTR). Positive controls for *Little cherry virus 1* was provided by Dr. Alison Dann (DPIPWE).

Table 3.1. List of DSMZ viral nucleic acid used as positive controls for RT-PCR assays for exotic viruses.

DSMZ Cat. #	Virus species	Abbr.	Source
PV.-0045	*Arabis mosaic virus*	ArMV	*Vitis vinifera*, grapevine
PV.-0046	*Arabis mosaic virus*	ArMV	*Ligustrum sp*.
PV.-0049	*Tomato ringspot virus*	ToRSV	*Pelargonium sp*., Denmark
PV.-0191	*Tomato black ring virus*	TBRV	*Rubus idaeus,* raspberry, Czechoslaovakia
PV.-0235	*Tobacco ringspot virus*	TRSV	*Eucharis candida*, Peru
PV.-0236	*Tobacco ringspot virus*	TRSV	*Phaseolus vulgaris* cv. Pinto, USA
PV.-0247	*Strawberry latent ringspot virus*	SLRSV	Scotland, UK
PV.-0275	*Cherry leaf roll virus*	CLRV	*Prunus avium*, Germany
PV.-0276	*Cherry leaf roll virus*	CLRV	*Sambucus nigra*, Germany
PV.-0380	*Tomato ringspot virus*	ToRSV	*Malus sylvestris*, USA
PV.-0429	*Raspberry ringspot virus*	RpRSV	*Vitus vinifera*, grapevine, Germany
PV.-0521	*Tomato black ring virus*	TBRV	*Pelargonium sp*., Russia

3.2.4 Synthetic positive controls

Synthetic positive controls were designed for the viruses and bacteria for which nucleic acid could not be obtained (Table3.2). Synthetic positive controls were designed using the GeneArt® Strings™ DNA Fragments gene synthesis services, available on the Invitrogen™, Life Technologies (Melbourne, Australia) website. GeneArt® Strings™ DNA Fragments are un-cloned, double-stranded linear DNA fragments and are assembled from synthetic oligonucleotides. For both RNA and DNA templates, a forward primer sequence, probe sequence (if the template was being used as a positive control for real time PCR), and the reverse complement of the reverse primer sequence were inserted into an artificial template for each assay. The size of the resulting PCR product was approximately 20% smaller or 20% larger than the original PCR product size to allow for direct visual identification of contamination with the positive control. The sequence for the T7 promoter (TAATACGACTCACTATAGGG) was also inserted at the beginning of the template for production of the synthetic RNA positive controls that were to be used for RT-PCR as this promoter is required to initiate the downstream process of RNA transcription.

For DNA assays, the synthetic positive control was re-suspended in RNase/ DNase-free water and used directly as a positive control. For RNA assays, the re-suspended synthetic positive control was used as a template for RNA transcription using an Ambion MEGAscript® Kit, the resulting complementary RNA (cRNA) was then treated with DNase and the resulting DNA-free cRNA was used as a synthetic positive control.

Most synthetic positive controls incorporated primers and/or probes sequence for more than one pathogen. Table 3.2 lists the ID No for each of the synthetic positive controls used in this project, the pathogen detected by the molecular test, the primer sets which detect each particular synthetic positive control, the expected PCR product size for the synthetic positive control, the expected PCR product size for samples and the size of the synthetic positive control fragment.

3.2.5 Nucleic acid extraction for detection of pathogens

Total nucleic acid (TNA) was extracted from plant material using a modified lysis buffer (MacKenzie et al 1997) and the QIAxtractor (QIAGEN Pty Ltd, Doncaster, VIC, Australia) as described previously (Constable et al 2012), except 200µl of lysate/ ethanol mixed sample was added to the filter plate instead of 500µl. Three separate TNA extractions were carried out for each sample and these three extractions were combined to ensure that there was ample TNA for all the PCR and RT-PCR assays.

3.2.6 Primers for RT-PCR and PCR

Housekeeping RT-PCR

Housekeeping primer sequences are listed in Table 3.3. Primers for the detection of NADH dehydrogenase ND2 subunit (ndhB gene, NAD) messenger ribonucleic acid (mRNA; Thompson et al 2003) were used to determine the quality of the extracted RNA. Primers for the detection of the 16S rDNA gene (Weisberg et al 1991) were used to determine the quality of the extracted DNA. These primers were used to ensure that nucleic acid was present and that there were no inhibitors in the nucleic acid extracts that retard the activity of the RT enzyme or DNA polymerase during the RT-PCR or PCR reactions.

Pathogen primers and probes

A literature review was conducted and international researchers were consulted to identify tests that are routinely used for virus detection. The primers of the selected tests were analysed using BlastN (Altschul et al 1997) to determine their suitability to detect published strains of each pathogen and the specificity for the pathogen species. In addition to selecting assays based on bioinformatic analysis, the selection of primers was also based on published information about the success of assays to detect virus isolates of each species in other countries. The primers that were selected for development for detection of endemic and exotic pathogens are listed in Table 3.3.

For all one step RT-PCR and PCR tests the final concentration for each primer was 0.4µM unless otherwise specified in the diagnostic protocol. The final primer concentrations were 0.2µM for the SLRSV, ArMV, TRSV RT-PCR tests and the *Xanthomonas arbicola* multiplex PCR test. Primer concentrations were exactly as published for the generic nested tests for viruses in the Family *Closteroviridae* or in the genera *Ampelovirus, Capillovirus, Foveavirus, Ilarivirus* and *Trichovirus* (Maliogka et al 2007, Dovas and Katis 2003, Maliogka et al 2008, Dovas and Katis 2003b, Dovas and Katis 2003a, Foissac et al 2001 and Liberti et al 2004).

Table 3.2. A list of synthetic positive controls that were designed in this project and used during PCR and RT-PCR assays for detection of exotic viruses and bacteria.

Synthetic positive control ID No.	Pathogen	Primer set	PCR product size (bp)	Original PCR product size (bp)	Total size of synthetic positive control (bp)
Synthetic positive 1	*Little cherry virus 2* *	LC2.13007F/ LC2.14545R	300	1080	484
		LC26L/ L26R	300	409	
		UP2/ LO2	300	438	
		01F/ 03R	300	180	
Synthetic positive 2	*Peach rosette mosaic virus*	PRMVF4321/ PRMVR5699	310	388	1190
	Apricot pseudo chlorotic virus	APCLSV-F2/ APCLSV-R2	1030	1288	
	Raspberry ringspot virus	RpRSV942F/ RpRSV1741R	640	800	
	Cherry leaf roll virus	CLRV-3/ CLRV-5	333	416	
	Cherry leaf roll virus qPCR *	CLRV-UTRF/ CLRV-3R/ CLRV-UTRP	187	N/A	
	Strawberry latent ringspot virus	SLRV-F/ SLRV-R	398	497	
Synthetic positive 3	*Apricot latent virus*	ApLV1/ ApLV2	1200	1500	1330
	American plum line pattern virus	APLPV VP340/ VP 339	450	563	
	Tomato ringspot virus	ToRSV U1/ D1	360	449	
	Tobacco ringspot virus	MF05-22-F/ MF-21-R	256	320	
Synthetic positive 4	*Tomato ringspot virus*	ToRSV UTRf/ UTRr/ UTRp	146	182	737
	Apricot latent virus	H-ALV1/ C-ALV1	160	200	
	Tomato black ring virus	TBRV 70F/ 70R/ 70P	90	72	
	Arabis mosaic virus	ArMV III D forward/ R reverse/ P probe	105	84	
Synthetic positive 5	*Cherry virus A*	CVA-fw1/ CVA-rev1	240	302	773
	Little cherry virus 1	6for sense/ 2rev antisense	550	670	
	Tomato ringspot virus	TORSV-62 F/ TORSV-738R	555	676	
	Cherry twisted leaf 1a	CTL-1a 218CPF/ NGRRM-TL CPR	450	562	
	Cherry necrotic rusty mottle virus	CNRM-7626F/ CNRMV-8210R	468	584	
Synthetic positive 7	*Agrobacterium*	UF f/ B1R r	151	184	592
	Pseudomonas sp	PsrpoD FNP1/ PsrpoDnprPCR1	560	700	
	Xanthomonas agricola pv. pruni	pXap41repA1-F/ pXap41repA1-R	274	343	
Synthetic positive 8	*Agrobacterium*	UF f/ B1R r	151	189	987
	Agrobacterium	UF f/ B2R r	847	1059	
	Agrobacterium	UF f/ AvR r	392	491	
	Agrobacterium	UF f/ ArR r	938	1173	

Synthetic positive 9	*Xanthomonas agricola pv. pruni*	pXap41repA1-F/ pXap41repA1-R	274	343	450
	Xanthomonas agricola pv. pruni	pXap41repA2-F/ pXap41repA2-R	361	451	
	Xanthomonas agricola pv. pruni	pXap41mob-F/ pXap41mob-R	196	245	
Synthetic positive 10	*Peach mosaic virus*	PM-AF1/ PM-AFR	310	383	734
	Cherry twisted leaf 1b	CTL-1b-235CPF/ NGRRM-TL CPR	435	545	
	Apricot latent virus (2)	H-ALV1/ C-ALV1	160	200	
	Cherry mottle leaf virus	CML13A/ CML4A	668	835	
Synthetic positive 11	*Xanthomonas agricola pv. pruni*	Xap-2F/ Xap-2P/ Xap-2R	86	72	814
	Xanthomonas agricola pv. pruni	Y17CoF/ Y17CoR	754	943	
	Xanthomonas agricola pv. pruni	Y17CoF3/ Xa ABC probe/ Y17CoR	90	108	
	Xanthomonas agricola pv. pruni	29F/ 29R	275	344	
Synthetic positive 12	*Pseudomonas sp.*	acn-Fp/ acn-Rp	545	682	825
	Pseudomonas sp.	acn-Fs/ acn-Rs	410	513	
	Pseudomonas sp.	cts-Fp/ cts-Rp	390	480	
	Pseudomonas sp.	cts-Fs/ cts-Rs	468	584	
	Pseudomonas sp.	pgi-Fp/ pgi-Rp	506	632	
	Pseudomonas sp.	pgi-Fs/ pgi-Fs	490	614	
Synthetic positive 13	*Asian Prunus viruses*	CP-PLV1/ CP-PLV2	294	367	750
	Cherry rusty mottle assoc. virus	CRM91CPF/ NGRRM-TL	556	695	
	Peach mosaic virus	PM-AF1/ PM-AFR	307	383	
	Peach latent mosaic viroid	cPLMVd/ hPLMVd	270	339	

3.2.7 Conventional and real time RT-PCR and PCR conditions

Cycling conditions for pathogen specific conventional and real time RT-PCR and PCR assays are given in table 3.4. Cycling conditions for generic conventional and real time RT-PCR and PCR assays are given in table 3.5.

One-step RT-PCR

The SuperScript™ One-Step RT-PCR System (Invitrogen, Life Technologies, Melbourne, Australia) was used for detection of NAD mRNA and most viruses. One step RT-PCR was done according to the manufacturer's instructions except that 0.5µl of SSIII/ Taq enzyme mixture was used in each RT-PCR reaction and the total reaction volume was 25 µl and 3 µl of nucleic acid template was added.

PCR

Platinum® *Taq* DNA Polymerase (Invitrogen, Life Technologies, Melbourne, Australia) was used for all PCR reactions according to the manufacturer's instructions except that 0.2µl of Platinum® *Taq* DNA

Polymerase was used in each reaction and the total reaction volume was 25 µl and 3 µl of nucleic acid template was added.

For nested PCR, 1 µl of the first round PCR reaction mixture was added to the PCR mixture containing the second primer set.

Real time PCR

The Rotor-Gene Probe PCR Kit (Qiagen, Melbourne, Australia) was used for all qPCR tests according to the manufacturer's instructions. Primer concentrations used for the real time PCR assays were exactly as published.

3.2.8 Gel electrophoresis

After amplification, 6X loading dye was added to each PCR reaction at a rate of 1µl loading dye to 5µl PCR reaction. 12.5 µL of the loading dye/ PCR reaction mixture was subjected to electrophoresis in a 1.0% agarose gel using 0·5 × TBE (0·045 M Tris-borate, 1 mM EDTA, pH 8·0) running buffer. Products were stained with SYBR®Safe DNA Gel Stain (Invitrogen, Life Technologies, Melbourne, Victoria, Australia) that was incorporated in the gel and visualized by UV transillumination. Water controls, in which no nucleic acid was added to the PCR mix, were also included. The 1Kb Plus DNA Ladder (Invitrogen, Life Technologies, Melbourne, Victoria, Australia) was used as a DNA marker.

3.2.9 Cloning and sequencing

PCR products were purified using the Qiaquick PCR purification kit (Qiagen) if only single PCR products were visible or by the Qiaquick Gel purification kit (Qiagen) if non-specific PCR products were present in addition to the expected PCR product. Purified PCR products generated by specific primers were sequenced directly. PCR products generated using degenerate primers were cloned using the pGEM-T Easy Vector system according to the manufacturer's protocol (Promega). Transformants were screened and selected using standard protocols for blue/white selection (Sambrook et al 1989). To confirm their identity, the cloned products were sequenced using primers SP6 and/or T7. Sequences were analysed using BlastN (Altschul et al 1997) to determine their identity (Joyce et al 2006).

Table 3.3. The list of endemic and exotic pathogens tested for, the type of PCR test, the primers used, the annealing temperature, the region amplified, expected product size and the reference for each test.

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
Housekeeping gene assays								
RNA- NADH dehydrogenase mRNA	One-step RT-PCR	AtropaNad2.1a	F	GGACTCCTGACGTATACGAAGGATC	55°C	NADH dehydrogenase ND2 subunit	188bp	Thompson et al 2003.
		AtropaNad2.2b	R	AGCAATGAGATTCCCCAATATCAT				
DNA- 16S rRNA gene	PCR	FD2	F	AGAGTTTGATCATGGCTCAG	45°C	16S rRNA gene	1400-1500bp	Weisberg et al 1991.
		RP1	R	ACGGTTACCTTGTTACGACTT				
Endemic viruses								
Apple chlorotic leaf spot virus	One-step RT-PCR	ACLSV A52	F	GCGAACCCTGGAACAGA	53°C	Coat protein gene	358bp	Candresse et al 1995.
		ACLSV A53	R	CAGACCCTTATTGAAGTCGAA				
Apple mosaic virus	One-step RT-PCR	ApMV1	F	TGGATTGGGTTGGTGGAGGAT	53°C	Coat protein gene	261bp	Petrzik and Svoboda 1997.
		ApMV2	R	TAGAACATTCGTCGGTATTTG				
Apricot pseudo chlorotic leaf spot virus	Nested RT-PCR	PDO-F1i	F	TiTTYATKAARWSiCARYWiTGiAC	42°C	RNA-dependent RNA polymerase gene	446bp, 631bp	Foissac et al 2001.
		PDO-R3i	R	GCRCACTRTCRTCiCCiGCRAAiiA				
		PDO-R4i	R	ARiYiCCATCCRCARAAMiTiGG				
		NT1	F	ARATACTYTCMARYTGTCTRC	58°C		218bp	Liberti et al 2004.
		NT3	R	ATKATTTTYTCATCCCABCCY				
Apple stem grooving virus	One-step RT-PCR	CTLV AM	F	CCTGAATTGAAAACCTTTGCTGCCACTT	60°C	Coat protein gene	456bp	Ito et al 2002.
		CTLV AP	R	TAGAAAAACCACACTAACCCGGAAATGC				
Apple stem pitting virus	One-step RT-PCR	Forward sense	F	ATGTCTGGAACCTCATGCTGCAA	55°C	Coat protein gene	370bp	Menzel et al 2002.
		Reverse antisense	R	TTGGGACAACTTTACTAAAAAGCATAA				
Cherry green	One-	CGRMV1	F	CCTCATTCACATAGCTTAGGTTT	55°C	Coat protein	958bp	Li and

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
ring mottle virus	step RT-PCR	CGRMV2	R	ACTTTAGCTTCGCCCCGTG		gene		Mock 2005.
Cherry necrotic rusty mottle virus	One-step RT-PCR	CNRMV-7626F	F	TCCCACCTCAAGTCCTAGCAG	58°C	Coat protein gene	584bp	Osman et al 2012.
		CNRMV-8210R	R	TGAACTTGGCCAGTTCTGCC				
Cherry virus A	One-step RT-PCR	CVA-6170 F	F	AGCCAGAAGGTATCATGCCAG	54°C	Coat protein gene	566bp	Osman et al 2012.
		CVA-6736 R	R	ATGACATGCCTGCTGGGAG				
Cucumber mosaic virus	One-step RT-PCR	Forward CMV1	F	TATGATAAGAAGCTTGTTTCGCGCA	55°C	Coat protein gene	501bp	Wylie et al 1993.
		Reverse CMV2	R	TTTTAGCCGTAAGCTGGATGGACAACCC				
Little cherry virus-2	One-step RT-PCR	LCV2 UP2	F	CTCGGCGTATATGGTGGATGTTTA	55°C	RdRp gene	438bp	Rott and Jelkmann 2001.
		LCV2 LO2	R	CCGAATGCAGTGGGGATAGG				
	One-step RT-PCR	LCH2-01F	F	AGACGCGCAGAGGAGGAC	55°C	RdRp gene	180bp	Jelkmann et al 2008.
		LCH2-03R	R	TCCAAACTCAACTTAAAGAAATCAAAATA				
Plum bark necrosis stem pitting associated virus	One-step RT-PCR	PBNSPaV ASP1	F	CGGTAGGGCTGTGACTACCG	52°C	HSP70 gene	290bp	Abou Ghanem-Sabaradzovic et al 2001.
		PBNSPaV ASP2	R	GTAGTCCGCTGGTACGCTACAAG				
	One-step RT-PCR	PBNSPaV detF	F	TACCGAAGAGGGTTTGGATG	56°C	HSP70 gene	400bp	Al Rwahnih et al 2007.
		PBNSPaV detR	R	AGTCGCACCACCAGTCTTCT				

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
Prune dwarf virus	One-step RT-PCR	PDV F	F	TAGTGCAGGTTAACCAAAAGGAT	62°C	Coat protein gene	172bp	Parakh et al 1995.
		PDV R	R	ATCGATGGGATGGATAAAATAGT				
Prunus necrotic ring spot virus	One-step RT-PCR	PNRSV F	F	ACGCGCAAAAGTGTCGAAATCTAAA	54°C	Coat protein gene	455bp	Mackenzie et al 1997.
		PNRSV R	R	TGGTCCCACTCAGAGCTCAACAAAG				
Exotic viruses and viroids								
Arabis mosaic virus	One-step RT-PCR	M2	F	YTRGATTTTAGGCTCAATGG	42°C	Movement protein gene	290bp	Wetzel et al 2002.
		M3	R	TGYAARCCAGGRAAGAAAAT				
Apricot latent virus	One-step RT-PCR	ApLV1	F	CCCGACCATGGCTACAAGC	50°C	Coat protein gene	1500bp	Garcia-Ibarra et al 2010.
		ApLV2	R	TTGCCGTCCCGATTAGGTTG				
American plum line pattern virus	One-step RT-PCR	VP 340	F	GGTCGTCAAGGGAGAGGC	50°C	Coat protein gene	563bp	Sanchez-Navarro et al 2005.
		VP 339	R	GGCCCCTAAGGGTCATTTC				
Asian Prunus virus(es)	One-step RT-PCR	CP-PLV1	F	KCRGTKATCAAAAAGCATAC	48°C	Partial CP gene	262bp	Marais et al 2006.
		CP-PLV2	R	AATCCATYTCCTTCCCCTTCAA				
Cherry leaf roll virus	One-step RT-PCR	CLRV-5	F	TGGCGACCGTGTAACGGCA	53°C	RNA2	416bp	Werner et al 1997.
		CLRV-3	R	GTCGGAAAGATTACGTAAAAGG				
Cherry mottle leaf virus	One-step RT-PCR	CML13A	F	GCCTGATCAGCAAAGTGAAG	60°C		848bp	James et al 1999.
		CML4A	R	CGGTCTGAAGCACAATGC				
Cherry rasp leaf virus	One-step RT-	JQ3D3FF	F	GCCAGTTTCTCCAGTGAACC	50°C	RNA-2	429bp	James et al 2001
		JQ3D3FR	R	CAGTTGAAACGGATTTAAAC				

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
	PCR							
Cherry rusty mottle associated virus	One-step RT-PCR	CRM 91CPF	F	GGGCCCGAYCCTGTCATTCC	60°C	Coat protein gene	695bp	Villamor and Eastwell 2013.
		NGRRM-TL CPR	R	ATNGGTTGAATTTGGCCAGT				
Cherry twisted leaf associated virus 1a	One-step RT-PCR	CTL-1a 218CPF	F	TCAGCAAGATTAAGGAGGTTG	60°C	Coat protein gene	562bp	Villamor and Eastwell 2013.
		NGRRM-TL CPR	R	CTNGGTTGAATTTGGCCAGT				
Cherry twisted leaf associated virus 1b	One-step RT-PCR	CT-1b 235CPF	F	TCGGACCCTACAACCCTCAATG	60°C	Coat protein gene	545bp	Villamor and Eastwell 2013.
Little cherry virus-1	One-step RT-PCR	LC1-9135F	F	TCTGCTGCTGCYATGCATCA	55°C	HSP70-like gene	723bp	Alison Dann, unpublished
		LC1-9858R	R	AWACACAAGCAGCAGTGGMA				
Peach mosaic virus	One-step RT-PCR	PM-AF1	F	TCACCTTCTGCAGATACGAAGTA	59°C	Replicase coding region	383bp	James et al 2006.
		PM-AFR	R	GCTGTTCTTCACAAAGAATCTA				
Peach rosette mosaic virus	One-step RT-PCR	PRMVF5321	F	ATTGGTCGCCGCTCTATTT	57°C	Polyprotein gene	388bp	Lebas and Ward 2012.
		PRMVR5699	R	CAACAACAAGCCCATTCTCC				
Plum pox virus	One-step RT-PCR	PPV P1	F	ACCGAGACCACTACACTCCC	60°C	Polyprotein gene	243bp	Wetzel et al 1991.
		PPV P2	R	CAGACTACAGCCTCGCCAGA				
Raspberry ring spot virus	One-step RT-PCR	RpRSV942F	F	CAGAGTATGGGTGATTTCTGG	55°C	Polyprotein gene	800bp	Lebas and Ward 2012.
		RpRSV1741R	R	TCCTTCTCCCAGGTCTGCAC				

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
Strawberry latent ring spot virus	One-step RT-PCR	SLRSV-5D	F	CCCTTGGTTACTTTTACCTCCTCATTGTCC	55°C	Coat protein gene	293bp	Faggioli et al 2002.
		SLRSV-3D	R	AGGCTCAAGAAAACACAC				
Tobacco ring spot virus	One-step RT-PCR	MF05-22-F	F	CAATACGGTAAGTGCACACCCCG	50°C	RdRp gene	320bp	Fuchs et al 2010
		MF05-21-R	R	CAGGGGCGTGAGTGGGGGCTC				
Tomato ringspot virus	One-step RT-PCR	U1	F	GAC GAA GTT ATC AAT GGC AGC	55°C	RNA-1 region	450 bp	Griesbach 1995
		D1	R	TCC GTC CAA TCA CGC GAA TA				
Tomato black ring virus	One-step RT-PCR	TBRV 70F	F	GCTCGTAACAGTTGCGGAGATAT	62°C	Polyprotein (P2) gene	73bp	Harper et al 2011.
		TBRV 70R	R	TGTCCACACTGTCATGGGA				
Tomato bushy stunt virus	One-step RT-PCR	TomCPF	F	CCGCCGTAGCATGACCAAGTA	55°C	Putative CP gene	1220bp	Russo et al 2002.
		TomCPR	R	CCATGAACTGGTCTTGTTCAA				
Apple scar skin viroid	One-step RT-PCR	VirPom C	F	CAGCACCACAGGAACCTGACGG	55°C	Whole genome	267bp	Faggioli and Ragozzino 2002.
		VirPom H	R	TCGTCGTCGACGAAGG				
Hop stunt viroid	One-step RT-PCR	HSV-83M	F	AACCCGGGGCTCCTTTCTCA	55°C	Complete genome	450bp	Sano et al 2001.
		HSV-78P	R	AACCCGGGGCAACTCTTCTC				
Peach latent mosaic viroid	One-step RT-PCR	cPLMVd	F	AACTGCAGTGCTCCGT	60°C	Whole genome	337bp	Shamloul et al 1995.
		hPLMVd	R	CCCGATAGAAAGGCTAAGCACCTCG				
	One-step RT-PCR	RF-43	F	CTGGATCACACCCCCTCGGAACCAACCGCT	60°C	Whole genome	340bp	Ambros et al 1998.
		RF-44	R	TGTGATCCAGGTACCGCCGTAGAAACT				

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
Exotic phytoplasma and bacteria								
Phytoplasma	Nested PCR	P1	F	AAGAGTTTGATCCTGGCTCAGGATT	56°C	16S-23S Ribosomal RNA gene	NA	Constable et al 2003.
		P7	R	CGTCCTTCATCGGCTCTT				
		R16F2n	F	GAAACGACTGCTAAGACTGG	56°C	16S-23S Ribosomal RNA gene	1600-2000bp	
		M23sr	R	TAGTGCCAAGGCATCCACTGT				
Agrobacterium	Multiplex PCR	UF f	F	CTAAGAAGCGAACGCAGGGACT	67°C	23S rDNA		Pulawska et el 2006.
		B1R r	R	GACAATGACTGTTCTACGCGTAA			189bp	
		B2R r	R	TCCGATACCTCCAGGGCCCCTCACA			1059bp	
		AvR640 r	R	AACTAACTCAATCGCGCTATTAAC			491bp	
		AvR1150 r	R	AAAACAGCCACTACGACTGTCTT			1173bp	
Erwinia amylovora	PCR	G1-F	F	CCTGCATAAATCAACCGCTGACAGCTCAATG	60°C	Hypothetical protein	187bp	Taylor et al 2001.
		G1-R	R	GCTACCACTGATCGCTCGAATCAAATCGGC				
	q-PCR	hpEaF	F	CCCGTGGAGACCGATCTTTTA	53°C	Hypothetical protein AMY1267	138bp	Gottsberger 2010.
		hpEaR	R	AAGTTTCTCCGCCCTACGAT				
		hpEaP	Probe	TCGTCGAATGCTGCCTCTCT				
Erwinia amylovora	LAMP	F3	F (Outer)	TCAAGATCGTGTGGCTATG	65°C	EAMY_3195	NA	Bühlmann et al 2013.
		B3	R (Outer)	CTAAAAACCGGGGCAAAC				
		loopF	F (Loop)	ACATTAGCGGCCCGACCAA				
		loopR	R (Loop)	CTRTTAAGATGGCATGCAGA				
		FIP	F (Inner)	ACGRTTCTACCCTTCCTGTCTACTTCTCTGGGGTTTCAGTC				
		BIP	R (Inner)	ATGTCACCTGATTCTACAGCCGCAATCATTCATGGTTCTGGAC				
Pseudomonas sp.	PCR	PsrpoD FNP1	F	TGAAGGCGARATCGAAATCGCCAA	55°C	RNA polymerase sigma factor rpoD gene	~700bp	Parkinson et al 2011.
		PsrpoDnprPCR1	R	YGCMGWCAGCTTYTGCTGGCA				
Xanthomona	Multipl	pXap41repA	F	GCGAGGACATGGCTTTCAC	55°C	pXap41- repA1	343bp	Pothier et

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
s arbicola pv. Pruni	ex PCR	1-F				gene		al 2011.
		pXap41repA 1-R	R	GCGGCCAAGGCGTGCATCTGC				
		pXap41repA 2-F	F	TACCAAGAGCGGCAACATCTGC		pXap41- repA2 gene	451bp	
		pXap41repA 2-R	R	TTTGGCCTTGCTGTAGAGCGT				
		pXap41mob-F	F	GCCTATCTGGCGAAGGTCGAG		pXap41-mobC gene	245bp	
		pXap41mob-R	R	GCTTGTAGCTCGGCCAGGATG				
Xylella fastidiosa	PCR	RST31	F	GCGTTAATTTTCGAAGTGATTCGA	55°C	RNA polymerase sigma factor gene	733bp	Minsavage et al 1994.
		RST33	R	CACCATTCGTATCCCGGTG				
	q-PCR	XF-F	F	CACGGCTGGTAACGGAAGA	62°C	rimM gene	70bp	Harper et al 2010.
		XF-R	R	GGGTTTGCGTGGTGAAATCAAG				
		XF-P	Probe	FAM-TCGCATCCCGTGGCTCAGTCC-BHQ				
Xylella fastidiosa	LAMP	XF-F3	F (Outer)	CCGTTGGAAAACAGATGGGA	65°C	rimM gene	149bp	Harper et al 2010.
		XF-B3	R (Outer)	GAGACTGGCAAGCGTTTGA				
		XF-LF	F (Loop)	TGCAAGTACACACCCTTGAAG				
		XF-LB	R (Loop)	TTCCGTACCACAGATCGCT				
		XF-FIP	F (Inner)	ACCCCGACGAGTATTACTGGGTTTTTCGCTACCGAGAACCACAC				
		XF-BIP	R (Inner)	GCGCTGCGTGGCACATAGATTTTTGCAACCTTTCCTGGCATCAA				
Generic tests								
Ampelovirus genus	Nested RT-PCR	dHSP up1	F	GGIHTIGAITTYGGIACIACITT	A	HSP70h gene	580-620bp	Dovas and Katis 2003.
		dHSP up1G	F	AGTTYGGGACGACGTT				

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
		dHSP do2	R	GTICCICCICCNAARTC				
		dHSP do2c	R	GTICCICCCCCNAARTC				
		dHSP nest2		TYGGGACGACGTTYTCNAC	A	HSP70 gene	490bp	Maliogka et al 2008.
		LR5 clusdoL		GGYTCRTTCACIACIGCYTGIAC				
Closterovirid ae family	Nested RT-PCR	dHSP up1	F	GGIHTIGAITTYGGIACIACITT	A	HSP70 gene	580-620bp	Dovas and Katis 2003b.
		dHSP up1G	F	AGTTYGGGACGACGTT				
		dHSP do2	R	GTICCICCICCNAARTC				
		dHSP do2C	R	GTICCICCCCCNAARTC				
		dHSP nest1	F	TTYGGGACGACGTTYAGYAC	A	HSP70 gene	500-535bp	
		dHSP nest2	F	TYGGGACGACGTTYTCNAC				
		dHSP nest3	R	SCIGCIGMISWIGGYTCRTT				
		dHSP nest3G	R	GCGGMGSWGGGPTCRTT				
Foveavirus genus	Nested RT-PCR	dRW up1	F	WGCIAARGCIGGICARAC		RNA-dependent RNA polymerase gene	363bp	Dovas and Katis 2003a.
		dRW do2	R	RMYTCICCISWRAAICKCAT				
		dRW do2G	R	GCCGSWRAAGCKCAT				Dovas and Katis 2003b.
		dRW nest1	F	GGGGCARACIHTIGCITGYTT			198bp	Dovas and Katis 2003a.
		dRW nest2	R	AAIGCYTCRTARTCIGAITCNGT				
Capillovirus Foveavirus Trichovirus genera	Nested RT-PCR	PDO-F1i	F	TiTTYATKAARWSiCARYWiTGiAC	42°C	RNA-dependent RNA polymerase gene	446bp, 631bp	Foissac et al 2001.
		PDO-R3i	R	GCRCACTRTCRTCiCCiGCRAAiiA				
		PDO-R4i	R	ARiYiCCATCCRCARAAMiTiGG				
		PDO-F2i	F	GCYAARGCiGGiCARACiyTKGCiTG	42°C		362bp	
		PDO-R1i	R	TCHCCWGTRAAiCKSATiAiiGC				
Ilarvirus genus	Nested RT-PCR	Ilapol up2	F	YTCIAMRTTYGAYAARTC	A	RNA2-encoded RdRp	381bp	Maliogka et al 2007.
		Ilapol do4	R	GGYTGRTTRTGIGGRAA				
		Ilapones up3	F	TCGAMRTTYGAYAARTCICA	A	RNA2-encoded	371bp	

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
		Ilapones d5	R	TGGGGRAAYTTIGYYTCRA		RdRp		
Trichovirus	One-step RT-PCR	Tricho F	F	GCCTGATCAAAATGTTCAAGAC	55°C	Coat protein	442bp	Renae Sarec unpublished
		Tricho R	R	CACTCCAATATTGGTTAGGTCC				

Table 3.4 The cycling conditions used for detection of each pathogen by specific RT-PCR and PCR assays

Housekeeping or pathogen assay	Pre-cycling conditions- 1 cycle		PCR-cycling conditions- 35 cycles			Post-cycling conditions- 1 cycle	
Step	**Reverse transcription**	**Initial denaturation**	**Denaturation**	**Annealing**	**Elongation**	**Final elongation**	**Hold**
Temperature	**48°C**	**94°C**	**94°C**	**See table x for temperatures**	**72°C**	**72°C**	**20°C**
Housekeeping gene assays							
NADH dehydrogenase mRNA	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	Indefinite
16S rRNA gene	NA	1 minute[1]	45 seconds[1]	30 seconds	30 seconds	10 minutes	Indefinite
Endemic viruses							
Apple chlorotic leaf spot virus	45 minutes	2 minutes	30 seconds	45 seconds	1 minute	10 minutes	Indefinite
Apple mosaic virus	45 minutes	2 minutes	30 seconds	45 seconds	1 minute	10 minutes	Indefinite
Apple stem grooving virus	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	10 minutes	Indefinite
Apple stem pitting virus	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	10 minutes	Indefinite
Cherry virus A- 6170F/ 6736R	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	10 minutes	Indefinite
Cucumber mosaic virus	45 minutes	2 minutes	30 seconds	45 seconds	1 minute	7 minutes	Indefinite
Cherry green ring mottle virus	45 minutes	2 minutes	30 seconds	1 minute	1 minute	10 minutes	Indefinite
Cherry necrotic rusty mottle	45 minutes	2 minutes	30 seconds	45 seconds	1 minute	7 minutes	Indefinite

Housekeeping or pathogen assay	Pre-cycling conditions- 1 cycle		PCR-cycling conditions- 35 cycles			Post-cycling conditions- 1 cycle	
Step	**Reverse transcription**	**Initial denaturation**	**Denaturation**	**Annealing**	**Elongation**	**Final elongation**	**Hold**
Temperature	**48°C**	**94°C**	**94°C**	**See table x for temperatures**	**72°C**	**72°C**	**20°C**
virus							
Little cherry virus 2- UP2/LO2 primers	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	5 minutes	Indefinite
Little cherry virus 2- 01F/ 03R primers	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	5 minutes	Indefinite
Plum bark necrosis stem pitting associated virus- ASP1/ASP2	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	7 minutes	Indefinite
Plum bark necrosis stem pitting associated virus- det-F/R	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	10 minutes	Indefinite
Prune dwarf virus	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	5 minutes	Indefinite
Prunus necrotic ring spot virus	45 minutes	2 minutes	30 seconds	45 seconds	1 minute	7 minutes	Indefinite
Exotic viruses and viroids							
American plum line pattern virus	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	7 minutes	Indefinite
Apricot latent virus	45 minutes	2 minutes	45 seconds	45 seconds	1 minute	10 minutes	Indefinite
Arabis mosaic virus	45 minutes	2 minutes	20 seconds	20 seconds	30 seconds	5 minutes	Indefinite
Asian Prunus virus(es)	45 minutes	2 minutes	30 seconds	30 seconds[2]	1 minute	7 minutes	Indefinite
Cherry leaf roll virus- CLRV-3/ CLRV-5 primers	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	7 minutes	Indefinite
Cherry mottle leaf virus	45 minutes	2 minutes	45 seconds	45 seconds	1 minute	10 minutes	Indefinite
Cherry rasp leaf virus	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	10 minutes	Indefinite
Cherry rusty mottle associated	45 minutes	2 minutes	45 seconds	45 seconds	45 seconds	5 minutes	Indefinite

Housekeeping or pathogen assay	Pre-cycling conditions- 1 cycle		PCR-cycling conditions- 35 cycles			Post-cycling conditions- 1 cycle	
Step	**Reverse transcription**	**Initial denaturation**	**Denaturation**	**Annealing**	**Elongation**	**Final elongation**	**Hold**
Temperature	**48°C**	**94°C**	**94°C**	**See table x for temperatures**	**72°C**	**72°C**	**20°C**
virus							
Cherry twisted leaf associated virus 1a	45 minutes	2 minutes	45 seconds	45 seconds	45 seconds	5 minutes	Indefinite
Cherry twisted leaf associated virus 1b	45 minutes	2 minutes	45 seconds	45 seconds	45 seconds	5 minutes	Indefinite
Little cherry virus-1	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	10 minutes	Indefinite
Peach mosaic virus	45 minutes	2 minutes	30 seconds[2]	45 seconds[2]	1 minute[2]	10 minutes	Indefinite
Peach rosette mosaic virus	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	7 minutes	Indefinite
Plum pox virus	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	Indefinite
Raspberry ring spot virus	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	7 minutes	Indefinite
Strawberry latent ring spot virus	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	Indefinite
Tobacco ring spot virus	45 minutes	2 minutes	30 seconds	1 minute	1 minute	10 minutes	Indefinite
Tomato ringspot virus	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	indefinite
Tomato black ring virus	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	Indefinite
Tomato bushy stunt virus	45 minutes	2 minutes	1 minute	2 minutes	2 minutes	10 minutes	Indefinite
Apple scar skin viroid	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	5 minutes	Indefinite
Peach latent mosaic viroid- cPLMVd/ hPLMVd	45 minutes	2 minutes	45 seconds[4]	1 minute[4]	2 minutes[4]	7 minutes	Indefinite
Peach latent mosaic viroid- RF-43/RF-44	45 minutes	2 minutes	40 seconds[4]	30 seconds[4]	2 minutes[4]	10 minutes	Indefinite
Hop stunt viroid	45 minutes	2 minutes	1 minute	2 minutes	2 minutes	10 minutes	Indefinite
Bacteria							

Housekeeping or pathogen assay	**Pre-cycling conditions- 1 cycle**		**PCR-cycling conditions- 35 cycles**			**Post-cycling conditions- 1 cycle**	
Step	**Reverse transcription**	**Initial denaturation**	**Denaturation**	**Annealing**	**Elongation**	**Final elongation**	**Hold**
Temperature	**48°C**	**94°C**	**94°C**	**See table x for temperatures**	**72°C**	**72°C**	**20°C**
Agrobacterium	NA	1 minute	1 minute	1 minute	1.5 minutes	10 minutes	Indefinite
Erwinia amylovora- hpEaf/ hpEaR/ hpEap primers and probe (qPCR)	15 minutes[3] (not an RT step)	5 minutes[1]	30 seconds[1, 2]	30 seconds[2]	30 seconds[2]	NA	NA
Erwinia amylovora- G1-F/ G2-R primers	NA	5 minutes[1]	30 seconds[1]	20 seconds	1 minute	5 minutes	Indefinite
Pseudomonas sp	NA	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	Indefinite
Xanthomonas	NA	5 minutes	30 seconds	30 seconds	30 seconds	7 minutes	Indefinite
Xylella fastidiosa- RST31/ RST33 primers	NA	1 minute[1]	45 seconds[1]	30 seconds	30 seconds	10 minutes	Indefinite
Xylella fastidiosa- XF-F/ XF-R/ XF-P primers and probe (qPCR)	NA	3 minutes[1]	10 seconds[1,2]	40 seconds[2]	NA	NA	NA

1. 95°C instead of 94°C
2. 40 cycles instead of 35 cycles
3. 50 minutes instead of 45 minutes
4. 30 cycles instead of 35 cycles
5. 50°C instead of 94°C

Table 3.5. The PCR cycling conditions used for the detection of pathogens using generic and nested PCR tests.

Generic/ nested assay	Pre-cycling conditions- 1 cycle			PCR cycling conditions						Post-cycling conditions- 1 cycle	
	Step 1	Step 2	Step 3	No of PCR cycles	Step 1	Step 2	Step 3	Step 4	Step 5	Final extension	Hold
Phytoplasma Round 1 RT-PCR	94°C for 1 min	NA	NA	35	94°C for 1 min	56°C for 1 min	72°C for 1.5 min			72°C for 5 min	20°C
Phytoplasma Round 2 PCR	94°C for 1 min	NA	NA	35	94°C for 1 min	56°C for 1 min	72°C for 1.5 min			72°C for 5 min	20°C
Ilarvirus Round 1 RT-PCR	42°C for 50 min	94°C for 4 min	NA	5	94°C for 30 sec	38°C for 30 sec	72°C for 20 sec			72°C for 2 min	20°C
				35	94°C for 30 sec	42°C for 20 sec	38°C for 10 sec	72°C for 20 sec			
Ilarvirus Round 2 PCR	94°C for 2 min	NA	NA	5	94°C for 30 sec	43°C for 30 sec	72°C for 20 sec			72°C for 2 min	20°C
				35	94°C for 30 sec	47°C for 20 sec	43°C for 10 sec	72°C for 20 sec			
Ampelovirus Round 1 RT-PCR	42°C for 55 min (RT)	50°C for 2 min	94°C for 4 min	10	94°C for 30 sec	38°C for 30 sec	72°C for 20 sec			72°C for 2 min	20°C
				30	94°C for 30 sec	49°C for 10 sec	44°C for 10 sec	38°C for 15 sec	72°C for 20 sec		
Ampelovirus Round 2 PCR	94°C for 4 min	NA	NA	5	94°C for 30 sec	55°C for 30 sec	72°C for 20 sec			72°C for 2 min	20°C
				39	94°C for 30 sec	59°C for 15 sec	55°C for 15 sec	72°C for 20 sec			
Closterovirus Round 1 RT-PCR	48°C for 30 min (RT)	50°C for 2 min	94°C for 4 min	5	94°C for 30 sec	43°C for 10 sec	38°C for 5 sec	72°C for 20 sec		72°C for 2 min	20°C
				35	94°C for 30 sec	43°C for 30 sec	72°C for 30 sec	NA			
Closterovirus	94°C for 3	48°C for	72°C for	39	94°C for	54°C for	72°C for 10 sec (+1s			72°C for 2 min	20°C

Generic/ nested assay	Pre-cycling conditions- 1 cycle			PCR cycling conditions						Post-cycling conditions- 1 cycle	
	Step 1	**Step 2**	**Step 3**	**No of PCR cycles**	**Step 1**	**Step 2**	**Step 3**	**Step 4**	**Step 5**	**Final extension**	**Hold**
Round 2 PCR	min	15 sec	15 sec		30 sec	15 sec	after each cycle)				
Foveavirus Round 1 RT-PCR	42°C for 60 min (RT)	50°C for 2 min	94°C for 4 min	5	95°C for 30 sec	43°C for 10 sec	38°C for 5 sec	72°C for 15 sec		72°C for 2 min	20°C
				35	95°C for 30 sec	43°C for 30 sec	72°C for 20 sec	NA			
Foveavirus Round 2 PCR	95°C for 3 min	48°C for 15 sec	72°C for 15 sec	39	95°C for 30 sec	54°C for 30 sec	72°C for 10 sec (+1s after each cycle)			72°C for 2 min	20°C
Trichovirus, Capillovirus and *Foveavirus* Round 1 RT-PCR	48°C for 30 min (RT)	95°C for 3 min	NA	35	95°C for 30 sec	42°C for 30 sec	72°C for 30 sec			72°C for 7 min	20°C
Trichovirus, Capillovirus and *Foveavirus* Round 2 PCR	95°C for 3 min	NA	NA	30	95°C for 45 sec	42°C for 30 sec	72°C for 30 sec			72°C for 10 min	20°C
Apricot pseudo chlorotic leaf spot virus Round 1 RT-PCR	48°C for 45 min (RT)	95°C for 3 min	NA	35	95°C for 30 sec	42°C for 30 sec	72°C for 30 sec			72°C for 7 min	20°C
Apricot pseudo chlorotic leaf spot virus	95°C for 3 min	NA	NA	35	95°C for 30 sec	58°C for 30 sec	72°C for 30 sec			72°C for 10 min	20°C

Generic/ nested assay	**Pre-cycling conditions- 1 cycle**			**PCR cycling conditions**						**Post-cycling conditions- 1 cycle**	
	Step 1	**Step 2**	**Step 3**	**No of PCR cycles**	**Step 1**	**Step 2**	**Step 3**	**Step 4**	**Step 5**	**Final extension**	**Hold**
Round 2 PCR											
Trichovirus Specific RT-PCR	48°C for 45 min (RT)	94°C for 2 min	NA	35	94°C for 30 sec	55°C for 1 min	72°C for 1 min			72°C for 8 min	20°C

3.2.10 LAMP

LAMP (Loop Mediated Isothermal Amplification) assays *for Xylella fastidiosa* (Harper et al 2012) and *Erwina amylovora* (Bühlmann et al 2013) were assessed using Isothermal Master Mix from OptiGene (GeneWorks, Adelaide, South Australia, Australia). Primer concentrations used for the LAMP assays were exactly as published. The primers are listed in Table 3.3 and the cycling conditions are given in Table 3.6

Table 3.6. LAMP cycling conditions used for the detection of housekeeping mRNA and DNA and pathogens.

(LAMP ASSAYS)	**Pre-cycling conditions- 1 cycle**			**Post-cycling conditions- 1 cycle**	
Step	**Reverse transcription**	**Initial denaturation**	**Annealing**	**Enzyme inactivation**	**Melting curve**
Temperature	**48°C**	**94°C**			
Erwinia amylovora	NA	NA	65°C for 60 minutes	NA	95 to 70°C
Xylella fastidiosa	NA	NA	65°C for 60 minutes	80°C for 2 minutes	

3.2.11 Detection of viruses in pollen

Sampling:

Flowers were collected from two ornamental cherry trees and two Satsuma plum trees of unknown disease status, one domestic plum infected with PDV and one rose plant that was infected with PNRSV. Anthers, containing pollen, were separated from the remainder of the flowers of each tree or plants The method described by was chosen. RNA was extracted from 1mg of anther/pollen tissue using the RNeasy Plant Mini Kit (Schiller et al 2010), using a modified lysis buffer (MacKenzie et al 1997) instead of the buffer supplied with the kit. Prior to RNA isolation each sample was ground in the lysis buffer using a Homex homogeniser.

All extracts were tested using the NADH RNA quality check RT-PCR protocols using a one-step RT-PCR kit with Platinum Taq DNA polymerase as described above. The cherry samples were also tested using the malate dehydrogenase mRNA quality check RT-PCR (Nassuth et al 2000, Constable et al 2007). The samples were then tested by RT-PCR for PNRSV (Mackenzie et al 1997).and PDV (Parakh et al 1995) as described above.

3.3 Results and discussion

3.3.1 Extraction

All samples, except pollen, were extracted on the QIAxtractor as described previously (Constable et al 2012) except that a lower sample volume (200µl of lysate/ ethanol mix instead of 500µl) was used. This volume modification allowed the sample homogenate to flow more freely and prevented blocking of the filter plate, which can lead to unsuccessful nucleic acid isolation. The Qiaextractor is a high throughput nucleic acid extraction platform, which can be used to extract total nucleic acid (containing DNA and RNA) from up to 96

samples simultaneously. Both the NAD (RNA) and 16S (DNA) quality check RT-PCR and PCR assays indicated that RNA and DNA were present in each extract of the 101 samples that were extracted using the QIAxtractor. The production of a single total nucleic acid extract containing both DNA and RNA can reduce the cost of testing because only one extract is required for downstream in RT-PCR and PCR assays for detection of RNA and DNA pathogens.

3.3.2 Pathogen testing – pathogens not detected

The following viruses and viroids which are of quarantine concern to almonds and/or summerfruit species in Australia were not detected during the survey: ApLV, ArMV, APLPV, APruV1, APruV2, APruV3, CLRV, CMLV, CRMaV, CTLaV, LChV1, PPV, PRMV, PcMV, RRSV, SLRSV, TBRV, TBSV, ToRSV and ASSVd. *Xylella fastidosa*, *Erwinia amylovora*, *Xanthomonas arboricola pv. Pruni* and Phytoplasmas were also not detected.

There have been reports of ASPV and CMV occurring in *Prunus* species (Appendix 2) in other countries. ASPV and CMV occur in Australia in other plant species but they were not detected in any *Prunus* species during the survey and they should not require testing within Australian high health programs. However the tests have been validated under Australian conditions and can be used for ASPV and CMV detection if infection is suspected in almonds and summerfruit species.

3.3.3 Pathogens present in Australia

The results of testing for pathogens present in Australia are given in tables 3.7. Viruses , viroids and/or bacteria were detected 71/101 samples. The pathogens that were detected include ACLSV, ApMV, APCLSV, ASGV, CGRMV, CNRMV, CVA , LChV2, PBNSPaV, PDV, PNRSV, an uncharacterised *Ilarvirus*, HSVd, PLMVd, *Pseudomonas syringae* and *Agrobacterium tumefaciens*. This is the first report of CGRMV, CNRMV, CVA and PBNSPaV in Australia. ASGV is endemic and infects pome and citrus fruit specis and this survey provides the first evidence that it can also infect *Prunus* species in Australia.

Mixed infections of viruses and/or viroids were observed in 24/101 trees as follows:

- 10/24 trees with two viruses/viroids: 4/24 PNRSV+ACLSV; 3/24 PNRSV+CVA; 2/24 PNRSV+PBNSPaV; 1/24 ACLSV+HSVd
- 10/24 trees with three viruses/viroids: 3/24 PNRSV+ACLSV+CVA; 2/24 PNRSV+PBNSPaV+HSVd; 2/24 PNRSV+CVA+HSVd; 1/24 PNRSV+ PDV+CVA; 1/24 PNRSV+ACLSV+HSVd; 1/24 PNRSV+LChV2+CGRMV
- 2/24 with four viruses/viroids: 1/24 PNRSV+ACLSV+ASGV+PBNSPaV; 1/24 PNRSV+CVA+CNRMV+CGRMV
- 2/24 with five viruses/viroids: 1/24 PNRSV+PDV+LChV2+CVA+PBNSPaV; 1/24 PNRSV+LChV2+CVA+CNRMV+CGRMV

Table 3.7 The number of each *Prunus* trees in which viruses and bacteria were detected using specific RT-PCR or PCR tests for each pathogen and the number in which an uncharacterised Ilarvirus were detected.

Crop type	Almond	Apricot	Cherry	Cherry plum	Nectarine	Peach	Peach almond hybrid	Plum	Rose	Total No samples
No of samples tested per crop	33	7	13	2	8	16	2	19	1	101
Pathogen*	The number of samples in which each pathogen was detected									Total No detections
ACLSV	0	2	3	0	1	2	0	5	0	13
ApMV	1	0	0	0	0	0	0	0	0	1
APCLSV	0	0	0	0	1	0	0	1	0	2
ASGV	0	0	0	0	0	0	0	1	0	1
PNRSV	17	6	8	1	4	3	2	10	1	52
PDV	0	0	1	1	0	1	0	0	0	3
CVA	0	4	10	1	0	0	0	1	0	16
CNRMV	0	0	2	0	0	0	0	0	0	2
CGRMV	0	0	3	0	0	1	0	0	0	4
LChV2	0	0	3	0	0	0	0	0	0	3
PBNSPaV	0	0	1	0	0	0	0	3	0	4

Crop type	Almond	Apricot	Cherry	Cherry plum	Nectarine	Peach	Peach almond hybrid	Plum	Rose	Total No samples
Unknown *Ilarvirus*	0	0	0	1	4	0	0	1	0	653
HSVd	1	3	0	0	1	0	0	4	0	9
PLMVd	0	0	0	0	4	2	0	0	0	6
Pseudomonas *syringae*	0	0	1	0	0	0	0	1	0	2
Agrobacterium tumefaciens	1	0	0	0	0	0	0	0	0	1

* Virus acronyms and full names: ACLSV: *Apple chlorotic leafspot virus*, ApMV: *Apple mosaic virus*, APCLSV: *Apricot pseudochlorotic leafspot virus*, ASGV: *Apple stem grooving virus*, CGRMV: *Cherry green ring mottle virus*, CNRMV: *Cherry necrotic rusty mottle virus,* CVA: *Cherry virus A*, LChV2 *Little cherry virus 2*, PBNSPaV: *Plum bark necrosis stem pitting associated virus*, PDV: *Prune dwarf virus*, PNRSV: *Prunus necrotic ringspot virus*, HSVd: *Hop stunt viroid*, PLMVd: *Peach latent mosaic viroid*

Most specific pathogen assays that were validated during the survey appeared to be reliable – generating very few false positive results. The exceptions were the specific assays for APLPV (Sanchez-Navarro et al 2005), HSVd (Sano et al 2001) and CNRMV (Osman et al 2012). These assays can generate a number of non-specific PCR products of various sizes, including some that are similar to the expected size. This result was confirmed by sequencing. Therefore the use of these assays is not recommended. Another assay for each pathogen will be validated.

Sequencing and analysis using BlastN confirmed the identity of Australian isolates of ACLSV, APCLSV, ASGV, CGRMV, CNRMV, CVA , LChV2, PBNSPaV, PDV, PNRSV, an uncharacterised *Ilarvirus* detected using the generic *Ilarvirus* test, HSVd, PLMVd, *Pseudomonas syringae* and *Agrobacterium tumefaciens* (Table 3.8). There was genetic diversity observed within Australian isolates of ACLSV, APCLSV, *A. tumefaciens*, CGRMV, CNRMV, CVA, LChV2, PDV, PLMVd and PNRSV, based on the region of each pathogen that was amplified and there was diversity between isolates the Australian isolates and isolates from other countries that have been sequenced. Australian isolates of PBNSPaV and HSVd appeared to be more conserved when they were compared with each other.

ASGV occurs in Australia in pome fruit and a strain also occurs in Citrus. ASGV was detected in one plum tree from Victoria and this is a first report of ASGV occurring in any *Prunus* species in Australia. Its economic impact in plum is unknown. The ASGV isolate from plum had a 99% sequence similarity over 456 bases of the coat protein gene with an ASGV isolate from apple in Germany (GenBank Accession JX080201).

3.3.5 Ilarvirus detection

PNRSV PDV and ApMV were detected in 50/101 trees, 3/101 trees and 1/101 trees respectively using specific RT-PCR assays (Table 3.7). All samples were tested for *Ilarvirus*, including ApMV, PDV, PNRSV and APLPV using a generic *Ilarvirus* test, which should detect all virus species in the genera (Maliogka et al 2008). PNRSV was also detected in 2/101 additional trees using the generic *Ilarvirus* test. PNRSV was not detected in these two trees using the specific test, indicating that genetic variation of the virus at the primer binding sites for the specific assay may affect its reliability. However the generic *Ilarvirus* assay is a nested PCR method in which a second PCR test is used to generate a PCR amplicon from the amplicon produced in the first PCR, which can increase sensitivity. It is possible that the nested generic assay amplified a PCR product from a virus that was below detectable levels if only one round of PCR is used. The generic assay did not detect the ApMV isolate that was detected with the specific assay nor did it detect three PNRSV isolates from peach/almond hybrid, almond and rose. These results indicate that the generic *Ilarvirus* assay and specific RT-PCR assays for PNRSV, PDV and ApMVshould be used to screen samples to ensure all strains of each virus are detected.

The generic assay also detected an uncharacterised *Ilarvirus* in peach, cherry plum and plum. Sequence analysis suggests that this virus is most closely related to *Parietaria mottle virus*

(Table 3.8). This virus has also been detected occasionally in almond, during routine diagnostic testing conducted by Crop Health Services. Further work, using next generation sequencing strategies will be done to determine the identity of this virus. The significance and impact of this virus in Australian *Prunus* species is not known.

Table 3.8 The results of sequence analysis using BlastN, including the pathogen and GenBank accession to which they matched and the percentage (%) nucleotide Identity of pathogens detected in *Prunus* samples during the survey using pathogen specific or generic RT-PCR and PCR assays

Sample ID	Host	Assay	BlastN match to pathogen	% nucleotide Identity	GenBank Accession match	Country of origin	Source crop
TAS 1	Cherry	ACLSV	ACLSV	97	AY677106	Hungary	Wild cherry
TAS 2	Cherry	ACLSV	ACLSV	97	AY677106	Hungary	Wild cherry
VIC 10	Cherry	ACLSV	ACLSV	97	AY677106	Hungary	Wild cherry
WA 1	Peach	ACLSV	ACLSV	95	AY730560	Turkey	Sweet cherry
WA 3	Nectarine	ACLSV	ACLSV	94	AY730560	Turkey	Sweet cherry
VIC 2	Plum	ASGV	ASGV	99	JX080201	Germany	Apple
TAS 16	Cherry	CGRMV	CGRMV	97	KF030874	USA	Sweet cherry
TAS 4	Cherry	CGRMV	CGRMV	98	KC432616	Chile	Sweet cherry
VIC 5	Peach	CGRMV	CGRMV	99	EU272822	Poland	Peach
TAS 12	Cherry	CNRMV	CNRMV	97	KC136843	Poland	Sweet cherry
TAS 4	Cherry	CNRMV	CNRMV	97	KC136843	Poland	Sweet cherry
TAS 1	Cherry	CVA	CVA	99	X82547	N/A	Sweet cherry
TAS 2	Cherry	CVA	CVA	99	X82547	N/A	Sweet cherry
TAS 7	Cherry	CVA	CVA	99	FR718888	India	Sweet cherry
TAS 9	Cherry	CVA	CVA	95	HQ267857	France	Peach
TAS 12	Cherry	CVA	CVA	99	X82547	N/A	Sweet cherry
VIC 10	Cherry	CVA	CVA	99	X82547	N/A	Sweet cherry
VIC12	Plum	CVA	CVA	94	JN676229	Czech republic	Plum
VIC15	Apricot	CVA	CVA	95	JN676229	Czech republic	Plum
VIC18	Apricot	CVA	CVA	94	JN676229	Czech republic	Plum
WA2	Apricot	CVA	CVA	95	JN676229	Czech republic	Plum
NSW 13	Almond	HSVd	HSVd	99	KJ754184	China	Peach

Sample ID	Host	Assay	BlastN match to pathogen	% nucleotide Identity	GenBank Accession match	Country of origin	Source crop
QLD 15	Apricot	HSVd	HSVd	99	KJ754184	China	Peach
VIC 1	Plum	HSVd	HSVd	99	KJ754184	China	Peach
VIC 11	Nectarine	HSVd	HSVd	99	KJ754184	China	Peach
VIC 18	Apricot	HSVd	HSVd	99	KJ754184	China	Peach
VIC 19	Plum	HSVd	HSVd	99	KJ754184	China	Peach
VIC 20	Plum	HSVd	HSVd	99	KJ754184	China	Peach
WA 2	Apricot	HSVd	HSVd	99	KJ754184	China	Peach
TAS 11	Cherry	LChV2	LChV2	97	AF333237	Germany	Cherry
TAS 12	Cherry	LChV2	LChV2	98	AF333237	Germany	Cherry
TAS 16	Cherry	LChV2	LChV2	98	AF333237	Germany	Cherry
QLD 13	Plum	PBNSPaV	PBNSPaV	99	AF195501	N/A	N/A
QLD 2	Plum	PBNSPaV	PBNSPaV	99	JF305307	China	Plum
TAS 11	Cherry	PBNSPaV	PBNSPaV	99	JF810189	China	Plum
VIC 2	Plum	PBNSPaV	PBNSPaV	98	JF810189	China	Plum
QLD 10	Peach	PDV	PDV	95	JN703168	Egypt	Plum
QLD16	Nectarine	PLMVd	PLMVd isolate SDP2	86	KJ754183	China	Peach
SA5	Peach	PLMVd	PLMVd isolate SDP2	86	KJ754183	China	Peach
VIC11	Nectarine	PLMVd	PLMVd isolate SDP2	89	KJ754183	China	Peach
VIC14	Nectarine	PLMVd	PLMVd isolate SDP2	86	KJ754183	China	Peach
WA1	Peach	PLMVd	PLMVd isolate SDP2	83	KJ754183	China	Peach
WA3	Nectarine	PLMVd	PLMVd isolate SDP2	86	KJ754183	China	Peach
QLD 2	Plum	*Ilarvirus*	PNRSV	97	JN416775	Peach	Canada
TAS 3	Plum	*Ilarvirus*	PNRSV	98	GQ865664	N/A	New Zealand
VIC 21	Peach	*Ilarvirus*	*Parietaria mottle virus*	82	GQ865652	Italy	NA
VIC 22	Peach	*Ilarvirus*	*Parietaria mottle virus*	84	GQ865652	Italy	NA

Sample ID	Host	Assay	BlastN match to pathogen	% nucleotide Identity	GenBank Accession match	Country of origin	Source crop
VIC 23	Cherry plum	*Ilarvirus*	*Parietaria mottle virus*	84	GQ865652	Italy	NA
VIC 24	Plum	*Ilarvirus*	*Parietaria mottle virus*	84	GQ865652	Italy	NA
VIC1 Clone 1	Plum	PDO RT-PCR *Capillovirus, Foveavirus* and *Trichovirus*	ACLSV	83	AF532166	New Zealand	Apricot
VIC1 Clone 2			ACLSV	81	AF413935	France	Peach
VIC3 Clone 1	Plum	PDO RT-PCR *Capillovirus, Foveavirus* and *Trichovirus*	APCLSV	83	AY713380	Italy	Apricot
VIC3 Clone 2			APCLSV	85	AY713380	Italy	Apricot
VIC5 Clone 1	Peach	PDO RT-PCR *Capillovirus, Foveavirus* and *Trichovirus*	CGRMV	99	KF534770	Montenegro	Peach
VIC5 Clone 2			CGRMV	99	KF534767	Montenegro	Peach
VIC8 Clone 1	Plum	PDO RT-PCR *Capillovirus, Foveavirus* and *Trichovirus*	ACLSV	98	KF534760	Montenegro	Peach
VIC8 Clone 2			ACLSV	80	AF413951	Japan	Apple
VIC11	Nectarine	PDO RT-PCR *Capillovirus, Foveavirus* and *Trichovirus*	APCLSV	82	AY713380	Italy	Apricot
TAS10	Apricot	PDO RT-PCR *Capillovirus, Foveavirus* and *Trichovirus*	CVA	74%	HE574818	India	Cherry
NSW 4	Almond	*Agrobacterium tumefaciens*	*A. tumefaciens*	94	CP011247	USA	Yarrow
QLD 1	Plum	*Pseudomonas*	*Pseudomonas syringae*	100	AB039493	N/A	N/A
TAS 9	Cherry	*Pseudomonas*	*Pseudomonas syringae*	99	AB039493	N/A	N/A

3.3.4 Capillovirus, Foveavirus and Trichovirus detection

All samples were tested with the generic PDO RT-PCR assay that detects viruses in the genera *Capillovirus, Foveavirus* and *Trichovirus (*Foissac et al 2001) and specific assays for ACLSV, ApLV, APruV-1, APruV-2, APruV-3, APCLSV, ASPV, ASGV, CVA, CMLV, CTLaV, CNRMV, CGRMV, PBNSPaV and PcMV. ApLV, APruV-1, APruV-2, APruV-3, ASPV, CMLV, CTLaV and PcMV were not detected with either the generic or the specific tests. Where ACLSV, ASGV, CNRMV CGRMV and PBNSPaV were detected with specific assays and a positive result was obtained with the generic PDO RT-PCR assay, it was assumed that the generic test had

detected the same virus species. However the generic assay sometimes returned a positive result when viruses were not detected with a specific assay. Conversely, in some instances the specific assays indicated the presence of a virus when the generic assay was negative.

ACLSV was detected in a total of 13/101 trees. ACLSV was detected in 7/101 trees that were tested with the specific ACLSV PCR test and five of these trees also tested positive using the generic PDO RT-PCR assay that detects viruses in the genera *Capillovirus, Foveavirus* and *Trichovirus* (Table 3.9). ACLSV was detected in an additional 6/101 tested using just the generic PDO RT-PCR assay. These six trees did not test positive using the specific ACLSV RT-PCR test. Only two of the 13 trees infected with ACLSV tested positive using the generic *Trichovirus* RT-PCR assay. These results indicate that genetic variation exists within ACLSV that affects the reliability of the generic *Trichovirus* assay and the specific RT-PCR assay. The generic *Trichovirus* assay and the ACLSV specific RT-PCR assay only use one round of PCR and the generic PDO RT-PCR assay is a nested PCR procedure in which the PCR amplicon of the first RT-PCR assay is used in a second round of PCR. It is possible that the nested generic PDO RT-PCR assay amplified a PCR product from a virus that was below detectable levels if only one round of PCR is used.

Sequence analysis of the 358 bp PCR products amplified by specific ACLSV RT-PCR assay indicated that one Cherry sample from Victoria and two Cherry samples from Tasmania shared 99-100% nucleotide similarity, suggesting a possible common origin. Two isolates from Western Australia, from peach and nectarine had 98% sequence similarity with each other but only shared a 76-79% similarity with the isolates from Victoria and Tasmania. The Victorian and Tasmanian ACLSV isolates had 97% sequence similarity with an isolate from wild cherry in Hungary (GenBank Accession AY677106). The Western Australian ACLSV isolates had 95% sequence similarity with and isolate from sweet cherry in Turkey (GenBank Accession AY730560)

Sequence analysis of the cloned 362 bp PCR products amplified only by the generic PDO RT-PCR assay indicated that one plum sample from Victoria and one plum sample each contained two distinct ACLSV isolates. One isolate from each of the plum sample from Victoria and the plum sample from Tasmania shared 97% nucleotide similarity. However when the isolates from each sample were compared it was evident that both trees were infected with a second more divergent isolate of ACLSV. When the two isolates within in each tree were compared with each other they only had 84-85% sequence similarity with each other. When the divergent isolates were compared between the two trees they also only had 84% sequence similarity with each other.

APCLSV was detected in a plum and a nectarine from Victoria using the generic PDO RT-PCR assay (Table 3.9; Foissac et al 2001). Sequencing of the PDO RT-PCR products indicated that the two Australian isolates had 98% sequence similarity with each other and both isolates had between 80-97% sequence similarity with strains of APCLSV published in GenBank. These samples also tested positive using the generic *Trichovirus* assay and it was assumed that the virus detected was APCLSV. Two additional trees known to be infected with APCLSV tested positive only with the generic *Trichovirus* RT-PCR assay. APCLSV was not detected in

any sample using the two virus specific assays that were selected for validation. There is limited sequence data for this virus on GenBank therefore it is difficult to develop a reliable diagnostic assay. Consequently further work is required to gather more genetic data for Australian APCLSV isolates so that an accurate test can be designed.

The four samples that tested positive for CGRMV using the specific test also tested positive using the generic PDO RT-PCR assay (Foissac et al 2001). Similarly two samples in which CVA were detected also tested positive using the generic PDO RT-PCR assay. It was assumed that CGRMV and CVA were detected by the generic assay.

The generic *Trichovirus* assay returned positive results in two plum samples in Victoria in specific assays for the Trichoviruses ACLSV, APCLSV, CMLV and PcMV were negative. The generic PDO RT-PCR assay also returned a negative result. There was also one cherry sample which tested positive with both the generic PDO RT-PCR assay and the *Trichovirus* assay. This suggests possible presence of another virus in the genus and further work is required to determine the identity of the virus. This result indicates that although the *Trichovirus* assay cannot be used reliably for detection of all virus species and strain in the genera it has value in detecting species and strains that might be missed with the specific assays.

CVA was detected for the first time in Australia, in aVictorian *P. cerasifera* tree, using next generation sequence analysis (Appendix 4). The presence of CVA in Victoria and in other states of Australia and CVA was detected in 14/101 samples using the specific RT-PCR assay and ten of these samples also tested positive using the generic PDO RT-PCR assay. Additionally one sample, an apricot from Tasmania, tested positive only with the generic PDO RT-PCR assay and sequence analysis of the resulting PCR product indicated that virus that had 74% sequence similarity with a CVA isolate from India, indicating that it is highly divergent. It is possible that the virus that was detected represents a previously uncharacterised species in the genus *Capillovirus.*

These results indicate that the generic PDO RT-PCR assay and the specific RT-PCR assays that were validated may not detect all *Prunus* infecting *Capillovirus, Foveavirus* and *Trichovirus* species and strains. However the generic PDO RT-PCR assay may detect some strains that the specific assays do not. It is also useful for virus species discovery. It is recommended that the generic and specific assays be used in conjunction with one another for reliable detection of these viruses. Further work is required to understand the genetic diversity of APCLSV and ACLSV in Australia and improve the specificity of specific RT-PCR assays.

Table 3.9. A list of *Prunus* samples in which ACLSV or APCLSV were detected using a virus specific assay, generic *Trichovirus* RT-PCR assay or the generic PDO RT-PCR assay (Foissac et al 2001).

State	Crop	ACLSV specific test	APCLSV specific tests	Foissac generic test	Trichovirus specific test
QLD8	Apricot	-	-	ACLSV	+
QLD15	Apricot	-	-	ACLSV	-
TAS1	Cherry	+	-	+	-
TAS2	Cherry	+	-	+	-

TAS3	Plum	+	-	+	-
TAS	Cherry	-	-	+	+
TAS8	Plum	-	-	ACLSV	-
TAS17	Peach	-	-	ACLSV	-
VIC1	Plum	-	-	ACLSV	-
VIC2	Plum	+	-	+	+
VIC3	Plum	-	-	APCLSV	+
VIC8	Plum	-	-	ACLSV	-
VIC10	Cherry	+	-	-	-
VIC11	Nectarine	-	-	APCLSV	+
VIC18	Apricot	-	-	+	+
VIC19	Plum	-	-	-	+
VIC20	Plum	-	-	-	+
WA1	Peach	+	-	+	-
WA3	Nectarine	+	-	-	-

3.3.6 Ampelovirus – Plum bark necrosis stempittng associated virus, Little cherry virus 1 and Little cherry virus 2

All samples were tested using two generic nested RT-PCR/PCR assays, one that detect species in the family *Closteroviridae* and another that detects species in the genus *Ampleovirus* (Dovas and Katis 2003; Maliogka et al 2008). The *Closteroviridae* assay should detect PBNSPaV, LChV1 and LChV2. the *Ampelovirus* generic assay should detect PBNSPaV and LChV2. All samples were also tested with specific test for each of the three viruses. LChV1 was not detected. LChV2 was detected in three chery samples using the specific assays. PBNSPaV was detected in one of the LChV2 infected cherry samples. PBNSPaV was detected in three plum samples. No positive results were obtained with the generic *Closterovirdae* assay. The ampleovirus generic assay returned a positive result for 2/3 LChV2 infected samples, including the sample co-infecetd with PBNSPaV. The *Ampelovirus* generic assay did not resturn a positive result for any of the PBNSPaV infected plum samples.

For grapevine it is recommended that the generic *Closteroviridae* and *Ampleovirus* assays are used for detection of strains *Grapevine leafroll associated virus* (GLRaV) species that are not able to be detected using species specific assays, however the tests are not reliable for GLRaV species detection on their own (Constable et al 2010). This strategy will also be useful for *Closteroviridae* and *Ampleovirus* detection in *Prunus* species. However, like grapevines, the results from this study suggest they are not reliable for the detection of all strains of a virus species within a genus or a family.

3.3.7 LAMP

The LAMP assays developed for detection of *X. fastidsioa* and *E. amylovora* were successfully used on purified total nucleic acid for the detection of both pathogens in the laboratory. The assays are simple, rapid, specific, cost-effective and can be used to confirm a positive result for each pathogen more quickly than PCR procedures. However they are not amenable to

high throughput as only 6-14 samples can be handled at any one time if the test is being monitored electronically in real time using the Genie thermocycler. However if the reaction is to be measured visually with a change in turbidity or colour, the test is not limited to these numbers as long as an 60°C incubator can hold a larger number of tubes.

The LAMP assays for both pathogens were not trialled on crude extracts that may be produced in the field and further validation is required o optimize this step. Harper et al (2010) suggested that the PickPen (Bio-Nobile) could be used in the field to extract DNA from plant material for LAMP detection of X. fastidiosa but noted that it was not as sensitive as laboratory based methods for DNA extraction. Similarly the LAMP assay for detection of *E. amylovra* is also less efficient that when used under laboratory conditions (Buhlmann et al 2013). However both could be used for surveillance in the field to determine a positive result. Negative results in symptomatic material would need to be confirmed in the laboratory.

3.3.8 Detection of viruses in pollen

Nucleic acid was successfully extracted from pollen of the two ornamental cherry trees, the two Satsuma plum trees , the PDV infected plum tree and the PNRSV infected rose plant from using the RNeasy Plant Mini Kit (Schiller et al 2010) with a modified lysis buffer (MacKenzie et al 1997). The NAD RT-PCR assay produced an amplicon of the expected size for RNA (188bp; Figure 3.1a and 3.1b). In the same assay an amplicon of approximately 700bp was generated (Figure 3.1a), which is a result of the amplification the ndhB gene chloroplast DNA containing the intron as well as part of exon 2. PNRSV was detected pollen of the PNRSV infected rose sample and one ornamental cherry (figure 3.1c). PDV was not detected in any sample. These results indicate that the procedure adopted in this project ca be used to detect viruses in pollen of *Prunus* species.

3.4 Conclusions

- The following viruses and viroids which are of quarantine concern to almonds and/or summerfruit species in Australia were not detected during the survey: ApLV, ArMV, APLPV, APruV1, APruV2, APruV3, CLRV, CMLV, CRMaV, CTLaV, LChV1, PPV, PRMV, PcMV, RRSV, SLRSV, TBRV, TBSV, ToRSV and ASSVd. *Xylella fastidosa*, *Erwinia amylovora*, *Xanthomonas arboricola pv. Pruni* and Phytoplasmas were also not detected.
- ACLSV, ApMV, APCLSV, ASGV, CGRMV, CNRMV, CVA , LChV2, PBNSPaV, PDV, PNRSV, an uncharacterised *Ilarvirus*, HSVd, PLMVd, *Pseudomonas syringae* and *Agrobacterium tumefaciens* were detected in Australian *Prunus* species.
- This is the first report of CGRMV, CNRMV and PBNSPaV in Australia. CVA was also detected for the first time in Australia using next generation sequencing (Appendix 4) and during the survey.
- ASGV is endemic and infects pome and citrus fruit species and this survey provides the first evidence that it can also infect *Prunus* species in Australia.

- An *Ilarvirus* most closely related to *Parietaria mottle virus* was detected and further work is required to characterise the virus and determine its impact on Australian *Prunus* species.
- Qiaextractor is a reliable high throughput nucleic acid extraction procedure for the isolation of DNA and RNA from *Prunus* species.
- Except for ACLSV, APCLSV, APLPV, CNRMV and HSVd, the RT-PCR and PCR assays were reliable and can be used for the detection of viruses, viroids, phytoplasmas and bacteria of *Prunus* species.
- Further work is required to gain knowledge of the genetic diversity of ACLSV and APCLV so that better molecular tests can be developed.
- A divergent strain of CVA was detected and further work is required to determine if this is in fact CVA or another *Capillovirus* species.
- The assays for APLPV, CNRMV and HSVd generate non-specific PCR products that could result in a false positive result and another assay for each pathogen requires validation.
- LAMP procedures show promise for field based detection of pathogens and further research could be done to develop similar field based strategies for detection of pathogens occurring in Australian and which are significant to certification schemes including ACLSV, ApMV, APCLSV, ASGV, CGRMV, CNRMV, CVA, LChV2, PBNSPaV, PDV, PNRSV, HSVd, PLMVd, *Pseudomonas syringae* and *Agrobacterium tumefaciens.*
- Pollen-borne viruses can be detected in pollen using the methods developed in this project.
- Since the conclusion of this work several new viruses of *Prunus* have been reported including *Apricot vein clearing associated prunevirus* and Caucasus *Prunus* Prunevirus and *Prunus virus T.* Molecular tests should be developed and validated for deection of these viruses and samples screened to determine their presence in Australia.

Appendix 4: Diversity of Ilarviruses in *Prunus* species in Australia

This work forms part of the PhD study of Wycliff Kinoti (La Trobe University)

4.1 Introduction

The existence of genetic variants in natural populations of *Ilarvirus* species, including PNRSV, PDV and ApMV, that infect almonds and summerfruit has been reported (Boulila, 2010). This variation may influence the reliability of molecular diagnostic tests, such as RT-PCR, for which development requires knowledge of the nucleotide sequence of the genome (Vašková et al 2000). Therefore, determining plant virus population diversity is important for development of rapid and reliable detection assays for almond and summerfruit Ilarviruses in Australia.

4.2 PCR detection of Ilarviruses

Detection of ilarviruses using PNRSV, PDV and ApMV species-specific RT-PCR tests and not by the *Ilarvirus* genus-specific RT-PCR tests and vice versa indicated genetic variation of ilarviruses in Australian *Prunus* trees (Appendix 3, table 3.8).

To further examine the variation of PNRSV selected samples were inoculated onto cucumber (*Cucumis sativus*) indicators and a variation in symptom expression was observed: some plants infected with PNRSV expressed symptoms whilst others did not (Figure 4.1). The PNRSV species specific and genus-specific RT-PCR products were sequenced directly or cloned and sequenced and phylogenetic analysis of the coat protein and RdRP sequences showed clustering of cucumber PNRSV isolates, which were distinct fromfrom the *Prunus* tree PNRSV isolates suggesting that the cucumber indicator plants were selecting for specific sequence variants. A phylogenetic analysis of the direct sequenced PNRSV species specific poducts and the cloned and sequenced genus-specific RT-PCR products also showed that variation of PNRSV isolates between *Prunus* trees in Australia. For further details see Appendix 1 (Kinoti et al 2014)

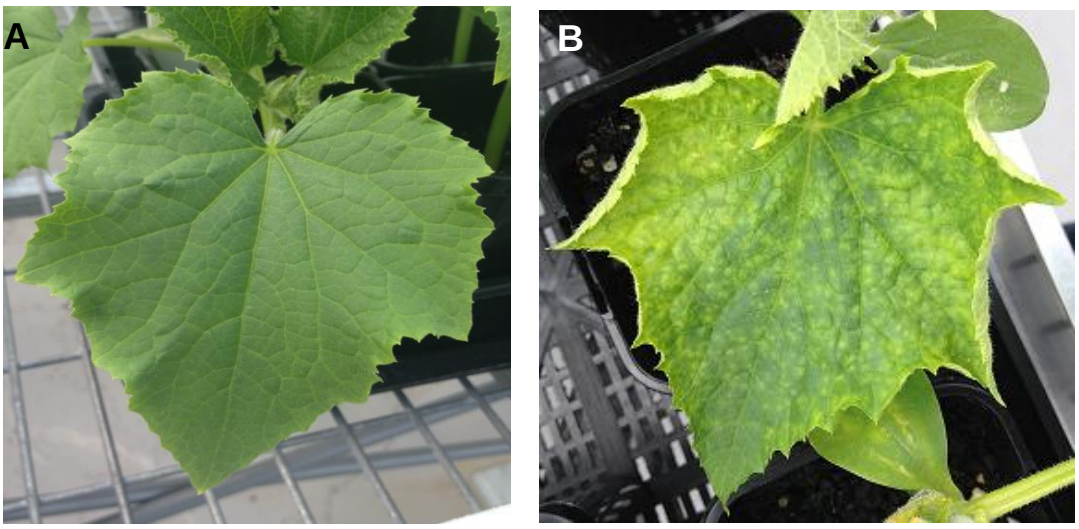


Figure 4.1. Symptomless (A) and symptomatic (B) cucumber indicators that tested positive for PNRSV.

4.3 Amplicon sequencing

Traditionally, cloning and sequencing of individual virus amplicons generated during PCR has been widely used to assess the genetic diversity of virus populations (Hadid et al 1995). However, this approach is time-consuming and labour intensive, thus limiting virus population studies to a few field samples. Depending upon the number of cloned amplicons that are sequenced, it is also likely that only the sequence variants occurring with high frequency will be detected and some infrequent but potentially important variants may be missed. This pool of infrequent viral sequence variants may be a reserve on which selection can occur and from which fitter more pathogenic virus isolates can emerge.

The development of next-generation sequencing (NGS) technologies allows direct sequencing of nucleic acid extracted directly from a plant sample or deep sequencing of PCR amplicons generated from a plant sample rather than cloning and sequencing of individual PCR amplicons (Egan et al 2012). This massively parallel sequencing approach has the potential to reduce laboratory time and cost and can overcome the low volume of information generated by clonal Sanger sequencing of individual PCR amplicons. This approach allows direct sequencing of all PCR amplicons generated in one PCR reaction and amplicons from multiple samples can be pooled thereby producing hundreds of thousands of sequence reads generated from the one gene region (Margulies et al 2005; Shendure et al 2011). Virus RT-PCR amplicon sequencing using NGS platforms provides the potential to detect individual virus sequence variants in a sample offering an in depth look at the population of both major and low-level virus sequence variants (Beerenwinkel & Zagordi, 2011).

Here, we report the *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV) and *Apple mosaic virus* (ApMV) RT-PCR test profiles of *Prunus* species samples collected from several regions of Australia and the diversity of these ilarviruses through amplicon sequencing by NGS.

4.3.1 Collection of samples and screening for PDV, PNRSV and ApMV.

A total of 156 *Prunus* tree samples, comprising of almond, peach plum, apricot, nectarine and cherry, were collected from various states in Australia between April 2013 and December 2014 (Table 4.1).

Table 4.1: Plant species and location (State) of 156 *Prunus* species samples screened for the presence of PDV, PBNRSV and ApMV.

State	Almond	Peach	Plum	Apricot	Nectarine	Cherry
Victoria	88	6	10	0	0	0
New South Wales	11	1	0	0	0	0
South Australia	6	2	0	0	2	0
Queensland	0	3	8	2	2	2
Tasmania	1	2	1	2	0	7
Total	**106**	**14**	**19**	**4**	**4**	**9**

The samples were screened for PDV, PNRSV and ApMV using three species-specific RT-PCR tests for each virus that target the coat protein (CP) gene on RNA 3 for (Table 4.2). Each of the plant samples were also tested using a genus-specific RT-PCR test (Maliogka et al 2007) targeting the RNA 2 of an *Ilarvirus* species that may be in the samples collected as described in Appendix 3. The generic test should detect most *Ilarvirus* species genome and could identify *Ilarvirus* species that have not been previously reported in Australian almond and summerfruit trees.

Table 4.2 The primers used for the detection of RNA1, RNA2 and RNA3 of PNRSV, PDV and ApMV, the primer annealing temperature, the region amplified, expected product size and the reference for each test.

Pathogen	Primer name	Orientation	Primer sequence (5'-3')	Tm	Amplified region	Expected product	Reference
PNRSV (RNA1)	PNRSV-MTv-1	F	TCGAGCGATCTATTCCTAAG	57° C	Methyl-transferase	218bp	This study
	PNRSV-MTv-2	R	ATCAATCACCGATTCTTCAG				
PNRSV (RNA2)	PNRSV-Rd-1	F	TTTTCACCGCTTTAGGTGCT	60° C	Polymerase	387bp	This study
	PNRSV-Rd-2	R	GAACCTTCTTTCCCCCACTC				
PNRSV (RNA3)	PNRSV-C537	F	ACGCGCAAAAGTGTCGAAATCTAAA	54° C	Coat protein	455bp	MacKenzie et al 1997
	PNRSV-H83	R	TGGTCCCACTCAGAGCTCAACAAAG				
PDV (RNA1)	PDV-MT-1	F	GCGCTGACGAGACTACTACC	55° C	Methyl-transferase	205bp	This study
	PDV-MT-2	R	GCGAAACTGTGTGAGGAACT				
PDV (RNA2)	PDV-Rd-1	F	CGTTTCTGGAAGGAAGTGGA	60° C	Polymerase	382bp	This study
	PDV-Rd-2	R	TTGCTTCGAAATTGAACAAAG				
PDV (RNA3)	PDV-F	F	TAGTGCAGGTTAACCAAAAGGAT	62° C	Coat protein	172bp	Parakh et al 1994
	PDV-R	R	ATGGATGGGATGGATAAAATAAT				
ApMV (RNA1)	ApMV-MT-1	F	AGTTTGTGTGATGTGAGATG	56° C	Methyl-transferase	222bp	This study
	ApMV-MT-2	R	ATTTCTAAGGCGTAACTTCC				
ApMV (RNA2)	ApMV-Rd-1	F	TCATTGGATCCCTTTGCTTC	59° C	Polymerase	383bp	This study
	ApMV-Rd-2	R	AAACTCGTCGTCCCTATCCA				
ApMV (RNA3)	ApMV-F*	F	TGGATTGGGTTGGTGGAGGAT	53° C	Coat protein	1261bp	Petrzik & Svoboda, 1997
	ApMV-R*	R	TAGAACATTCGTCGGTATTTG				

After PCR amplification (as described in Appendix 3, section 3.2.7), 10 µL of each PCR reaction was run on a 2% agarose gel in 0·5 ×Tris-borate-EDTA buffer, stained with SYBR®Safe DNA Gel Stain (Invitrogen, Life Technologies, Melbourne, Victoria, Australia) and visualised on a UV transilluminator. Table 4.3 gives the number of samples for which a positive result was obtained using the species specific test for PNRSV, PDV, ApMV and the number in which a positive results was obtained using the *Ilarvirus* generic test.

Table 4.3: The total number of samples of each *Prunus* species that tested positive with the PNRSV, PDV and ApMV species-specific assays which deect the coat protein located on RNA3 and the *Ilarvirus* genus-specific RT-PCR test which detects the RNA dependent RNA polymerase on RNA 2.

***Prunus* species (number of samples)**	**Species-specific RT-PCR**			**Genus-specific RT-PCR**
	PNRSV	**PDV**	**ApMV**	***Ilarvirus* genus**
Almond (106)	44	3 (2)*	1	31 (8)[#]
Peach (14)	4	6 (1)*	-	6
Plum (19)	6	-	2	9 (2)[#]
Apricot (4)	3	-	-	2
Nectarine (4)	2	1	-	1
Cherry (9)	6	-	-	-
Total (156)	**65**	**10 (3)***	**3**	**49 (10)[#]**

*Indicates samples with PNRSV-PDV mixed infections.
# Indicates samples that tested positive only with the generic *Ilarvirus* RT-PCR test species-specific.

Ilarviruses were detected in a total of 85/156 (54%) samples using the species and/or *Ilarvirus* generic RT-PCR tests. Based on detection using the species-specific RT-PCR assays PNRSV was the most frequently detected virus. PNRSV was detected in all the *Prunus* species and almonds had the highest incidence of the virus (41%; 44/106). Peach had the highest incidence of PDV (42%; 6/10). Three samples had a mixed infection of PNRSV and PDV. ApMV had the lowest frequency (3/156) of detection which may be due to low incidence of the virus in the field.

Ideally, all samples that tested positive with species-specific RT-PCR test should also test positive with the *Ilarvirus* genus-specific RT-PCR test. However, only 49/156 samples were positive using this test. Using the *Ilarvirus* genus-specific test, positive results were obtained for 36/75 samples that also tested positive with the PDV, PNRSV or ApMV species-specific RT-PCR tests. Ten samples tested positive only with *Ilarvirus* genus-specific test. These results suggest that sequence variants of PDV, PNRSV and ApMV and/or other *Ilarvirus* may exist in Australian *Prunus* trees.

4.3.2 Amplicon sequencing

Prior to next generation amplicon sequencing, new species-specific RT-PCR primers were designed for the amplification of methyltransferase (MT) gene on RNA 1 and RNA dependent RNA polymerase (Rd) gene on RNA 2 for PDV, PNRSV and ApMV (Table 4.2). These primers were used to amplify a region of the RNA 1 and RNA 2 virus segments in the 75 samples in which the PNRSV, PDV and/or ApMV CP genes (RNA3) had already been detected (Table 4.4). Amplicons from the three viral RNA segments of 65 PNRSV positive samples, ten PDV positive samples and ten ApMV positive sampes (234 amplicons in total) were generated. There were a total of 234 RT-PCR amplicons (Table 4.4).

Table 4.4: Total number of PNRSV, PDV and ApMV positive samples from which amplicons from RNA1, RNA2 and RNA3 were generated and the expected size of each amplicon.

Ilarvirus specie	Positive trees	RNA 1 (MT)	RNA 2 (Rd)	RNA 3 (CP)
PNRSV	65	218 bp	387 bp	455 bp
PDV	10	205 bp	383 bp	172 bp
ApMV	3	222 bp	382 bp	261 bp

The 234 RT-PCR amplicons generated were purified using the Promega Wizard® PCR clean-up kit (Promega, Auburn, VIC) according to manufacturer's instructions. NGS libraries were prepared for each of the purified amplicons by ligation of adapters, addition of unique barcodes to each amplicon, PCR enrichment and finally a purification step using Agencourt AMPure XP beads (Beckman Coulter Australia Pty Ltd, Lane Cove NSW). The 234 sample amplicons were pooled into one sample and sequenced using the Illumina Miseq (Illumina, San Diego, CA, USA) with a paired read length of 300 base pairs. The sequenced amplicons were then quality checked and reads with low quality and shorter than the required amplicon size were removed. After quality and size trimming, the number of sequence reads generated from NGS of each of the 234 amplicons ranged from 600 reads to as much as 30000 reads per sample.

No pipeline has been described for bioinformatics analysis of next generation amplicon sequencing data generated from plant virus amplicons. Therefore a preliminary analysis of the RNA1, RNA2 and RNA3 amplicon sequence reads from three PNRSV positive samples, M5_PNRSV, M6_PNRSV and NS9_PNRSV (Table 4.5), were used to develop a sequence variant bioinformatics analysis pipeline. The process of developing the pipeline is described below.

Sequence reads of amplicons of RNA1, RNA2 or RNA3 from the three samples (Table 4.5) were first combined into clusters of 100% sequence identity using the Uclust pipeline (Edgar, 2010). This reduced the number of sequence reads of each RNA segment from each of the samples. However, there were a high proportion (up to 88%) of sequence singletons which are clusters containing only a single sequence read (Table 4.5).

Table 4.5 Total number of clusters, including singletons, generated from combining amplicon reads with 100% sequence identity in each of the three PNRSV positive samples.

Sample	**M5_PNRSV**			**M6_PNRSV**			**NS9_PNRSV**		
RNA segment	**RNA 1**	**RNA 2**	**RNA 3**	**RNA 1**	**RNA 2**	**RNA 3**	**RNA 1**	**RNA 2**	**RNA 3**
Total reads	5,000	646	7,923	2,804	606	21,044	31,809	721	2,782
Total clusters	289	249	2,455	234	265	5,109	967	319	180
% singletons	50.9	87.6	79.5	65.4	86.8	82.5	61.6	88.1	85.7

Sequence errors may be generated during PCR amplification and these may not be distinguished from naturally occurring variants arising from mutations. The amplicons generated from each RNA segment are from coding regions and therefore are unlikely to contain a stop codon if the virus is functional. It was assumed that stop codons would not be present unless introduced by PCR errors and this assumption was used to filter out sequences with putative PCR errors. To apply this filter, all the generated nucleotide sequence clusters were translated into amino acid sequences and scanned for stop codons. More than 80% of singletons contained stop codons and more than 95% of clusters containing ≤ 10 sequence reads also had stop codons. These sequence variants were removed from the data set. The number of remaining clusters after filtering (filtered clusters) is considered more likely to contain naturally occurring sequences of each of the viral RNA segment clusters (Table 4.6). It is possible that filtering resulted in the removal of some naturally occurring variants of low incidence.

Table 4.6 Total number of filtered clusters remaining in each of the three PNRSV positive samples after nucleotide sequences containing stop codons were removed.

Sample	**M5_PNRSV**			**M6_PNRSV**			**NS9_PNRSV**		
RNA segment	**RNA 1**	**RNA 2**	**RNA 3**	**RNA 1**	**RNA 2**	**RNA 3**	**RNA 1**	**RNA 2**	**RNA 3**
Total reads	5,000	646	7,923	2,804	606	21,044	31,809	721	2,782
Total clusters	289	249	2,455	234	265	5,109	967	319	180
Filtered clusters	13	11	25	8	12	154	151	7	8

Singletons and clusters with ≤ 10 reads that did not contain stop codons were also removed as it was assumed that many of these sequence variants may also be a result of PCR errors. Many of these Singletons and clusters with ≤ 10 reads did not result in a change in amino acid sequence and would have been difficult to distinguish them from true biological variants based on amino acid sequences.

To determine how many of the nucleotide variants might result in a biological change associated to a change in the encoded protein, the filtered variant clusters were translated to amino acid sequences and clustered using Uclust pipeline. Less amino acid variant clusters

for each of the PNRSV viral RNA segments were observed (Table 4.7) indicating that some of the nucleotide variations that were observed were inconsequential to the overall function of the encoded protein. This final filer on the sequence variants from each amplicon resulted in fewer amino acid variants of each PNRSV RNA segment within a tree (Table 4.7).

Table 4.7 Final number of sequence variants/"strains" for each PNRSV positive sample RNAs

Sample	**M5_PNRSV**			**M6_PNRSV**			**NS9_PNRSV**		
RNA segment	**RNA 1**	**RNA 2**	**RNA 3**	**RNA 1**	**RNA 2**	**RNA 3**	**RNA 1**	**RNA 2**	**RNA 3**
Variants	2	5	8	1	6	10	7	5	5

The occurrence of different PNRSV amino acid sequence variants/"strains" for each RNA segment in each of the three samples (Table 4.8) indicates that each of the trees were infected with multiple strains of PNRSV at the same time or through repeat infections during the lifetime of the tree. However it may also be a result of evolution of PNRSV within each tree. When the amino acid cluster sequences were compared between the three trees the MT region that was amplified (RNA 1) had 96.1% to 98.3% identity, the RDRP region (RNA2) had 96% to 99% identity and the CP region (RNA3) had 81% to 98% identity. This indicated that none of the three trees hosted identical PNRSV RNA1, RNA2 or RNA 3.

4.3.3 Summary

Ilarviruses, including PNRSV,have a tripartite genome consisting of RNA1, RNA2 and RNA3, which are each encapsidated separately. The analysis showed that multiple variants of each RNA segment can occur in a single tree and that the number of each variant of RNA1, RNA2 and RNA3 may not be the same. It is not known which of each of RNA1, RNA2 and RNA3 segments interact to define a strain. It is possible that they all interact as a population of variants to form the strain.

The presence of these multiple PNRSV strains as observed above across all the three RNAs of PNRSV may be of great biological impact in case of re-infection and mixing of different RNAs strains of PNRSV which can lead to more severe manifestations of PNRSV infections in *Prunus* trees. This study indicated that variability of Ilarviruses can occur between trees and within a tree at the amino acid and nucleotide level. It also confirmed that this variation can affect diagnosis of PNRSV, PDV and ApMV infection using molecular methods.

Species-specific primers were developed for this study that might be adopted for routine diagnosis of PNRSV, PDV and ApMV infection. The sequence information could be used to develop additional species or genus specific RT-PCR assays.

This study showed that amplicon sequencing is a high throughput means of studying population diversity of viruses within a plant and between plants. A next generation amplicon sequencing and bioinformatics pipeline was developed to analyze the sequencing data. This pipeline will be used to analyze the remaining amplicons 225 amplicons. It will also be used to analyze the amplicons produced by the *Ilarvirus* genus specific RT-PCR assay, which may identify additional PNRSV, PDV and ApMV variants and other *Ilarvirus* species in the samples.

4.4 Genome sequencing

Next generation sequencing (NGS) technologies are being developed to gather nucleotide sequence data and characterise the *Ilarvirus* strains/variants of *Prunus* species in Australia. Preliminary work was undertaken using the recommended method for purification of double stranded viral RNA (replicative form) on cf11 cellulose (Coetzee et al 2010; Kreuze 2014). However this method did not produce a high yield of viral nucleic acid sequence data (~ 0.4% of total reads) and most data comprised of plant host nucleic acid sequence data (>95%).

To improve the yield of viral sequence data from *Prunus* trees eight extraction and extraction/enrichments methods were compared using an ornamental plum, *Prunus cerasifera*, in which PDV and PNRSV had been detected. The methods that were compared included extractions of 1) total nucleic acid (TNA); 2) total RNA (TRNA) ; 3) total RNA from partially purified virus (TRNApv); and 4) exraction of viral dsRNA using CF11. An enrichment procedure using J2 monoclonal antibodies (mAb; (Scicons Budapest, Hungary) that specifically bind to dsRNA was also tried on the same four extracts (Schonborn et al 1991). All eight methods were carried out in triplicate.

NGS Libraries were prepared from the nucleic acid extracts using NEBNext® Ultra™ RNA Library Prep Kit (Genesearch, QLD, Australia) following manufacturer's instructions. The library was sequenced using the Illumina Miseq (Illumina, San Diego, CA, USA) with a paired read length of 301 base pairs. The sequence reads were quality trimmed using Trim Galore! (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). The trimmed reads (8,055,106) were analyzed against Genbank non-redundant database by BLASTn (Altschul et al 1997), which confirmed PNRSV and PDV infection. This analysis also revealed the presence of CVA in the sample. Reference mapping using Geneious version 7.1.7 (http://www.geneious.com, Kearse et al 2012) was used to confirm the presence of PDV, PNRSV and CVA. CVA infection was also confirmed by RT-PCR (Osman et al 2012)

The total number of viral sequence reads per extraction/enrichment method compared to total sequence reads was determined by reference mapping. The TNA and TRNA extractions yielded the greatest viral sequence data (~4% viral reads) and this was improved (>6%) when further enrichment was carried out using dsRNA mAb. The CF11-dsRNA yielded less than 0.4% viral sequence reads and this was not improved with the use of the dsRNA mAb enrichment procedure.

A coverage of 99% was obtained for the RNA3 sequences of PDV and PNRSV by NGS. Approximately 97% coverage was obtained for RNA1 and RNA 2 of PDV and PNRSV. The sequence similarity between the isolate sequenced by NGS and the type isolates of each RNA segment of PDV and PNRSV are shown in Table 4.8. The results indicate that the PDV and PNRSV isolates that were detected in the *P. cerasifera* tree are distinct form the type isolates described overseas.

Table 4.8 The sequence similarity between the RNA1, RNA2 and RNA 3 segemnts of the PDV and PNRSV isolates that were sequenced in this study and the type isolates published in Genbank

Virus	RNA geneome segment	Genbank Accession of the type isolate	Sequence similarity
PDV	RNA1	U57648	97%
PDV	RNA2	AF277662	98%
PDV	RNA3	L28145	95%
PNRSV	RNA1	AF278534	96%
PNRSV	RNA2	AF278535	97%
PNRSV	RNA3	U57046	94%

4.5 Conclusions

The nucleic acid extraction/enrichment procedures and NGS pipeline developed in this study will be used to investigate interesting *Ilarvirus* isolates observed during amplicon sequencing. The methods will also be used to investigate the detection of a putative and previously uncharacterised *Ilarvirus* with detected during the survey (Appendix3) and to investigate the cause of a decline of almonds in the Sunraysia district.

The results of this study indicate that significant diversity exists between the Ilarviruses infecting *Prunus* trees in Australia compared to those occurring overseas. There is also significant *Ilarvirus* diversity occurring in Australia between different *Prunus* species and within a species. The results also show that there is a population of strains or quasispecies within a tree.

4.6 First detection of *Cherry virus A* in Australia

Cherry virus A (CVA), was first described in sweet cherry (*Prunus avium*) in Germany presenting little cherry disease symptoms (Jelkmann, 1995). Molecular characterization of CVA revealed a genome organization similar to *Apple stem grooving virus* (ASGV), and CVA was classified as a member of the genus *Capillovirus* in the family *Flexiviridae* (Jelkmann, 1995). CVA has been previously reported in Europe, North America and Asia with no reports of its detection in Australia (Marais et al 2011). In spring 2014, an ornamental plum tree, *Prunus cerasifera*, was observed exhibiting chlorotic mottling, distortion and ringspot with severely infected branches having dieback symptoms. NGS and sequence analysis was carried out as described in section 4.2. The full genome coverage for CVA was obtained with more than 100 times sequence reads coverage. The sequence reads mapping to CVA were then assembled into a single continuous contig of 7106 nucleotides long which was 98% identical (96% coverage) to CVA type isolate (Genbank Accession No. NC_003689). The sequence will be deposited in Genbank. CVA infection was also confirmed by RT-PCR (Osman et al 2012). This is the first report of CVA in Australia and the first report of CVA infecting *P. cerasifera*.

Appendix 5: Develop a post entry quarantine diagnostic manual for *Prunus* species

The provision of high health certified almond and summerfruit planting material has traditionally been supported by the best available diagnostic tools for pathogen and pest detection. In Australia nucleus collections are tested annually in spring by molecular methods for *Prunus necrotic ringspot virus* (PNRSV)*, Prune dwarf virus* (PDV)*, Apple mosaic virus* (ApMV) and *Apple chlorotic leafspot virus* (ACLSV). Plants in the nucleus collection are visually inspected on a routine basis for evidence of other diseases. Biological indexing is still used during post entry quarantine for detection of quarantine diseases and after heat treatment for pathogen eradication.

An outcome of this research project is a world's best practice, diagnostic capability for the detection of economically significant pathogens of almonds and summerfruit. The diagnostic tools will be used by the Australian almond and summerfruit industries for the production and maintenance of high health planning material and by the Department of Agriculture during Post Entry Quarantine (PEQ). The output from this research is a draft diagnostic manual that incorporates the endemic and exotic protocols developed in these projects. The protocols in the manual will form the basis of a national certification standard to support the production of pathogen tested almonds and summerfruit propagation material in Australia.

Scope of the manual

- The manual is a technical document for "certification" programs, PEQ, and other users who wish to produce and maintain high health almonds and summerfruit using industry approved standards.
- This manual is based on world's best practice validated diagnostic tests for almond and summerfruit pathogens and incorporates existing and new technology.
- Through research and development, protocols have been identified which are efficient and increase biosecurity for the Australian almonds and summerfruit industries and these protocols have been incorporated into the manual.
- The format of the manual is prescriptive and designed for easy interpretation of the methodologies/techniques and interpretation of the results of the various diagnostic tests.
- The protocols in the manual allow the various stakeholders/users to adapt a standardized approach to pathogen testing almond and summerfruit including certification schemes and PEQ.
- The intention of this manual is that the protocols will be integrated into the conditions/standards of certification schemes.
- This manual will become part of a standards based approach to almonds and summerfruit certification in Australia.

OVERVIEW OF INDEXING PROCEDURES

This procedure for pathogen testing of the almond and summerfruit nucleus plants is based on world's best practice validated diagnostic tests for almond and summerfruit pathogens and incorporates existing and new technology.

1. Plants entering the nucleus collection must have returned two seasons of negative pathogen testing results which included two years of biological indexing and molecular indexing. If all tests are negative the plants can be included into the nucleus collection.

2. Almond nucleus plants are tested annually for PNRSV, PDV, *Plum bark necrosis stem pitting-associated ampleovirus* (PBNSPaV), ACLSV and ApMV in spring by molecular indexing. Summerfruit nucleus plants are tested annually in spring for *Apricot pseudochlorotic leaf spot trichovirus* (APCLSV), *Apple stem grooving virus capillovirus* (ASGV), ACLSV, ApMV, *Cherry green ring mottle foveavirus* (CGRMV), *Cherry necrotic rusty mottle virus* (CNRMV), PBNSPaV, PDV and PNRSV.

3. Nucleus plants must be routinely screened for symptoms of disease and for insect pests during the growing season.

    - Pathogens and insects must be treated as soon as is practical.

4. Pathogen testing for viruses by biological indexing and/or molecular indexing is conducted during October-January of each year. Sampling and diagnostic testing is carried out according to the protocols in this manual. Testing will be done by a diagnostic laboratory.

    - Repeat molecular indexing for viruses can be carried out in April and May if required.

5. If required biological indicators will be maintained and propagated by a diagnostic laboratory in an approved glasshouse according to the protocols outlined in this manual.

6. If required biological indexing by graft or chip bud inoculation of woody indicators and rub inoculation of herbaceous indicators will be carried by a diagnostic laboratory in screenhouse and glasshouse conditions according to the protocols in this manual.

7. If negative pathogen testing results are obtained the plants can be certified as pathogen tested and used as nucleus plants in the following year.

8. If a positive result is obtained for any prescribed pathogen the affected variety will be removed immediately from the collection and placed in an isolated facility.

TECHNICAL MANUAL

Pathogen testing of *Prunus* Plants

This manual was prepared by:

- Fiona Constable, Narelle Nancarrow, Wycliff Kinoti, Geoff Kelly and Brendan Rodoni of the Department of Economic Development, Jobs, Transport and Resources (DEDJTR)Victoria and La Trobe University.

ACKNOWLEDGMENTS

This manual is an outcome of research project that has been funded by Horticulture Innovation Australia Limited using the almond and summerfruit levies with co-investment from Department of Economic Development, Jobs, Transport and Resources and funds from the Australian Government. We would like to acknowledge and thank ABA and Summerfruit Australia. Many thanks also to Mark Whattam (Department of Agriculture - Biosecurity), Ben Brown, Project and Technical Manager at Select Harvests Ltd (Formerly Almond Baord Australia, ABA), Brett Rozenzweig (ABA), Ross Skinner (ABA) and John Moore (Summerfruits Australia) for their input and helpful discussions. We would also like to thank the following overseas researchers who provided helpful discussions and information for the tests that were developed.

Contents

SECTION 1: Introduction

The Australian Almond and summerfruit industries have identified a number of high priority pests and diseases that pose a quarantine risk to the local industry. (Table 1). New varieties of Almonds and summerfruit are imported into Australia as budwood or tissue culture and these plants are grown and tested for the presence of these fungi, bacteria and viruses.

The almond certification scheme supply certified pathogen-testedbudwood throughout Australia. There is no formal certification program in place for the summefruit industry, however some nurseries choose to undertake pathogen testing of their rootstocks and varieties to ensure they are delivering quality planting material to industry. The budwood and nursery stock of almond and summerfruit are certified on the basis of their high health status and, in ther case of almonds,are derived from collections of nucleus–plants maintained in isolated and high security facilities. Nuclear collections are tested annually for viruses listed in Table 2. They are also visually inspected for signs of disease and samples may be tested for confirmation of the presence of a pathogen. The certification schemes and pathogen-testing in operation in Australia have contributed greatly to the biosecurity of the Almond and summerfruit industry and contribute to improved yields and quality of nuts and fruit for growers by excluding viral, fungal and bacterial pathogens.

This manual describes the methods that can be used for detection and identification of the endemic and exotic pathogens that are important to the biosecurity of the Australian almond and summerfruit industry (Constable et al 2011; Constable et al 2015). The methods include visual observations, biological indexing onto sensitive indicator plants, culturing onto agar plates for bacterial pathogens and molecular methods such as polymerase chain reaction (PCR) tests and reverse transcription (RT)-PCR tests. Fungal pathogens of quarantine singnificance are of lower risk of transmission in propagation material entering PEQ and are not included in this manual.

Table 1.1 Bacteria phytoplasmas, viruses and viroids that are known to infect almonds and summerfruit and are of quarantine significance.

ALMONDS			
Bacteria	**Phytoplasmas and phytoplasma groups**	**Viruses**	**Viroids**
Pseudomonas amygdali *Xylella fastidiosa* *Erwinia amylovora*	*Candidatus Phytoplasma prunorum* *X-Disease phytoplasma* *Candidatus Phytoplasma pyri* *Candidatus Phytoplasma phoenicium'* *Peanut witches' broom group phytoplasmas (16SrII - Candidatus*	*Little cherry virus 1 (unassigned genus, LCHV1)* *Peach mosaic trichovirus (PcMV)* *Peach rosette mosaic nepovirus (PRMV)* *Plum pox potyvirus (PPV)* *Raspberry ringspot Nepovirus (RpRSV)*	*Hop stunt viroid*

	Phytoplasma aurantifolia related strains) *Clover proliferation group phytoplasmas (16SrVI - Ca. P. trifolii related strains)* *Stolbur (16SrXII-A) group phytoplasmas*	*Tomato black ring nepovirus* *Tomato ringspot nepovirus*	

SUMMERFRUIT			
Bacteria	**Phytoplasmas and phytoplasma groups**	**Viruses**	**Viroids**
Xylella fastidiosa *Erwinia amylovora* *Pseudomonas syringae pv. persicae* *Pseudomonas syringae pv. avii*	*Candidatus* Phytoplasma prunorum X-Disease phytoplasma *Candidatus* Phytoplasma pyri *Candidatus* Phytoplasma phoenicium Peanut witches' broom group phytoplasmas (16SrII - *Candidatus* Phytoplasma aurantifolia related strains) Clover proliferation group phytoplasmas (16SrVI - *Ca.* P. trifolii related strains) Stolbur (16SrXII-A) group phytoplasmas *Candidatus* Phytoplasma mali Elm yellows (16SrV) group phytoplasmas Aster yellows group (I-B, I-F, I-Q)	*American plum line pattern Ilarvirus (*APLPV*)* *Apricot latent ringspot nepovirus (ALRSV)** *Apricot latent virus foveavirus (ApLV)* *Apricot vein clearing associated prunevirus (AVCaV)* *Asian Prunus virus 1 foveavirus (APruV-1)* *Asian Prunus virus 2 foveavirus (APruV-2)* *Asian Prunus virus 3 foveavirus (APruV-3)* *Arabis mosaic nepovirus (ArMV)* *Carnation Italian ringspot tombusvirus (CIRV)** *Cherry leaf roll nepovirus (CLRV)* *Cherry mottle leaf trichovirus (CMLV)* *Cherry rasp leaf cheravirus (CRLV)* *Cherry rosette nepovirus (ChRV)** *Cherry twisted leaf foveavirus* *Epirus cherry ourmiavirus (EpCV)** *Little cherry virus 1 (unassigned genus, LCHV1)* *Myrobalan latent ringspot nepovirus (MLRSV)** *Peach chlorotic mottle foveavirus (PCMV)* *Peach enation nepovirus (PEV)** *Peach mosaic virus trichovirus (PcMV)* *Peach rosette mosaic nepovirus (PRMV)* *Petunia asteroid mosaic tombusvirus* *Plum pox potyvirus (PPV)* *Prunus virus T (PrVT)*	*Apple scar skin viroid* *Hop stunt viroid*

	phytoplasmas	*Raspberry ringspot nepovirus (RpRSV)* *Strawberry latent ringspot virus (SLRSV)* *Stocky prune cheravirus (StPV)** *Tobacco ringspot nepovirus (TRSV)* *Tomato black ring nepovirus (TBRV)* *Tomato ringspot nepovirus (ToRSV)* *Tomato bushy stunt tombusvirus (TBSV)*

Table 1.2 A list of pathogens that are known to infect almonds and/or summerfruit that occur in Australia and may require testing at the certification level.

Pathogen group	Pathogens known to infect almond	Pathogens known to infect other *Prunus* species
Bacteria	*Agrobacterium tumefaciens* *Pseudomonas syringae pv. syringae* *Xanthomonas arboricola pv. pruni*	*Agrobacterium tumefaciens* *Pseudomonas syringae pv. mors-prunorum* *Pseudomonas syringae pv. syringae* *Xanthomonas arboricola pv. pruni*
Viruses	*Apple chlorotic leaf spot trichovirus* (ACLSV) *Apple mosaic virus Ilarvirus* (ApMV) *Prune dwarf Ilarvirus* (PDV) *Prunus necrotic ringspot Ilarvirus* (PNRSV) *Plum bark necrosis stem pitting-associated ampleovirus* (PBNSPaV)	*Apricot pseudochlorotic leaf spot trichovirus* (APCLSV) *Apple stem grooving virus capillovirus* (ASGV) *Apple chlorotic leaf spot trichovirus* (ACLSV) *Apple mosaic virus Ilarvirus* (ApMV) *Cherry green ring mottle foveavirus* *Cherry necrotic rusty mottle virus* *Cherry virus A* (CVA) *Little cherry virus 2* (LChV2) *Plum bark necrosis stem pitting-associated ampleovirus* (PBNSPaV) *Prune dwarf Ilarvirus* (PDV) *Prunus necrotic ringspot Ilarvirus* (PNRSV) on stone fruit in Australia
Viroids	*Hop stunt viroid* (Australian strains) *Peach latent mosaic viroid*	*Hop stunt viroid* (Australian strains) *Peach latent mosaic viroid*

1.1 OVERVIEW OF INDEXING PROCEDURES

Biological indexing

Biological indexing takes advantage of a sensitive plant response to the presence of pathogens. Indicator plants are inoculated with material of another source and are observed for characteristic symptoms. Two biological indexing methods are used for the detection of grapevine viruses: herbaceous indexing by sap inoculation and leaf grafting.

Herbaceous indicators can be used within the Australian almond and summerftruit certification schemes for detection of PNRSV, PDV, ACLSV and ApMV and in Australian post entry quarantine for detection of ArMV, CRLV, PeAMV, PcMV, PRMV, RpRSV, SLRV, TBRV, TBSV, TRSV and ToRSV (Table 3). Minor viruses might also be detected and include *Apricot latent ringspot nepovirus* (ALRSV), *Carnation Italian ringspot tombusvirus* (CRIV), *Cherry rosette nepovirus* (ChRV), *Epirus cherry ourmiavirus* (EpCV), *Myrobalan latent ringspot nepovirus* (MLRSV), *Peach enation nepovirus* (PEV) and *Stocky prune cheravirus* (StPV).

Herbaceous indicator plant species that are sensitive to these viruses include *Chenopodium* spp., in particular *C. quinoa*. Cucumber, var. Crystal apple, can be used to detect strains of PNRSV, PDV and ApMV Plant tissue is ground in an appropriate buffer and rubbed onto the leaves of the indicator plants that have been dusted with an abrasive powder. Symptoms can

develop within seven days of inoculation, however plants are often observed for up to six weeks post inoculation. Although this method is useful, not all virus strains cause symptoms on indicators.

Biological indexing for viruses is also done by grafting the a bud of a candidate plant onto sensitive *Prunus* species. Alternatively it is possible to graft buds of the indicator inot the candidate. In both methods the sensitive indicator is observed for symptoms indicative of virus infection. ontot he host plant. This method is currently used in Australian PEQ and certification schemes for diagnosis of virus associated diseases. Biological indexing onto woody indicators is dependent on the successful transmission of associated viruses from the chip bud via the graft union to the indicator plant. This virus inoculation process is affected by graft take and presence of viruses in the grafted chip buds. Disease expression in the indicators is also affected by strain variation amongst the associated virus species, and some viruses may not induce obvious symptoms on the selected biological indictors. It is possible that environmental factors, such as temperature, light and nutrition, will affect symptom expression. Some indicators develop symptoms that cannot be readily attributed to a known causal agent, or combination of causal agents.

- Biological indexing by rub inoculation onto herbaceous indicators is carried by a diagnostic laboratory in glasshouse conditions in October and November according to the protocols in this manual.

- Biological indexing by graft inoculation of biological indicators is carried by a diagnostic laboratory in screenhouse conditions in during dormancy according to the protocols in this manual.

Molecular testing

Reverse transcription polymerase chain reaction (RT-PCR) and PCR techniques are rapid and sensitive molecular methods that detect the genetic material of a target orgnaisim. These methods can be used to detect a number of viruses, viroids, phytoplasmas and bacteria of that infect Almonds and summerfruit. RT-PCR or PCR methods require sequence information of at least part of the pathogen genome so that primers can be designed and used in for its specific detection. For detection of RNA viruses the RT step is required to make a DNA copy (cDNA) of the viral RNA before the PCR can proceed. During PCR a thermostable enzyme (e.g. *Taq* DNA polymerase) is used to generate multiple copies of a specific nucleotide sequence from DNA or cDNA. These methods are dependent on accurate sampling and high quality nucleic acid extracts to increase the chance of pathogen detection reduce the risk of false negative results. Molecular tests can be used for detection of most viruses, viroids, phytoplamsas and bacteria.

- Molecular indexing of prescribed pathogens is carried out in October to December according to the protocols described in this manual. Testing can be done at other times of the year, although the reliability for some viruses, including ApMV, PNRSV and PDV, may be reduced.

SECTION 2: Preparing facilities and indicator plants for biological indexing and maintenance of positive controls

2.1 Maintaining security and plant productivity

The same level of security applies to areas for uninoculated and inoculated biological indicator plants and the virus positive control plants.

- The uninoculated indicator plants, the inoculated indicator plants and the virus positive control plants are maintained in a separate secure glasshouses or screenhouses or isolated compartments within a glasshouse or screenhouse facility.
- Dedicated, isolated and signed secure areas are required for uninoculated indicators, inoculated indicators and positive controls.
- Where necessary, additional signs inside each area should identify the type of plant present and specify the handling requirements of these plants.
- Access to the facility is controlled and limited to specified personnel.
- The facility is enclosed with polythene, glass or quarntine approved mesh (maximum aperture 0.6mm).
- All windows and vents are screened with a quarantine approved insect proof mesh
- Access to the facility is through tight fitting doors to an insect proof anteroom or airlock
- Doors should remain closed at all times except when approved personnel enter or leave the facility through the airlock. In each case only one door should be open and then closed before opening the other door.
- The anteroom should be large enough to permit entry of personnel and plant material.
- Personnel are to routinely dip footwear in a foot bath that is located in the anteroom utilizing an effective disinfectant:
    - The footbath must be replenished with an approved disinfectant once a week or sooner if required.
    - All personnel using quarantine approved facilities should disinfect footwear
- The facility should have a concrete floor and should not flood.
- Where practical the facility will be maintained at 18-25°C.
- Appropriate plant and screenhouse/glasshouse hygiene measures shall be maintained at all times:
    - Regular cleaning to remove dirt and rubbish on benches and floors.
    - Disinfection of cutting tools.
- A 1m wide weed free buffer is recommended around facilities in which the indicator plants are grown. Also the facility and the anteroom shall be maintained free of weeds, lichen and moss.
- Ventilation will be controlled to ensure low humidity which discourages foliar disease such as powdery mildew.
- Yellow sticky insect traps shall be appropriately installed in the facility at a minimum rate of one per 15 square meters of planted area and one shall be installed in the anteroom to monior for insect vectors of plant pathogens:
    - Sticky traps will be inspected 2-3 times per week.

- Sticky traps will be replaced every two weeks.
- If potential pathogen vectors are detected in the facility they will be sent to a diagnostic laboratory for identification.
- If pathogen vectors are detected on the sticky traps or on plants, all plants within the facility shall be treated with an appropriate insecticide.

- Employees of the diagnostic laboratory and visitors must not enter the facility after they have been working amongst/visiting field grown plants on the same day unless they have changed into clothing that has not been exposed to field conditions and ensured that any exposed body parts are not harbouring potential insect vectors of viruses and phytoplasmas such as whiteflies, thrips, aphids or leaf or plant hoppers.

2.2 Propagation and maintenance of biological indicators

PEQ and Australian certification programs use the woody biological indicator *P. persica* cv. GF305 for detection of virus associated diseases. PEQ also uses *Chenopodium quinoa.* Other useful indicators include cucumber (*Cucumis sativus*) cv crystal apple.

The viruses that are detected are listed in Table 2.1.

Table 2.1 A list of viruses that infect almond and/or summerfruit and the indicators that can be used to detect them

Virus	GF305	C. quinoa	Cucumber
ACLSV	✓	✓	
ALRSV	✓	✓	
ApMV	✓		✓
ApLV	✓		
ArMV	✓	✓	
APCLSV	✓		
APLPV	✓		✓
APruV1, 2 &3	✓ - Requires further confirmation, but symptoms that can't be attributed to another cause should be investigated for these viruses		
ASGV		✓	
AVCaV			
CGRMV	✓ - not all strains		
CIRV		✓	
CLRV	✓	✓	✓
CMLV	✓		
CNRMV			
CRLV	✓	✓	✓
CTLaV	✓		
CVA			
EpMV			✓
LChV1			
LChV2			
MLRSV	✓	✓	
PeAMV		✓	
PBNSPaV	✓		
PcMV		✓	
PCMV	✓		
PDV	✓		✓
PEV		✓	
PNRSV	✓		✓
PPV	✓		
PRMV	✓	✓	✓
RRSV	✓	✓	✓
SLRSV	✓	✓	✓
StPV	✓	✓	
TBRV	✓	✓	✓
TBSV		✓	
TRSV	✓	✓	✓
ToRSV	✓	✓	✓
Phytoplasma – X-disease, ESFY	✓		

2.2.1 GF305 Indicator plants

- GF305 is the minimum requirement for biological indexing during certification and isreccommended during PEQ. .
- All indicator plants must be free of virus and other pathogens prior to inoculation.
- Biological indexing requires two GF305 indicators plants of per nucleus variety. Additional plants should be produced each season to ensure there are ample plants for inoculation.
- GF305 should be produced from seed collected from pathogen tested trees. GF305 must be tested prior to grafting.
- Production of GF 305 indicator plants will commence no earleir than two seasons prior to graft inoculation in to ensure that there are adequate numbers of each indicator cultivar with at sufficient girth for grafting.
- Propagation and maintenance of indicators uses methods of high hygiene that comply with standards for the quarantine-approved facility in which plants are held throughout.
- Indicator plants to be used as mother plants will be grown in pasteurised standard-type potting mix with a medium-term, slow-release fertiliser (e.g. Osmocote®) in new pots with a minimum size of 1.1 L.
- Legible waterproof labels are hand written on the outside of each pot with the name or code of the indicator and date of establishment. Space is left for additional labels for the diagnostic test and target,
- All indicator plants will be grown on raised benches and be easily accessible for inspection.
- Plants will be adequately spaced on benches to physically isolate plants and aide visual inspection.
- GF305 indicator plants must be maintained in a healthy, vigorous state.
- The indicator plants will be inspected daily for flower buds and evidence of non target disease and insect pests:
    - Flower buds are removed to prevent flowering of any indicator plant during the growing season
    - Pathogens and insects must be treated as soon as is practical.
    - If the plants are infected with a prescribed pathogen they must be isolated then destroyed.
    - Positive results from diagnostic tests mean that the plant in the holding area, or Nucleus Mother plant is infected. This triggers a client consultation process.

2.2.2 Herbaceous indicator plants

- All herbaceous indictor plants are grown from seed that has been produced from a virus free source.
- Seed is planted into a sterile 0.5L pot with a sterile seed raising mix with fertiliser.

- When the seed has reached the two leaf stage (approx. 10 days) they are pricked out and three seedlings are potted into a 0.25L pot containing a standard-type potting mix with a medium-term, slow-release fertiliser (e.g. Osmocote®).
- One pot of three *C quinoa* seedlings or cucumber seedlings is produced for each candidate variety.
- One of the three seedlings is removed from the pot at the time of inoculation.
- Plants must be grown at 18-25°C before and after inoculation
- The indicator plants will be inspected daily for flower buds and evidence of non target disease and insect pests:
    - Pathogens and insects must be treated as soon as is practical.
    - If the plants are infected with a prescribed pathogen they must be isolated then destroyed.
    - Positive results from diagnostic tests mean that the plant in the holding area, or Nucleus Mother plant is infected. This triggers a client consultation process.

2.3 PROPAGATION AND MAINTENANCE OF VIRUS POSITIVE CONTROL PLANTS

- The virus isolates for use as positive controls for biological and molecular testing are maintained in *Prunus* sp. plants.
- The virus positive control plants are maintained in a separate facility that is isolated from two other secure facilities in which uninoculated and inoculated indicators plants are held to reduce the risk of virus transmission between plants.
- Normal horticultural protocols are used to grow each *Prunus* species that is used as a positive control plant.
    - New positive control plants are propagated from vegetative production or by chip bud grafting whereby diseased tissue from the original plant is grafted to sensitive GF305 .
    - Newly inoculated plants are isolated on a glasshouse bench and standard water proof labels applied that identify the original source and disease or virus with which they were inoculated and date of inoculation.
    - In addition to the presentation of disease symptoms each plant is tested by molecular methods to determine confirm the transmission of virus.
    - Follow-up labels identify the viruses that are detected.
- Inspect virus positive control plants daily for presence of flower buds and evidence of disease and insect pests
    - Flower buds are removed to prevent transmission of pollen born viruses during flowering
    - Pathogens and insects must be treated as soon as is practical.

2.4 LABORATORY FACILITIES

2.4.1 Molecular laboratories:

- Three laboratories are required for molecular indexing:
  .1. Nucleic acid extraction laboratory – gel electrophoresis can be done in this laboratory. The pipettes used for eelctrophorieses must not be used for nucleic acid extraction.
  .2. Molecular laboratory for PCR reaction set up – no plant material or nucleic acid extracted from plant material is allowed in this laboratory.
  .3. Molecular laboratory for the addition of nucleic acid to the PCR reactions.
    - A fourth laboratory for gel electrophoresis is desirable to reduce the risk of contamination of nucleic acid extracts by PCR products.
- The equipment used is specific to each laboratory and must not be removed.
- If three laboratories are not available PCR reaction set up can be done in a PCR workstation in the same laboratory where nucleic acid is extracted:
    - The pipettes, boxes of tips and bags of tubes used in this work station must not be used for other purposes.
    - The workstation must have filter system in place to reduce contamination through aerosols and an ultraviolet lamp for irradiation of the interior of the cabinet to degrade potential contaminating nucleic acid.

2.4.2 Bacterial culturing laboratories:

- All cultures of bacteria are located in a secure facility for growth and identification

2.4.3 Disposal of samples and cultures:

- All infective material, laboratory materials that have been in contact with samples or their extracts; and fungal and bacterial cultures should be destroyed by steam sterilisation or by an accredited waste disposal facility.

SECTION 3: Diagnostic protocols

3.1 Detection of endemic pathogens and pathogens present in Australia

The detection methods for exotic pathogens and pathogens present in Australia that are given in this manual are litsed in table 1.

Table 3.1. A list of endemic/present and exotic pathogens of Almonds and summerfruit and methods used for detection of each pathogen.

Pathogen	Detection method			
	Visual inspection[1]	Culturing	Biological	Molecular
Virus: Exotic				
American plum line pattern Ilarvirus (APLPV)	Yes		GI[2], HI[3]	RT-PCR[4]
Apricot latent virus f*oveavirus* (ApLV)			GI	RT-PCR
Apricot vein clearing associated prunevirus (ACVaV)	Yes			RT-PCR
*Asian Prunus virus 1 foveavirus (*APruV-1)				RT-PCR
*Asian Prunus virus 2 foveavirus (*APruV-2)				RT-PCR
Asian Prunus virus 3 foveavirus (APruV-3)				RT-PCR
Arabis mosaic nepovirus (ArMV)	Yes		GI, HI	RT-PCR
Cherry leaf roll nepovirus (CLRV)	Yes		GI, HI	RT-PCR
Cherry mottle leaf trichovirus (CMLV)	Yes			RT-PCR
Cherry rasp leaf cheravirus (CRLV)	Yes		GI, HI	RT-PCR
Cherry twisted leaf foveavirus (CTLaV)	Yes			RT-PCR
Little cherry virus 1 (unassigned genus, LCHV1)	Yes			RT-PCR
Peach chlorotic mottle foveavirus (PCMV)	Yes		GI	RT-PCR
Peach mosaic virus trichovirus (PcMV)	Yes		HI	RT-PCR
Peach rosette mosaic nepovirus (PRMV)	Yes		GI, HI	RT-PCR
Petunia asteroid mosaic tombusvirus (PeAMV)	Yes		HI	RT-PCR
Plum pox potyvirus (PPV)	Yes		GI	RT-PCR
Prunus virus T (PrVT)				RT-PCR
Raspberry ringspot nepovirus (RpRSV)	Yes		GI, HI	RT-PCR
Strawberry latent ringspot virus (SLRSV)	Yes		GI, HI	RT-PCR
Tobacco ringspot nepovirus (TRSV)	Yes		GI, HI	RT-PCR
Tomato black ring nepovirus (TBRV)	Yes		GI, HI	RT-PCR
Tomato ringspot nepovirus (ToRSV)	Yes		GI, HI	RT-PCR

Pathogen	Detection method			
	Visual inspection[1]	Culturing	Biological	Molecular
Tomato bushy stunt tombusvirus (TBSV)	Yes			RT-PCR
Virus - present				
Apricot pseudochlorotic leaf spot trichovirus (APCLSV)	Yes		GI	RT-PCR
Apple stem grooving virus capillovirus (ASGV)			HI	RT-PCR
Apple chlorotic leaf spot trichovirus (ACLSV)	Yes		GI, HI	RT-PCR
Apple mosaic virus Ilarvirus (ApMV)	Yes		GI, HI	RT-PCR
Cherry green ring mottle virus (CGRMV)	Yes		GI –not all strains	RT-PCR
Cherry necrotic rusty mottle foveavirus (CNRMV)	Yes			RT-PCR
Cherry virus A (CVA)				RT-PCR
Little cherry virus 2 ampelovirus (LChV2)				RT-PCR
Plum bark necrosis stem pitting associated ampelovirus (PBNSPaV)	Yes		GI	RT-PCR
Prune dwarf Ilarvirus (PDV)	Yes		GI, HI	RT-PCR
Prunus necrotic ringspot Ilarvirus (PNRSV)	Yes		GI, HI	RT-PCR
Viroids - Exotic				
Apple scar skin viroid (ASSVd)				RT-PCR
Hop stunt viroid (HSVd)				RT-PCR
Viroid - present				
Hop stunt viroid (HSVd)				RT-PCR
Peach latent mosaic viroid (PLMVd)			GI	RT-PCR
Bacteria - Exotic				
Xylella fastidiosa	Yes	Yes		PCR[5]
Erwinia amylovora	Yes	Yes		PCR
Pseudomonas amygdali	Yes	Yes		PCR
Pseudomonas syringae pv. persicae	Yes	Yes		PCR
Pseudomonas syringae pv. avii	Yes	Yes		PCR
Bacteria - present				
Agrobacterium tumefaciens	Yes	Yes		PCR
Pseudomonas syringae pv. mors-prunorum	Yes	Yes		PCR
Pseudomonas syringae pv. syringae	Yes	Yes		PCR
Xanthomonas arboricola pv. pruni	Yes	Yes		PCR
Phytoplasmas - exotic	Yes			PCR

[1] Active surveillance by visual inspection may not be reliable – symptom expression is dependent upon the interaction between virus strain and cultvar

[2] GI = Biological indexing by graft inoculation onto the susceptible *GF305*

[3] HI = Biological indexing by rub inoculation onto *Chenopodium quinoa and/or cucumber* (herbaceous indexing)

[4] RT-PCR = Detection of pathogen RNA by reveres transcription (RT) PCR

[5] PCR = Detection of pathogen DNA by polymerase chain reaction (PCR)

3.2 Pathogen testing methods

3.2.1 Visual Inspection for disease

- Table 3.1 lists the pathogens for which may be detected in Almond and summerfruit plants by visual inspection of the associated diseases and the alternative methods of detection which can be used to confirm infection.
- Symptom expression may be depedent on the interaction between the pathogen, cultivar and environment and therefore may not be a reliable method of detection.
- Almond or summerfruit plants that are not adequately fertilised may exhibit nutrient deficiencies that resemble virus associated diseases, e.g. chlorosis and necrosis.
- Infection by viruses, bacteria and phytoplasmas should be confirmed by molecular methods by the diagnostic laboratory. The methods are detailed in appendix 2.

3.2.2 Active pathogen testing for viruses, viroids, Phytoplasmas and Bacteria

3.2.2.1 Sampling and transport of samples to diagnostic facilities for pathogen testing

Generic sampling guidelines for active pathogen detection

- A record will be kept by the diagnostic laboratory that states:
    - The identifying code or name for the sample and the variety from which it was collected.
    - The date and time that the sample was collected.
    - The date and time that the sample arrived at the diagnostic laboratory.
    - The specific diagnostic tests that have been ordered.
- Hands will be washed and cutting tools will be sterilised before use and between taking samples from plants that are to be tested separately.
- The samples are placed in a separate zip-lock bags that is clearly labelled with a code or name that is traceable to the plant that is being tested:
    - Remove as much air as possible from the zip-lock bags.
    - The code or name will be used to identify the sample during all diagnostic testing and reporting.
- The bagged samples are placed into a ice-box on ice at approximately 4°C after sampling and during transport to the diagnostic facility.

- The samples will be logged to the diagnostic laboratory's database immediately upon arrival.
- Samples that are not used immediately upon arrival at the diagnostic facility will be stored in a cool room at 4°C.
- Do not collect samples on a Friday (specify Mon to Thurs).

Sampling for virus detection

- Sampling for virus testing by biological indexing onto GF305 occurs in spring for grafting or winter for budding
- Sampling for virus testing by biological onto *C. quinoa* and cucumber and molecular methods occurs during October – December.
- A minimum of 10 shoot tips are required for graft indexing for viruses onto GF305
- A minimum if 3 shoots, 20cm long, with new leaves are required for hebcaoeus indexing and molecular methods.
- A minimum of 10 bud sticks with 3-4 buds/stick are required for graft indexing for viruses onto GF305
- Any samples that are are not used immediately upon arrival will be stored at 4°C until required.
    - For molecular indexing and herbaceous indexing on *C. quinoa* the samples must be used as soon as possible after removal from the host
    - If samples require storage before testing, the leaves must be kept whole, all surface water removed and the material stored in a plastic bag at 4°C for no more than 20 days.
    - Samples that have partially decayed or become mouldy should not be tested, and further samples must be collected.

Sampling for detection of bacteria

- For bacterial testing by culturing or molecular methods, subsamples of the petioles and leaflets are collected from the samples taken for molecular or biological indexing of viruses:
    - The subsamples are labelled with a code or name that is traceable to the variety that is being tested.
    - Depending upon the extraction technique used it may be possible to use the same nucleic acid that is used for the molecular indexing of viruses to test for bacteria using PCR. The nucleic acid extractions that can be used for testing of bacteria by molecular methods is detailed in appendix 3.

3.2.4 PATHOGEN TESTING PROTOCOLS

3.2.4.1 Biological indexing

Biological indexing is done according to the protocols in this manual. Biological indexing by graft inoculation of *Prunus* sp. onto sensitive GF305 biological indicators and rub inoculation of *C. quinoa* and cucumber is carried by in a secure glasshouse in October and November Budding of GF305 is done in a secure glasshouse in summer

Diagnosis by grafting test samples to biological GF305 indicator plants

- Graft inoculation of GF305 indicators will be conducted in October-November.
- Chip bud incolauation of GF305 indicators will be conducted in Summer.
- Each variety must be tested by grafting or chip budding a minimum of one shoot or two buds respectively on each of two replicate indicator GF305. Table 3.2 list the symptoms that may be observed.
- Chip buds should be grafted that are taken from bud sticks of similar diameter to the indicator
- Clearly label the pot of each indicator that will be grafted with a particular sample/variety with the code or name assigned to the sample and the date of grafting.
- The method of graft and chip bud inoculation is described in detail in appendix 2.
- The indicator plants must be maintained in a vigorous state of growth before and after grafting and must be grown under moderate temperatures and light intensities.
- Single plants GF305 must be left ungrafted and subjected to the same horticultural practices and environmental conditions as the inoculated plants.
- GF305 indicators must be grafted with tissue sampled from virus positive control plants containing PNRSV, PDV, ApMV and/or ACLSV as a minimum.
- Check the graft union on each indicator weekly after inoculation. At least one graft per indicator plant must have survived. If both grafted buds have not survived the graft must be repeated.
- The inoculated indicator plants will be held in a secure facility as described in section 2.2.1 of this manual.
- The indicator plants will be examined once per week for symptom expression over a four month period.

Table 3.2. A list of endemic/present and exotic pathogens of Almonds and summerfruit and methods used for detection of each pathogen.

Pathogen	Symptoms on GF305
Virus: Exotic	
American plum line pattern Ilarvirus (APLPV)	Chlorotic lines, rings and oak-leaf patterns, yellow net
Apricot latent virus f*oveavirus* (ApLV)	Chlorotic lesions, green spots asteroid ringspots
Arabis mosaic nepovirus (ArMV)	Stunting, short internodes and rosetting
Cherry leaf roll nepovirus (CLRV)	Rosetting and slight leaf rolling
Cherry rasp leaf cheravirus (CRLV)	
Peach chlorotic mottle foveavirus (PCMV)	Chlorotic mottling and ring pattern symptoms
Peach rosette mosaic nepovirus (PRMV)	Yellowing and inward folding of leaves, rosetting
Plum pox potyvirus (PPV)	Chlorotic blotches, deformation of leaf tips and margins
Raspberry ringspot nepovirus (RpRSV)	Chlorotic blotches and leaf deformation
Strawberry latent ringspot virus (SLRSV)	Stunting, short internodes and rosetting
Tobacco ringspot nepovirus (TRSV)	Stunting, short internodes and rosetting
Tomato black ring nepovirus (TBRV)	Stunting, short internodes and rosetting
Tomato ringspot nepovirus (ToRSV)	Stunting, short internodes,chlorotic leaves curling upwards and turning red in autumn
Virus - present	
Apricot pseudochlorotic leaf spot trichovirus (APCLSV)	Dark green sunken mottle
Apple chlorotic leaf spot trichovirus (ACLSV)	Dark green sunken mottle
Apple mosaic virus Ilarvirus (ApMV)	Chlorotic line and/or and banding patterns
Cherry green ring mottle virus (CGRMV)	Vein yellowing in young leaves
Plum bark necrosis stem pitting associated ampelovirus (PBNSPaV)	Chlorotic rings and mottling, and line patterns
Prune dwarf Ilarvirus (PDV)	Stunted growth and mild mottle
Prunus necrotic ringspot Ilarvirus (PNRSV)	Chlorotic mottle or rings, necrotic rings or spots, and tatter leaf symptom
Viroids - present	
Peach latent mosaic viroid (PLMVd)	Chlorotic blotches, mosaic or chlorotic creamy calico on leaves

Diagnosis by herbaceous indexing

- Herbaceous indexing by rub inoculation of *Chenopodium quinoa* and/or cucumber indicators will be conducted in October-November.
- Each variety in the nucleus collection must be tested by rub inoculation onto two replicate *C. quinoa* and cucumber indicator plants in each year.
- The indicator plants are ready for rub inoculation when they have two or more fully expanded leaves.
- Clearly label the pot of each indicator that will be rub inoculated with a particular sample/variety with the code or name assigned to the sample and the date of inoculation.
- Two fully expanded leaves of each indicator plant will be rub inoculated.
- The method of herbaceous indexing by rub inoculation is described in appendix 2. Table 3.3 lists the symptoms that may be observed.
- The *C. quinoa* and cucumber indicator plants must be maintained in a vigorous state of growth before and after inoculation and must be grown under moderate temperatures and light intensities.
- A single pot containing two *C. quinoa* plants and a single pot containing two cucumber plants must be left uninoculated and subjected to the same horticultural practices and environmental conditions as the inoculated plants.
- A single pot containing two *C. quinoa* plants and a single pot containing two cucumber plants must be inoculated with a PNRSV, PDV, ApMV and/or ACLSV virus positive control and subjected to the same horticultural practices and environmental conditions as the inoculated plants. In PEQ at least one ne*pv.*irus species should eb used, if available.
- The inoculated indicator plants will be held in a secure facility as described in section 2.1 for a minimum of eight weeks.
- The indicator plants will be examined at least twice per week for symptom expression.
- When biological indexing is completed all inoculated indicator plants are destroyed.

Table 3.3. A list of endemic/presnet and exotic pathogens of Almonds and summerfruit and methods used for detection of each pathogen.

Pathogen	Indicator	symptoms
Virus: Exotic		
American plum line pattern Ilarvirus (APLPV)	Cucumber	Small chlorotic local lesions in inoculated cotyledons
Arabis mosaic nepovirus (ArMV)	*C. quinoa*	Chlorotic local lesions and systemic chlorotic mottle
	Cucumber	Chlorotic local lesions, systemic yellow spots or veinbanding , which fading. Plants stop growing

Pathogen	Indicator	symptoms
Cherry leaf roll nepovirus (CLRV)	*C. quinoa*	Chlorotic or necrotic local lesions, systemic mottle or necrosis and distortion
	Cucumber	Chlorotic local lesions in cotyledons, occasional systemic mosaic
Cherry rasp leaf cheravirus (CRLV)	*C. quinoa*	Fine mottle and vein clearing on the third or fourth leaf above the inoculated leaf, Wilting of axillary shoots
	Cucumber	Faint chlorotic primary lesions in cotyledons; fine systemic mottle
Peach mosaic virus trichovirus (PcMV)	*C. quinoa*	Mild chlorotic mosaic on the young expanding, which become chlorotic and epinastic. Mild stunting.
Peach rosette mosaic nepovirus (PRMV)	*C. quinoa*	Cream to yellow chlorotic lesions in inoculated leaves, which dissappear. Systemic mottling, leaf deformity, and epinasty. Apical necrosis may occur.
Petunia asteroid mosaic tombusvirus (PeAMV)	*C. quinoa*	Necrotic lesions
Raspberry ringspot nepovirus (RpRSV)	*C. quinoa*	Necrotic local lesions, systemic necrosis
Strawberry latent ringspot virus (SLRSV)	*C. quinoa*	Chlorotic or necrotic local lesions, systemic chlorosis and deformation, necrosis or faint chlorotic mottle
Tobacco ringspot nepovirus (TRSV)	*C. quinoa*	Necrotic local lesions
	Cucumber	Chlorotic or necrotic local lesions, systemic mottling , dwarfing with apical distortion
Tomato black ring nepovirus (TBRV)	*C. quinoa*	Chlorotic or necrotic local lesions , systemic necrosis or chlorotic mottle
Tomato ringspot nepovirus (ToRSV)	*C. quinoa*	Small chlorotic or necrotic local lesions, systemic apical necrosis
	Cucumber	Local chlorotic spots, systemic chlorosis and mottle
Tomato bushy stunt tombusvirus (TBSV)	*C. quinoa*	Chlorotic/necrotic local lesions surrounded by chlorotic haloes
Virus - present		
Apple stem grooving capillovirus (ASGV)	*C. quinoa*	Systemic leaf epinasty, distortion, and mottle and stunting. Necrotic lesions on inoculated leaves caused by some strains
Apple chlorotic leaf spot trichovirus (ACLSV)	*C. quinoa*	Chlorotic and necrotic spots in inoculated leaves 3-4 days after inoculation, systemic f chlorotic spots, mottling, ring and line patterns in upper leaves

Pathogen	Indicator	symptoms
Apple mosaic virus Ilarvirus (ApMV)	Cucumber	Prominent chlorotic primary lesions on inoculated cotyledons, extreme stunting
Prune dwarf Ilarvirus (PDV)	Cucumber	Chlorotic spots; systemic mosaic, which may be restricted to parts of leaves
Prunus necrotic ringspot Ilarvirus (PNRSV)	Cucumber	Prominent chlorotic primary lesions, systemic tip killing, stunted and compact growth of axillary buds

3.2.4.2 Diagnosis by molecular indexing

Molecular indexing is carried out by in October and December according to the protocols in this manual.

Sub-sampling for molecular detection of pathogens.

- Samples should be processed as soon as possible after receipt by the diagnostic laboratory:
    - If samples cannot be processed immediately they should be stored as described in section 3.2.2.1.
- A 0.5g tissue is required for nucleic acid extraction. Samples comprise of at least three 20cm shoots, with leaves from replicate plant(s) from each variety:
    - Return all unused tissue to the sample bag for biological indexing on *C. quinoa* and cucumber and bacterial and fungal culturing if required,.

Nucleic acid extraction

- Nucleic acid extraction is required for molecular indexing for the prescribed viruses, viroids, phytoplasmas and bacteria.
- Nucleic acid extraction is carried out in the nucleic acid extraction laboratory.
- Nucleic acid extraction will be carried out according to the protocols detailed in appendix 3:
    - If there are more that eight samples a QiaExtractor® robot can be used to extract total nucleic acid from each sample.
    - If the QiaExtractor® is not available or if there are eight samples or less RNA and/or DNA are extracted using the Qiagen RNeasy® and DNeasy® kits respectively.
- All vials containing samples or extracts from plant samples should be labelled with the sample code, the date and initials of diagnostic scientist or a code that can be traced to the sample information date of extraction and name of the diagnostic scientist.

Housekeeping PCR for nucleic acid quality

- Prior to molecular indexing for pathogens all nucleic acid extracts will be subject to “housekeeping” RT-PCR and PCR assays to ensure that the nucleic acid is of adequate

quality for pathogen detection by molecular methods and does not result in a false negative result. The protocol is detailed in appendix 3:

- The housekeeping PCR requires:
    - A positive control: RNA of known good quality.
    - A no template control: Sterile distilled water.
- The housekeeping RT-PCR or PCR assay will only be considered valid if:
    - the positive control produces the correct size product.
    - no bands are produced in the no template control.
- If a positive result is obtained the extract can be tested for the specified pathogens.
- A negative result indicates that nucleic acid has failed to amplify and is not of sufficient quality for pathogen detection:
    - Failure of the samples to amplify with the housekeeping primers suggests that the nucleic extraction has failed, compounds inhibitory to PCR are present in the nucleic acid extract or the nucleic acid has degraded.
    - Dilution or re-extraction of the nucleic acid may be required.
    - Do not use this nucleic acid for pathogen detection.
- If a negative result is returned the extract can be diluted 1/5 and 1/10 and the dilutions re-tested using the housekeeping assay.
    - If a positive result is returned the diluted nucleic acid extract can be tested for the specified pathogens.
    - If the diluted nucleic acid extracts return a negative result the sample must be re-extracted and the new extract must be tested using the housekeeping RT-PCR and/or PCR assay.

RT-PCR and PCR assays

- The viruses, viroids, bacteria and phytoplasmas of Almonds and summerfruit that can be detected by molecular methods are listed in Table 3.1.
- All PCR tests must include:
    - Positive control: Nucleic acid extracted directly from the pathogen or from an infected plant.
    - No template control: Sterile distilled water.
    - (Optional) Negative control: Nucleic acid extracted from uninfected plant.
- The diagnostic laboratory will ensure that positive control for each of the specified viruses is available before molecular indexing is conducted.
- Specific protocols for the molecular detection of each pathogen are detailed in appendix 3.
- The results of molecular indexing will be examined by a qualified diagnostician to verify the results and determine that a positive result in a test sample is the same molecular size as the positive control for that pathogen.

3.2.4.3 Diagnosis of bacteria by culturing

- Bacterial cultures are stored on appropriate media, assigned an identifying number and located in a secure incubator or facility
- Bacterial cultures are identified by a qualified taxonomist using appropriate reference material and illustrated guides.

Bacterial culturing

- Protocols for culturing depend upon the bacteria requiring isolation and will be determined by the diagnostic laboratory and are not given in this manual.

If available other methods, as determined by the diagnostic laboratory, may be used to detect and identify specific bacteria.

3.2.5 Interpretation of pathogen testing results

Biological testing

- Pathogen detection by biological indexing is based on symptom expression.
- Biological indexing will only be considered positive if:
    - Symptoms typical of a prescribed pathogen are detected on an indicator during the observation period.
    - Symptoms for virus resemble those observed on indicators inoculated with virus positive controls or described in this manual.
    - Indicators inoculated with the virus positive controls express symptoms indicative of virus infection.
- Images of virus infection in the GF305, *C. quinoa* and cucumber biological indicators are given in appendix 2
- A result from biological indexing is considered negative if symptoms of a prescribed pathogen are not detected during the observation period.

Note: Mixed virus infections could lead to symptoms which are difficult to interpret and PCR should be used to verify the presence of virus in the indicator and in the candidate. A negative result indicates that the pathogen was not detected and PCR reaction to specific virus in the test sample indicates positive providing the positive and negative controls give correct results. The test is repeated to confirm presence and mixed infection.

Molecular testing

- The PCR tests will only be considered valid if:
    - the positive control produces the correct size product.
    - no bands are produced in the no template control and the negative control (if used).
- A sample result from valid molecular indexing is considered negative for a pathogen if the PCR assay does not return a PCR product of the expected size.
- A sample result from valid molecular indexing is considered positive for a pathogen if the PCR assay returns a PCR product of the expected size.

Bacterial culturing

- Bacterial culturing is considered negative if colonies do not exhibit typical taxonomic morphology of the bacteria when compared to a type specimen .
- Bacterial culturing is considered positive if colonies do exhibit typical taxonomic morphology of the bacteria when compared to a type specimen.

SECTION 3.2.6: Recording results

A database (electronic and/or notebook) must be kept to record all experimental data, in such a way that it is verifiable by other people.

- All activities must be recorded in the database on a daily basis:
    - the date at the start of each session.
    - underline, sign and date at the completion of each session.
- Images can be attached to the database to show results.
- Record all experimental data, results and observations in the database.

SECTION 3.2.6: Reporting results

- Records including the date of examination, the identity of the sample and any specific details of the sample, the tests that were conducted, the results of testing, including images were appropriate, and the name of diagnostician are retained on confidential file.
- When diagnostic testing is completed the diagnostic laboratory will inform the client via a written report which includes:
    - A list of the samples that were tested using the code or label that is traceable to the plants that were tested.
    - The tests that were used to detect pathogens in each sample.
    - The results that were obtained.
- The report will be transmitted to the client by an appropriate science manager.

SECTION 4: ACTION BY THE DIAGNOSTIC LABORATORY FOLLOWING POSITIVE PATHOGEN TESTING RESULTS

The client will be informed immediately of any positive results by an appropriate science manager.

Biological indexing

- If symptoms of a prescribed pathogen are observed on any indicator plant the infection must be verified in both the affected indicator plant and the variety that was inoculated:
    - The affected symptomatic indicator plant will be tested for pathogens using molecular methods as described in this manual if available.
    - Images of virus infection in the GF305, *C. quinoa* and cucumber biological indicators are given in appendix 2.
- If *an* infection is suspected the affected indicator plants and the variety that was screened will be tested by PCR and culturing protocols where available, as detailed in sections 3.2 and appendix 3 to confirm infection.
- If a pathogen is detected in the indicator plant but not the nucleus plant using molecular (all pathogens) and/or culturing methods the tests must be repeated on all plants in question.

Molecular indexing for pathogens

- If a positive result is obtained the client must be informed and the result must be verified in the affected variety:
    - The original or second PCR product will be sequenced to verify its relationship to the pathogen and ensure that a false positive result was not obtained.
- If a pathogen is not detected in any of the re-tested nucleus plants of the affected variety the molecular tests must be repeated on all plants in question.
- If molecular indexing returns a PCR product similar but not identical to the expected size and the result is considered “strong” the PCR product may be sequenced by the diagnostic laboratory to determine its origin.

Bacterial culturing

- If a positive result is obtained the client must be informed and the result must be verified in the affected variety:
    - Samples will be collected from each of the plants of the candidate nucleus variety and tested separately for the prescribed pathogens using molecular methods and culturing as described in this manual.

Appendix 1 – DESCRIPTION OF PATHOGENS

1.1 Bacteria

QUARANTINE

Organism: *Erwinia amylovora*

Disease: Associated with fire blight disease in plants species of *Rosaceae* including apple and pear. Associated with shoot blight on European plum, *Prunus domestica* L. 'd'Agen' and apricot .

Distribution: The Americas, Europe, Asia (Armenia, Azerbajhan, India Israel, Iran, Jordon, Lebanon, Turkey), Africa (Egypt and Morocco) and New Zealand.

Host range: *E. amylovora* mainly infects members of the sub-family Pomoideae of the family *Rosaceae*: Primary hosts include*: Malus* (apple), *Pyrus* (pears), *Crataegus* (hawthorns), *Cotoneaster*, *Cydonia*, *Eriobotrya* and *Pyracantha* species. Other hosts include *Amelanchier*, *Mespilus*, *Chaenomeles*, *Rubus* (blackberry, raspberry), chokeberry (*Aronia melanocarpa*), strawberry and *Sorbus.* It has been reported to infect almond under experimental conditions. References to infections in almond in general non-refereed publications may be associated with flowering almond (*Prunus triloba*). Natural infections are reported for other *Prunus* sp. in the field, including apricot, peach, Japanese and European plum, sour cherry and cherry plum. Other research has shown that all *Prunus* sp., except *P. domestica*, can support epiphytic growth of the bacterium on flowers.

Economic impact: The disease can cause severe economic impact in *Maloideae* host species such as pear and apple, although the effect is dependent on environment and cultivar. Although disease is observed the impact on summerfruit production is not known, yet these hosts could represent a source of inoculum for other more susceptible species such as pome fruits.

Pathway: Contaminated plant material

Diagnostic tests:

- Isolation and culturing onto general and selective media
- Morphological methods: ooze test and moist incubation
- Hypersensitivity assay in *Nicotiana tabacum.*
- Pathogenicity tests onto pear fruitlets and apple and pear seedlings
- Fatty acid analysis
- Molecular diagnostics: Many conventional and real time PCR tests are available, however some tests may not detect all strains and other tests may detect *Erwinia* sp. other than *E. amylovora.* The currently accepted SPHDS protocol utilises primers that are known to cross react with other bacterial sp. and which have not been validated for summerfruit or almonds. The EPPO protocol recommends to use two conventional PCR tests to detect plasmid and chromosomal DNA (EPPO 2103) especially as some studies show that not all PCR tests will detect all strains (Powney et al 2007). qPCR assays have been developed that may be more sensitive than

conventional assays (EPPO 2013, Gottsberger 2010 and Pirc et al 2009). A LAMP based assay has also been developed (Buhlmann et al 2013).

Notes: The bacterium enters plants through blossoms and the open stomata of new shoots and leaves in spring and then systemically invades the hosts, where it can persist in parenchyma and xylem tissue. It can also enter though wounds. It is transmitted by wind, rain and water splash, insects and birds. It can also be transmitted in plants and propagation material.

Symptoms include: flower or shoot tip necrosis, blight of fruitlets, shepherd's crook shape of affected shoots, red or reddish brown streaks of the vascular tissue, cankers on larger branches, bacterial ooze may be present on shoot tips, blossoms and at the margins of cankers and blighted blossoms and leaves may remain attached after leaf fall.

References: Atanasova et al 2005, Berger et al 2000, EPPO 2013, Johnson et al 2006, Korba and Sillerova 2010, Mohan and Thomson 1996, Mohan and Bijman 1999, Mohan et al 2001, Mohan 2007, Moltmann and Viehrig 2008, Pirc et al 2009, Snow, 1922, Vanneste et al 2002, Végh et al 2012, Végh and Palkovic 2013, Vojinovic 2010.

Organism: *Pseudomonas amygdali*

This bacterium is listed on the PHA Almond Industry biosecurity plan.

Disease: Bacterial (Hyperplastic) canker of almond.

Distribution: Greece, Afghanistan and Turkey.

Host range: *S*pecific to almond. Some almond varieties are more susceptible to *P. amygdali* than others.

Economic impact: Infection by *P. amygdali* can result in yield loss and decline and death of trees, therefore having a significant negative economic impact to industry.

Pathway: Propagation material

Diagnostic tests: No specific diagnostic tool exists.

- Symptom expression, bacterial culturing onto selective media and biochemical tests together should suggest the presence of this pathogen.
- It is specific to almond; therefore pathogenicity onto other hosts may assist diagnosis. An HR response is induced on inoculated *Nicotiana tabacum*.
- A combination of culturing and the use of PCR and sequencing of the *rpoD* locus should distinguish *P. amygdali* from other Pseudomonads that may be isolated from *Prunus* species. This should allow isolates to be assigned to a specific assign phylogroup or genomospecies. Further confirmation could be done using PCR and sequencing of the *acn, pgi* and *cts* loci.

Notes: *P. amygdali* overwinters in cankers and cankers are perennial. It is spread by wind and rain/water splash from active cankers to other parts of a tree and to other trees. It enters the host though leaf scars on twigs and shoots or through wounds. Infection of the wounds results in cankers that consist of swollen bark that cracks open longitudinally around infection points on shoots, twigs and branches. Cankers forming around buds can result in

no bud burst. Cankers can girdle shoots, twigs and branches resulting in dieback. Affected trees decline. The canker symptoms are distinct to *P.* amygdali and differ significantly from cankers associated with *P. syringae pv. syringae* infection.

References: Ercolani and Ghaffer 1985, Gundogdu and Demir 1990, Janse 2010, Psallidas 1997, Sarkar & Guttman 2004, Parkinson et al 2011.

Organism: *Pseudomonas syringae pv. persicae*

Disease: Bacterial dieback of peach and nectarine. It also infects plum in NZ.

Distribution: New Zealand, France, Germany and Portugal Possibly Yugoslavia, but not confirmed. It was isolated once in the UK from *P. cerasifera*.

Host range: Peach, nectarine and Japanese plum. It is not known to infect almond.

Economic impact: The bacterium may have serious economic impacts as it can reduce quality and yield of fruit and quickly cause decline and death of trees

Pathway: Infected propagation and planting material. Mechanically transmitted by pruning.

Diagnostic tests:

- Characteristic symptoms on affected plants should indicate possible infection.
- Isolation and culturing onto semi-selective media
- Biochemical analyses
- PCR tests for the detection of genes associated with toxin production.
- *P. s. pv. persicae* can be distinguished from *P. s. pv. syringae* and *P. s. pv. mors-prunorum* which occur in Australia, by biochemical tests: GATTa (gelatine liquefaction, aesculin hydrolysis, tyrosinase activity, Na-tartrate utlization)test; lack of acid production from sorbitol, erythritol, inositol; non-utilization of lactate, D(-) and L(+) tartrate.); slow growth and non-fluorescence on Kings B media
- A combination of culturing and the use of PCR and sequencing of the *rpoD* locus should distinguish *P. s. pv. persicae* from other Pseudomonads that may be isolated from *Prunus* species. Sequencing of the *rpoD* locus PCR product may not distinguish this species from *P. s. pv. avii.* This should allow isolates to be assigned to a specific assign phylogroup or genomospecies. Further confirmation could be done using PCR and sequencing of the *acn, pgi* and *cts* loci.
- Repetitive PCR, multi-locus sequence typing and melting profile PCR may also be used to distinguish between some *P. syringae* pathovars.

Notes: *P. s. pv. persicae* is spread from epiphytic populations on leaves and enters through wounds such as leaf scars, pruning wounds and water soaked areas on bark that occur due to freeze- thaw cycles. It is spread by pruning, wind and rain.

Symptoms develop through winter as the bacteria spreads systemically within the plant. In spring infection can lead to wilting and dieback of shoots, death of larger branches and in severe infections death of the entire tree. The bacterium can cause necrotic spots on leaves which fall out causing a shot hole appearance. Necrotic spots can also form on fruit, especially Nectarine, which can be covered in a transparent gum that browns quickly.

References: EFSA 2014, Eppo 2005, Janse 2010, Kaluzna et al 2012, Parkinson et al 2011, Prunier et al 1970, Young 1988.

Organism: *Pseudomonas syringae pv. avii*

Disease: Bacterial canker

Distribution: France

Host range: Wild and cultivated cherries. It is not known to occur on other *Prunus* sp. including almond

Economic impact: *P.s. pv. avii* can cause severe dieback and death of affected trees and therefore can have a significant negative economic impact on Cherry production.

Pathway: Propagation material.

Diagnostic tests:

- Characteristic cankers on affected plants should indicate possible infection.
- Isolation and culturing onto semi-selective media
- Biochemical analyses
- PCR tests for the detection of genes associated with toxin production.
- *P. s. pv. avii* can be distinguished from other pathovars, including those which occur in Australia, though biochemical tests: GATTa test and using the Toxin PCR tests
- A combination of culturing and the use of PCR and sequencing of the *rpoD* locus should distinguish *P. s. pv. avii* from other Pseudomonads that may be isolated from *Prunus* species. Sequencing of the rpoD locus PCR product alone may not distinguish the species from *P. s. pv. persicae.* This should allow isolates to be assigned to a specific assign phylogroup or genomospecies. Further confirmation could be done using PCR and sequencing of the *acn, pgi* and *cts* loci.
- Repetitive PCR, multi-locus sequence typing and melting profile PCR may also be used to distinguish between some *P. syringae* pathovars.

Notes: It is spread by pruning, wind and rain. It invades plants through leaf scars and wounds from pruning, feeding etc. and can spread systemically within the plant. It can also spread in propagation material and plants as it can over-winter in cankers and buds

References: Janse et al 2010, Kaluzna et al 2012, Menrad et al 2003, Parkinson et al 2011, Santi et al 2004.

Organism: *Xylella fastidiosa*

This bacterium is listed on the PHA Industry biosecurity plan for almonds

Disease: *X. fastidiosa* causes almond leaf scorch disease (ALSD), phony peach disease, plum leaf scald and leaf scorch of the ornamental Purple leafed plum (*Prunus cerasifera*). Schipka laurel (*Prunus laurocerasus* 'Schipkaensis'), and Japanese flowering cherry (*Prunus serrulata* 'Kwanzan') are also reported as hosts.

Distribution: Asia (Taiwan, Turkey), North America (Canada, Mexico, USA), Central America and Caribbean (Costa Rica), and South America (Argentina, Brazil, Paraguay, Venezuela). There are unconfirmed reports of its occurrence in Kosovo and France in Europe in imported grapevine propagation material from the USA. Almond leaf scorch disease has been observed in India and the presence of *X. fastidiosa* was presumed based on the use of a chemical (acid based) test; its presence has not been confirmed by other methods.

Host range: Broad host range (more than150 plant species in 30 families) that includes both woody and herbaceous plants such as: Alfalfa, Almond, Blueberry, Citrus, Coffee, Elm, Grape, Maple, Mulberry, Oak, Oleander, Peach, Pear, Sycamore. Some strains are specific to specific hosts e.g. almond strains do not infect grapevine but grapevine strains can infect almond.

Economic impact: Infection by *X. fastidiosa* can result in yield loss and decline and death of trees, therefore having a significant negative economic impact to industry.

Pathway: Propagation material. Infectious vectors

Diagnostics tests:

- Isolation and culturing.
- Dark field microscopy.
- A bio-assay on Nicotiana tabacum cv SR-1 has been developed.
- ELISA is available however this technique is not as sensitive as molecular methods.
- Many PCR tests are available, including conventional and real-time techniques. Many PCR tests are generic and should pick up all strains of *X. fastidiosa*. A specific conventional PCR test is available for strains associated with almond leaf scorch disease. A qPCR and a LAMP assay have been developed and are used by Ministry for Primary Industries in New Zealand; these assays could be used for routine detection and during an incursion. The LAMP based assay is colourmetric and could be used in the field.
- The current SPHDS accepted PCR test for detection of *X. fastidiosa* in Australian grapevines requires validation for use in summerfruit and almonds under Australian conditions. Conventional PCR tests using the primer pairs RST31/RST33 (Minesavage et al 1994) and XF1-F/XF6-R (Firrao and Bazzi 1994) are included in this protocol and the former primer pair still recommended for detection of *X. fastidiosa* in Almonds and summer fruit. This protocol should be updated with the most currently used PCR tests available:
    - Conventional tests could use: HL5/HL6 (Francis et al 2006).
    - qPCR tests could use: HL5/HL6 and probe (Francis et al 2006); ITS and 16S primers and probes (Schaad et al 2002); (LAMP) and Real-time PCR assays targeted to the *rim*M gene (Harper et al 2010)

Notes: *X. fastidiosa* is a xylem limited, gram-negative bacterium.

There are several subspecies: *X. f.*subsp *fastidiosa* (infects grape, almond, alfalfa and maple), *X. f. multiplex* (infects peach, plum, almond, sycamore, elm and pigeon grape) and *X. f. pauca* (infects Citrus spp. and possibly coffee) *X. f.* subsp. *Sandyi* (infects *Nerium oleander)* and *X. f.* subsp. *taske (*infects *Chitalpa tashkentensis*, this subspecies needs confirmation)

Susceptible hosts may exhibit symptoms such as wilting, leaf scorching, premature leaf fall, chlorosis, stunting, early shooting and flowering, decline and dieback. *X. fastidiosa* has a serious impact on production in almonds, peach and plum as well as other host species such as citrus, coffee and grape due to loss in quality and yield of the crop and decline and death of infected host plants.

Symptom expression is dependent on the bacterial strain and plant host species and cultivar. Differences in susceptibility are observed amongst almond cultivars. Not all infected plants host species exhibit symptoms, but these may serve as a source of inoculum.

X. fastidiosa is transmitted by xylem feeding insects. It has been suggested that most sucking insects which feed predominantly on xylem fluid are potential vectors. The most important vectors are species of *Cicadellidae* and *Cercopidae* (Insecta: Hemiptera: Homoptera) and include. *Homalodisca vitripennis* (glassy winged sharpshooter), *Xyphon* (formerly *Carneocephala*) *fulgida* (red-headed sharpshooter), *Draeculacephala minerva* (green sharpshooter) and *Graphocephala atropunctata* (blue-green sharpshooter).

X. fastidiosa is also spread via infected planting material.

References: Cabrera-la *Rosa* et al 2008, Chang et al 2009, Connell et al 2011, Davis et al 1981,Doddapaneni et al 2007, EFSA 2015 , Firrao and Bazzi 1994, Francis et al 2008, Harper et al 2010, Hernandez-Martinez et al 2006a, 2006b, Janse 2010, Janse and Obradovic 2010, Janse et al 2012, Jindal and Sharma 1987, Luck et al 2002, Mircetich et al 1976, Purcell, 1989, Qi 2007, Rodrigues et al 2003, Schaad et al 2002 , Schaad et al 2004.

Present in australia

Organism: *Agrobacterium tumefaciens and A. rhizogenes*

Disease: Crown gall, root knot and hairy root

Distribution: Worldwide

Host range: Many hosts. *Prunus* spp including almond

Economic impact: *A. tumefaciens* is not considered to be a significant pathogen of *Prunus* sp. in Australia if effective management practices are used.

Pathway: Propagation material, Soil.

Diagnostic tests:

- Characteristic symptoms of galls on affected plants should indicate possible infection.
- Isolation and culturing on selective media.
- Pathogenicity tests.
- Biochemical tests: these can assist in discriminating between *Agrobacterium* sp.
- Several specific PCR tests are available (Lopez et al 2010) including a multiplex PCR that allows detection and differentiation between 4 different agrobacterium species including *A. tumefaciens* and *A. rhizogenes* (Pulawska et al 2006). These have not been validated for *Prunus* species. Using and in silico analysis several primers were

developed for the specific detection of *A. tumefaciens* but these have not been validated in vivo (Albuquerque et al 2012)

Notes: The bacteria invade the tissue of roots through wounds and move systemically.

A. tumefaciens causes large tumour-like growths of affected *Prunus* sp. on roots and at the collar just above the soil. It may be more problematic in young plants and rootstocks, where it can cause significant losses at the nursery level. Affected plants may be stunted, decline and die due to disruption to the vascular tissue and girdling of the trunk. Fissures within the galls can lead to secondary infections by other pathogens. Older trees are often less affected by the disease and may have no economic loss. However the occurrence of the bacterium in trees used for nursery stock production could represent a risk for contamination of propagation material.

A. rhizogenes causes proliferation of the roots and root hairs. It can be used to promote root production in almonds and other plant species undergoing micropropagation. An avirulent strain is used to cross protect against other *Agrobacterium sp.* in many plant hosts but is not reported on almond.

Both species have a broad host range. They are soil-borne, where they can last for several years in the absence of a host. They can be transmitted through planting material and may be transmitted from plant to plant on pruning equipment.

References: Cubero et al 1999, Cubero et al 2006, Escobar and DanLopes et al 1997, Janse et al 2010, Lopez et al 2010, Pulawska et al 2006, Albuquerque et al 2012.

Organism: *Pseudomonas syringae pv. syringae* and *P. s. pv. mors-prunorum*

Disease: Bacterial canker and blast

Distribution: Worldwide.

Host range: *P.s. pv. syringae* infects many *Prunus* sp. and causes bacterial canker. It is associated with bacterial canker and blast of almond in Italy, Iran, Algeria, USA. *P. s. pv. mors-prunorum* infects several *Prunus* spp but is not reported on almond.

Economic impact: Both pathovars can cause yield loss due to blast and death of buds and decline and death of *Prunus* trees and therefore can have negative impact on production of summerfruits.

Pathway: Transmitted on infected propagation material and plants. Mechanical transmission during pruning.

Diagnostic tests:

- Characteristic symptoms on affected plants should indicate possible infection.
- Isolation and culturing onto semi-selective media.
- Biochemical analyses.
- PCR tests for the detection of genes associated with toxin production.

- *P. s. pv. syringae and P. s. pv. mors-prunorum* can be distinguished from one another and from *P. s. pv. avii* and *P. s. pv. persica*e, which do not occur in Australia, using biochemical and PCR based tests.
- A combination of culturing and the use of PCR and sequencing of the *rpoD* locus should distinguish *P. s. pv. avii* from other Pseudomonads that may be isolated from *Prunus* species. Sequencing of the rpoD locus PCR product alone may not distinguish the species from *P. s. pv. persicae*. This should allow isolates to be assigned to a specific assign phylogroup or genomospecies. Further confirmation could be done using PCR and sequencing of the *acn, pgi* and *cts* loci.
- Repetitive PCR, multi-locus sequence typing and melting profile PCR may also be used to distinguish between some *P. syringae* pathovars.

Notes: These bacteria are spread by pruning, wind and rain. They can also spread in propagation material and plants as it can overwinter in cankers and buds, survive epiphytically on leaf and bud surfaces in spring and it can invade the vascular system.

Both pathovars prefer wet cool conditions for growth and disease development. In less favourable conditions they may live as saprotrophs. They overwinter in cankers and on infected leaves and buds.

Both pathovars are distributed worldwide but are often managed through production of high health planting material in certification programs.

References: Harzallah et al 2004, Janse 2010, Kaluzna et al 2012, Parkinson et al 2012, Samavatian, 2006, Scortichini 2010, Vavaro 1983

Organism: *Xanthomonas arboricola pv. pruni*

Disease: Bacterial leaf spot, shot-hole and black spot of almond, apricot, cherries, nectarine, peach, plum and *P. salicina*.

Distribution: Occurs in Australia, Europe, the Americas, New Zealand, Africa, Asia

Host range: It infects only *Prunus* species including *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry) *P. cerasus* (sour cherry) *P. persica* (nectarine, peach) and *P. domestica* (plum). It also infects the ornamental *species P. davidiana, P. japonica* and *P. laurocerasus* and *P. salicina*

Economic impact: This bacterium affects yield and quality of fruit and therefore can have negative impact on production of summerfruits.

Pathway: Disseminated through rain and wind from cankers. Entry is through wounds. Transmitted mechanically on pruning equipment. Transmitted in plants and propagation material and infected fruit.

Diagnostic tests:

- Characteristic symptoms on affected plants should indicate possible infection.
- Pathogenicity tests on young shoots or leaves and fruit.
- Isolation/culturing onto general and specific media.
- Fatty acid and protein profiling

- Specific conventional and real time PCR diagnostic tests are available for detection. Most recently a Bio-PCR has been developed in Australian conditions that can detect the pathogen in symptomless and symptomatic tissues in plum (Ballard et al 2011). This test could be used to support Australian certification programs for almond and summerfruit.
- A conventional PCR (Pagani et al 2004) was further modified to improve detection of this bacterium (Lopez et al 2012)
- Several other PCR tests have been developed including conventional and real-time assays but all require further evaluation for specificity on different *Prunus* species and in with Xap isolates world-wide (Palacio-Bielsa et al 2011, Ballard et al 2011, Pothier et al 2011a, Pothier at al 2011b, Palacio-Bielsa et al 2012).

Notes: Severe infections can result in defoliation of trees. Severely affected trees may decline and die back. Fruit may be small and unmarketable. Infected fruit may develop spotting, cracks and lesions resulting poor quality. The bacterium affects production in warm and moist environments.

X. arboricola pv. pruni can overwinter on peach in the intercellular spaces of the cortex, phloem and xylem parenchyma towards the tips of twigs. It can overwinter in cankers formed in summer on plum and apricot providing a source of inoculum in the following spring. It can also overwinter in buds of plum and fallen leaves.

References: Ballard, 2008, Ballard et al 2011, EFSA 2014, EPPO Bulletin 2006, Hetherington et al 2005, Janse 2010, Lopez et al 2011, Pagani, 2004, Palacio-Bielsa et al 2010, Park et al 2010, Palacio-Bielsa et al 2011, Pothier et al 2011a, Pothier et al 2011b, Palacio-Bielsa et al 2012

1.2 Phytoplasmas

Quarantine

Organism: *Candidatus* Phytoplasma prunorum

Synonyms: European stone fruit yellows phytoplasma

This phytoplasma is listed on the PHA Industry biosecurity plan for almonds

Disease: European stone fruit yellows and decline in almond**,** Apricot chlorotic leaf roll, Apricot witches broom, Plum leptonecrosis, Molieres disease of cherry, Peach yellows, Plum chlorotic leaf roll

Distribution: Europe, Azerbaijan and Turkey

Host range: Natural Hosts*:* apricot (*Prunus armeniaca)*, Japanese (flowering) cherry (*P. serrulata)*, black cherry *(P. mahaleb),* peach *(P. persica),* Japanese plum *(P. salicina),* European plum (*P.domestica),* cherry (myrobalan) plum (*P. cerasifera*) and almond (*P. dulcis* syn. *P. amygdalus* Batsch). Rootstocks can be infected: including *P. marianna, P. domestica*, *P. cerasifera, P. domestica* x *P. cerasifera,, P. salicina* x *P. spinosa, and P. persica* x *P. cerasifera*.

Natural alternative hosts include Hackberry *(Celtis australis),* Ash *(Fraxinus excelsior*), Dog rose *(Rosa canina),* Wild cherry *(Prunus avium) and* Blackthorn *(Prunus spinosa*). Non-*Prunus* species may be symptomless. These hosts are important in the epidemiology of the phytoplasma as they act as a source of inoculum for orchards.

Ca. P. prunorum has been experimentally inoculated to several other *Prunus* sp.

Economic impact: Causes significant reduction in yield and quality of fruit and decline and death of trees. Therefore this phytoplasma has negative economic impact in *Prunus* sp. including almond.

Pathway: Propagation material, infection vectors

Diagnostic tests: Universal and Specific PCR. A national diagnostic protocol for Australia has been developed but the PCR tests require validation (Constable et al 2009a). A real time PCR using the *Ca.* P. prunorum specific primers ECA1/ECA2 has been developed (Jarausch et al 2010); these primers can be used in conventional PCR for detection of this phytoplasma. A specific probe based assay has been developed for detection of this phytoplasma (Nikolic et al 2010). Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). The Christensen and Hodgetts qPCR assays have been validated by ring testing in several European laboratories (Euphesco 2011). LAMP based assay for generic detection of phytoplasmas have also been developed (Tomlinson et al 2010, Hodgetts et al 2011).

Notes: Bacteria; Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16SrX (Apple proliferation group). *Candidatus* Phytoplasma prunorum

Many strains exist and may be associated with biological differences such as host and symptom expression.

The severity of symptom expression in *Prunus* sp. is dependent on the species and the variety and phytoplasma strain.

Cacopsylla pruni (Scopoli) is the vector of *Ca. P.* prunorum. It is persistent and propagative in these insects. There is some indication of transovarial transmission but this needs to be confirmed. Blackthorn is a preferred host of the vector.

References: Berges et al 2000, Carraro et al 1998, 2001, 2004a, 2004b, Castelain et al 1997, Christensen et al 2004, Conci et al 1992, Constable et al 2009a, Deng and Hiruki 1991, Domenichini, 1967, Ermacora etal., 2009, Green et al 1999, Jarausch et al 1998, Jarausch et al 1999, Jarausch , et al 2000, Jarausch et al 2009, Kirkpatrick, 1991 , Lee et al 1993, Loi et al 2008, Lorenz et al 1995, Lorenz et al 1994, Marcone et al 1996, Marcone et al 2010, Marzachì et al 2004, McCoy, 1984, Morvan et al 1986,1991, Necas and Krska, 2006, Necas et al 2008, Nemeth, 1986. Ossiannilsson, 1992, Pignatta et al 2008, Poggi Pollini et al 2002, Schaub and Monneron, 2003, Schneider et al 1995, Seemuller and Schneider, 2004, Seemüller and Foster 1995, Seemuller et al 1998, Seemüller et al 1998c, Sertkaya et al 2005, Tedeschi et al 2002, Tedeschi et al 2006, Thebaud 2005, Thebaud et al 2009, Varga et al 2001, Yvon et al 2009

Organism: X-Disease phytoplasma

Suggested scientific name of X-disease phytoplasma is *Candidatus* Phytoplasma pruni, Synonyms:**Western X (WX) mycoplasma-like organism (MLO),** Western X phytoplasma**, Peach –X** phytoplasma**, Cherry-X** phytoplasma**, Cherry buckskin** MLO**,** Eastern peach X disease phytoplasma, Western peach-X phytoplasma, Green Valley X (GVX) phytoplasma, Napa Valley X (NVX) phytoplasma, Peach yellow leafroll 1 (WX/PYLR1) phytoplasma

This phytoplasma is listed on the PHA Industry biosecurity plan for almonds

Disease: Cherry-X disease, Decline of almond, Cherry blossom anomaly, Cherry albino, Cherry buckskin , Cherry Western X, Peach little peach, Peach yellows , Peach yellow leafroll, Peach rosette, Peach red suture

Distribution: North America.

There are reports of this phytoplasma in declining cherry in Italy. Related stains in subgroup IIIB of the 16SrIII group of phytoplasmas have been detected in declining sweet and sour cherry trees in Lithuania and Italy.

Host range: *Prunus* hosts: *Prunus avium* (sweet cherry), *P. cerasus* (sour cherry), *P. persica* (peach), *P. dulcis* (almond), *P. virginiana* (wild chokecherry), *P. emarginata* (bitter cherry), Japanese plum (*Prunus salicina), Prunus angustifolia, P.injucunda, P. Mexicana, P. munsoniana*, *P. glandulosa* (flowering almond), *P. armeniaca* (apricot), *P. instititia* (Damson plum), *P. domesticca* (European plum), *P. mahaleb* (Mahaleb cherry), *P. tomentosa* (Manchu cherry), *P. avium* (Mazzard cherry), *P. pumila* (sand cherry), *P. besseyi* (western sand cherry).

Alternative non-*Prunus* hosts: red maple, burclover (*Medicago polymorpha*), clovers (Trifolium sp.), and dandelion (*Taraxacum officinale*). The last three alternative host plants may also act as hosts for the vectors

Economic impact: Causes significant reduction in yield and quality of fruit and decline and death of trees. Therefore this phytoplasma has negative economic impact in *Prunus* sp. including almond.

Pathway: Infected propagation material, infectious vectors.

Diagnostic tests: Universal and Specific PCR. A national diagnostic protocol for Australia has been developed but the PCR tests require validation (Constable et al 2009b). Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009).

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16Sr III (X-disease group).

Four X-disease phytoplasma strains are reported: Green Valley (GVX), Napa Valley (NVX), Peach yellow leafroll 1 (WX/PYLR1) and the Seibe strain The GVX strain is most common. GVX and NVX strains are associated with slightly different symptoms in cherry

Transmitted by leafhoppers *Colladonus montanus, C. clitellarius*, *C.geminatus, Fieberiella florii, Graphocephala confluens, Gyponana lamina*, *Macropsis trimaculata, Norvellina seminuda, Paraphlepsius irroratus* and *Scaphytopius acutus.* It is persistent and propagative in these insects.

Risk of transmission through propagation material is considered low by USA researchers

References: Berges et al 2000, Christensen et al 2004, Constable et al 2009b, Deng and Hiruki 1991, Gilmer and Blodgett, 1976, Gilmer et al 1966, Green et al 1999, Guerra and Eastwell, 2006, IRPCM 2004, Kirkpatrick et al 1995, Landi et al 2007, Lee et al 1993, Lee et al 1994, Lukens et al 1971, McClure 1980, McCoy, 1984, Necas and Krska, 2006 , Nemeth, 1986, Paltrinieri et al 2001, Paltrinieri et al 2007, Rosenburger and Jones 1978, Schneider 1946, Schneider et al 1995, Scott and Zimmerman 2001, Seemuller et al 1998 , Suslow and Purcell 1982, Uyemoto, 1989, Valiunas et al 2009, Wolfe 1955.

Organism: *Candidatus* Phytoplasma pyri

This phytoplasma is listed on the PHA Industry biosecurity plan for almonds

Disease: Almond brown line – symptomatic trees die, almond shrivelled kernal disease, yellow canopy of almond, Peach yellow leaf roll, Decline of Cherry

Distribution: Europe, North America and Libya

Host range: *Pyrus communis, P. pyrifiolia and P. usseriensis, Cydonia oblonga, Prunus dulcis and P. persiCa.*

Economic impact: This phytoplasma has a serious economic impact on all host species due to loss of yield and quality of fruit and decline and death of trees.

Pathway: Propagation material, infectious vectors

Diagnostic tests: Universal and specific PCR. A 16Sr X group PCR conventional PCR test has been developed for detection of all phytoplasma in this group (Lorenz et al 1995). . A specific probe based assay has been developed for detection of this phytoplasma (Nikolic et al 2010). Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). The Christensen and Hodgetts qPCR assays have been validated by ring testing in several European laboratories (Euphesco 2011).

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16Sr X (Apple proliferation group) strains of *Candidatus* Phytoplasma pyri

Vectored by psyllids: *Cacopsylla pyri* and *Cacopsylla pyricola*. *C. pyricola* vectors PYLR associated strains. The phytoplasma is persistent and propagative in its vectors.

References: Blomquist and Kirkpatrick, 2002, Carraro et al 1998a, Carraro et al 2001, Cieslinka and Morgas 2010, Del Serrone et al 1998, Guerra, 1997, Jensen et al 1964, Kison & Seemuller, 2001, Kison et al 1997, Lee et al 1995, Lorenz et al 1995, Marcone et al 1996a Mehle et al 2007, Navratil et al 2001, Paltineri et al 2001, Seemuller, 1992, Seemuller and Schneider 2004, Seemuller et al 1998a Topchiiska et al 2001, Uyemoto 1997, Uyemoto 1998, Uyemoto et al 2000,

Organism: *Candidatus* Phytoplasma phoenicium'

Disease: Almond witches' broom, Shoot proliferation diseases of Nectarine and Peach (trees decline and die), Apricot chlorotic leafroll

Distribution: Iran and Lebanon

Host range: *P. dulcis* (Almond), *P. persica* (peach and nectarine).

Economic impact: High economic impact due to loss of yield and decline and death of trees.

Pathway: Propagation material, infectious vectors

Diagnostic tests: Universal PCR. A probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Christensen et al 2004).

Notes: Bacteria; Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16SrIX (Pigeon pea witches'-broom group). *Candidatus* Phytoplasma phoenicium

A vector is unknown but there is evidence for spread. The phytoplasma was detected in the following leafhopper sp. *Fieberiella macchiae, Euscelidius mundus, Asymmetrasca decedens, Thamnottetix seclusis, Balclutha sp., Lylatina inexpectata, Allygus sp., Annoplotettix danutae,* and Empoasca *decipiens* indicating their ability to acquire the phytoplasma. Further work is required to determine their transmission ability

Transmitted in propagation material

References: Abou-Jawdah et al 2002, Abou-Jawdah et al 2003, Abou-Jawdah et al 2009a, 2009b, Bove et al 1999, Choueiri et al 2001, Dahkil et l 2011, Salehi et al 2005, Salehi et al 2006, Verdin et al 2003, Zirak et al 2009b

Organism: Peanut witches' broom group phytoplasmas *(*16SrII - *Candidatus* Phytoplasma aurantifolia related strains)

Disease: Almond little leaf, Shoot proliferation of almond, yellowing of almond, Peach rosetting, peach yellowing

Distribution: Group II Phytoplasma species and strains occur worldwide, including in Australia; however they have only been reported from Iran on summerfruit.

Host range: *P. dulcis,* (almond) *P. persica* (peach)

Economic impact: May have some economic impact in almond and peach in Iran.

Pathway: Propagation material, infectious vectors

Diagnostic tests: Universal nested PCR. Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). A group II specific qPCR has also been developed (Hodgetts et al 2009)

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16SrII (Peanut witches' broom group) *Candidatus* Phytoplasma aurantifolia related strains. Phytoplasma strains and species in this group can have a broad host range

(e.g. Tomato big bud [TBBp] in Australia is found Australia wide in many plant host species). However Australian Group II phytoplasmas have not been reported in summerfruit, including almond. It is likely the group II phytoplasmas reported elsewhere differ genetically and biologically to the Australian phytoplasmas and should be considered of quarantine significance.

Although several vectors are reported for various species and strains in some plants hosts (e.g. *Orosius orientalis* tansmits TBBp in Australia) none are reported to transmit this phytoplasma to summerfruit

References: Bagheri et al 2009, Perez et al 2010, Zirak et al 2009a, Zirak et al 2009b

Organism: Clover proliferation group phytoplasmas (16SrVI - *Ca. P.* trifolii related strains)

Disease: Shoot proliferation in Almond, Peach rosetting, yellowing

Distribution: *Species* and strains of 16SrVI group phytoplasmas are found in North America, Europe and Asia. However they have only been found in almonds and peaches in Iran.

Host range: *P. dulcis* and *P. persica*

Economic impact: May have some economic impact in almond and peach in Iran.

Pathway: Propagation material

Diagnostic tests: Universal PCR. Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma group (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009).

Notes: Bacteria; Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16SrVI (Clover proliferation group)

The leafhoppers *Macrosteles fascifrons* and *Cirulifer tenellus* transmitted *Ca. P.* trifolli strains in some hosts. A vector is not reported for peach and almond in Iran.

References: Hiruki and Wang 2004, Shaw et al 1993, Zirak et al 2009a, Zirak et al 2009b

Organism: Stolbur (16SrXII-A) group phytoplasmas

Disease: Strains associated with yellowing or little leaf in almond, Moliere's disease of cherry in Europe and decline in Cherry in Italy, Peach leaf rolling, bronzing, tattering and shot hole in Iran, Peach reddening, yellowing decline witches' broom in Serbia, and Japanese Plum leaf rolling, rosetting yellowing and shoot proliferation.

Distribution: Europe and Asia (Lebanon, Iran, Armenia, Azerbaijan, Cyprus, Israel, Kyrgyzstan, Tajikistan, Turkey, Uzbekistan)

Host range: Broad host range. Strains of this phytoplasma are reported from *P. dulcis* (almond) in Iran and from *P. avium* (sweet cherry), *P. cerausus* (sour cherry), *P. persica* (peach) and *P. salacina* (Japanese plum) in Europe.

Economic impact: Strains of phytoplasmas in this group have a serious economic impact on their hosts.

Pathway: Propagation material, infectious vectors

Diagnostic tests: Universal and specific PCR. Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). A group XII specific qPCR has also been developed (Hodgetts et al 2009)

Notes: Bacteria; Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; Candidatus Phytoplasma; 16SrXII-A (Stolbur group).

Vectors include planthopper species, including *Hyalesthes obsoletus, Oncopsis alni, Reptalus panzeri* and *Pentastiridius* leporinus and the leafhopper *Macrosteles quadripuntulatas.*

References: Batle et al 2008, Bressan et al 2009, Duduk et al 2008, Gattineau et al 2001, Jovic et al 2009, Marcone et al1999, Maixner 1994, Paltrinieri et al 2001, Paltrinieri et al 2008, Schneider et al 1993, Sforza et al 1998, Valiunas et al 2009a, Zirak et al 2009a, Zirak et al 2009b, Zirak et al 2009c

Organism: *Candidatus* Phytoplasma mali

Disease: Cherry: decline, floral and phloem necrosis, and wilting. Apricot trees: stem necrosis and leaf wilting; Plum tree: blooming

Distribution: Europe

Host range: Primary hosts are *Malus* sp.(apple) but other plant host species can be infected including *P. armeniaca* (apricot), *P. domestica* (plum) and *P. avium* (cherry). Not known in almond

Economic impact: Strains of this phytoplasma have a serious economic impact in their hosts.

Pathway: Propagation material, infectious vectors

Diagnostic tests: Universal and specific PCR. A specific probe based assay has been developed for detection of this phytoplasma (Nikolic et al 2010). Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). The Christensen and Hodgetts qPCR assays have been validated by ring testing in several European laboratories (Euphesco 2011).

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; *Candidatus* Phytoplasma; 16Sr X (Apple proliferation group) strains of *Candidatus* Phytoplasma mali

Cacopsylla picta and *C. melanoneura are confirmed vectors and Fieberiella florii*, is a likely vector.

References: Bliefernicht and Krczal, 1995; Cieslinka and Moras 2011, Carraro et al 2008, Krczal et al 1988, Mehle et al 2006, Mattedi et al 2008, Mayer et al 2009, Schaper and Seemuller 1982, Seemuller et al 1984, Tedeschi and Alma, 2004, Tedeschi and Alma, 2006

Organism: Elm yellows (16SrV) group phytoplasmas

Disease: Cherry lethal yellows (16SrV-B phytoplasma); Plum: Leafroll, proliferation and little leaf in Plum (16SrV-B phytoplasma); Peach decline. Not known in almond

Distribution: Phytoplasmas in the 16SrV group occur in North America, Europe and Asia.

Host range: The host range for this phytoplasma group is diverse, including *P. avium* (cherry), *P. domestica* (plum) and *P. persica* (peach)

Economic impact: Strains of this phytoplasma have a serious economic impact in their hosts.

Pathway: Propagation material, infectious vectors.

Diagnostic tests: Universal PCR. Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma group (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). A group V specific qPCR test has also been developed (Hren et al 2007).

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; *Candidatus* Phytoplasma; 16Sr V (Elm yellows group)

Various leafhopper species transmit phytoplasma in the group but none are reported from summerfruit. *Hishimonoides chinesis is a vector of Ca. Phytoplasma* ziziphi, a phytoplasma very closely related to those detected in plum and cherry in Asia.

References: Cieslinka et al 2004, Jovic et al 2011, Jung te al 2003, Hong et al 2010, Lee et al1995, Lee et al 2004, Paltinieri et al 2004, Paltinieri et al 2006, Thakur et al 1998, Zhung et al 2003

Organism: Aster yellows group (I-B, I-F, I-Q) phytoplasmas

Disease: Apricot chlorotic leafroll, Decline of Sweet and Sour cherry with decline in Lithuania,

Distribution: North America, Africa, Europe and Asia

Host range: Strains and species of Aster yellows group phytoplasmas infect a broad host range. AY group phytoplasma in the subgroups I-B, I-F and I-Q have been detected in *Prunus* sp. in Europe including *P. armeniaca* (apricot) *P. avium* (sweet cherry), *P. cerasus* (sour cherry), *P domestica* (plum) and *P. persica* (peach). Not known in almond

Economic impact: Strains of this phytoplasma have a serious economic impact in their hosts.

Pathway: Propagation material

Diagnostic tests: Universal PCR. Probe based qPCR assays have been developed for universal detection of all phytoplasma species and could detect this phytoplasma group (Hren et al 2007; Christensen et al 2004; Hodgetts et al 2009). A group 1 specific qPCR tests have also been developed (Hodgetts et al 2009).

Notes: Firmicutes; Mollicutes; Acholeplasmatales; Acholeplasmataceae; *Candidatus* Phytoplasma; 16Sr I (Aster yellows group). This is the largest group of phytoplasmas and strains and species infect a broad range of hosts. Leafhopper vectors are reported for various AY group phytoplasma but none were reported in summerfruit. Aster yellows group phytoplasmas occur in North America, South America, Africa, Europe and Asia.

References: Lee et al 1998, Navratil et al 2001, Paltrinieri et al 2001, Valiunas et al 2009b, Varga et al 2001

1.3 Viruses

Quarantine

Organism: *American plum line pattern Ilarvirus* (APLPV**)**

Disease: Plum line pattern, peach line pattern.

Distribution: Canada, USA, Palestine, Albania, Tunisia, Lebanon and Italy.

Because this virus is associated with symptoms that are also caused by PNRSV and ApMV its distribution may be wider than has been reported.

Host range: APLPV is thought to occur naturally on many *Prunus* sp. It is known to occur in *P. domestica* (Plum), *P. persica* (peach) *P. salicina* (Japanese Plum) and *P. serrulata* (Japanese flowering cherry). Not formally reported on almond but pathogen testing schemes in the USA actively test for this virus in Almond. It has been experimentally transmitted to at least 85 plant species,

Economic impact: It is not considered a serious pathogen on its own but it may have greater impact when found in combination with other viruses

Pathway: Planting material

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR

Notes: APLPV is an *Ilarvirus* in the family *Bromoviridae*.

APLPV causes similar symptoms to strains of *Prunus necrotic ringspot virus* (PNRSV) or *Apple mosaic virus* (ApMV) in plum. It is possible that previous reports of viruses associated with diseases in *Prunus* sp. for which PNRSV and ApMV could not be detected might be associated with APLPV

No vector is known Transmitted in propagation material.

A dot blot hybridisation approach has also been developed using a polyprobe for APLPV in combination with PNRSV, PDV, ApMV, PPV and ACLSV. This approach may be useful for screening in PEQ or certification programs.

References: Alayasa et al 2003, Al Rwahnih et al 2004, Fulton 1984, Herranz et al 2008, Herranz et al 2005, Kirkpatrick and Fulton 1976, Sanchez-Navarro et al 2005, Scott and Zimmerman 2001.

Organism: *Apricot latent virus foveavirus* (ApLV)

(= Peach sooty ringspot virus)

Disease: Chlorosis and leaf deformation in apricot cultivars Tirynthos and Haward, sooty ringspot in experimentally inoculated peach.

Distribution: France, Turkey, Palestine, Lebanon, Egypt, Italy and Spain.

Host range: Natural host is *P. armeniaca* (apricot). Experimentally transmitted to *Prunus persica* (peach), *P. domestica* European (plum), *P. salicina* (Japanese plum), *P. avium* (sweet cherry) and *P. cerasifera.*

Economic impact: Little information is available on the economic impact of this virus. As it is latent in many apricot cultivars ApLV may not be a significant pest.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- PCR. This virus can be detected by specific conventional RT-PCR (Nemchinov and Hadidi, 1998, Ghanem-Sabanadzovic et al 2005, Garcia-Ibarra et al 2010). A polyprobe has also been designed for simultaneous detection of ApLV, seven additional viruses and two viroids of *Prunus* species (Peiro et al 2012).

Notes: ApLV is a member of the genus *Foveavirus* in the family *Betaflexiviridae*.

It is known to naturally infect apricot and is symptomless in many cultivars except cvs Tirynthos and Haward.

Experimental transmission indicated that cherry and plums were symptomless but the virus was associated with asteroid or sooty ringspot symptoms on the leaves and therefore could be a causal agent of Peach sooty ringspot and peach asteroid spot diseases. The peach sooty ringspot strain of ApLV causes sooty ringpsot symptoms on inoculated GF305 indicators

Reference: Ghanem-Sabanadzovic et al 2005, Barone et al 2008, El-Maghraby et al 2007, Garcia-Ibarra et al 2010, Gentit et al 2001, Grimova et al 2010, Gümüs et al 2004, Jarrar et al 2006, Jarrar et al 2007, Nemchinov and Hadidi, 1998, Nemchinov et al 2000, Zemtchik and Verdevskaya, 1993, Zemtchik et al 1998.

Organism: *Apricot latent ringspot nepovirus* (ALRSV)

Disease: Unclear.

Distribution: France: limited distribution.

Host range: *P. armeniaca* (apricot).

Economic impact: The economic impact of this virus is unknown, although it is likely to cause a significant reduction in yield in susceptible varieties.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: ALRSV is a member of subgroup C of the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* It is only reported to naturally infect apricot trees, which appeared bare due to reduced foliage and had reduced yield. The rootstock scion combination affected symptom expression in some varieties. Cultivar differences were observed. The virus was also experimentally transmitted to peach, cherry and plum, which also showed a range of foliar symptoms and stunting.

ALRSV is transmitted in propagation material and plants. No vector is reported.

Reference: Anon 2011, Gentit et al 2001.

Organism: *Apricot vein clearing associated virus* (AVCaV)

Disease: Vein clearing in apricot cv. Jameloppis

Distribution: Europe

Host range: *P. domestica (*plum), *Prunus armeniaca* (apricot)

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- PCR

Notes: Member of the genus in the family *Betflexiviridae.* No symptoms reported on plums or other infected apricot cultivars.

References: Elbeaino et al 2014, Abou-Kabaa et al 2014

Organism: *Arabis mosaic nepovirus* (ArMV)

Disease: European rasp leaf of cherry.

Distribution: Europe, North America, Asia, South Africa, Chile, New Zealand and Australia (Victoria and Tasmania). Australian reports arte from hops and likely to have been eradicated.

Host range: Broad host range. In Europe it is reported on *P. persica* (peach), *P. armeniaca* (apricot) and *P. avium* (cherry).

Economic impact: Unknown, however ArMV has significant economic impact in other crops such as grapevine.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR: Probe based real time PCR assays have been developed (Bertolini et al 2010, Wei et al 2012). A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: ArMV is a member of the genus subgroup A in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.*

In cherry ArMV causes "European rasp leaf" in combination with *Prunus* necrotic ringspot or *Prune dwarf virus*es. It has also been associated with decline in Cherry. The association with disease in peach and apricot is unknown.

It is transmitted by *Xiphinema divericaudatum*. It is transmitted in propagation material and plants. Seed transmission is reported in other hosts.

Reference: Cropely 1964, Cropely 1961, Digiaro et al 2007, Gambino and Gibraudo et al 2006, Jenser et al 1984, Maligoka et al 2004, Munro 1987, Vuittenez and Kuszala 1971, Wei and Clover 2008, Wetzel et al 2002. Anon 2011, Bertolini et al 2010, Wei et al 2012

Organism: *Asian Prunus virus 1 Foveavirus (*APruV-1), *Asian Prunus virus 2 Foveavirus (*APruV-2) and *Asian Prunus virus 3 Foveavirus* (APruV -3)

Disease: Unknown.

Distribution: USA and Europe.

Host range: *Prunus* sp. of Asian origin: *Prunus mume* and *Prunus persica* (Ta Tao 24).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR. Degenerate primers have been designed to amplify the coat protein gene of these viruses (Marais et al 2006). Degenerate primers that amplify member of the genera (*Trichovirus*, *Capillovirus* and *Foveavirus* may detect strains of these viruses but are not reliable for routine detection. A generic RT-PCR test might be used to detect these viruses but needs development and validation (Dovas and Katis 2003)

Notes: APruV-1, -2 and -3 are members of the genus *Foveavirus* in the family *Betaflexiviridae*.

APruV 1 was detected in both *Prunus* mume and *Prunus persica* (Ta Tao 24). APruV 2 was detected in *P. mume*, and APruV 3 was detected in *P. persiCa.*

Little is known about the association between these viruses and disease. However, it is possible that APruV-3 is associated with blossom delay in peach cultivars. This effect is used to reduce the risk of damage and reduced yield associated with frost, which will improve production in environments where flowering trees are at risk of frost damage.

They can be transmitted in propagation material. No vector is reported.

These viruses have the potential to cross react with some PPV antisera.

Reference: Foissac et al 2005, Gibson et al 2001, Gibson et al 2008, Hari et al 1995, James et al 1994, James et al 1996, Marais et al 2006, Marini et al 2009. Dovas and Katis 2003.

Organism: *Cherry leaf roll Nepovirus* (CLRV)

Disease: Leafroll disease in cherry

Distribution: Widespread in Europe and North America. It also occurs in Asia in Turkey, China and Japan. It occurs with limited distribution in Peru, Chile and infects *Rubus idaeus* in New Zealand but is not reported on summerfruit in these countries. It has been reported to occur in Australia but specific details of host and location are unknown.

Host range: CLRV has a wide host range. In *Prunus* it is known to infect *P. myrobalan* (myrobalan plum), *P. avium* (sweet cherry), *P. serotina* (black cherry), *P. persica* (peach) and *P. armeniaca* (apricot).

Economic impact: CLRV is of economic importance in cherry as it causes reductions in yield and decline of trees

Pathway: Seed, pollen, propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR. A National Diagnostic Manual for Australia has been prepared (Rodoni and Thomas 2011, SPHDS) but the methods require validation in Australia. A specific probe based real time RT-PCR has been developed in NZ (Anon 2012, Tang unpublished) for detection of this virus in grapevine in quarantine. Specific primers were developed for detection of CLRV in the North America (Osman et al 2012). A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: CLRV is a member of subgroup C in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* There are many strains, which have apparently diversified according to plant host species. Strain variation could impact on detection by ELISA and PCR.

In *Prunus* spp it is transmitted in propagation material, seed and pollen.

References: Anon 2011, Anon 2012, Cropley, 1961, Crosslin et al 2010, Eastwell and Howell 2010, Herrera and Madariaga, 2001, Jones, 1985 , Keglar 1972, Kurcman 1977, Kumari 2009, Murant, 1983, Osman et al 2012, Posnette and Cropley 1955, Rodoni and Thomas 2011, Schimanski et al 1975, Schimanski et al 1976, Sipahioglu et al 2011, Rebenstorf et al 2006, Walker 2004, Werner et al 1997

Organism: *Cherry mottle leaf trichovirus* (CMLV)

This virus is listed on the PHA Industry biosecurity plan for almonds, although it is not formally reported on almond in the literature.

Disease: Mottle leaf of cherry, Peach wart disease on peach fruit.

Distribution: USA, Europe, China and South Afri*Ca.*

Host range: *P. avium* (sweet cherry)*, P. cerasus (*sour cherry), some cherry hybrids, *P. persica* (peach), *Prunus emarginata* (bitter cherry)*, P. armeniaca (apricot), P. serrulata* (Japanese flowering cherry) and *P. yedoensis.*

CMLV has been experimentally transmitted to *P. dulcis* (almond) but natural infections are not known to occur (D. James Pers. comm.).

Economic impact: The virus is of economic importance in cherry as it reduces quality and yield.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA - not commercially available
- PCR. Conventional RT-PCR tests are available (James and Upton 2001, Rott and Jelkmann 2001). Generic RT-PCR tests for detection of Foveaviruses and Tricohviruses may detect PcMV (Dovas and Katis 2003, Foissac et al 2005).

Notes: CMLV is a *Trichovirus* in the family *Betaflexiviridae*. It causes mottle leaf symptoms in sweet cherry. It is symptomless on sour cherry, some cherry hybrids, peach, bitter cherry, apricot, flowering cherry and *P. yedoensis.* It is associated with peach wart disease of peach fruit

It is transmitted in propagation material and by the bud mite, *Eriophyes inaequalis.*

The listing of CMLV in the PHA biosecurity plan for almonds may be due to confusion with reports regarding the occurrence of the closely related *Peach mosaic trichovirus* which is reported in almond and may cause significant disease.

References: James et al 2000, James and Upton 1999, James et al 1999, James and Mukerji 1993, James and Mukerji 1996, Nemeth 1986 , Rott and Jelkmann 2001, Mekuria et al 2013, Ma et al 2014.

Organism: *Cherry rasp leaf cheravirus* (CRLV)

Disease: Rasp leaf of cherry.

Distribution: North America, China. There are unconfirmed reports from South Africa and New Zealand. These reports may be associated with a description of disease rather than detection of a specific virus. Rasp leaf symptoms in cherry may be caused by other viruses such as *Strawberry latent ringspot virus*.

Host range: *P. avium (*sweet cherry) *P. cerasus* (sour cherry), *P. mahaleb* (rootstock) and *P. persica* (peach). It has been detected in other plant species in infected orchards. It also causes flat apple disease and is known to infect potato.

Economic impact: In sweet and sour cherries and peach it causes loss in yield and quality of fruit and stunting and decline of trees. Therefore it may have significant economic impact.

Pathway: Propagation material

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR. A specific conventional RT-PCR has been developed (James and Upton 2005). A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: CRLV is a member of the family *Secoviridae*, subfamily *Comovirinae*, genus *Cheravirus*.

CRLV is named for the enations it causes on the underside of cherry leaves.

CRLV is seed and pollen borne in some herbaceous hosts. It has been detected in cherry pollen but transmission was not confirmed. Seed collected from the infected branches of cherry trees did not grow. It is also transmitted by nematodes in the complex *Xiphinemea Americanum, sensu lato*.

References: Anon 2011, Bobine et al 1942, Fry and wood 1973, Hansen et al 1974, James et al 2001, James and Upton 2002, James and Upton 2005, Ma et al 2014b, Nyland 1974, Nyland et al 1969, Thomson et al 2004, Wagnon et al 1968, Wood and Fry 1972

Organism: *Cherry rosette nepovirus* (ChRV)

Disease: Rosetting disease of cherry.

Distribution: Switzerland.

Host range: *P. avium* (sweet cherry).

Economic impact: The economic impact of the virus is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011)

Notes: ChRV is a tentative member of the genus *Nepovirus*

ChRV is reported from Switzerland Europe and associated with a rosetting disease of cherry. The disease in the USA could be a mix of RRSV + CLRV

ChRV is transmitted by *Longidorus arthensis.* It is likely to be transmitted in propagation material and plants

Reference: Brown et al 1994, Brown et al 1998, Kunz and Bertschinger 1998, Kunz 1998.

Organism : Cherry rusty mottle asssociated virus

Disease: Rusty mottle diseae

Distribution: North America.

Host range: *Prunus avium* (sweet cherry), *Prunus* lusitanica (Portugese laurel)

Economic impact: This virus may have a negative economic impact in sweet cherry

Pathway: Propagation material.

Diagnostic tests:

- Woody indexing
- PCR. A specific conventional RT-PCR has been developed (Villamor and Eastwell 2013).

Notes:

References: Villamor and Eastwell 2013, Villamor et al 2015. Villamor et al 2014, Villamor et al 2013

Organism: Cherry twisted leaf associated virus

(= Apricot ring pox virus).

Disease: Cherry twisted leaf and Apricot ring pox diseases

Distribution: Cherry twisted leaf disease is reported in North America, Denmark and Romania. Although its distribution is limited. Apricot ring pox has been reported in North America, Europe (Italy) and there is one report of this disease in NSW.

Host range: Natural hosts are *P. avium* (Sweet cherry) *and P. armeniaca* (apricot). The associated virus has been experimentally transmitted to *P. mahaleb (*rootstock), *P. serotina* (black cherry), *P. cerasus* (sour cherry), *P. persica* (peach), *P. dulcis* (almond) and *P. salacina* (Japanese plum) and *P.* besseyi.

Economic impact: It is not considered to be of economic importance due to its limited distribution. However it may have an impact on quality and yield in sensitive cherry and apricot varieties.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- PCR

Notes: CTLaV is a distinct virus forming part of a proposed new genus (Robigovirus) in the family *Betaflexiviridae*. Variation in sweet cherry and apricot cultivar susceptibility is observed and some maybe symptomless. The associated virus has been experimentally transmitted to mahaleb, black and sour cherry, apricot, peach, almond and Japanese plum, which were symptomless hosts. Previous work has suggested that the agent(s) associated with Apricot ring pox and Cherry twisted leaf diseases in the USA naturally infect western chokecherry and hybrid plums (*P. salicina* x *P. simomi*). The virus is transmitted in propagation material and plants. It may be transmitted by root grafting between trees. The distribution patterns within an orchard are suggestive of a vector, although none is known.

References: Fidlund 1964, Foissac et al 2005, Hansen 1976, Hansen and Cheney 1976, James 2011, James et al 1995, Keane and May 1963, Liberti et al 2003, Nemeth 1986, Zhang et al 1992, Villamor and Eastwell 2013, Villamor et al 2014, Villamor et al 2013, Villamor et al 2015.

Organism: *Little cherry Velarivirus 1* (LCHV1)

Disease: Little cherry disease

Distribution: Europe and Asia and with limited distribution in North America. The disease is reported in New Zealand and there is an unconfirmed report of the disease occurring in Australia. The occurrence of associated viruses such as LCHV1 in Australia and New Zealand is unknown

Host range: Naturally infects *P. serrulata* (flowering cherry), *P. avium* (sweet cherry), *P. cerasus* (sour cherry), *P. dulcis* (almond), *P. persica* (peach), and *P. domestica* (plum). The disease was transmitted from symptomless *Prunus emarginata* var. mollis to cherry but the associated virus is unknown.

Economic impact: The disease may have significant economic impact in sweet and sour cherry.

Pathway: Propagation material

Diagnostic tests:

- Woody indexing
- PCR. A generic *Closteroviridae* RT-PCR has been developed for detection of viruses in the family *Closteroviridae* and can be used to detect this virus (Dovas and Katis

2003). Specific conventional tests are available (Rott and Jelkmann 2001, Osman et al 2012, Matic et al 2010),

Notes: LCHV1 is a member of the genus *Velarivirus*, family *Closteroviridae*. A virus described as LChV3 may be a variant of this species. LCHV1 is one of two viruses associated with little cherry disease. The other virus is *Little cherry virus 2*. The disease is associated with a reduction in yield and quality of fruit in sweet and sour cherry and may be associated with stunting in some flowering cherry varieties. A divergent strain of LChV1 is associated with shirofugen stunt disease. It is not associated with symptoms in almond, peach and plum. Symptomless hosts could represent a source of infection for susceptible cherry varieties.

Transmitted in propagation material. There is no known vector of LCHV1.

References: Candresse et al 2013, EPPO 1992, Jelkmann 2010, Martelli et al 2012, Matic 2007, Matic 2010, Matic et al 2009 a, Matic et al 2009b, Rott and Jelkmann 2001, Rott and Jelkmann 2005, Theilmann et al 2001, Vitushkina et al 1997, Wilks and Welsh 1961, Wilkes and Reeves 1960, Wood and Fry 1970

Organism: *Myrobalan latent ringspot nepovirus* (MLRSV)

Disease: peach rosetting. Leaf enation in cherry.

Distribution: France.

Host range: *Prunus cerasifera* (myrobalan plum).*Prunus persica* (peach) *P. avium* (sweet cherry).

Economic impact: MLRSV is not considered to be economically important.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: MLRSV is a member of subgroup C in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* MLRSV is latent in myrobalan (*Prunus cerasifera*). It causes short internodes and rosetting in peach (*Prunus persica*) and enations on the leaves of sweet cherry (*P. avium*).

The mode of transmission is unknown but a nematode vector is suspected.

References: Anon 2011, Dunez and Delbos 1976, Dunez et al 1971, Diekmann and Putter 1996, Gallitelli et al 1981, Polak 2008.

Organism: *Peach enation nepovirus* (PEV)

Disease: Enation on peach leaves.

Distribution: Japan.

Host range: *P. persica* (Peach).

Economic impact: The economic importance of this virus is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: PEV is a member of the genus *Nepovirus* in the family *Comoviridae*, subfamily *Comovirinae.* Spread is observed but a vector is unknown. A nematode vector is presumed.

References: Anon 2011, Kishi et al 1973.

Organism: *Peach rosette mosaic nepovirus* (PRMV)

Disease: Rosetting, stunting, chlorosis, mottling.

Distribution: PRMV infects peach in Turkey, Egypt, Canada and the USA. It is reported on almond from Turkey.

Host range: Peach, almond *Vitis labrusca, V. vinifera*, French-American *Vitis* sp. Hybrids, highbush blueberry (*Vaccinium corymbosum) Rumex crispus, Solanum carolinense* and *Taraxacum officinale*

Economic impact: PRMV can cause serious economic impact in peach and grapevines. The full economic impact of this virus in almonds is not known. Introduction in any host may pose a risk to all three horticultural crops, especially in areas where vectors occur. The ability of other Australian *Xiphinema* and *Longidorus* species to transmit this virus is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR. Specific primers have been developed in NZ (Anon 2012, Tang unpublished) for detection of grapevine in quarantine. A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: PRMV is member of subgroup C in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.*

Almond trees infected with PRMV showed one or a combination of the following symptoms: chlorotic spots, mosaic and narrowing of the leaves. Leaves on affected peach trees may be delayed or develop slowly. Symptoms on peach leaves include chlorotic mottling, wavy leaf margins and severe distortion of leaves. Shoots on affected peach trees have severe shortening of the internodes resulting into rosette type of growth. Infected peach trees can die. PRMV is also a serious pathogen of grapevines in which it causes degeneration and decline.

PRMV is transmitted in planting material. PRMV is transmitted by two nematode species: *Xiphinema Americanum* and *Longidorus diadecturus.* Although not reported, transmission is likely to be in a semi-persistent manner. *X. Americanum is* reported in Australia in Victoria and South Australia although the extent of its distribution *is not known. The occurrence of L. diadecturus* is not reported. Several other *Xiphinema* and *Longidorus* species have been shown to transmit the virus experimentally. PRMV is seed transmitted in *V. labrusca* cv. Concord. It is not known to be transmitted by seed in summerfruit or almonds.

References: Anon 2011, Anon 2012; Allen et al 1984, Allen, et al 1999, Azery and Cycek 1997, Brown et al 1994, Brown et al 1995, Digiaro et al 2007, Kheder et al 2004, Nicol et al 1999 , Quader et al 2003, Ramsdell and Meyers 1974, Ramsdell and Gillett 1981, Sanafaçon et al 2009.

Organism: *Peach chlorotic mottle foveavirus* (PCMV)

Disease: Chlorotic mottle on GF305.

Distribution: USA.

Host range: *P. persica* (peach).

Economic impact: The economic impact of this virus is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- PCR - Generic *Foveavirus* PCR tests may detect this virus (Dovas and Katis 2003, Foissac et al 2005)

Notes: PCMV is a member if the genus *Foveavirus* in the family *Betaflexiviridae.*

It occurs in the USA on a peach cultivar originally imported from Mexico. PCMV causes chlorotic mottle symptoms in the indicator GF305. It can cross react with PPV antisera.

It is likely to be transmitted in propagation material. No vector is reported.

Reference: Foisssac et al 2005, James et al 2007, James et al 1996, James et al 1994.

Organism: *Peach mosaic trichovirus* (PcMV)

Disease: peach mosaic disease.

Distribution: North America, Europe (Greece, Italy), Asia (India)

Host range: *P. persica,* The disease also affects *P. dulcis* (almond), *P. armeniaca* (apricot), nectarine and *P. domestica* (plum). Further elucidation of the host range for PcMV is required.

Economic impact: PcMV has a negative economic impact in susceptible Peach varieties. Some varieties of almond are symptomless hosts of the disease.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA - but not commercially available
- PCR. Generic RT-PCR tests for detection of Foveaviruses and Tricohviruses may detect PcMV (Dovas and Katis 2003, Foissac et al 2005). A specific conventional RT-PCR test is also available (James et al 2006)

Notes: PcMV is a member of the genus *Trichovirus* in the family *Betaflexiviridae*

PcMV is associated with peach mosaic disease, which can also affect almond, apricot, nectarine and plum. A graft transmissible agent from almond can cause mosaic symptoms in peach, indicating that almond is a likely host of the virus.

PcMV is transmitted by the eriophyid mite *Eriophyes insidiosus* in peach. The virus is transmitted in propagation material.

References: Bodine and Durell 1941, Cochran and Hutchins 1938, , Gispert et al 1998a, Gispert et al 1998b, James and Howell 1998, James and Upton 1999, James et al 2006, Larsen et al 1998, Oldfield et al 1994, Pine 1965

Organism: *Plum pox potyvirus* (PPV)

This virus is listed on the PHA Industry biosecurity plan for almonds.

Disease *Sharka, Plum pox*

Distribution: Europe, Turkey, Syria, Egypt, India, the United Kingdom, Africa, the former USSR and parts of the United States, Canada and South America.

Host range: Primary hosts of PPV include apricots (*P. armeniaca*), peaches (*P. persica*), nectarines (*P. persica var. nucipersica*), plums (*P. domestica* and *P. salicina*) and sweet (*P. avium*) and sour cherry (*P. cerasus*). Almond (*P. dulcis*) can be infected and express mild symptoms. Other wild and ornamental *Prunus* species are also hosts of PPV. Cultivated or weedy annual plant species have been reported as hosts for PPV but they are unlikely to act as a reservoir of the virus.

Economic impact: In susceptible commercial *Prunus* species and cultivars fruit quality and yield are severely affected. PPV is considered to be one of the most economically important pathogens of summerfruit worldwide.

Pathway: Propagation material**.** Viruliferous aphids.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA: The latest draft ISPM protocol for PPV detection recommends the DASI-ELISA kit based on the 5B-IVIA monoclonal antibody which is available from AC Diagnostics, Inc.(Fayetteville, USA), Agritest (Valenzano, Italy), AMR Lab (Barcelona, Spain) and Real/Durviz (Valencia, Spain) as the most reliable ELISA method for PPV detection as it detects all known strains.
- PCR: The ISPM protocol (Cambra et al 2012) recommends several molecular tests for PPV detection and conventional and real-time assays are available for the universal detection of PPV strains. Some assays can also differentiate between strains. Several molecular tests have also been identified in the National Diagnostic Protocol for Australia. A LAMP assay has also been designed and may be useful for detection in the field during an incursion (Hadersdorfer et al 2011, Hadersdorfer et al 2012). A polyprobe has also been designed for simultaneous detection of PPV, seven additional viruses and two viroids of *Prunus* species (Peiro et al 2012). Melt Peak analysis may also be useful for detection of this virus by RT-PCR without the need for traditional gel based technology (Winder et al 2011)

Notes: PPV is a member of the genus *Potyvirus* in the family *Potyviridae*. There are nine strains of PPV: An (Albania), D (Dideron), M (Marcus), C (Cherry), CR (Cherry Russian), EA (El Amar), W (Winona), Rec (Recombinant) and T (Turkish).

PPV causes plum pox disease in several *Prunus* sp. The disease is also called Sharka. Symptoms appear on leaves, fruits, flowers, and seeds. Symptom expression varies depending on the PPV strain, host species and cultivar. Other wild or ornamental *Prunus* sp. such as *P. cerasifera, P. insititia, P. spinosa, P. salicina* may be symptomless.

It is transmitted in a non-persistent manner by aphids. There are more than 20 aphid species that can transmit PPV. Some of the most important vectors are *Aphis spiraecola, Brachycaudus cardui, B. helichrysi, Myzus persicae* and *Phorodon humuli.* The virus is transmitted in propagation material.

References: Cambra et al 2012 (ISPM 27;2012), Candresse and Cambra, 2006, Damsteegt et al 2007, Dosba et al 1987, EPPO 2004, Festic,1978, Garcia et al 2014, James and Glasa, 2006; Kunze and Krczal, 1971, Leclant, 1973, Levy et al 2000, Minoiu 1975, Olmos et al 2002, Olmos et al 2005, Schneider et al 2004, Ulubaş Serçe et al 2009, Van Oosten 1970, Varga and James 2006, Wetzel et al 1991, Wetzel et al 1992, Hadersdorfer et al 2011, Hadersdorfer et al 2012, Peiro et al 2012, Winder et al 2011.

***Prunus* Tepovirus T (PrVT)**

Disease: Unknown as has PrVT has only been reported in mixed infections with other viruses

Distribution: Europe

Host range: *P. domestica (*plum), *P. avium* (sweet cherry), *P. cerasifera* (cherry plum and myrobalan plum).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- PCR

Notes: Member of the genus in the family *Betflexiviridae.* Reported with low prevalence (1%) in Italy and Azberjain and may be a minor pest.

References: Marais et al 2015

Organism: *Raspberry ringspot nepovirus* (RpRSV)

This virus is listed on the PHA Industry biosecurity plan for almonds.

Disease: Chlorosis, mosaic, decline death in *Prunus* sp.

Distribution: Europe and Asia: (Kazakhstan, Turkey).

Host range: It is known to infect *Prunus avium* (sweet cherry*)* and *P. domestica (*Plum*)* and has been reported in almond in Turkey. RRSV also infects many other plant species, including grapevine, currant, raspberry and strawberry.

Economic impact: The economic impact of this virus in almonds is unknown however it can cause yield loss in cherry due to decline and dieback. Plum trees infected by RpRSV also showed stunting and decline and necrosis was observed at the graft union.

Pathways: Propagation material. Seed transmission in plum.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR. Specific primers have been developed in NZ (Anon 2012, Tang unpublished) for detection of this virus in grapevine in quarantine. A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011). An RT-LAMP assay has also been designed for detection of this virus and may be useful for laboratory and field based applications (Morimoto et al 2011).

Notes: RpRSV is a member of subgroup A in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* Serologically two strains can be differentiated: Scottish strain RRSV-S and English strain RRSV-E. Strain variation can impact on detection by ELISA and PCR detection

Almond trees infected with RRSV showed one or a combination of the following symptoms: chlorotic spots, mosaic and narrowing of the leaves.

RpRSV is transmitted in propagation material and plants. There is evidence for seed transmission in plum. RpRSV is seed transmitted in other host species. Nematode vectors include *Longidorus macrosoma, L. elongata* and *L. arthensis*. There are also reports of transmission by *Xiphinema sp.* and *Paralongidorus maximus*

References: Anon 2011, Anon 2012; Azery and Cycek 1997, EPPO 1995, Hubschen et al 2004, Jones et al 1984, Ochoa-Corona 2006, Trudgill et al 1983, Wei et al 2008, Zawadzka 1985, Morimoto et al 2011

Organism: *Stocky prune cheravirus* (StPV)

Disease: Stunting.

Distribution: France: limited distribution.

Host range: *P. domestica* (plum and prune cultivars). Experimentally inoculated to other *Prunus* sp.

Economic impact: It is not considered to be of economic importance due to its limited distribution. It can cause yield loss in infected trees.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: StPV is a member of genus *Cheravirus* in the family *Secoviridae.*

StPV causes stunting in prune/plum. In transmission experiments it caused rosetting on peach.

There is evidence for field transmission and a nematode vector is suspected.

Reference: Candresse et al 1998, Candresse et al 2006, Desvinges 1990, LeGall et al 2007, Sanafacon et al 2009.

Organism: *Strawberry latent ringspot virus* (SLRSV)

Disease: Peach rosetting, decline poor growth and graft union failure in combination with other viruses on peach, bare twig and unfruitfulness in apricot.

Distribution: Europe, North America, Israel, New Zealand and Turkey. There is one report of the virus occurring in Australia – no details were available about the host species.

Host range: *Broad host range.* It has been detected in flowering *P. avium* (sweet cherry), *P. cerasus* (sour cherry), *P. persica* (peach), *P. armeniaca* (apricot), *P. domestica* (plum) and *P. lusitaniCa. P. dulcis* (almond) and *P. laurocerasus* (cherry laurel) are also reported.

Economic impact: This virus may be associated with reduced yield and quality in peach and apricot and therefore may have an economic impact to industry.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- ELISA
- PCR. Conventional assays exist (Postman et al 2004, Faggioli et al 2005, Martin et al 2004, Tzanetakis et la 2006) and could be converted to a Sybr based realt time RT-PCR assay.

Notes: SLRSV *is* a tentative member of the genus *Sadwavirus* in the family *Secoviridae*.

SLRSV is associated with rosetting disease in peach, bare twig and unfruitfulness in apricot, poor growth and graft union failure in combination with other viruses on peach. Associated with decline of peach in combination with PDV.

Experimentally inoculated to *P. dulcis* (almond) but these appeared tolerant. There is little other information to support the report in almond therefore SLRSV is likely to be a minor pest.

It is transmitted by the nematode vector *Xiphinema diversicaudatum*. It is transmitted in propagation material and plants.

Reference: Belli et al 1980, Blattny and Janeckova 1980, Brown 1985, Faggioli et la 2005, Elbeaino et al 2007, Everett et al 1994, Fry and wood 1973, Huguet et al 1977, Lamberti et al 1986, Lamberti et al 1993, Lister 1964, Nemeth 1980, Polak et al 2004, Postman et al 2004, Richter and Kegler 1967, Saric and Velagic 1980, Scotto la Massese et al 1973, Sweet 1980.

Organism: *Tobacco ringspot nepovirus* (TRSV)

Disease: Associated with Eola rasp leaf of cherry, stem pitting of peach

Distribution: Europe, North America, Central America (Cuba), South America (Brazil, Uruguay), Africa, Australia, PNG and New Zealand.

Host range: Broad host range. *P. avium* (sweet cherry), *P. serrulata, P. incisa* and *P. serrula* (ornamental cherries) and *P.persica* only in the USA.

Economic impact: It is not considered to have an economic impact in summerfruit species.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR. A Probe based real time RT-PCR assays has been developed (Yang et la 2007). A degenerate primer pair for detection of *Comovirinae* species has also been

developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).

Notes: TRSV is a member of the genus *Nepovirus* subgroup A in the family *Secoviridae*, subfamily *Comovirinae*.

Reports of an association with disease in *Prunus* species are mixed and TRSV may be symptomless in some sweet cherry varieties. Transmitted by *Xiphinema Americanum sensu lato* in some hosts; seed in some hosts; in propagation and planting material

Reference: Anon 2011, Digiaro et a 2007, Fuchs et al 2010, Liu and Allen 1965, MacNish 1963, Martin et al 2009, Randles and Franco 1965, Reynolds and Teakle,1976, Shiller et al 2010, Stace-Smith 1985, Stace-Smith and Hansen 1974, Uyemoto et al 1977, Watson 1949, Wilkinson 1952, Yang et al 2007.

Organism: *Tomato black ring nepovirus (TBRV)*

This virus is listed on the PHA Industry biosecurity plan for almonds.

Disease: yellow peach bud and peach shoot stunting

Distribution: Europe, Japan, India and Chile.

Host range: TBRV has many horticultural, wild and weed hosts. *Prunus* sp. include *P. dulcis* (almond), *P. avium* (sweet cherry) and *P. persica* (peach).

Economic impact: TBRV is considered an economically important pathogen of peach. Its economic impact on almond is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA - Multiple strains mean that more than one test may be required
- PCR. A specific probe based real time RT-PCR has been developed in NZ (Harper et al 2011) for detection of this virus. A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).
-

Notes: TBRV is a member of subgroup B in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* There are many TBRV strains.

In Peach TBRV has been associated with enations, yellow peach bud and peach shoot stunting and it was associated with yield loss. In almond it has been associated with leaf enations, but its impact on quality and yield of fruit is unknown. It has also been isolated from sweet cherry with ringspot symptoms, although its association with these symptoms was inconsistent.

TBRV is transmitted by nematode vectors including: *Longidorus attenuatus* and *L. elongatus* (infrequently). Transmissibility may be affected by virus strain. TBRV is seed transmitted in

some plant host species (e.g. lettuce) but its seed transmission in *Prunus* sp. is unknown. It is transmitted in propagation material and plants.

References: Anon 2011, Bercks and Mishcke 1964, Digiaro et al 2007, Jacob 1974, Harrison and Murant 1977, Harper et al 2010, Harper et al 2011, Le Gall et al 1995, Martelli and Savino 1997, Mischke and Bercks 1963, Mischke and Bercks 1965, Wei and Clover 2008

Organism: *Tomato ringspot nepovirus (ToRSV)*

This virus is listed on the PHA Industry biosecurity plan for almonds.

Disease: Yellow bud mosaic of almond and peach stem pitting and decline in peach, cherry and apricot, brown line disease in plum.

Distribution: USA. It also occurs with limited distribution in other hosts in Europe, Asia, South and Central America, Australia and New Zealand. ToRSV is not reported on *Prunus* sp. in the EPPO regions in Europe, Central America, Australia and New Zealand.

Host range: ToRSV has a broad host range. ToRSV infects various *Prunus* sp., including *P. dulcis* (almond) in North America. ToRSV is associated with diseases of almond in Turkey.

Economic impact: This virus is economically important in summerfruit species including peach and almond as it reduces production in peach and seriously affects quality and yield in almond**.**

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA - Multiple strains mean that more than one test may be required
- PCR - Some evidence to suggest that current D1/U1 primers recommended for use in many countries may not detect all isolates. A specific probe based real time RT-PCR has been developed in NZ (Anon 2012, Tang unpublished) for detection of this virus in grapevine in quarantine. Specific primers were developed for detection of ToRSV in the North America (Li et al 2011; Osman et al 2012). A degenerate primer pair for detection of *Comovirinae* species has also been developed for the Xiamen Entry Exit Inspection and Quarantine Bureau (Anon, 2011).
-

Notes: ToRSV is a member of subgroup C in the genus *Nepovirus* of the *Secoviridae* subfamily *Comovirinae.* There are several ToRSV strains. Several species/strains within the nematode complex *Xiphinema Americanum sensu lato* are vectors

Strain variation can affect detection by ELISA and the RT-PCR.

References: Auger et al 2009, Azery and Cycek 1997, EPPO 2005, Griesbach 1995, Kommineni and Ramsdell 1997, Mircetich and Moller 1977, Li et al 2011, Moini et al 2009,, Anon 2012, Li et al 2011, Osman et al 2012, Anon, 2011.

Tomato bushy stunt tombusvirus **(TBSV) and** ***Petunia asteroid mosaic tombusvirus*** **(PeAMV).**

Disease: Twig necrosis in sweet and sour cherry and plum.

Distribution: Europe, North America Argentina, Morocco and Tunisia.

Host range: Various plants species including *P. persica* (peach) and *P. domestica* (plum) PeAMV and TBSV have not been reported in almond.

Economic impact: Has a negative economic impact due to reduction in yield and quality of fruit.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR

Notes: TBSV and PeAMV are members of the genus *Tombusvirus* in the family *Tombusviridae*. A *Tombusvirus* reported from sour and sweet Cherry in the USA and Europe and Plum in Europe where it was associated twig necrosis and fruit pitting. Although initial studies suggested TBSV was associated with this disease in cherry and plum subsequent studies showed that the virus was PeAMV. The occurrence of TBSV in cherries cannot be dismissed; however it is likely this virus occurs with lower frequency than PeAMV.

Tombusvirus species are transmitted in propagation material and may be transmitted in seed. TBSV (= PeAMV) was detected in cherry pollen. *Tombusvirus* species have been isolated from soil and water

References: Albechtova et al 1980, Allen and Davidson 1966, Hansen and Yorsten 1975, Hollings and Stone 1975, Jelkmann 2011, Kegler et al 1983, Kegler and Kegler 1980, Keldysh et al 2005, Koenig and Kunze 1982, Koenig and Lesemann 1985, Koenig et al 2004, Novak and Lanzova 1977, Novak and Lanzova 1980, Pfeilstetter et al 1996, Tomlinson and Faithful 1984, Tremaine 1969.

Minor quarantine viruses

Organism: ***Carnation Italian ringspot tombusvirus*** **(CIRV)**

Disease: Viral twig necrosis of cherry.

Distribution: Europe and North America.

Host range: *P. avium* (cherry). Carnation and some forest trees.

Economic impact: The economic impact of this virus is minor.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing

- ELISA
- PCR - PCR primers used to amplify part of the genome for dot blot probe development

Notes: CIRV is a member of the genus *Tombusvirus* in the family *Tombusviridae*.

Soil-borne and has been isolated from surface water.

Reference: Allen and Davidson 1967, Buttner et al 1987, Jelkmann 2011, Koenig et al 2004, Lesemann et al 1989, Pfeilstetter et al 1992, Rubino et al 1995, Sanchez Navarro et al 1999, Tremaine 1970.

Organism: *Epirus cherry ourmiavirus* (EpCV)

Disease: Rasp leaf of cherry.

Distribution: Greece.

Host range: *P. avium* (sweet cherry).

Economic impact: There are no reports of economic impact.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing?

Notes: EpCV is a member of the genus *Ourmiavirus* for which there is no assigned family.

It is transmitted in seed. There is no evidence for spread in affected orchards.

Reference: Accotto et al1997, Avgelis et al 1989, Rastgou et al 2009.

Viruses in the genus *Marafivirus*

Disease: Unknown

Distribution: Europe

Host range: *P. persica* (peach)

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- None described

Notes: uncharacterised viruses of the genus Marafivirus, family *Tymoviridae*. Preliminary report based on an analysis RNA transcriptomic data of gf305 deliberately inoculated with PPV. Possible multiple novel species present.

References: Candresse et al 2015, Rubio et al 2015.

Present in Australia

Organism: ***Apple chlorotic leaf spot trichovirus*** **(ACLSV)**

Disease: Pseudopox disease of plum and apricot, apricot viruela disease and plum bark split.

Distribution: Worldwide.

Host range: *P. dulcis, P persica (*peach), *P. domestica* (plum), *P. avium* (sweet cherry), *P. cerasus* (sour cherry), *P. armeniaca* (apricot), *Prunus cerasoides, Prunus glandulosa. P orientalis, P korschinskii, Malus* spp and *Pyrus* spp.

Economic impact: ACLSV may be of economic significance in apricot and plum. May not be important in almond as a single infection.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR. Several specific conventional RT-PCR tests are available (Osman et al 2012, Constable et al 2007, Nakahara et al 2011). A probe based real-time RT-PCR assay has also been developed (Salmon et al 2002)

Notes: ACLSV is a member of the genus Trichovirus in the family *Betaflexiviridae*.

It has been associated with pseudopox disease of plum and apricot, apricot viruela disease and plum bark split. Possibly associated with chlorotic leafroll of almond in combination with PDV

Transmitted in propagation material

References: Al Rwahnih et al 2004, Candresse et al 1995, Canizares et al 2001, Constable et al 2007, Dunez and Marenaud 1969, Foissac et al 2001, Garcia_Ibarra 2010, German et al 1990; German et al 1997, Jelkmann 1996, Kanaan-Atallah et al 2000, LLacer et al 1985, Marini et al 2008, Martelii and Savino 1997, Menzel 2002, Nemeth, 1986, Rana et al 2007, Rana et al 2008, Rana et al; 2009, Salmon et al 2002, Sato *etal.* 1993, Spiegel et al 2005, Sutic et al 1999, Ulubas and Ertunc 2005, Youseff and Shalaby 2009.

Organism: ***Apricot pseudochlorotic leaf spot trichovirus*** **(APCLSV)**

Disease: decline, stem-grooving, butteratura (pockmark) in apricot.

Distribution: Europe and Australia.

Host range: *P. ameniaca (*Apricot) *P. domestica* (plum), *P. salicina* (Japanese plum) and *P. persica* (peach).

Economic impact: If it is the cause of the diseases with which has been associated APCLSV could have serious economic impact in plum and apricot. It is not reported in almond.

Pathway: Propagation material.

Diagnostic tests:

- PCR specific RT-PCR tests are available (Niu et al 2012, Liberti et al 2004). A generic RT-PCR test can be used to detect this virus but needs development and validation (Foissac et al 2005, Dovas and Katis 2003)
-

Notes: APCLSV is a member of the genus *Trichovirus* in the family *Betaflexiviridae.*

It has been associated with various symptoms including decline of plum, stem-grooving symptoms in plum, butteratura symptoms on apricot fruit. It is also associated with symptoms similar to those caused by ACLSV and symptoms of apricot ringpox disease.

No vector is reported. Transmitted in propagation material.

References: Barone et al 2006, Barone et al 2008, Liberti et al 2005, Sarec et al 2003.

Organism: *Apple mosaic virus Ilarvirus* (ApMV)

Disease: European plum line pattern. Line pattern in various other *Prunus* sp.

Distribution: Worldwide,

Host range: Broad host range, including *Prunus* spp: *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry) *P. cerasus* (sour cherry), *P. persica* (peach) and *P. domestica* (plum).

Economic impact: In some instance this virus, alone or in combination with other viruses, can impact on quality and yield of fruit, therefore this virus can have a negative economic impact.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA
- PCR

Notes: ApMV is a member of the genus *Ilarvirus* in the family *Bromoviridae*. Genetic variants exist that may be associated with plant host species.

In Almond it is associated with mosaic symptoms. It is associated with line pattern in plum, almond and other *Prunus* sp. ApMV may be symptomless in some varieties of various *Prunus* sp.

ApMV is transmitted in propagation material. It is seed and pollen borne in some plant host species. It has been detected neither in the ovules of one cultivar of almond but not in pollen nor in seedlings.

References: Barba et al 1985, Ciccarone, 1958, Digiaro et al 1992, Digiaro and Savino, 1992, Digiaro et al 1992, Fulton 1972, Fulton 1983, Garau et al1989, Gilmer 1956, Hamilton, 1985, Imed et al 1997, Llacer et al 1997, Maliogka et al 2010, Marenaud and Lansac, 1977, Martelli and Savino 1997, Menzel et al 2002, Petrzik and Svoboda 1997, Petzrik and Lenz 2002, Saade et al 2000, Savino et al 1989a, Tirro and Catara, 1982, Uyemoto and Scott 1992, Youseff and Shalaby 2009.

Organism: *Cherry necrotic rusty mottle foveavirus* (CNRMV)

Disease: Rusty mottle of Cherry, Lambert mottle

Distribution: Australia, North America, Europe and Japan. The associated disease is also reported in Chile, Israel and New Zealand.

Host range: *P. avium* (sweet cherry), *P. serrulata* (flowering cherry), *P. cerasus* (sour cherry, *P. armeniaca* (apricot), *P. persica* (peach) and *P. domestica* (plum). Experimental hosts include peach, apricot and almond.

Economic impact: This virus is of economic importance on cherry where it is associated with a reduction in fruit quality and yield.

Pathway: Propagation material.

Diagnostic tests:

- Woody indexing
- ELISA
- PCR. Various conventional RT-PCR tests are available (Osman et al 2012, Li and Mock 2005, Rott and Jelkmann 2001). A generic RT-PCR test can be used to detect this virus (Dovas and Katis 2003)

Notes: CNRMV is a member of the genus *Foveavirus* in the family *Betaflexiviridae*. Strains infect sweet cherry, flowering cherry, sour cherry and apricot. The flowering cherry strain has been experimentally transmitted to peach, apricot and almond and all three experimental hosts did not show symptoms. In 1960 a cherry necrotic rusty mottle strain of Lambert mottle virus was transmitted to sour cherry, peach and, *P. mahaleb*; these experimental hosts were symptomless.

Surveys of symptomless host are not reported so their natural occurrence in these hosts is unknown.

No vector is reported. It is likely to be spread in propagation material and plants.

References: Fry and Wood 1973, Isogai et al 2004, Li and Mock 2005, Li and Mock 2008, Mandic et al 2005, Sabandsovic et al 2005, Rott and Jelkmann 2001a, Rott and Jlekmann 2001b, Zhou et al 2013

Organism: *Cherry green ring mottle foveavirus* (syn. Sour cherry green ring mottle virus, CGRMV)

Disease: Green ring mottle disease.

Distribution: Australia, North America North America, Europe, New Zealand, Africa, and Asia.

Host range: *Prunus cerasus* (sour cherry), *P. avium* (sweet cherry), *P. serrulata* (flowering cherry), *P. persica* (peach), *P. domestica* (plum) and *P. armeniaca* (apricot). Symptom expression is dependent of the virus strain and *Prunus* species and cultivar.

Economic impact: This virus may have a negative economic impact in sweet and sour cherry.

Pathway: Propagation material.

Diagnostic tests:

- Woody indexing
- PCR. A specific conventional RT-PCR has been developed (Li and Mock 2005). A generic RT-PCR test might be used to detect these viruses but needs development and validation (Dovas and Katis 2003)

Notes: CGRMV is a member of the genus *Foveavirus* in the family *Betaflexiviridae.*

This virus is associated with green ring mottle disease of flowering and sour cherry.

Sweet cherry and peach may be symptomless hosts.

It is transmitted in propagation material. A vector is not reported.

Reference: Desvignes, 1999, Li and Mock 2005, Liberti et al 2005, Nemeth, 1986, Parker et al 1976, Rott and Jelkmann 2001, Sipahioglu et al 2007, Wang et al 2009, Zhang et al 1998, Zhang et al 2000, Zhou et al 2011, Villamor et al 2015.

Organism: *Cherry A capillovirus* (CVA)

Disease: CVA is considered a latent virus in most infected hosts. In *Prunus domestica* subsp. Insititia it may be associated with Krikon necrosis disease in which symptoms include chlorotic mottling of leaves and stem necrosis**.**

Distribution: Australia, Asia, Europe and North America.

Host range: It infects several *Prunus* sp. including *P. avium* (sweet cherry) and *P. cerasus* (sour cherries), *P. mume* (Japanese apricot), *P. armeniaca* (apricot), *P. domestica* (plum)

Economic impact: Unknown

Pathway: Propagation material.

Diagnostic tests:

- PCR. Specific RT-PCR tests are available (Jelkamnn 1995, Noorani et al 2010, Osman et al 2012, Marais et al 2012). Generic RT-PCR tests for detection of Foveaviruses and Tricohviruses may detect PcMV (Dovas and Katis 2003, Foissac et al 2005).

Notes: CVA is a member of the genus *Capillovirus*, family *Betaflexiviridae.*

It has been detected in symptomless plants.

It is graft transmissible and may be transmitted in propagation material.

It is possible that this virus is present in regions where it has not been reported as it could be transmitted in symptomless material.

Cherry virus A has been associated with Krikon necrosis disease in *Prunus domestica* subsp. Insititia (http://agris.fao.org/aos/records/US201302277539 and https://www.bordeaux.inra.fr/cherry/docs/dossiers/Activities/Meetings/02%2009%202013_WG3%20Small%20Group%20Meeting_Olomouc/Candresse_Olomouc.pdf)

References: Barrone et al 2008, Jelkmann 1995, Marais et al 2008, Noorani et al 2010, Rao et al 2009, Sabanadzovic et al 2005

Organism: *Little cherry virus 2 ampelovirus* (LChV2)

Disease: Little cherry disease.

Distribution: Australia, North America and Europe.

Host range: *P. avium, P. serrulata, P emarginata, P. domestica.*

Economic impact: LChV2 has affects the quality of fruit and may have a negative economic impact.

Pathway: Propagation material. Infectious vectors.

Diagnostic tests:

- Woody indexing
- PCR A generic *Closteroviridae* RT-PCR has been developed for detection of viruses in the family *Closteroviridae* and can be used to detect this virus (Dovas and Katis 2003). Specific conventional tests are available (Rott and Jelkmann 2001, Osman et al 2012, Matic et al 2010),

Notes: LChV2 is a member of the genus *Ampelovirus* in the family *Closteroviridae.*

It is associated with little cherry disease in sweet cherry. It may be symptomless in flowering cherry. *Prunus emarginata* may be a natural host.

LChV2 is vectored by the apple mealybug (*Phenacoccus aceris*) and transmitted in propagation material and plants.

Reference: Bajet et al 2008, Eastwell and Bernardy 2001, Isogai et al 2004, Jelkmann et al 1997, Kiem-Konrad and Jelkmann 1996, Matic et al 2010, Osman et al 2012, Raine et al 1986, Rott and Jelkmann 2005, Yorston et al 1981.

Organism: *Plum bark necrosis stem pitting-associated ampelovirus* (PBNSPaV)

Disease: Plum bark necrosis, stem pitting

Distribution: Australia, North America, Europe, Jordan, Turkey, Morocco and Egypt

Host range: *P. dulcis (*almond), *P. domestica* (plum), *P. mume* (Japanese plum) *P. persica* (peach), *P. avium* (sweet cherry) *P. cerasus* (sour cherry) and *P. armeniaca* (apricot). It was also experimentally transmitted to *P. serrulata* and colt cherry (*P. avium* x *P. pseudocerasus*).

Economic impact: Its economic importance is unclear however it has been associated with decline.

Pathway: Propagation material.

Diagnostic tests:

- Woody indexing
- PCR. A generic *Closteroviridae* RT-PCR has been developed for detection of viruses in the family *Closteroviridae* and might be used to detect this virus although this needs validation (Dovas and Katis 2003). Specific conventional tests are available (Abou Ghanem-Sabanadzovic et al 2001, Al Rwahnih et al 2007, Matic et al 2010, Garcia-Ibarra et al 2010). This Virus can also be detected along with eight other viruses and two viroids using a polyprobe (Peiro et al 2012)

Notes: PBNSPaV is a member of the genus *Ampelovirus* in the family *Closteroviridae.*

It is associated bark necrosis and stem pitting disease in susceptible almond, plum, prune, peach, cherry, and apricot varieties. In sensitive cultivars can also cause gummosis and graft union failure. Symptomless infections occur. It was also experimentally transmitted to. *P. serrulata* and colt cherry (*P. avium* x *P.* pseudo*cerasus*).

It is transmitted in propagation material. No vector is reported although natural spread occurs

References: Al Rwahnih et al 2007, Amenduni et al 2005, Amenduni et al 2004a, Amenduni et al 2004b, Bouani et al 2004, Di Terlizzi and Savino 1994, El Maghraby et al 2006, Ghanem-Sabanadzovic et al 2001, Garcia-Ibarra et al 2010, Gumus et al 2007, Mandic et al 2005, Marais et al 2009, Marini et al 2002, Matic et al 2010, Sánchez-Navarro et al 2005, Usta et al 2007, Uyemoto and Teviotdale, 1996, Dovas and Katis 2003, Peiro et al 2012.

Organism: *Prune dwarf Ilarvirus* (PDV)

Disease: Prune dwarf, almond mosaic.

Distribution: Worldwide.

Host range: Several *Prunus* species, including *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry) *P. cerasus* (sour cherry), *P. persica* (peach) and *P. domestica* (plum).

Economic impact: PDV alone or in combination with other viruses can have a serious economic impact in many *Prunus* sp., including almond.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing

- ELISA
- PCR. Several conventional RT-PCR tests exist that could be assessed in this project in comparison to those already used (Massart et al 2008, Youssef and Shalaby 2009, Osman et al 2012). A real time RT-PCR assay has been developed (Jarasova and Kundu 2010). A polyprobe assay has been developed to detect PNRSV as well as seven other viruses and two viroids (Peiro et al 2012). Two generic *Ilarvirus* tests are also available (Untiveros et al 2010, Maliogka et al 2007) but may not detect all strains of PDV. Further work is being undertaken to develop RT-PCR assays for detection of Australian isolates of PDV,

Notes: PDV is a member of the genus *Ilarvirus* in the family *Bromoviridae*. Genetic variants exist that may be associated with plant host species and region.

Associated with almond mosaic disease. It causes stunting in peach and plum, sour cherry yellows, chlorotic spots, shot hole, defoliation fruit cracking in sweet cherry.

Symptom expression in some *Prunus* sp. can vary from year to year depending on climate. Some cultivars may be symptomless.

Transmission occurs through seed and pollen and in propagation material, including almond. Some evidence for spread by vectors including mite (*Vasates fockeui*),

References: Al-Chaabi and Darwesh 2008, Bertozzi et al 2002, Boulila 2009, Boulila and Marrakchi, 2001, Boulila, 2002, Brunt et al 1996, Di Terlizzi et al 1994, Digiaro and Savino 1992, Fridlund, 1965; Foneseca et al 2005, Greber et al 1992, Helguera et al 2002, Jarasova and Kundu 2010, Johnstone et al 1995, Kelly and Cameron 1986, Martelli and Savino 1997, Mekuria et al 2005, Mekuria et al 2003, Nemeth 1986, Parakh et al 1995, Raquel et al 1998, Saade et al 2000, Savino et al 1994, Spiegel et al 1996, Spiegel et al 1998, Ulubas Serce et al 2009, Uyemoto et al 1992, Vaskova et al 2000, Waterworth and Fulton, 1964, Youssef et al 2002, Youseff and Shalaby 2009.

Organism: *Prunus necrotic ringspot Ilarvirus* (PNRSV)

Disease: Almond calico, sweet cherry rugose mosaic, necrotic ringspot, European plum line pattern.

Distribution: Worldwide.

Host range: Many commercial, wild and ornamental *Prunus* species, including almond, apricot, cherry, peach and plum. Also many *Rosa* sp., and other plants species.

Economic impact: Alone and in combination with other viruses PNRSV can have a serious economic impact due to reduction in yield.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA

- PCR Several conventional RT-PCR tests exist (Massart et al 2008, Youssef and Shalaby 2009, Osman et al 2012). A real time RT-PCR assay has been developed (Jarasova and Kundu 2010). A polyprobe assay has been developed to detect PNRSV as well as seven other viruses and two viroids (Peiro et al 2012). Two generic *Ilarvirus* tests are also available (Untiveros et al 2010, Maliogka et al 2007) but may not detect all strains of PDV.

Notes: PNRSV is a member of the genus *Ilarvirus* in the family *Bromoviridae*. Genetic variants exist that may be associated with plant host species and region.

In Almond it has been associated with necrotic shock, bud failure, calico and chlorotic mottling. It may be symptomless in some almond cultivars. In Other *Prunus* species PNRSV may be associated with more serious disease and especially when occurring in mixed infection with other viruses.

Spread in pollen and seed and propagation material. Some evidence for spread by vectors including mite (*Vasates fockeui*), nematode (*Longidorus macrosoma*) and thrips (*Frankliniella occidentailis*)

References: Amari et al 2009, Aparicio et al 1999, Barba 1986, Bertozzi et al 2002, Boulila 2002, Boulila and Marrakchi et al 2001, Cole et al 1992, Crosslin and Mink 1992, Digiaro and Savino 1992, Greber et al 1992, Heleguera et al 2001, Howell and Mink 1988, Lansac et al 1980, Martelli and Savino 1997, Massart et al 2008, Mekuria et al 2003, Mink 1983, Mink et al 1987, Moury et al 2000, Nyland and Lowe 1964, Nyland et al 1976, Saade et al 2000, Salem et al 2003, Savino et al 1994, Sanchez-Navarro et al 1998, Spiegel et al 1998, Sweet 1976, Uyemoto, 1996, Uyemoto et al 1989, Varveri et al 1997, Williams et al 1970, Youseff and Shalaby 2009.

Minor viruses of *Prunus* sp. present in Australia

Organism: *Apple stem grooving virus capillovirus* (ASGV)

Disease: Unknown in *Prunus* sp.

Distribution: Worldwide.

Host range: Naturally infects citrus, lily and pome fruits. Natural infections are also reported from *P. persica* (nectarine), *P. domestica* (plum), *P. armeniaca* (apricot), Japanese apricot (*Prunus mume*) and *P. avium* (cherry).

Economic impact: Unknown

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- ELISA

- PCR (several conventional RT-PCR assays have been developed for the detection of ASGV (Ito et al 2002, Menzel et al 2002). The primers developed by Menzel et al (2002) were successfully used in a Sybr based RT-qPCR assay.

Notes: ASGV is the type species of the genus *Capillovirus* in the family *Betaflexiviridae*.

The fungus *Talaromyces flavus* is reported as a vector of ASGV in Korea. It is transmitted in propagation material.

References: Constable et al 2007, Fuchs and Grntzig 1994, Hassan et al 2006,Hilf 2008, Ito et al 2002, James 1999, James 2008, Kinard et al 1996, Marinho et al 1998, Massart et al 2008, Negi et al 2010, Nickel et al 2004, Roy et al 2005, Takahashi et al 1990, Yoshikawa et al 1992, Yoshikawa et al 1996.

Organism: *Apple stem pitting associated Foveavirus* (ASPV)

Disease: It is primarily a pathogen of pome fruit but has close relationships to viruses in summerfruit. Associated yellow vein disease in sweet and sour cherry in India needs confirmation.

Distribution: Worldwide.

Host range: Pome fruit species. Possibly *P. cerasus* (sour cherry) *and P. avium* (sweet cherry).

Economic impact: The economic impact of this virus in summerfruit is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- ELISA
- PCR. Many conventional assays exist (Komorowska et al 2010, Mathioudakis et al 2009). A probe based real time RT-PCR assay has also been developed (Salmon et al 2002)

Notes: ASPV is the type member of the genus *Foveavirus* in the family *Betaflexiviridae*

There is a recent report of ASPV in cherry trees with vein yellows symptoms (P *avium* and *P. cerasus*) in India. Further work to confirm this result is required. It is possible that closely related summerfruit foveaviruses, such as ApLV, have cross reacted with both antisera and RT-PCR primers for ASPV

References: Cameron 1989, Dhir 2009, Gugerli and Ramel 2004, Komorowska et al 2009, Mackenzie et al 1997, Malinowski et al 1998, Menzel et al 2002, Schwarz and Jelkmann 1998, Stouffer 1989, Yousseff et al 2011, Komorowska et al 2010, Mathioudakis et al 2009, Salmon et al

Organism: *Carnation ringspot dianthovirus* (CRSV)

Disease: Unknown.

Distribution: Worldwide.

Host range: Naturally infects *Dianthus sp. P. domestica (* plum), *P. cerasus* (sour cherry) and *P. avium* (sweet cherry).

Economic impact: Is likely to be of little economic significance.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- ELISA
- PCR

Notes: CRSV is the type member of the genus *Dianthovirus* in the family *Tombusviridae.*

It has been detected in summerfruit orchards in plum, sour cherry, and sweet cherry in Germany: an associated disease is unknown.

Likely to be transmitted in propagation material, Spreads in soil in the presence and absence of nematodes however transmission by *Longidorus elongatus, L. macrosoma and Xiphinema diversicaudatum* is reported and questioned.

References: Brown and Trudgill 1984, Fritzsche et al 1979, Kegler et al 1983, Kleinhempel et al 1980, Koenig et al 1988, Koenig et al 1989, Jelkmann 2011, Sanchez-Navarro et al 1999, Sit et al 2001

Organism: Citrus enation - woody gall virus (CVEV)

Disease: Unknown

Distribution: Libya, Spain, Turkey, China, India, Iran, Japan, Kenya, Libya, Réunion, South Africa, Tanzania, USA Peru, Australia, Kiribati and New Zealand.

Host range: Primarily infects Citrus. One report on *P. domestica* (plum).

Economic impact: Unlikely to be of economic significance in *Prunus* sp.

Pathway: Propagation material.

Diagnostic tests: None

Notes: CVEV is a graft transmissible agent in citrus. It may be a member of the genus *Luteovirus* but is not a recognised virus species.

It is primarily infects Citrus and is not considered of economic importance as it is symptomless on commercial cultivars. The occurrence on plum was reported from NSW, Australia and probably needs confirmation.

Aphid transmitted: *Toxoptera citricidus*, *Myzus persica*e and *Aphis gossypii* .Transmitted in propagation material.

References: EPPO 1997, Fraser and Broadbent 1979, Maharaj and da Graca, 1989, Mali et al 1976, Wallace and Drake, 1960.

Organism: *Cucumber mosaic cucumovirus* (CMV)

Disease: Associated with pseudopox disease of plum and chlorotic mottle of cherry.

Distribution: Worldwide.

Host range: Broad host range. *Prunus* sp. include *P. dulcis* (almond), *P. serrulata* (flowering cherry), *P. avium* (sweet cherry) *P. cerasus* (sour cherry), *P. mume* and *P.domestica* (plum).

Economic impact: May have a significant impact on susceptible *Prunus* sp. in combination with other viruses. The economic impact when CMV occurs on its own is unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- ELISA
- PCR

Notes: CMV is the type member of the genus *Cucumovirus* in the family *Bromoviridae*.

In Japan it is associated with a severe disease of *P. mume* when found in combination with PNRV. In China it was detected in sweet cherry with deformed, chlorotic mottled leaves. Detected in *Prunus* sp. in Russia, almond, flowering cherry, sour cherry, and plum. CMV was associated with pseudopox disease of plum in Germany

It is transmitted by many aphid species. It is transmitted in propagation material.

References: Bashir et al 2006, Berniak et al 2010, Bertolini et al 2003, Casper 1977, Keldish et al 1998, Kishi et al 1973, Kurihara et al 1998, Tan et al 2010, Topchiiska and Topchiiski 1976, Tremain 1968, Waterworth and Kaper 1980, Zitikaite and Stanliulis 2006.

Organism: *Sowbane mosaic sobemovirus* (SoMV)

Disease: Unknown.

Distribution: Worldwide.

Host range: Many plant hosts including *P. cerausus* (sour cherry) and *P. domestica* (plum).

Economic impact: Economic significance is unknown. Likely to be low.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- ELISA
- PCR

Notes: SoMV is the type member of the genus *Sobemovirus*, which is not yet assigned to virus family.

SMoV is transmitted by insects (thrips, leafminer fly, beet leafhopper, fleahopper, aphid) and pollen and seed in some plant hosts.

References: Bennett and Costa, 1961, Eastwell et al 2010, Hull and Fargette 2005, Saric and Velagic 1980, Sutic and Juretic 1976.

Organism: *Tobacco mosaic tobamovirus* (TMV)

Disease: Detected in cherry with mottle leaf, and peach with red leaf.

Distribution: Worldwide.

Host range: Broad host range. Including *P. domestica* (plum), *P. persica* (peach) and *P. avium* (cherry).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- ELISA
- PCR

Notes: TMV is the type member of the genus Tobamovirus in the family *Virgaviridae.*

Transmitted mechanically and in propagation material.

References: Babovic et al 1980, Burgyan et al 1980, Gilmer 1967, Jacobi et al 1998, Letschert et al 2002, Niu et al 2009.

Organism: *Tobacco necrosis necrovirus* (TNV)

Disease: Considered symptomless in *Prunus* sp.

Distribution: Worldwide.

Host range: Broad host range including; *P. domestica (*plum), *P. armeniaca* (apricot), *P. persica* (peach), *P. cerasus* (sour cherry).

Economic impact: Unknown.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- ELISA
- PCR

Notes: TNV is a member of the genus *Necrovirus* in the family *Tombusviridae.*

Found in water. Transmitted by *Olipidium brassicae* and propagation material.

References: Albrechtova et al 1980, Kegler et al 1969, Paulechova 1983, Paulechova and Baumgartnerova 1980, Mitrofanova and Teslenko 1982, Uyemoto and Gilmer 1972, Zitikaite et al 2005, Zitikaite and Staniulis 2006.

1.4 Viroids

Organism: *Peach latent mosaic viroid* (PLMVd)

Disease: plum spotted fruit, peach mosaic, peach yellow mosaic, peach calico

Distribution: Strains occur worldwide, including Australia.

Host range: *Many hosts including P. dulcis (almond), P. armeniaca (apricot), P. avium (sweet cherry), P. domestica (plum)* and *P. persica (peach)*

Economic impact: Loss of quality and yield in affected crops.

Pathway: Propagation material. Pollen

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- PCR

Notes: PLMVd is a member of the genus *Pelamoviroid* in the family *Avsunviroidae*. Many genetic variants exist. Latent infections occur that may be associated with strain variation and/or host. In peach infection may be latent for many years

References: Barba et al 2007, Boubouakas et al 2009, Desvignes 1986, Di Serio et al 1999, Flores et al 2006, Hadid et al 1997. Hassen et al 2006, Hassen et al 2009, Hernandez and Flores 1992, Luigi and Faggioli 2011, Parisi et al 2011, Ragozzino et al 2004,

Organism: *Hop stunt viroid* (HSVd)

Disease: dapple fruit.

Distribution: Strains occur worldwide, including Australia in grapevine and citrus. Not known to occur in *Prunus* species in Australia.

Host range: *Many hosts including P. dulcis (almond), P. armeniaca (apricot), P. avium (sweet cherry), P. domestica (plum)* and *P. persica (peach)*

Economic impact: Loss of quality and yield in affected crops.

Pathway: Propagation material.

Diagnostic tests:

- Herbaceous indexing
- Woody indexing
- PCR

Notes: HSVd is a member of the genus *Hostuviroid* in the family *Pospoviroidae*. Many genetic variants exist. Latent infections occur that may be associated with strain variation and/or host. HSVd is noted in the PEQ conditions for almonds as being present in crops in Australia and therefore not of quarantine significance. However hop strains are not reported on hops in Australia and are considered quarantine pathogens. Consequently it may be necessary to actively test imported almonds and other *Prunus* species for HSVd.

References: Amari et al 2007, Astruc et al 1996, Biosecurity Australia 2010, Cañizares et al 1999, Gillings et al 1988, Hadidi et al 1992, Hadidi et al 2003, Kofalvi et al 1997,Koltunow et al 1988, Pallas et al 2002, Pallas et al 2003, Pethybridge et al 2008, Ragozzino et al 2004, Sano et al 1989, Sano 2003, Zhou et al 2006

Organism: *Apple scar skin viroid* (APSVd)

Disease: Cherry mosaic and fruit spot. Epinasty and distortion in apricot.

Distribution: Europe, North America, Asia. In Cherry in Greece and peach and apricot in China.

Host range: This viroid is primarily a pathogen of *Malus sp.* and *Pyrus sp*. *Prunus* species that are are known hosts include *P. avium, P. cerasoides P. persica* and *P. armeniaCa.* Other Pomoideae hosts include *Cydonia oblonga*, *Pyracantha coccinea*, *Chaenomeles japonica, Sorbus aucuparia, S. domestica, S. mougeotii, S. prattii and × Pyronia veitchi*.

Economic impact: May have an economic impact in cherry due to a reduction in the quality of fruit. Its impact on apricot and peach is unknown.

Pathway: Propagation material.

Diagnostic tests:

- PCR

Notes: ASSVd belongs to the genus *Apscaviroid* of the *Pospiviridae* family. It is seed borne in apples. Seed transmission in summerfruit species is unknown.

Reference: Behl et al 1998, Campbell and Sparks, 1976, Desvignes et al 1999 Hadidi et al 1991, Handa et al 1998, Kaponi et al 2010, Kyriakopoulou and Hadidi, 1998, Lee et al 2001, Osaki et al 1996, Tharkur et al 1995, Walia et al 2012, Zhao and Niu 2006, Zhao and Niu 2008, Zhu et al 1995.

Appendix 2 – Biological indexing Protocols

- **Graft indexing**
- **Herbaceous indexing**
- **Examples of of symptoms on biological indicators**

2.1. Graft indexing of GF305 biological indicators

Biological indexing by graft inoculation of GF305 biological indicators is done in October and November and chip bud inoculation to GF305 biological indicators is done in summer. Inoculations will be carried by a diagnostic laboratory in glasshouse or screenhouse conditions according to the following protocol.

Equipment

- two GF305 indicator plants per variety
- stericrepe tape, parafilm or grafting tape.
- Scalpel blades
- Grafting/budding knife
- Scissors
- Beakers
- Labels
- cotton wool
- 70% ethanol
- distilled water
- mist bed or plastic bags

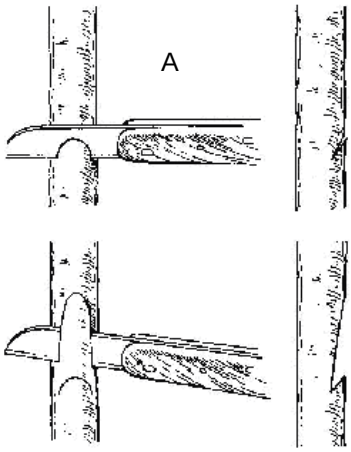
- The indicator plants must be maintained in a vigorous state of growth before and after grafting and must be grown under moderate temperatures and light intensities.
- A single GF305 plant must be left ungrafted and subjected to the same horticultural practices and environmental conditions as the inoculated plants.
- Each indicator variety must be grafted with virus positive controls containing PNRSV, PDV, ApMV and/or ACLSV at a minimum.

Method

1. Sterilize the grfating/budding knife between each variety.
2. Clearly label the pot of each indicator that will be grafted with a particular sample/variety with the code or name assigned to the sample by the managing organisation and the date of grafting.
3. Chip bud inoculation is carried out as shown in figure 1 or by grafting as shown in figure 2.

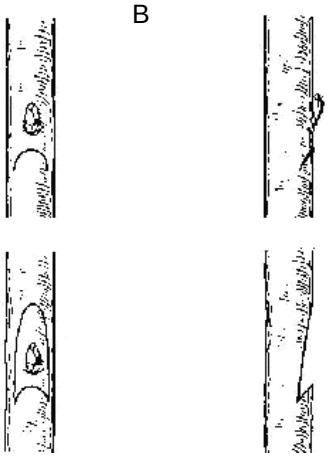
4. Bind the graft firmly with self-adhesive medical tape (e.g. stericrepe), parafilm, grafting tape or similar (Fig. 1).
5. Repeat this process for each of the remaining indicator plants.
6. Check the graft union on each indicator weekly after inoculation:
    - Grafts are successful if they are still alive after eight weeks.
    - At least one graft per indicator plant must have survived.
    - If both grafted buds have not survived the graft must be repeated.
7. Grafted plants are examinedweekly for symptoms over a four month period:
    - Symptom expression may not be observed in the first season. The plants should be examined during two spring seasons at a minimum

Figues 3-5 are examples of symptoms that may be observed after graft inoculation of *GF305* indicators.



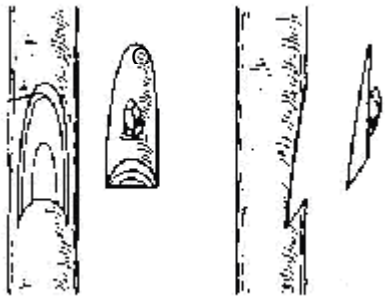
Prepare the indicator

A. A cut is made at a 30°angle about 1/4 the way through the indicator stem.
B. About 2-3cm. above the first cut a second is made going downward and inward until it connects with the first cut.

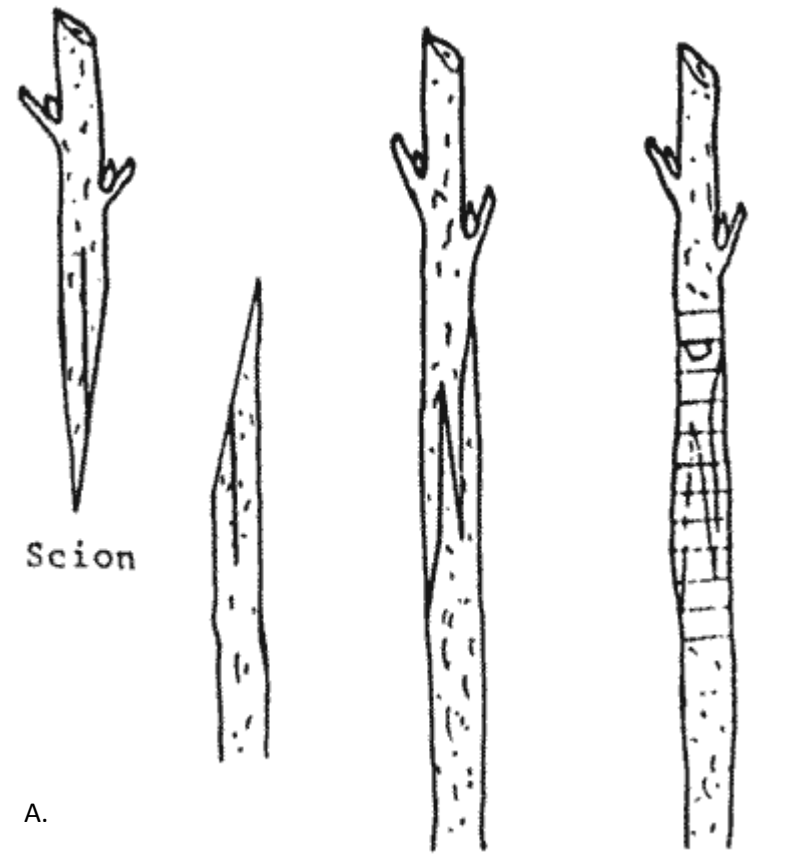


Prepare the bud

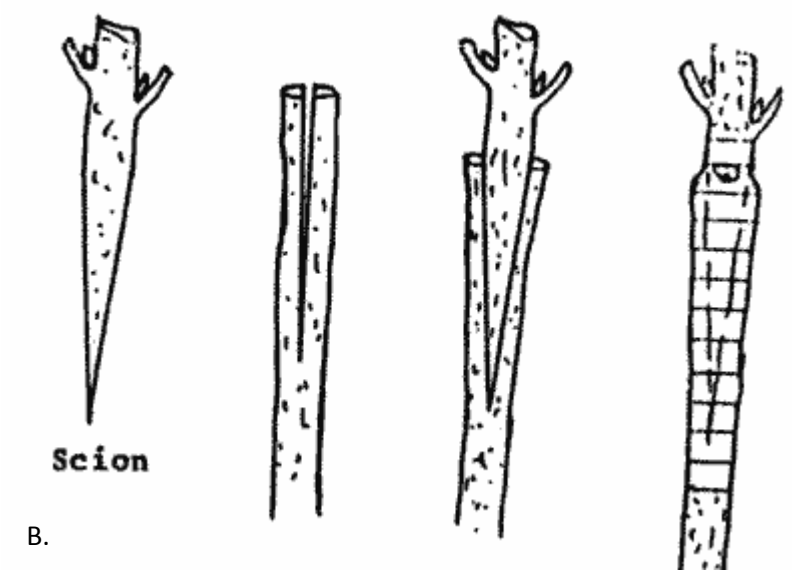
C. A cut is made at a 30°angle about 1/4 the way through the bud stick . The lower cut is made about 0.5-1cm below the bud.
D. About 2-3cm above the first cut a second is made going downward and inward until it connects with the first cut.



E. Inserting the Bud Into the Stock. Match the cambial layers
F. The bud chip is wrapped with grafting tape or similar



A.



B.

Figure 2. Procedure for grafting a bud stick of acandidate cultivar to a GF305 indictaor. A. Whip and tongue grafting. B. Cleft grafting. Either method can be used. Secure the graft with grafting tape or similar. It is important that there is contact between cambial layers of the candidate material and the indicator to ensure virus transmission. Source of images: http://rfcarchives.org.au/Next/CaringForTrees/Grafting11-92.htm

2.2 Herbaceous indexing

Biological indexing by rub inoculation of *C. quinoa* and cucumber will be carried by a diagnostic laboratory in glasshouse conditions in October and November according to the following protocol.

Equipment

- *Chenopodium quinoa* and *Cucumis sativus* (PEQ only) indicator plants (3 plants per indicator)
- Chilled extraction buffer (0.05M phosphate buffer pH 7.0 containing 0.1% sodium sulphite)
- Homex grinder and bags or mortar and pestle
- Distilled water
- 300μm carborundum powder
- Labels

Method

1. Grind sample leaf tissue at a rate of 1g tissue/5ml Phosphate buffer (0.05M sodium phosphate pH 7.0 containing 2% poly vinyl pyrrolidone – *PV*.P) using a mortar and pestle:
    - a Homex bag and the Homex grinder or a mortar and pestle can be used to grind the sample.
2. Label the pot with the sample code with which the plants will be inoculated.
3. To identify the inoculated leaves at a later time, pierce two young fully expanded leaves, preferably opposite leaves, to be inoculated on each plant with a pipette tip (or similar).
4. Lightly dust the two pierced leaves with carborundum powder.
5. Dip a gloved finger or a cotton swab in the tissue/phosphate buffer slurry and apply the slurry to the pierced leaves of the indicator plants, gently rubbing the leaf 5-6 times from the petiole towards the leaf tip while supporting the leaf below with the other hand.
    - change gloves between or cotton swabs between samples to prevent cross contamination.
    - To improve virus transfer, indicator plants may be placed in the dark 12hrs prior to inoculation.
6. Rinse inoculated leaves with water after the slurry has been applied.
7. Grow plants in a glasshouse (18-25°C) for 4-8 weeks.
8. Observe the inoculated indicator plants daily for symptom development:
    - Symptoms may begin to appear 4-5 days after inoculation. Symptoms associated with viruses infecting almond and summerfruit species is given in Table 3.3
        - Symptoms of *Prunus necrotic shock virus* (SPNRSV): Chlorotic systemic lesions, on cucumber (Figure 6).
        - Symptoms of *Arabis mosaic virus* (ArMV) on *C. quinoa* include - local lesions, systemic chlorotic mottling (Figure 7).

- Symptoms of *Raspberry ringspot virus* (RpRSV) on *C. quinoa* include - chlorotic or necrotic local lesions, systemic chlorotic mottle or apical necrosis (Figure 8).
- Symptoms of *Apple stem grooving virus capillovirus* (ASGV) on *C. quinoa* include: Epinasty, distortion and systemic mottling (Figure 9)..

- Dead tissue appearing on the inoculated leaf within the first few days of inoculation indicates excessive pressure has been applied during rub inoculation – the inoculation must repeated on uninoculated indicators.

EXAMPLES OF SYMPTOMS ON BIOLOGICAL INDICATORS

Figure 3. Symptoms of *Plum pox virus* on GF305 (INRA Bordeaux, France).

Figure 4. Symptoms of *American plum line pattern virus* on GF305 (A. Myrta, IAM Bari, Italy)

Figure 5. Symptoms of *Peach latent mosaic viroid on* GF305
(http://www.dpvweb.net/dpv/showdpv.php?dpvno=362)

Figure 6. Symptoms of *Prunus necrotic ringspot virus on* cucumber var. Crystal apple (Wycliff Kinoti, La Trobe University)

Figure 7. Symptoms of *Arabis mosaic virus* infection on *Chenopodium quinoa* (A. Eppler, Justus-Liebig Universität, Bugwood.org)

Figure 8. Symptoms of *Raspberry ringspot virus* infection on *Chenopodium quinoa* (SCRI-Dundee Archive, Scottish Crop Research Institute, Bugwood.org)

Figure 9. Symptoms of *Epinasty, distortion and mottling* on *Chenopodium quinoa* infected with *Apple stem grooving virus* (http://lclane.net/text/tsvsympt.html)

APPENDIX 3 – MOLECULAR DIAGNOSTIC PROTOCOLS

Extraction protocols

- Extract RNA for RT-PCR using the MacKenzie buffer and the RNeasy® Plant Mini Kit.
- Extract DNA for PCR using the DNeasy® Plant Mini Kit.
- Extract total nucleic acid using the QiaExtractor.

POLYMERASE CHAIN REACTION

- PCR materials and equipment.
- RT-PCR and PCR reaction set up.
- Cycling conditions for RT-PCR and PCR.
- Gel electrophoresis.
- Interpretation of results.

1. Extraction protocols

1. Extract RNA for RT-PCR using the MacKenzie buffer and the RNeasy® Plant Mini Kit (section 1.1).
2. Extract DNA for PCR using the DNeasy® Plant Mini Kit (Section 1.2).
3. Extract total nucleic acid for RT-PCR or PCR using the QiaExtractor – this method can be used if sample numbers exceed eight (Section 1.3).
4. All nucleic acid extraction is done in a nucleic acid extraction laboratory.
5. After extraction all nucleic acid extracts that are not used immediately must be stored in a freezer at -18°C.

1.1 RNA Extraction Protocol Using Guanidine Thiocyanate Buffer and the RNeasy® Plant Mini Kit (Qiagen, MacKenzie et al 1997).

Materials and equipment

1. QIAGEN RNeasy® Plant mini kit
2. 1.5 ml centrifuge tubes or 2 ml screw cap centrifuge tubes
3. 20-200 µl and 200-1000 µl pipettes
4. 20-200 µl and 200-1000 µl sterile filter pipette tips
5. Autoclave
6. Balance
7. Bench top centrifuge
8. Distilled water
9. Ice machine
10. Freezer
11. Homex grinder and bags or mortar and pestle
12. Scalpel handle
13. Sterile scalpel blades
14. Vortex
15. Water bath at 60°C
16. Buffers

Guanidine thiocyanate buffer for total nucleic acid extraction (MacKenzie *et al* 1997)

Reagent	Final concentration	Amount needed for 1L
Guanidine thiocyanate	4M	472.64 g
3M Sodium Acetate	0.2M	66.67 ml
0.5M EDTA pH 8.0	25mM	50 ml
*PV.*P-40	2.5%(w/v)	25 g

Make up to volume with sterile distilled water. Store at room temperature. Just before use, add 1% (v/v) β- mecaptoethanol.

Reference: MacKenzie, DJ, McLean, MA, Murkerji, S and Green, M. 1997. Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription-polymerase chain reaction. *Plant Disease* **81(2),** 222-226.

Method:

1. With a sterile scalpel, cut 500 mg of tissue from the petioles of the plant sample and place in a grinding bag (containing mesh to assist homogenisation) or mortar.
2. Add 1980 µl of extraction buffer to the bag or mortar.
3. In a fume hood, add 20 µl of β-mercaptoethanol to the bag or mortar.
4. Place bag in Homex homogeniser and grind sample. If using a mortar, grind in the fume hood with a pestle until a slurry has formed.
5. In a fume hood, transfer 1 ml of the sample to a 1.5 ml centrifuge tube.
6. In a fume hood, add 100 µl of 20% Sarkosyl (lauryl sarcosine sodium salt) to the tube and shake to mix.
7. Place the tube in a 70˚C water bath and incubate the samples for 15 minutes. Agitation in water bath is preferable but not necessary.
8. Transfer a maximum of 750 µl of the plant extract onto the lilac QIAshredder™ column. Close the tube and centrifuge at maximum speed for 2 minutes.
9. Mix 450 µl of the flowthrough with 225 µl of ethanol (96-100%), mix by pipetting and transfer all the mixture to the pink RNeasy® mini spin column, which has been placed in a 2 ml collection tube. Close the tube and centrifuge at ≈ 8000 × G (≈ 10 000 rpm) for 15 s.
10. Discard flow-through and replace column back into the same 2 ml collection tube.
11. Add 700ul of QIAGEN buffer RW1 to the pink RNeasy® mini spin column, close the tube and centrifuge at ≈ 8000 × G (≈ 10 000 rpm) for 15 s.
12. Discard flow-through and place column into a new 2 ml collection tube.
13. Add 500ul of QIAGEN buffer RPE (wash buffer) to the pink RNeasy® mini spin column, close the tube and centrifuge at ≈ 8000 × G (≈ 10 000 rpm) for 15 s.
14. Discard flow-through and replace column back into the same 2 ml collection tube.
15. Add another 500 µl of QIAGEN buffer RPE (wash buffer) to the pink RNeasy® mini spin column, close the tube and centrifuge at ≈ 8000 × G (≈ 10 000 rpm) for 15 s.
16. Discard flow-through and replace column back into the same 2 ml collection tube. Centrifuge at maximum speed for 1 minute.
17. Place the pink RNeasy® mini spin column in an appropriately labelled 1.5 ml centrifuge tube. Add 200 µl of RNase-free sterile water directly to the filter (don't apply down the side of the tube), close the tube and centrifuge at ≈ 8000 × G (≈ 10 000 rpm) for 1 minute to elute the RNA.

1.2 Modified DNA extraction procedure using the DNeasy® Plant mini kit (Qiagen, Green et al 1999).

Materials and equipment

1. QIAGEN DNeasy® Plant mini kit
2. 1.5 ml centrifuge tubes
3. 20-200 µl and 200-1000 µl pipettes
4. 20-200 µl and 200-1000 µl sterile filter pipette tips
5. Autoclave
6. Balance
7. Bench top centrifuge
8. Distilled water
9. Ice machine
10. Freezer
11. Mortars and pestles or Homex grinder and bags
12. Scalpel handle
13. Sterile scalpel blades
14. Vortex
15. Water bath at 60°C
16. Buffers

CTAB extraction buffer for DNA extraction (Green et al 1999)

Reagent	Final concentration	Amount needed for 1L
CTAB (cetylmethylammonium bromide)	2.5%	25 g
Sodium Chloride	1.4 M	56 g
1M Tris, pH 8.0 (sterile)	100 mM	100 ml
0.5M EDTA, pH8.0 (sterile)	20 mM	40 ml
*PV.*P-40	1%	10g

Make up to volume with sterile distilled water. Store at room temperature. Just before use, add 0.2% 2-mercaptoethanol (v/v) to the required volume of buffer

95% Ethanol

Reference: Green MJ, Thompson DA and MacKenzie DJ, 1999. Easy and efficient DNA extraction from woody plants for the detection of phytoplasmas by polymerase chain reaction. *Plant Disease* **83,** 482-485

Method

1) Pre-heat Qiagen buffer AE buffer to 65°C.
2) Weigh approximately 500 mg of leaf petioles.
3)
    a) Place the material in a sterile mortar with 5 ml of CTAB extraction buffer containing 0.2% 2 – mercaptoethanol and grind thoroughly with the sterile pestle.

or

    b) Place the material in a grinding bag (containing mesh to assist homogenisation) with 5 ml of CTAB extraction buffer containing 0.2% 2 – mercaptoethanol. Place the bag in Homex homogeniser and grind the sample.

4) With a sterile plastic transfer pipette transfer 500ul of extract to a 1.5 ml centrifuge tube and add 4 μl of RNase A (Supplied with the DNeasy® kit), cap tube and incubate at 65°C for 25-35 minutes, mixing gently several times.

5) Add 162 μl of QIAGEN buffer AP2 to extract. Invert 3 times to mix and place on ice for 5 minutes.

6) Pour extract into a QIAshredder™ column and centrifuge at maximum speed for 2 minutes.

7) Transfer 450 μl of flowthrough from QIAshredder™ column to a 1.5 ml centrifuge tube containing 675μl QIAGEN buffer AP3. Mix by pipetting.

8) Transfer 650 μl of extract onto a DNeasy® spin column and centrifuge at 10000rpm for 1 minute.

9) Discard flow-through and add the rest of the sample to the column and centrifuge at 8000 rpm for 1 minute.

10) Place DNeasy® column in a new 2 ml collection tube and add 500 μl of QIAGEN buffer AW (wash buffer) and centrifuge at 8000 rpm for one minute.

11) Discard flowthrough and add another 500 μl of QIAGEN buffer AW and centrifuge at maximum speed for 2 minutes.

12) Discard flowthrough and collection tube. Ensure that the base of the column is dry (recentrifuge the column if it remains a little wet) and place in an appropriately labelled 1.5 ml centrifuge tube. Add 200 μl of pre-warmed AE buffer directly to the filter (don't apply down the side of the tube) and centrifuge at 8000 rpm for 1 minute. Discard column and store DNA in at -18°C.

1.3. Nucleic acid extraction using the QIAxtractor

Materials and equipment

1. QIAxtractor
2. 96 Square-Well,1.2 ml, lysis plate
3. Adhesive plastic film to cover Lysis plate and unused wells of the capture plate
4. 3mL transfer pipettes
5. 96-well 800 µl long drip Unifilter capture plate (Whatman)
6. Caps or adhesive foil to cover the elution plate
7. 96 well elution plate (Qiagen)
8. 20-200 µl and 200-1000 µl pipettes
9. 20-200 µl and 200-1000 µl sterile filter pipette tips
10. Autoclave
11. Balance
12. Bench top centrifuge
13. Distilled water
14. Freezer
15. Homex grinder and bags or mortar and pestle
16. Scalpel handle
17. Sterile scalpel blades
18. Vortex
19. Water bath at 70°C
20. Buffers:

Guanidine thiocyanate buffer for total nucleic acid extraction (MacKenzie et al 1997)

Reagent	Final concentration	Amount needed for 1L
Guanidine thiocyanate	4M	472.64 g
3M Sodium Acetate	0.2M	66.67 ml
0.5M EDTA pH 8.0	25mM	50 ml
*PV.*P-40	2.5%(w/v)	25 g

Make up to volume with sterile distilled water. Store at room temperature. Just before use, add 1% (v/v) β- mecaptoethanol.

Propanol Wash buffer for the Corbett X-Tractor

Reagent	Final concentration	Amount needed for 700ml
Ethanol	25%	175ml
2-Propanol	25%	175ml
1M Tris-Cl, pH 8	10mM	7ml
NaCl	100 mM	4.09g

Dissolve salts in water first before adding alcohols then make up to 700mL with dH_2O. Store at room temperature.

Ethanol (absolute)

1.4 Total nucleic acid extraction using the QIAxtractor and guanidine thiocyanate extraction buffer

1. With a sterile scalpel, cut 500 mg of tissue from the petioles of the plant sample and place in a grinding bag (containing mesh to assist homogenisation) or mortar.
2. Add 5 ml of Guanidine thiocyanate extraction buffer to the bag or mortar.
3. In a fume hood, add 30 µl of β-mercaptoethanol to the bag or mortar.
4. Place bag in Homex homogeniser and grind sample. If using a mortar, grind in the fume hood with a pestle until a slurry has formed.
5. In a fume hood, transfer 1 ml of the homogenate to one well of pre-racked 1.1ml strip minitubes arranged in standard 96-well format (Pathtech), each containing 100 µl of 20% N-lauroylsarcosine containing 100 µl of 20% N-lauroylsarcosine sodium salt (Sarkosyl).
6. Aspirate the sample to mix to mix.
7. Place the tube in a 70˚C water bath and incubate the samples for 15 minutes. Agitation in water bath is preferable but not necessary.
8. Close the tube and centrifuge at maximum speed for 2 minutes.
9. Transfer a maximum of 500 µl of the plant extract into one well of pre-racked 1.1ml strip minitubes arranged in standard 96-well format.
10. Place the second lysis plate in the QIAxtractor.
11. Add ethanol, propenol wash buffer and RNase-free sterile water tot e appropriate tubs and program the QIAxtractor to do the following:
    a. Add 500 µl of 100% ethanol to each sample.
    b. Mix the samples by aspiration.
    c. Transfer 500 µl of the mixed samples to the 96-well 800 µl long drip Unifilter capture plate (Whatman).
    d. Apply a vacuum pressure of 70 Kpa for 5 minutes to draw samples through the filter.
    e. Transfer 500 µl of the 500 µl propanol wash buffer to the capture plate.
    f. Apply a vacuum pressure of 50 Kpa for 5 minutes to draw through wash buffer.
    g. Repeat 11.e and 11.f
    h. The capture plate was then washed twice under vacuum with (Sigma) at 50 Kpa for 5 minutes.
    i. Transfer 500 µl of 100% ethanol to the capture plate.
    j. Apply a vacuum pressure of 45 Kpa for five minutes to draw through ethanol wash.
    k. Apply a vacuum pressure of 40 Kpa for 5 minutes to remove all traces of ethanol.
    l. Transfer the capture plate to a 96 well elution plate (Qiagen).
    m. Transfer 200 µl of RNase, DNase free water to each well of the capture plate.
    n. Incubate at room temperature for 2 minutes.
    o. Apply a vacuum pressure of 45 Kpa for 5 minutes to elute the RNA.

p. Transfer the capture plate to it's original position.

12. Remove the elution plate and seal tubes with caps or adhesive foil.
13. Store at -18°C.

Reference: MacKenzie, DJ, McLean, MA, Murkerji, S and Green, M. 1997. Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription-polymerase chain reaction. *Plant Disease* **81(2),** 222-226.

2 POLYMERASE CHAIN REACTION

2.1 Laboratory requirements

- To reduce the risk of contamination and possible false positive results, particularly when nested PCR is used for pathogen detection, RT-PCR and PCR reactions must be set up in a different laboratory to where nucleic acid extractions are routinely done.
- RT-PCR and PCR reagent stocks and RT-PCR and PCR reaction set up must be done in a separate clean molecular laboratory or PCR workstation with dedicated pipettes, PCR tubes and tips that have not been exposed to nucleic acid extracts.
- Use a separate pipette for the addition of nucleic acids to the PCR reactions.
- Do not add nucleic acid to reactions in the same clean room or PCR workstation in which PCR stocks are handled.
- The RT-PCR and PCR assays developed in this project have been validated using the SuperScript® III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase kit (Invitrogen) and the Platinum® *Taq* DNA Polymerase kit (Invitrogen) respectively. The use of different kits has not been validated and cannot be guaranteed for pathogen detection.

2.2 PCR materials and equipment

1. PCR reagents t
   - SuperScript® III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase kit (Invitrogen)
   - Platinum® *Taq* DNA Polymerase kit (Invitrogen)
2. Primers for the pathogen being tested (Table 2.1)
3. Positive controls
4. PCR grade water
5. 0-2 μl, 2-20 μl, 20-200 μl and 200-1000 μl pipettes
6. 0-2 μl, 2-20 μl, 20-200 μl and 200-1000 μl sterile filter pipette tips
7. 200 μl yellow pipette tips
8. 1.5 ml centrifuge tubes to store reagents
9. PCR tubes (volume depends on thermocycler)
10. Bench top centrifuge– with adapters for small tubes
11. Freezer

12. Ice machine
13. Gel tank, casting plate and combs
14. Power pack
15. Latex or nitrile gloves
16. Themocycler
17. DNA molecular weight marker
18. UV transilluminator and camera
19. Buffers:

5 × TBE - 1L

Reagent	Final concentration	Amount needed for 1L
Tris-Cl	445mM	54.0 g
Boric acid	445mM	27.5 g
0.5M EDTA pH 8.0	10mM	20 ml

Make up to volume with distilled water. Store at room temperature. Dilute 1/10 for gel elctrophoresis.

1% agarose gel – 100ml

1. Add 1g agarose per 100 ml 0.5 × TBE
2. Melt the agarose in a microwave
3. Add 10 µl Ethidium bromide (1 mg/ml) per 100 ml agaose gel.

6X gel Loading Buffer:

Bromophenol Blue	0.25% (w/v)
Glycerol	30% (w/v)

Make up to volume with sterile distilled H_2O.

Use 1 µl of loading buffer to 5 µl PCR sample.

Table 2.1. The list of endemic and exotic pathogens tested for, the type of PCR test, the primers used, the annealing temperature, the region amplified, expected product size and the reference for each test.

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
Housekeeping gene assays								
RNA- NADH dehydrogenase mRNA	One-step RT-PCR	AtropaNad2.1a AtropaNad2.2b	F R	GGACTCCTGACGTATACGAAGGATC AGCAATGAGATTCCCCAATATCAT	55° C	NADH dehydrogenase ND2 subunit	188bp	Thompson et al 2003.
DNA- 16S rRNA gene	PCR	FD2 RP1	F R	AGAGTTTGATCATGGCTCAG ACGGTTACCTTGTTACGACTT	45° C	16S rRNA gene	1400-1500bp	Weisberg et al 1991.
Endemic viruses								
Prunus necrotic ring spot virus	One-step RT-PCR	PNRSV F PNRSV R	F R	ACGCGCAAAAGTGTCGAAATCTAAA TGGTCCCACTCAGAGCTCAACAAAG	54° C	Coat protein gene	455bp	Mackenzie et al 1997.
Prune dwarf virus	One-step RT-PCR	PDV F PDV R	F R	TAGTGCAGGTTAACCAAAAGGAT ATCGATGGGATGGATAAAATAGT	62° C	Coat protein gene	172bp	Parakh et al 1995.
Apple mosaic virus	One-step RT-PCR	ApMV1 ApMV2	F R	TGGATTGGGTTGGTGGAGGAT TAGAACATTCGTCGGTATTTG	53° C	Coat protein gene	261bp	Petrzik and Svoboda 1997.
Apple chlorotic leaf spot virus	One-step RT-PCR	ACLSV A52 ACLSV A53	F R	GCGAACCCTGGAACAGA CAGACCCTTATTGAAGTCGAA	53° C	Coat protein gene	358bp	Candresse et al 1995.
Apple stem	One-	CTLV AM	F	CCTGAATTGAAAACCTTTGCTGCCACTT	60°	Coat protein	456bp	Ito et al

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
grooving virus	step RT-PCR	CTLV AP	R	TAGAAAAACCACACTAACCCGGAAATGC	C	gene		2002.
Little cherry virus-2	One-step RT-PCR	LCV2 UP2	F	CTCGGCGTATATGGTGGATGTTTA	55° C	RdRp gene	438bp	Rott and Jelkmann 2001.
		LCV2 LO2	R	CCGAATGCAGTGGGGATAGG				
	One-step RT-PCR	LCH2-01F	F	AGACGCGCAGAGGAGGAC	55° C	RdRp gene	180bp	Jelkmann et al 2008.
		LCH2-03R	R	TCCAAACTCAACTTAAAGAAATCAAAATA				
Cherry virus A	One-step RT-PCR	CVA-fw1	F	CAACTGCCGCAGATGTTGG	54° C	Movement protein gene	281bp	Marais et al 2012.
		CVA-rev1	R	AGMCCTACATGAATTTGACCT				
Cherry virus A	One-step RT-PCR	CVA-6170 F	F	AGCCAGAAGGTATCATGCCAG	54° C	Coat protein gene	566bp	Osman et al 2012.
		CVA-6736 R	R	ATGACATGCCTGCTGGGAG				
Exotic viruses and viroids								
Cherry green ring mottle virus	One-step RT-PCR	CGRMV1	F	CCTCATTCACATAGCTTAGGTTT	55° C	Coat protein gene	958bp	Li and Mock 2005.
		CGRMV2	R	ACTTTAGCTTCGCCCCGTG				
Cherry necrotic rusty mottle virus	One-step RT-PCR	CNRMV-7626F	F	TCCCACCTCAAGTCCTAGCAG	58° C	Coat protein gene	584bp	Osman et al 2012.
		CNRMV-8210R	R	TGAACTTGGCCAGTTCTGCC				
Plum bark necrosis stem	One-step RT-	PBNSPaV ASP1	F	CGGTAGGGCTGTGACTACCG	52° C	HSP70 gene	290bp	Abou Ghanem-

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
pitting associated virus	PCR	PBNSPaV ASP2	R	GTAGTCCGCTGGTACGCTACAAG				Sabaradzovic et al 2001.
	One-step RT-PCR	PBNSPaV detF	F	TACCGAAGAGGGTTTGGATG	56°C	HSP70 gene	400bp	Al Rwahnih et al 2007.
		PBNSPaV detR	R	AGTCGCACCACCAGTCTTCT				
Tomato black ring virus	One-step RT-PCR	TBRV 70F	F	GCTCGTAACAGTTGCGGAGATAT	62°C	Polyprotein (P2) gene	73bp	Harper et al 2011.
		TBRV 70R	R	TGTCCACACTGTCATGGGA				
Plum pox virus	One-step RT-PCR	PPV P1	F	ACCGAGACCACTACACTCCC	60°C	Polyprotein gene	243bp	Wetzel et al 1991.
		PPV P2	R	CAGACTACAGCCTCGCCAGA				
Little cherry virus-1	One-step RT-PCR	LC1-9135F	F	TCTGCTGCTGCYATGCATCA	55°C	HSP70-like gene	723bp	Alison Dann, unpublished
		LC1-9858R	R	AWACACAAGCAGCAGTGGMA				
Strawberry latent ring spot virus	One-step RT-PCR	SLRSV-5D	F	CCCTTGGTTACTTTTACCTCCTCATTGTCC	55°C	Coat protein gene	293bp	Faggioli et al 2002.
		SLRSV-3D	R	AGGCTCAAGAAAACACAC				
Arabis mosaic virus	One-step RT-PCR	M2	F	YTRGATTTTAGGCTCAATGG	42°C	Movement protein gene	290bp	Wetzel et al 2002.
		M3	R	TGYAARCCAGGRAAGAAAAT				
Apricot latent virus	One-step RT-PCR	ApLV1	F	CCCGACCATGGCTACAAGC	50°C	Coat protein gene	1500bp	Garcia-Ibarra et al 2010.
		ApLV2	R	TTGCCGTCCCGATTAGGTTG				

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
American plum line pattern virus	One-step RT-PCR	VP 340 VP 339	F R	GGTCGTCAAGGGAGAGGC GGCCCCTAAGGGTCATTTC	50° C	Coat protein gene	563bp	Sanchez-Navarro et al 2005.
Asian Prunus virus(es)	One-step RT-PCR	CP-PLV1 CP-PLV2	F R	KCRGTKATCAAAAAGCATAC AATCCATYTCCTTCCCCTTCAA	48° C	Partial CP gene	262bp	Marais et al 2006.
Cherry rusty mottle associated virus	One-step RT-PCR	CRM 91CPF NGRRM-TL CPR	F R	GGGCCCGAYCCTGTCATTCC ATNGGTTGAATTTGGCCAGT	60° C	Coat protein gene	695bp	Villamor and Eastwell 2013.
Cherry mottle leaf virus	One-step RT-PCR	CML13A CML4A	F R	GCCTGATCAGCAAAGTGAAG CGGTCTGAAGCACAATGC	60° C		848bp	James et al 1999.
Cherry twisted leaf associated virus 1a	One-step RT-PCR	CTL-1a 218CPF NGRRM-TL CPR	F R	TCAGCAAGATTAAGGAGGTTG CTNGGTTGAATTTGGCCAGT	60° C	Coat protein gene	562bp	Villamor and Eastwell 2013.
Cherry twisted leaf associated virus 1b	One-step RT-PCR	CT-1b 235CPF NGRRM-TL CPR	F R	TCGGACCCTACAACCCTCAATG CTNGGTTGAATTTGGCCAGT	60° C	Coat protein gene	545bp	Villamor and Eastwell 2013.
Peach mosaic virus	One-step RT-PCR	PM-AF1 PM-AFR	F R	TCACCTTCTGCAGATACGAAGTA GCTGTTCTTCACAAAGAATCTA	59° C	Replicase coding region	383bp	James et al 2006.
Tomato	One-	TomCPF	F	CCGCCGTAGCATGACCAAGTA	55°	Putative CP	1220bp	Russo et al

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
bushy stunt virus	step RT-PCR	TomCPR	R	CCATGAACTGGTCTTGTTCAA	C	gene		2002.
Peach rosette mosaic virus	One-step RT-PCR	PRMVF5321 PRMVR5699	F R	ATTGGTCGCCGCTCTATTT CAACAACAAGCCCATTCTCC	57° C	Polyprotein gene	388bp	Lebas and Ward 2012.
Raspberry ring spot virus	One-step RT-PCR	RpRSV942F RpRSV1741R	F R	CAGAGTATGGGTGATTTCTGG TCCTTCTCCCAGGTCTGCAC	55° C	Polyprotein gene	800bp	Lebas and Ward 2012.
Cherry leaf roll virus	One-step RT-PCR	CLRV-5 CLRV-3	F R	TGGCGACCGTGTAACGGCA GTCGGAAAGATTACGTAAAAGG	53° C	RNA2	416bp	Werner et al 1997.
Apple scar skin viroid	One-step RT-PCR	VirPom C VirPom H	F R	CAGCACCACAGGAACCTGACGG TCGTCGTCGACGAAGG	55° C	Whole genome	267bp	Faggioli and Ragozzino 2002.
Peach latent mosaic viroid	One-step RT-PCR	cPLMVd hPLMVd	F R	AACTGCAGTGCTCCGT CCCGATAGAAAGGCTAAGCACCTCG	60° C	Whole genome	337bp	Shamloul et al 1995.
	One-step RT-PCR	RF-43 RF-44	F R	CTGGATCACACCCCCCTCGGAACCAACCGCT TGTGATCCAGGTACCGCCGTAGAAACT	60° C	Whole genome	340bp	Ambros et al 1998.
Hop stunt viroid	One-step RT-PCR	HSV-83M HSV-78P	F R	AACCCGGGGCTCCTTTCTCA AACCCGGGGCAACTCTTCTC	55° C	Complete genome	450bp	Sano et al 2001.
Exotic phytoplasma and bacteria								
Phytoplasma	Nested	P1	F	AAGAGTTTGATCCTGGCTCAGGATT	56°	16S-23S	NA	Constable

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
	PCR	P7	R	CGTCCTTCATCGGCTCTT	C	Ribosomal RNA gene		et al 2003.
		R16F2n	F	GAAACGACTGCTAAGACTGG	56° C	16S-23S Ribosomal RNA gene	1600-2000bp	
		M23sr	R	TAGTGCCAAGGCATCCACTGT				
Pseudomonas sp.	PCR	PsrpoD FNP1	F	TGAAGGCGARATCGAAATCGCCAA	55° C	RNA polymerase sigma factor rpoD gene	~700bp	Parkinson et al 2011.
		PsrpoDnprpcr1	R	YGCMGWCAGCTTYTGCTGGCA				
Agrobacterium	Multiplex PCR	UF f	F	CTAAGAAGCGAACGCAGGGACT	67° C	23S rDNA	189bp 1059bp 491bp 1173bp	Pulawska et el 2006.
		B1R r	R	GACAATGACTGTTCTACGCGTAA				
		B2R r	R	TCCGATACCTCCAGGGCCCCTCACA				
		AvR640 r	R	AACTAACTCAATCGCGCTATTAAC				
		AvR1150 r	R	AAAACAGCCACTACGACTGTCTT				
Xylella fastidiosa	PCR	RST31	F	GCGTTAATTTTCGAAGTGATTCGA	55° C	RNA polymerase sigma factor gene	733bp	Minsavage et al 1994.
		RST33	R	CACCATTCGTATCCCGGTG				
	q-PCR	XF-F	F	CACGGCTGGTAACGGAAGA	62° C	rimM gene	70bp	Harper et al 2010.
		XF-R	R	GGGTTTGCGTGGTGAAATCAAG				
		XF-P	Probe	FAM-TCGCATCCCGTGGCTCAGTCC-BHQ				
	LAMP	XF-F3	F (Outer)	CCGTTGGAAAACAGATGGGA	65° C	rimM gene	149bp	Harper et al 2010.
		XF-B3	R (Outer)	GAGACTGGCAAGCGTTTGA				
		XF-LF	F (Loop)	TGCAAGTACACACCCTTGAAG				
		XF-LB	R (Loop)	TTCCGTACCACAGATCGCT				

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
Erwinia amylovora		XF-FIP	F (Inner)	ACCCCGACGAGTATTACTGGGTTTTTCGCTACCGAGAACCACAC				
		XF-BIP	R (Inner)	GCGCTGCGTGGCACATAGATTTTTGCAACCTTTCCTGGCATCAA				
	PCR	G1-F	F	CCTGCATAAATCAACCGCTGACAGCTCAATG	60° C	Hypothetical protein	187bp	Taylor et al 2001.
		G1-R	R	GCTACCACTGATCGCTCGAATCAAATCGGC				
	q-PCR	hpEaF	F	CCCGTGGAGACCGATCTTTTA	53° C	Hypothetical protein AMY1267	138bp	Gottsberger 2010.
		hpEaR	R	AAGTTTCTCCGCCCTACGAT				
		hpEaP	Probe	TCGTCGAATGCTGCCTCTCT				
	LAMP	F3	F (Outer)	TCAAGATCGTGTGGCTATG	65° C	EAMY_3195	NA	Bühlmann et al 2013.
		B3	R (Outer)	CTAAAAACCGGGGCAAAC				
		loopF	F (Loop)	ACATTAGCGGCCCGACCAA				
		loopR	R (Loop)	CTRTTAAGATGGCATGCAGA				
		FIP	F (Inner)	ACGRTTCTACCCTTCCTGTCTACTTCTCTGGGGTTTCAGTC				
		BIP	R (Inner)	ATGTCACCTGATTCTACAGCCGCAATCATTCATGGTTCTGGAC				
Xanthomonas arbicola pv. Pruni	Multiplex PCR	pXap41repA1-F	F	GCGAGGACATGGCTTTCAC	55° C	pXap41-repA1 gene	343bp	Pothier et al 2011.
		pXap41repA1-R	R	GCGGCCAAGGCGTGCATCTGC				
		pXap41repA2-F	F	TACCAAGAGCGGCAACATCTGC		pXap41-repA2 gene	451bp	
		pXap41repA2-R	R	TTTGGCCTTGCTGTAGAGCGT				

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
		pXap41mob-F	F	GCCTATCTGGCGAAGGTCGAG				
		pXap41mob-R	R	GCTTGTAGCTCGGCCAGGATG		pXap41-mobC gene	245bp	
Generic tests								
Ilarvirus	Nested RT-PCR	Ilapol up2	F	YTCIAMRTTYGAYAARTC	A	RNA2-encoded RdRp	381bp	Maliogka et al 2007.
		Ilapol do4	R	GGYTGRTTRTGIGGRAA				
		Ilapones up3	F	TCGAMRTTYGAYAARTCICA	A	RNA2-encoded RdRp	371bp	
		Ilapones d5	R	TGGGGRAAYTTIGYYTCRA				
Ampelovirus	Nested RT-PCR	dHSP up1	F	GGIHTIGAITTYGGIACIACITT	A	HSP70h gene	580-620bp	Dovas and Katis 2003.
		dHSP up1G	F	AGTTYGGGACGACGTT				
		dHSP do2	R	GTICCICCICCNAARTC				
		dHSP do2c	R	GTICCICCCCCNAARTC				
		dHSP nest2		TYGGGACGACGTTYTCNAC	A	HSP70 gene	490bp	Maliogka et al 2008.
		LR5 clusdoL		GGYTCRTTCACIACIGCYTGIAC				
Closterovirus	Nested RT-PCR	dHSP up1	F	GGIHTIGAITTYGGIACIACITT	A	HSP70 gene	580-620bp	Dovas and Katis 2003b.
		dHSP up1G	F	AGTTYGGGACGACGTT				
		dHSP do2	R	GTICCICCICCNAARTC				
		dHSP do2C	R	GTICCICCCCCNAARTC				
		dHSP nest1	F	TTYGGGACGACGTTYAGYAC	A	HSP70 gene	500-535bp	
		dHSP nest2	F	TYGGGACGACGTTYTCNAC				

Pathogen	Test	Primer name	Orientation	Primer sequence (5'-3')	Tm	Region amplified	Expected product size	Reference
		dHSP nest3	R	SCIGCIGMISWIGGYTCRTT				
		dHSP nest3G	R	GCGGMGSWGGGPTCRTT				
Foveavirus	Nested RT-PCR	dRW up1	F	WGCIAARGCIGGICARAC		RNA-dependent RNA polymerase gene	363bp	Dovas and Katis 2003a.
		dRW do2	R	RMYTCICCISWRAAICKCAT				
		dRW do2G	R	GCCGSWRAAGCKCAT				Dovas and Katis 2003b.
		dRW nest1	F	GGGGCARACIHTIGCITGYTT			198bp	Dovas and Katis 2003a.
		dRW nest2	R	AAIGCYTCRTARTCIGAITCNGT				
Tricho, capillo, foveavirus	RT-PCR	PDO-F1i	F	TiTTYATKAARWSiCARYWiTGiAC	42° C	RNA-dependent RNA polymerase gene	446bp, 631bp	Foissac et al 2001.
		PDO-R3i	R	GCRCACTRTCRTCiCCiGCRAAiiA				
		PDO-R4i	R	ARiYiCCATCCRCARAAMiTiGG				
	Nested PCR	PDO-F2i	F	GCYAARGCiGGiCARACiyTKGCiTG	42° C		362bp	
		PDO-R1i	R	TCHCCWGTRAAiCKSATiAiiGC				
Apricot pseudo chlorotic leaf spot virus	RT-PCR	PDO-F1i	F	TiTTYATKAARWSiCARYWiTGiAC	42° C	RNA-dependent RNA polymerase gene	446bp, 631bp	Foissac et al 2001.
		PDO-R3i	R	GCRCACTRTCRTCiCCiGCRAAiiA				
		PDO-R4i	R	ARiYiCCATCCRCARAAMiTiGG				
	Nested PCR	NT1	F	ARATACTYTCMARYTGTCTRC	58° C		218bp	Liberti et al 2004.
		NT3	R	ATKATTTTYTCATCCCABCCY				
Trichovirus	One-step RT-PCR	Tricho F	F	GCCTGATCAAAATGTTCAAGAC	55° C	Coat protein	442bp	Renae Sarec unpublished
		Tricho R	R	CACTCCAATATTGGTTAGGTCC				

Pathogen	Test	Primer name	Orientation	Primer sequence (5’-3’)	Tm	Region amplified	Expected product size	Reference

See separate table of cycling conditions for the various cycling conditions for the generic/ nested tests

2.3 RT-PCR and PCR amplification of pathogens

- Set up all PCR and RT-PCR reactions on ice.
- Prior to the addition to RT-PCR or PCR reactions all nucleic acid must be fully defrosted and mixed thoroughly to reduce the risk of false negative results.
- Table 2.3 gives the components and concentrations for one generic one-step RT-PCR reaction using the SuperScript® III One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase kit (Invitrogen):
    - 12.5 µl reactions are used for the RNA housekeeping PCR.
    - 20µl reactions are used for virus detection.
- Table 2.4 gives the components and concentrations for one generic PCR reaction using the Platinum® *Taq* DNA Polymerase kit (Invitrogen):
    - 20µl reactions are used for the DNA housekeeping assay and pathogen detection.
    - The Lamp assay reaction is set up according to the manufacturer's instructions
- For more than one reaction, make a master mix for the number of reactions required plus one extra reaction and aliquot the amount of each reaction required into individual labelled tubes.
- Prior to pathogen detection conduct a housekeeping RT-PCR or PCR to determine if the nucleic extract is of sufficient quality for pathogen detection.
- The cycling conditions for the housekeeping assays and pathogens is given in table 2.5 for the specific RT-PCR and PCR assays, table 2.6 for the4 generic virus genera RT-PCR and PCR assays and Table 2.7 for the LAMP assay for PCR
- Run the PCR products on a gel as described in section 2.4.

Table 2.3. The volumes of components required for one RT-PCR reaction for the detection of Housekeeping RNA (12.5 µl reaction) or viruses (20 µl reaction) using the Invitrogen SuperScript™ III One-Step RT-PCR system with Platinum® Taq DNA Polymerase.

Reagent	Volume for a 12.5 µl reaction – Housekeeping RT-PCR	Volume for a 20 µl reaction – Pathogen RT-PCR
Sterile (RNase, Dnase free) water	4.5µl	6µl
10 µM forward primer	0.25µl	0.8µl
10 µM reverse primer	0.25µl	0.8µl
2 × reaction mix (Contains buffer, dNTPs and $MgCl_2$)	6.25µl	10µl
SuperScript™ III RT/Platinum® Taq mix	0.25µl	0.4µl
RNA template	1µl	2µl
Total reaction volume		20 µl

Table 4. The volumes of components required for one 20 μl PCR reaction for the detection of Housekeeping DNA or DNA pathogens using the Invitrogen Platinum® Taq DNA Polymerase kit.

Reagent	Volume
Sterile (RNase, Dnase free) water	18.05μl
10 × reaction buffer	2μl
50 mM $MgCl_2$	0.75μl
10 mM dNTP mixture	0.5μl
10 μM forward primer	0.8μl
10 μM reverse primer	0.8μl
Platinum® Taq (5 units/μl)	0.08μl
DNA template or control	2μl
Total reaction volume	20 μl

Table2.5 PCR cycling conditions used for the detection of housekeeping mRNA and DNA and pathogens.

Housekeeping or pathogen assay	Pre-cycling conditions- 1 cycle		PCR-cycling conditions- 35 cycles			Post-cycling conditions- 1 cycle	
Step	**Reverse transcription**	**Initial denaturation**	**Denaturation**	**Annealing**	**Elongation**	**Final elongation**	**Hold**
Temperature	**48°C**	**94°C**	**94°C**	**See table x for temperatures**	**72°C**	**72°C**	**20°C**
Housekeeping gene assays							
NADH dehydrogenase mRNA	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	Indefinite
16S rRNA gene	NA	1 minute[1]	45 seconds[1]	30 seconds	30 seconds	10 minutes	Indefinite
Endemic viruses							
Prunus necrotic ring spot virus	45 minutes	2 minutes	30 seconds	45 seconds	1 minute	7 minutes	Indefinite
Prune dwarf virus	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	5 minutes	Indefinite
Apple mosaic virus	45 minutes	2 minutes	30 seconds	45 seconds	1 minute	10 minutes	Indefinite
Apple chlorotic leaf spot virus	45 minutes	2 minutes	30 seconds	45 seconds	1 minute	10 minutes	Indefinite
Apple stem grooving virus	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	10 minutes	Indefinite
Apple stem pitting virus	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	10 minutes	Indefinite
Little cherry virus 2- UP2/LO2 primers	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	5 minutes	Indefinite
Little cherry virus 2- 01F/ 03R primers	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	5 minutes	Indefinite
Cherry virus A- fw1/ rev1 primers	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	10 minutes	Indefinite
Cherry virus A- 6170F/ 6736R	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	10 minutes	Indefinite
Cucumber mosaic virus	45 minutes	2 minutes	30 seconds	45 seconds	1 minute	7 minutes	Indefinite
Exotic viruses and viroids							
Cherry green ring mottle virus	45 minutes	2 minutes	30 seconds	1 minute	1 minute	10 minutes	Indefinite
Cherry necrotic rusty mottle virus	45 minutes	2 minutes	30 seconds	45 seconds	1 minute	7 minutes	Indefinite
Plum bark necrosis stem pitting associated virus- ASP1/ASP2	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	7 minutes	Indefinite
Plum bark necrosis stem pitting	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	10 minutes	Indefinite

Housekeeping or pathogen assay	Pre-cycling conditions- 1 cycle		PCR-cycling conditions- 35 cycles			Post-cycling conditions- 1 cycle	
Step	**Reverse transcription**	**Initial denaturation**	**Denaturation**	**Annealing**	**Elongation**	**Final elongation**	**Hold**
Temperature	**48°C**	**94°C**	**94°C**	**See table x for temperatures**	**72°C**	**72°C**	**20°C**
associated virus- det-F/R							
Tomato black ring virus	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	Indefinite
Plum pox virus	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	Indefinite
Little cherry virus-1	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	10 minutes	Indefinite
Strawberry latent ring spot virus	45 minutes	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	Indefinite
Arabis mosaic virus	45 minutes	2 minutes	20 seconds	20 seconds	30 seconds	5 minutes	Indefinite
Apricot latent virus	45 minutes	2 minutes	45 seconds	45 seconds	1 minute	10 minutes	Indefinite
American plum line pattern virus	45 minutes	2 minutes	30 seconds	30 seconds	1 minute	7 minutes	Indefinite
Asian Prunus virus(es)	45 minutes	2 minutes	30 seconds	30 seconds[2]	1 minute	7 minutes	Indefinite
Cherry rusty mottle lassociated virus	45 minutes	2 minutes	45 seconds	45 seconds	45 seconds	5 minutes	Indefinite
Cherry mottle leaf virus	45 minutes	2 minutes	45 seconds	45 seconds	1 minute	10 minutes	Indefinite
Cherry twisted leaf associated virus 1a	45 minutes	2 minutes	45 seconds	45 seconds	45 seconds	5 minutes	Indefinite
Cherry twisted leaf associated virus 1b	45 minutes	2 minutes	45 seconds	45 seconds	45 seconds	5 minutes	Indefinite
Peach mosaic virus	45 minutes	2 minutes	30 seconds[2]	45 seconds[2]	1 minute[2]	10 minutes	Indefinite
Tomato bushy stunt virus	45 minutes	2 minutes	1 minute	2 minutes	2 minutes	10 minutes	Indefinite
Peach rosette mosaic virus	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	7 minutes	Indefinite
Raspberry ring spot virus	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	7 minutes	Indefinite
Cherry leaf roll virus- CLRV-3/ CLRV-5 primers	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	7 minutes	Indefinite
Apple scar skin viroid	45 minutes	2 minutes	30 seconds	30 seconds	45 seconds	5 minutes	Indefinite
Peach latent mosaic viroid- cPLMVd/ hPLMVd	45 minutes	2 minutes	45 seconds[4]	1 minute[4]	2 minutes[4]	7 minutes	Indefinite

Housekeeping or pathogen assay	Pre-cycling conditions- 1 cycle		PCR-cycling conditions- 35 cycles			Post-cycling conditions- 1 cycle	
Step	**Reverse transcription**	**Initial denaturation**	**Denaturation**	**Annealing**	**Elongation**	**Final elongation**	**Hold**
Temperature	**48°C**	**94°C**	**94°C**	**See table x for temperatures**	**72°C**	**72°C**	**20°C**
Peach latent mosaic viroid- RF-43/RF-44	45 minutes	2 minutes	40 seconds[4]	30 seconds[4]	2 minutes[4]	10 minutes	Indefinite
Hop stunt viroid	45 minutes	2 minutes	1 minute	2 minutes	2 minutes	10 minutes	Indefinite
Erwinia amylovora- hpEaf/ hpEaR/ hpEap primers and probe (qPCR)	15 minutes[3] (not an RT step)	5 minutes[1]	30 seconds[1, 2]	30 seconds[2]	30 seconds[2]	NA	NA
Pseudomonas sp	NA	2 minutes	30 seconds	30 seconds	30 seconds	5 minutes	Indefinite
Agrobacterium	NA	1 minute	1 minute	1 minute	1.5 minutes	10 minutes	Indefinite
Xylella fastidiosa- RST31/ RST33 primers	NA	1 minute[1]	45 seconds[1]	30 seconds	30 seconds	10 minutes	Indefinite
Xylella fastidiosa- XF-F/ XF-R/ XF-P primers and probe (qPCR)	NA	3 minutes[1]	10 seconds[1,2]	40 seconds[2]	NA	NA	NA
Erwinia amylovora- G1-F/ G2-R primers	NA	5 minutes[1]	30 seconds[1]	20 seconds	1 minute	5 minutes	Indefinite
Erwinia amylovora- hpEaF/ hpEaR/ hpEaP	NA	15 minutes[5]	30 seconds[1,2]	30 seconds[2]	30 seconds[2]	NA	NA
Xanthomonas	NA	5 minutes	30 seconds	30 seconds	30 seconds	7 minutes	Indefinite

6. 95°C instead of 94°C
7. 40 cycles instead of 35 cycles
8. 50 minutes instead of 45 minutes
9. 30 cycles instead of 35 cycles
10. 50°C instead of 94°C

Table 2.6. PCR cycling conditions used for the detection of pathogens using generic and nested PCR tests.

	Pre-cycling conditions- 1 cycle			PCR cycling conditions						Post-cycling conditions- 1 cycle	
Generic/ nested assay	**Step 1**	**Step 2**	**Step 3**	**No of PCR cycles**	**Step 1**	**Step 2**	**Step 3**	**Step 4**	**Step 5**	**Final extension**	**Hold**
Phytoplasma Round 1 RT-PCR	94°C for 1 min	NA	NA	35	94°C for 1 min	56°C for 1 min	72°C for 1.5 min			72°C for 5 min	20°C
Phytoplasma Round 2 PCR	94°C for 1 min	NA	NA	35	94°C for 1 min	56°C for 1 min	72°C for 1.5 min			72°C for 5 min	20°C
Ilarvirus Round 1 RT-PCR	42°C for 50 min	94°C for 4 min	NA	5	94°C for 30 sec	38°C for 30 sec	72°C for 20 sec			72°C for 2 min	20°C
				35	94°C for 30 sec	42°C for 20 sec	38°C for 10 sec	72°C for 20 sec			
Ilarvirus Round 2 PCR	94°C for 2 min	NA	NA	5	94°C for 30 sec	43°C for 30 sec	72°C for 20 sec			72°C for 2 min	20°C
				35	94°C for 30 sec	47°C for 20 sec	43°C for 10 sec	72°C for 20 sec			
Ampelovirus Round 1 RT-PCR	42°C for 55 min (RT)	50°C for 2 min	94°C for 4 min	10	94°C for 30 sec	38°C for 30 sec	72°C for 20 sec			72°C for 2 min	20°C
				30	94°C for 30 sec	49°C for 10 sec	44°C for 10 sec	38°C for 15 sec	72°C for 20 sec		
Ampelovirus Round 2 PCR	94°C for 4 min			5	94°C for 30 sec	55°C for 30 sec	72°C for 20 sec			72°C for 2 min	20°C
				39	94°C for 30 sec	59°C for 15 sec	55°C for 15 sec	72°C for 20 sec			
Closterovirus Round 1	48°C for 30 min	50°C for 2 min	94°C for 4 min	5	94°C for 30 sec	43°C for 10 sec	38°C for 5 sec	72°C for 20 sec		72°C for 2 min	20°C

	Pre-cycling conditions- 1 cycle			PCR cycling conditions						Post-cycling conditions- 1 cycle	
Generic/ nested assay	**Step 1**	**Step 2**	**Step 3**	**No of PCR cycles**	**Step 1**	**Step 2**	**Step 3**	**Step 4**	**Step 5**	**Final extension**	**Hold**
RT-PCR	(RT)			35	94°C for 30 sec	43°C for 30 sec	72°C for 30 sec	NA			
Closterovirus Round 2 PCR	94°C for 3 min	48°C for 15 sec	72°C for 15 sec	39	94°C for 30 sec	54°C for 15 sec	72°C for 10 sec (+1s after each cycle)			72°C for 2 min	20°C
Foveavirus Round 1 RT-PCR	42°C for 60 min (RT)	50°C for 2 min	94°C for 4 min	5	95°C for 30 sec	43°C for 10 sec	38°C for 5 sec	72°C for 15 sec		72°C for 2 min	20°C
				35	95°C for 30 sec	43°C for 30 sec	72°C for 20 sec	NA			
Foveavirus Round 2 PCR	95°C for 3 min	48°C for 15 sec	72°C for 15 sec	39	95°C for 30 sec	54°C for 30 sec	72°C for 10 sec (+1s after each cycle)			72°C for 2 min	20°C
Trichovirus, Capillovirus and *Foveavirus* Round 1 RT-PCR	48°C for 30 min (RT)	95°C for 3 min		35	95°C for 30 sec	42°C for 30 sec	72°C for 30 sec			72°C for 7 min	20°C
Trichovirus, Capillovirus and *Foveavirus* Round 2 PCR	95°C for 3 min			30	95°C for 45 sec	42°C for 30 sec	72°C for 30 sec			72°C for 10 min	20°C
Apricot pseudo chlorotic leaf	48°C for 45 min	95°C for 3 min		35	95°C for 30 sec	42°C for 30 sec	72°C for 30 sec			72°C for 7 min	20°C

	Pre-cycling conditions- 1 cycle			PCR cycling conditions						Post-cycling conditions- 1 cycle	
Generic/ nested assay	**Step 1**	**Step 2**	**Step 3**	**No of PCR cycles**	**Step 1**	**Step 2**	**Step 3**	**Step 4**	**Step 5**	**Final extension**	**Hold**
spot virus Round 1 RT-PCR	(RT)										
Apricot pseudo chlorotic leaf spot virus Round 2 PCR	95°C for 3 min			35	95°C for 30 sec	58°C for 30 sec	72°C for 30 sec			72°C for 10 min	20°C
Trichovirus Specific RT-PCR	48°C for 45 min (RT)	94°C for 2 min		35	94°C for 30 sec	55°C for 1 min	72°C for 1 min			72°C for 8 min	20°C

Table 2.7. LAMP cycling conditions used for the detection of housekeeping mRNA and DNA and pathogens.

Housekeeping or pathogen assay	**Pre-cycling conditions- 1 cycle**			**Post-cycling conditions- 1 cycle**	
Step	**Reverse transcription**	**Initial denaturation**	**Annealing**	**Enzyme inactivation**	**Melting curve**
Temperature	**48°C**	**94°C**			
(LAMP ASSAYS)					
Erwinia amylovora	NA	NA	65°C for 60 minutes	NA	95 to 70°C
Xylella fastidiosa	NA	NA	65°C for 60 minutes	80°C for 2 minutes	

2.4 Gel electrophoresis of PCR products

1. Use gloves for all steps.
2. Prepare a 1% agarose gel in 0.5 x TBE buffer. Dissolve the agarose in the buffer by heating in a microwave, swirl to mix and check to ensure all agarose has dissolved.
3. When the gel solution is cool enough to be held, add 5μl of 10mg/ml ethidium bromide to 50 ml 1% agarose and mix. Avoid bubbles – do not stir vigorously.
4. Tape both ends of the gel tray or place the gel tray into a horizontal gel castor and pour the agarose mixture. Remove any bubbles present; add a comb and leave to set.
5. Once the agarose has set remove the tape from the gel tray or remove the gel tray from gel castor, remove the comb from the agaraose gel and place the agarose gel in the electrophoresis tank.
6. Add 0.5 x TBE buffer until the agarose gel is just covered.
7. Add 6 × loading buffer to the DNA sample at a rate of 1μl loading buffer to 5μl of the DNA sample and mix thoroughly.
8. Make a well plan in your laboratory book, including standards, and load DNA samples in the wells of the agarose gel accordingly.
9. Run gel at 100V for 30-60 minutes depending of the length of the agarose gel or until the bromophenol blue has reached 1cm from the end of the agarose gel.
10. View under UV illumination.

Appendix 6: Identify optimal pest and disease management strategies for maintaining summerfruit budwood repository blocks.

6.1 Background

The aim of certification schemes is to reduce the threat of spread of endemic (primarily) pathogens and pests, to provide traceability, and to ensure provision of high quality planting material that is true to type. Budwood repository blocks established with such material, if properly maintained, should remain sustainable and productive for many years. An additional benefit of a well-managed repository is the reduced risk of incursion of a quarantine pest or pathogen.

Many of the endemic viruses and viroids and some of the quarantine viruses, and bacteria are transmitted in the field by insect vectors. Most of the pathogens are also transmitted in budwood. Some of the viruses may be transmitted via pollen. Some viruses may be transmitted by a vector.

6.2 Recommended management practices

Introduction of new material

Importation of new varieties: Where possible select material from recognised certification programs that provide high-health material. Such phytosanitary and certification programs should undertake pathogen testing and preventive measures to minimize the risk of graft-transmissible diseases caused by viruses, viroids and some bacteria.

Pathogen testing: Where material has been obtained from an accredited source it should have been tested for the pathogens listed in table 1 prior to introduction and they should not have been detected. Viruses should have had at least two rounds of negative test during two growing seasons (minimum testing time of 18 months).

Heat therapy for pathogen elimination

Heat therapy – *incubation of plants at high temperatures (36° to 40°C) for 4-12 weeks combined with the excision and shoot tip/meristem culture to eradicate viruses from infected varieties or clones.*
In some instances varieties infected with an unwanted pathogen may be acquired from an overseas source or locally. In such cases these varieties should be subjected to pathogen elimination methods including heat treatment and shoot tip or meristem culture. This is one of the few methods by which some viruses can be removed from infected plants. Heat therapy should also reduce the risk of transmission of bacteria and fungi through planting material. It cannot be used for eradication of viroids.
Material that has been produced through heat therapy for virus eradication should be pathogen-tested during at least two growing seasons after establishment in a screenhouse. Pathogen testing should include both RT-PCR methods and biological indexing to confirm the virus status.
It is important to note that removal of a pathogen may affect the character of the variety. It may be important to monitor the performance of the variety after heat therapy to ensure the desirable traits are still present.

Table 1. A list of pathogens that are known to infect almonds and/or other Prunus species that occur in Australia and may be significant to high health budwood repository blocks.

Pathogen group	Pathogens known to infect almond	Pathogens known to infect other *Prunus* species
Bacteria	*Agrobacterium tumefaciens* *Pseudomonas syringae pv. syringae* *Xanthomonas arboricola pv. pruni*	*Agrobacterium tumefaciens* *Pseudomonas syringae pv. mors-prunorum* *Pseudomonas syringae pv. syringae* *Xanthomonas arboricola pv. pruni*
Viruses	*Apple chlorotic leaf spot trichovirus* (ACLSV) *Apple mosaic virus Ilarvirus* (ApMV) *Prune dwarf Ilarvirus* (PDV) *Prunus necrotic ringspot Ilarvirus* (PNRSV) *Plum brak necrosis stem pitting associated virus* (PBNSPaV)	*Apricot pseudochlorotic leaf spot trichovirus* (APCLSV) *Apple stem grooving virus capillovirus* (ASGV) *Apple chlorotic leaf spot trichovirus* (ACLSV) *Apple mosaic virus Ilarvirus* (ApMV) *Prune dwarf Ilarvirus* (PDV) *Cherry green ring motle virus* (CGRMV) *Cherry necrotic rusty mottle virus* (CNRMV) *Prunus necrotic ringspot Ilarvirus* (PNRSV) *Little cherry virus 2* (LChV-2) *Plum brak necrosis stem pitting associated virus* (PBNSPaV)
Viroids	*Hop stunt viroid* (Australian strains) *Peach latent mosaic viroid*	*Hop stunt viroid* (Australian strains) *Peach latent mosaic viroid*

Establishment of the repository

The high health repository plants should be maintained under conditions ensuring freedom from (re)infection.

Aerial infections: Several pathogens represent a risk of aerial infection, these include PNRSV and PDV which are transmitted in pollen. Pollen may be carried to trees by wind or by insects such as bees.

Pest control: Some viruses are at risk of spread by insect vectors or transmission is insect mediated. There is no known vetor of PBNSPaV

Screenhouse: Ideally high health repository (mother) plants should be maintained in an aphid-proof screenhouse and grown in containers of sterilized growing medium, isolated from soil. In practice this may not be feasible.

Buffer zone: Buffer zones reduce the risk of infection by pollen-borne and naturally transmitted viruses in open-field planted repositories. Where high health-stock plants are maintained in the

open they should be separated by buffer zone of approximately 1 km from any cultivated or wild *Prunus* spp.

Climate: Repositories should be located in areas of low rain fall and less prone to frost and hail, which can predispose the trees to bacterial infections. Avoid overhead irrigation.

Sanitation and Cultural Practices

Dedicated equipment: If possible dedicated equipment should be used in high health budwood repository blocks to prevent movement of mechanically transmitted pathogens and virus vectors.

Shared equipment: If dedicated equipment is not possible then cultivation, spray and other equipment used in the block should washed down with a high pressure wash and free of soil prior to entering the block.

Disinfection: Pruning equipment such as secateurs and saws should be disinfected with hypochlorite or other adequate disinfectant between trees to prevent spread of mechanically transmitted pathogens.

People movement: Where possible movement to and within the repository should be restricted to staff. Visitors should be accompanied at all times and follow the phytosanitary management procedures.

Production and maintenance activities must be planned so that workers start with the high health repository trees before proceeding to other orchards with less stringent isolation and control measures to minimize the movement of pests and pathogens that can be transmitted on equipment, clothes and hands, such as mealybug, thrips and viroids.

Growing conditions: All plantings entered should be kept in good growing condition and pests and weeds should be kept under effective control.

Deblossoming: It is suggested that the trees be deblossomed, either by hand or by chemical spray, to reduce the spread of pollen-borne viruses

Block/Field Monitoring: The repository should be monitored for visual symptoms of viruses and other diseases at least once a month during the growing season. If signs or symptoms of a virus or other pests of significance are found during these visual inspections, pathogen testing to determine the cause of the symptoms should be undertaken so that adequate control measures can be applied.

Pathogen testing:

Annual testing: Repository trees should be tested for PNRSV, PDV, ACLSV, ApMV, PBNSPaV, PLMVd and HSVd annually.

Every three years: The following *Prunus* species should be tested every three years for APCLSV, ASGV and/or LChV-2

P. mume: ASGV

*P. persica (*peach/nectarine): APCLSV, ASGV

P. domestica (plum): APCLSV, ASGV, LChV-2

P. cerasifera (myrobalan plum): APCLSV, LChV-2

P. armeniaca (apricot); APCLSV, ASGV

P. salicina (Japanese plum) APCLSV, ASGV, LChV-2

Positive result: If a positive result is obtained the infected tree should be removed. The remaining trees should be monitored by visual inspection and active testing further testing should be undertaken to determine the extent of the infection, especially if more than one tree was bulked into a sample for virus testing.

Training of Facility Staff

Training program: All employees should have a general understanding of the facility's phytosanitary management system and specific knowledge related to those components for which each employee has responsibility. All staff should be aware of the specific pathogens for which the material is being managed and/or certified and the risk of infection associated with natural spread and spread through cultural and management practices.

6.3 Pathogen Information

Pseudomonas syringae pv. syringae* and *P. s. pv. mors-prunorum

Host range: *Pseudomonas syringae pv. syringae* infects many *Prunus* sp and causes bacterial canker. *P. s. pv. mors-prunorum* infects several *Prunus* sp but is not reported on almond.

Pathway: Transmitted on infected propagation material and plants through infected cankers, buds and systemically infected tissue.

Disease: Cause bacterial canker or blast. Both pathovars can cause yield loss due to blast and death of buds and decline and death of *Prunus* trees and therefore can have negative impact on production. Infection may result in death of young trees in the first or second year of planting.

The bacteria overwinter in cankers and buds, where they multiply. Both are spread by wind and rain to blossom and leaf surfaces in spring, where they can survive epiphytically. Infection takes place in cool wet periods during autumn and winter through wounds on any part of the plant. Both bacteria can colonize then subsequently enter the host through the leaf scars, particularly if early defoliation in occurs in autumn occurs. They also colonise and enter the host through wounds caused by physical damage including winter and spring frost, hail and pruning. The bacteria can systemically invade the vascular system. Dormant plants may be more susceptible that actively growing plants.

Outbreaks of bacterial canker may occur in spring after prolonged periods of cold, frosty, wet weather or with severe storms that injure the emerging blossom and leaf tissues. Both pathovars prefer wet cool conditions for growth and disease development. In less favourable conditions they may live as saprotrophs.

P. syringae pathovars have ice nucleation activity and can promote the formation of ice crystals on and within plant tissues. They can raise the temperature at which a host is sensitive to freezing, thus making the plant more susceptible to frost damage and through which the bacteria can invade the plant. Free water on leaf surfaces and high relative humidity are required for at least 24 hours after a frost event for leaf infection to take place. Symptoms appear about 5 days later at temperatures between 21°C and 27°C.

The ring nematode *Mesocriconema (Criconemella) xenoplax,* can predispose *Prunus* trees to infection by *P. s. pv. syringae.* There is no direct interaction between the nematode and bacterium. The nematode causes stress by feeding on feeder roots and reducing the uptake of nutrients which in turn may favour *P. s. pv. syringae* infection. Nemaguard roostock is not resistant to ring nematode.

Sandy or clay soils with low calcium content may increase the susceptibility of some apricot cultivars to *P. s. pv. syringae*.

Many *P. syringae* pathovars are reported to exist in non-agricultural habitats as a part of epilithic biofilms. They have detected in wild plants, irrigation water, streams, lakes and rain.

Surveillance: Trees should be inspected regularly for symptoms of bacterial canker and blast.

Trunks, limbs, branches: Depressed areas in the bark that become darkened; bark tissue under the depressions may be orange to brown and can have a strong sour smell; cankers that exude gum (gummosis) in spring and summer;
Buds (leaf and flower): Failed bud break. Dead dormant buds which are brown. Cankers may form at the base of these buds.
Flowers: Flower blast. Infected blossom clusters may collapse. Buds and blossoms become brown or black.
Shoots: Wilting/death of terminal shoots/leaves on limbs and branches severely affected with cankers. Cankers on damaged shoots, associated with wounds or leaf scars. Infected buds that open may collapse in early summer resulting in wilted leaves and shriveled fruit.
Leaves: Water soaked spots on younger leaves, which become necrotic (brown) and drop out, giving a shot hole appearance. Brown spots may be surrounded by a yellow halo. Midseason leaf fall may occur.
Fruit: Sunken spots that may have pockets of gum underneath.

Preferred location of the repository block: Areas of low rain fall and less prone to frost and hail.
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.
Minimise damage to trees in the orchard: Take care when moving large machinery. Take care when tying trees against supports.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease. Control ring nematode.
Removed diseased material: Remove severely affected trees. Remove diseased branches/limbs on less affected trees, cut back to uninfected wood.
Pruning: Prune during summer-early autumn, during periods of little or no rain, so that pruning wounds can heal. Seal pruning wounds.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Chemical treatment: Copper treatments have been recommended for control of *P. syringae* pathovars however resistant isolates are reported.. The spray schedule depends upon disease severity in the orchard. Where disease is not observed epiphytic populations may be controlled by spraying after leaf fall and again at bud swell. Copper sprays may cause defoliation.
References: Davis and English 1969, Lownsberry et al 1973, 1977, Lindow 1983, Ferris et al 2004,Harzallah et al 2004, Janse 2010, Samavatian 2006, Scortichini 2010, Vavaro 1983, Morris et al 2007, 2008, Spotts et al 2010, Wenneker et al 2012, Gutiérrez-Barranquero et al 2013.

Xanthomonas arboricola pv. pruni

Host range: Only *Prunus* species including *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry) *P. cerasus* (sour cherry) *P. persica* (nectarine, peach) and *P. domestica* (plum). It also infects the ornamental *species P. davidiana, P. japonica* and *P. laurocerasus* and *P. salicina*
Pathway: Disseminated through rain and wind from cankers, infected leaf debris and mummified fruits. Entry is through wounds and natural openings. Transmitted mechanically on pruning equipment. Transmitted in plants and propagation material and infected fruit.
Disease: Bacterial leaf spot, shot-hole and black spot of almond, apricot, cherries, nectarine, peach, plum and *P. salicina*. Severe infections can result in defoliation of trees. Severely affected trees may

decline and die back. Fruit may be small and unmarketable. Infected fruit may develop spotting, cracks and lesions resulting poor quality. Frequent rainfall favors the dispersal of bacteria.
X. arboricola pv. pruni can overwinter in the intercellular spaces of the cortex, phloem and xylem parenchyma towards the tips of twigs and in cankers formed in summer and in buds, leaf scars and protected areas on the tree such as cracks in the bark. It can also overwinter in buds and fallen leaves and may overwinter on fruit mummies of almonds. Ooze from spring cankers serve as a primary source of inoculum
The bacteria multiply in late winter as daily temperatures increase. It is spread by water splash or wind driven rain. Primary infections on emerging leaves and fruit take place when frequent rainfall occurs from the end of the flowering and until some weeks after petal fall. The bacterium affects production in warm and moist environments. Optimal temperatures for disease development are between 24°C and 29°C. Infection through leaves requires leaf wetness for several hours, which could be caused through heavy dew, overhead irrigation, rainfall and/or high humidity. If wet conditions continue secondary infections can occur during the growing season.
The disease may be more severe on sandy soils compared to heavier soils.
Surveillance: Trees should be inspected regularly for symptoms:

Shoots: Spring cankers are observed during bloom and occur on the previous year's growth, extending a few centimeters either side of flower or leaf buds which often fail to open. Spring cankers referred to as "Black tip" can be observed in in late winter: the black canker extends from the terminal bud down the shoot. The terminal bud does not enlarge and open and appears sunken and black. The canker can lengthen and the bark surface cracks during the growing season. Summer cankers occur on current-season growth and are observed as very small lesions from early to midsummer. Summer cankers develop into spring cankers.
Leaves: Water soaked spots near mid ribs, which become enlarged, necrotic (brown) and drop out, giving a shot hole appearance. Leaf fall may occur with severe infection.
Fruit: Water soaked lesions appear in late spring and become sunken and darker as fruit enlarges and develops. . The lesions can crack and ooze gum. Corky tissue may develop around the edges of lesions.

Preferred location of the repository block: Protected areas with little wind, low rain fall and humidity. Avoid sandy soils.
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.
Minimise damage to trees in the orchard: Take care when moving large machinery. Take care when tying trees against supports.
Minimise stress: Provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove severely affected trees.
Pruning: Prune trees to facilitate canopy drying and reduce humidity within the canopy. Prune during summer-early autumn, during periods of little or no rain, so that pruning wounds can heal. Seal pruning wounds.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Chemical treatment: Copper treatments may assist control. Copper sprays may cause defoliation.

References: Battilani et al 1999, Ballard, 2008, Ballard et al 2011, EPPO Bulletin 2006, Hetherington et al 2005, Janse 2010., Pagani, 2004, Palacio-Bielsa et al 2010, Park et al 2010, Richie 1995, Zaccardelli et al 1998, Zehr and Shepard 1996.

Agrobacterium tumefaciens and A. rhizogenes

Host range: Many hosts including *Prunus* spp

Pathway: Propagation material, soil, irrigation water. They can be transmitted through planting material and may be transmitted from plant to plant on pruning equipment.

Disease: Crown gall, root knot and hairy root

The bacterium exists in soil and may survive for several years even in the absence of a host. They multiply in the root zone of susceptible plants. The bacteria invade the plant through wounds caused by mechanical injury (equipment, insects and animals) to crown or roots. Infection can occur through grafting and budding scars.

A. tumefaciens causes large tumour-like growths of affected *Prunus* sp. on roots and at the collar just above the soil. Secondary tumours can form on the stem/trunk and branches. It may be more problematic in young plants and rootstocks, where it can cause significant losses at the nursery level. Affected plants may be stunted, decline and die due to disruption to the vascular tissue and girdling of the trunk. Fissures within the galls can lead to secondary infections by other pathogens. Older trees are often less affected by the disease and may have no economic loss. However the occurrence of the bacterium in trees used for nursery stock production could represent a risk for contamination of propagation material.

A. rhizogenes causes proliferation of the roots and root hairs. It can be used to promote root production in almonds and other plant species undergoing micropropagation. An avirulent strain is used to cross protect against other *Agrobacterium* sp in many plant hosts.

The bacteria can affect graft take. Severely affected trees may be less vigorous and can die, particularly young trees. Infection may allow secondary infection by other pathogens, which further weakens trees.

Surveillance: Trees should be inspected regularly for galls:

Galls vary in size and can grow more than 300mm in diameter depending on the age of the tree. They are frequently found on rots or at the collar. They may also be associated with grafts. Secondary falls can form on trunks,/stems and branches.

Early galls appear as pale, soft, spongy, or wart-like growths developing on the crown or on roots.

Older galls darken and are often hard and woody.

Preferred location of the repository block: Agrobacterium free soils. Soil solarisation may assist control.

Control:

Diseased trees should not be used for propagation material.

Minimise damage to trees: Try to minimise damage to roots crowns and stems associated with cultivation and damage by insect pests and animals.

Removed diseased material: Remove severely affected trees.

Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.

Biological control: An avirulent strain is used to cross protect against other *Agrobacterium* sp in many plant hosts and is used to prevent infection at wound sites. It does not cure infection.

References: Cubero et al 1999, Cubero et al 2006, Escobar and DanLopes et al 1997, Eskobar and Dandekar 2003, Janse et al 2010.

Apple chlorotic leaf spot virus* (ACLSV), Genus *Trichovirus*, Family *Betaflexiviridae

Host range: *P. dulcis, P. persica (*peach), *P. domestica* (plum), *P. avium* (sweet cherry), *P. cerasifera* (myrobalan plum), *P. cerasus* (sour cherry), *P. armeniaca* (apricot), *P. cerasoides, P. glandulosa. P. orientalis, P. korschinskii, P. salicina* (Japanese plum), *P. spinosa* (blackthorn), *P. tomentosa* (Nanking cherry tree),
Cydonia oblonga (quince), *Malus* (ornamental species apple), *Malus domestica* (apple), *Malus platycarpa*, *Pyrus communis* (European pear), *Pyrus pyrifolia* (oriental pear tree)
Minor hosts: *Amelanchier canadensis* (thicket serviceberry), *Chaenomeles* (flowering quinces), *Crataegus monogyna* (hawthorn), *Crataegus rhipidophylla* (Midland hawthorn), *Mespilus germanica* (medlar), *Sorbus aucuparia* (mountain ash)
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting, including root grafting. Mechanically transmitted by inoculation to herbaceous indicators, however mechanical transmission by contaminated pruning or harvesting equipment is not reported. There is one unconfirmed report of transmission by nematodes.
Disease: Pseudopox disease of plum and apricot, apricot viruela disease and plum bark split.
It has been associated with pseudopox disease of plum and apricot, apricot viruela disease and plum bark split. Possibly associated with chlorotic leafroll of almond in combination with PDV
Symptom expression/disease is dependent of the host species and variety and the virus strain. Some virus strains may be symptomless. Some hosts may be symptomless hosts.
In sensitive hosts ACLSV can have a significant impact on fruit quality, particularly associated with fruit size and shape, and on yield.
Can cause graft union incompatibilities.
May enhance severity of disease in combination with other viruses and/or abiotic stress such as nutrient deficiencies.
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of virus disease.

> **Trunks, limbs, Branches**: Bark split in plum: brownish-red areas followed by cracking and splitting of the bark; branch dieback can occur if necrosis progresses to the cambium; slowed tree development; production of suckers at the base of the tree. Graft union incompatibilities, leading to decline.
> **Buds (leaf and flower):** Bud necrosis in apricot.

Fruit: Deformation, yield loss. Deformation particularly occurs in apricot and plums associated with depressions in the fruit. In apricot yellow/cream spots and/or depressions are sometimes observed on the stones.
Leaves: Leaf deformation and chlorosis. Dark green sunken mottle on some peach varieties such as GF305.
Preferred location of the repository block: At least 30 metres from ACLSV infected trees
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.
Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.

Removed diseased material: Remove severely affected trees. Remove diseased branches/limbs on less affected trees, cut back to uninfected wood.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Heat therapy: Heat therapy following by meristem tip culture can be used to successfully eradicate ACLSV from infected varieties.
References: Al Rwahnih et al 2004, Candresse et al 1995, Canizares et al 2001, Constable et al 2007, Dunez and Marenaud 1969, Foissac et al 2001, Garcia_Ibarra 2010, German et al 1990; German et al 1997, Gilmer 1971, Jelkmann 1996, Kanaan-Atallah et al 2000, LLacer et al 1985, Marini et al 2008, Martelii and Savino 1997, Menzel 2002, Nemeth, 1986, Rana et al 2007, Rana et al 2008, Rana et al; 2009, Salmon et al 2002, Sato *etal.* 1993, Spiegel et al 2005, Sutic et al 1999, Ulubas and Ertunc 2005, Youseff and Shalaby 2009.

Apple mosaic virus* (ApMV), Genus *Ilarvirus*, Family *Bromoviridae
Host range: Broad host range, including *Prunus* spp: *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry) *P. cerasus* (sour cherry), *P. persica* (peach) and *P. domestica* (plum). Many other members of the family *Rosaceae* (*Malus, Pyrus, Rosa, Fragaria* etc)
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting. Mechanically transmitted by inoculation to herbaceous indicators, however mechanical transmission by contaminated pruning or harvesting equipment is not reported. It is seed and pollen borne in some plant host species, but this is not known for most *Prunus* species. It has been detected in the ovules of one cultivar of almond but not in pollen nor in seedlings.
Disease: European plum line pattern. Line pattern or mosaic diseases in various *Prunus* sp. Symptom expression/disease is dependent of the host species and variety and the virus strain. Some virus strains may be symptomless. Some hosts may be symptomless hosts.
In Almond it is associated with mosaic symptoms. It is associated with line pattern in plum, almond and other *Prunus* sp. ApMV may be symptomless in some varieties of various *Prunus* sp.
In sensitive hosts ApMV can have a significant impact on fruit quality, particularly associated with fruit size and shape, and on yield. In almond ApMV is associated with the failure of flower and leaf buds to grow.
May enhance severity of disease in combination with other viruses and/or abiotic stress such as nutrient deficiencies.
The symptoms associated with ApMV may be similar to those caused by other *Ilarvirus* species (e.g. PNRSV and PDV) in *Prunus* species.
ApMV is transmitted in propagation material.
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of virus disease. Symptoms appear from early summer and may only be present on a few leaves.

> **Buds (leaf and flower):** Failure to grow
> **Leaves:** Leaf chlorosis in the form of blotching, mottling, mosaic, line patterns, oakleaf patterns, yellow vein clearing. Dark green sunken mottle on some peach varieties such as GF305.

Preferred location of the repository block: Isolated from other orchards.
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.

Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove infected trees
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Heat therapy: Heat therapy following by meristem tip culture can be used to successfully eradicate ApMV from infected varieties.
References: Barba et al 1985, Ciccarone, 1958, Digiaro et al 1992, Digiaro and Savino, 1992, Digiaro et al 1992, Fulton 1972, Fulton 1983, Garau et al1989, Gilmer 1956, Hamilton, 1985, Imed et al 1997, Llacer et al 1997, Maliogka et al 2010, Marenaud and Lansac, 1977, Martelli and Savino 1997, Menzel et al 2002, Petrzik and Svoboda 1997, Petzrik and Lenz 2002, Saade et al 2000, Savino et al 1989a, Tirro and Catara, 1982, Uyemoto and Scott 1992, Youseff and Shalaby 2009.

Organism: ***Cherry green ring mottle foveavirus (syn. Sour cherry green ring mottle virus, CGRMV)***
Host range: *Prunus cerasus* (sour cherry), *P. avium* (sweet cherry), *P. serrulata* (flowering cherry), *P. persica* (peach), *P. domestica* (plum) and *P. armeniaca* (apricot). Symptom expression is dependent of the virus strain and *Prunus* species and cultivar.
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting, including root grafting.
Disease: Rusty mottle of Cherry, Lambert mottle
It has been associated with vein yellowing, ringspot, mosaic, necrosis, shot hole, leaf distortion and stunting symptoms in peach and plum that were also infected with ACLSV, PBNSPaV and/or CNRMV. Symptom expression/disease is dependent of the host species and variety and the virus strain. Some virus strains may be symptomless. Some hosts may be symptomless hosts.
In sensitive hosts CGRMV can have a significant impact on fruit quality, particularly associated with fruit size and shape, and on yield.
May enhance severity of disease in combination with other viruses and/or abiotic stress such as nutrient deficiencies.
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of virus disease.

Fruit: Yield loss.

Leaves: Vein yellowing, ringspot, mosaic, necrosis, shot hole, leaf distortion and stunting

Preferred location of the repository block: At least 30 metres from CGRMV infected trees
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.
Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove severely affected trees. Remove diseased branches/limbs on less affected trees, cut back to uninfected wood.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Heat therapy: Heat therapy following by meristem tip culture can be used to successfully eradicate CGRMV from infected varieties.

Reference: Desvignes, 1999, Li and Mock 2005, Liberti et al 2005, Nemeth, 1986, Parker et al 1976, Rott and Jelkmann 2001, Sipahioglu et al 2007, Wang et al 2009, Zhang et al 1998, Zhang et al 2000, Zhou et al 2011, Villamor et al 2015.

Organism: *Cherry necrotic rusty mottle foveavirus* (CNRMV)
Host range: *P. avium* (sweet cherry), *P. serrulata* (flowering cherry), *P. cerasus* (sour cherry, *P. armeniaca* (apricot), *P. persica* (peach) and *P. domestica* (plum). Experimental hosts include apricot and almond.
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting, including root grafting.
Disease: Rusty mottle of Cherry, Lambert mottle
It has been associated with vein yellowing, ringspot, mosaic, necrosis, shot hole, leaf distortion and stunting symptoms in peach and plum that were also infected with ACLSV, PBNSPaV and/or CGRMV. Symptom expression/disease is dependent of the host species and variety and the virus strain. Some virus strains may be symptomless. Some hosts may be symptomless hosts.
In sensitive hosts CNRMV can have a significant impact on fruit quality, particularly associated with fruit size and shape, and on yield.
May enhance severity of disease in combination with other viruses and/or abiotic stress such as nutrient deficiencies.
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of virus disease.

Fruit: Yield loss.

Leaves: Vein yellowing, ringspot, mosaic, necrosis, shot hole, leaf distortion and stunting
Preferred location of the repository block: At least 30 metres from CNRMV infected trees
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.
Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove severely affected trees. Remove diseased branches/limbs on less affected trees, cut back to uninfected wood.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Heat therapy: Heat therapy following by meristem tip culture can be used to successfully eradicate CNRMV from infected varieties**References:** Fry and Wood 1973, Isogai et al 2004, Li and Mock 2005, Li and Mock 2008, Mandic et al 2005, Sabandsovic et al 2005, Rott and Jelkmann 2001a, Rott and Jlekmann 2001b, Zhou et al 2013

Organism: *Plum bark necrosis stem pitting-associated ampelovirus (PBNSPaV)*
Host range: *P. dulcis (*almond), *P. domestica* (plum), *P. mume* (Japanese plum) *P. persica* (peach), *P. avium* (sweet cherry) *P. cerasus* (sour cherry) and *P. armeniaca* (apricot). It was also experimentally transmitted to *P. serrulata* and colt cherry (*P. avium* x *P. pseudocerasus*).
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting, including root grafting. Natural spread occurs although no vector is reported. Other virus species in the genus are transmitted by mealybug and scale insects.

Disease: Plum bark necrosis, stem pitting
Symptom expression/disease is dependent of the host species and variety and the virus strain. Some virus strains may be symptomless. Some hosts may be symptomless hosts.
In sensitive hosts PBNSPaV can have a significant impact on yield.
May enhance severity of disease in combination with other viruses and/or abiotic stress such as nutrient deficiencies.
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of virus disease.

> **Trunks, limbs, Branches**: Bark gummosis (formation of gumballs) and bark necrosis (die-off of bark), pitting of the vascular tissues along branches and trunks, flattening of the scaffold branches. Graft union incompatibilities, leading to decline.
> **Fruit:** Yield loss.
> **Leaves:** Chlorotic mottle, ringspots or line patterns.

Preferred location of the repository block: At least 30 metres from PBNSPaV infected trees
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.
Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove severely affected trees. Remove diseased branches/limbs on less affected trees, cut back to uninfected wood.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Heat therapy: Heat therapy following by meristem tip culture can be used to successfully eradicate PBNSPaV from infected varieties.
References: Al Rwahnih et al 2007, Amenduni et al 2005, Amenduni et al 2004a, Amenduni et al 2004b, Bouani et al 2004, Di Terlizzi and Savino 1994, El Maghraby et al 2006, Ghanem-Sabanadzovic et al 2001, Garcia-Ibarra et al 2010, Gumus et al 2007, Mandic et al 2005, Marais et al 2009, Marini et al 2002, Matic et al 2010, Sánchez-Navarro et al 2005, Usta et al 2007, Uyemoto and Teviotdale, 1996, Dovas and Katis 2003, Peiro et al 2012.

Prune dwarf virus* (PDV), Genus *Ilarvirus*, Family *Bromoviridae
Host range: Several *Prunus* species, including *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry) *P. cerasus* (sour cherry), *P. persica* (peach) and *P. domestica* (plum), *P. besseyi* (bessey cherry), *P. cerasifera* (myrobalan plum), *P. mahaleb* (mahaleb cherry), *P. salicina* (Japanese plum), *P. serotina* (black cherry), *P. serrulata* (Japanese flowering cherry), *P. tomentosa* (Nanking cherry tree)
Other hosts: *Crataegus* spp., *P. padus* (bird cherry), *P. spinosa* (blackthorn)
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting. Mechanically transmitted by inoculation to herbaceous indicators, however mechanical transmission by contaminated pruning or harvesting equipment is not reported. It is seed and pollen transmitted. Thrips feeding on infected pollen may transmit the virus. Some evidence for spread by vectors including mite (*Vasates fockeui*).
Disease: Prune dwarf, peach stunt, almond mosaic.
Associated with almond mosaic disease. It causes stunting in peach and plum, sour cherry yellows, chlorotic spots, shot hole, defoliation fruit cracking in sweet cherry.

Symptom expression in some *Prunus* sp can vary from year to year depending on climate. Some cultivars may be symptomless.
Symptom expression/disease is dependent of the host species and variety and the virus strain. Some virus strains may be symptomless. Some hosts may be symptomless hosts.
The symptoms associated with PDV may be similar to those caused by other *Ilarvirus* species (e.g. PNRSV and ApMV) in *Prunus* species.
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of virus disease. Symptoms appear from early summer and may only be present on a few leaves.

Buds (leaf and flower): Failure to grow

Leaves: Chlorosis, mosaic symptoms, chlorotic spots, shot hole, stunting. In combination with PNRSV rosetting of the shoots may be observed.

Preferred location of the repository block: Isolated from other orchards to limit transmission via infected pollen (wind or insect borne). Mother trees maintained in a screenhouse or glasshouse.
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.
Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove infected trees. Flower removal of mother trees to prevent infection via pollen.
Vector control: Monitor for thrips and control if necessary.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Heat therapy: Heat therapy following by meristem tip culture can be used to successfully eradicate PDV from infected varieties.
References: Al-Chaabi and Darwesh 2008, Bertozzi et al 2002, Boulila 2009, Boulila and Marrakchi, 2001, Boulila, 2002, Brunt et al 1996, Di Terlizzi et al 1994, Digiaro andSavino 1992, Fridlund, 1965; Foneseca et al 2005, Greber et al 1992, Helguera et al 2002, Jarasova and Kundu 2010, Johnstone et al 1995, Kelly and Cameron 1986, Martelli and Savino 1997, Mekuria et al 2005, Mekuria et al 2003, Nemeth 1986, Pallas et al 2012, Parakh et al 1995, Raquel et al 1998, Saade et al 2000, Savino et al 1994, Smith and Stubbs 1976, Spiegel et al 1996, Spiegel et al 1998, Ulubas Serce et al 2009, Uyemoto et al 1992, Vaskova et al 2000, Waterworth and Fulton, 1964, Youssef et al 2002, Youseff and Shalaby 2009.

Prunus necrotic ringspot Ilarvirus* (PNRSV), Genus *Ilarvirus*, Family *Bromoviridae
Host range: Many commercial, wild and ornamental *Prunus* species, including almond, apricot, cherry, peach and plum. Also many *Rosa* sp and other plants species.
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting. Mechanically transmitted by inoculation to herbaceous indicators, however mechanical transmission by contaminated pruning or harvesting equipment is not reported. It is seed and pollen transmitted. Thrips feeding on infected pollen may transmit the virus. Some evidence for spread by vectors including mite (*Vasates fockeui*), nematode (*Longidorus macrosoma*) and thrips (*Frankliniella occidentailis*).
Disease: Almond calico, sweet cherry rugose mosaic, necrotic ringspot, European plum line pattern. Genetic variants exist that may be associated with plant host species and region.

Symptomless infections occur. Symptoms may be observed 12 months after infection, After the initial "shock" period when symptoms expressed trees may recover and become symptomless.
In Almond it has been associated with necrotic shock, bud failure, calico and chlorotic mottling. It may be symptomless in some cultivars. In Other *Prunus* species PNRSV may be associated with more serious disease and especially when occurring in mixed infection with other viruses.
Spread in pollen and seed and propagation material.
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of virus disease. Symptoms appear from early summer and may only be present on a few leaves.

Buds (leaf and flower): Bud failure

Leaves: Leaf chlorotic mottling, calico, necrosis – shot hole, line pattern. . In combination with PDV rosetting of the shoots may be observed.

Preferred location of the repository block: Isolated from other orchards to limit transmission via infected pollen (wind or insect borne). Choose site with low numbers of nematodes. Mother trees maintained in a screenhouse or glasshouse.
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.
Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove infected trees. Flower removal of mother trees to prevent infection via pollen.
Vector control: Monitor for thrips and control if necessary.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Heat therapy: Heat therapy following by meristem tip culture can be used to successfully eradicate PNRSV from infected varieties.
References: Amari et al 2009, Aparicio et al 1999, Barba 1986, Bertozzi et al 2002, Boulila 2002, Boulila and Marrakchi et al 2001, Cole et al 1992, Crosslin and Mink 1992, Digiaro and Savino 1992, Greber et al 1992, Heleguera et al 2001, Howell and Mink 1988, Lansac et al 1980, Martelli and Savino 1997, Massart et al 2008, Mekuria et al 2003, Mink 1983, Mink et al 1987, Moury et al 2000, Nyland and Lowe 1964, Nyland et al 1976, Pallas et al 2012, Saade et al 2000, Salem et al 2003, Savino et al 1994, Sanchez-Navarro et al 1998, Spiegel et al 1998, Sweet 1976, Uyemoto, 1996, Uyemoto et al 1989, Varveri et al 1997, Williams et al 1970, Youseff and Shalaby 2009.

Apple stem grooving virus* (ASGV) Genus *Capillovirus*, Family *Betaflexiviridae
Host range: Naturally infects citrus, lily, *Actinidia chinensis* and pome fruits. Natural infections are also reported from *P. persica* (nectarine), *P. domestica* (plum), *P. armeniaca* (apricot), Japanese apricot (*Prunus mume*) and *P. avium* (cherry).
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting, including root grafting. The fungus *Talaromyces flavus* is reported as a vector of ASGV in Korea.
Disease: Unknown in *Prunus* sp.
Surveillance: Active virus testing using RT-PCR.
Preferred location of the repository block: Isolated from other orchards.
Control:
Infected trees should not be used for propagation material.

Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove infected trees.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Heat therapy: Heat therapy following by meristem tip culture can be used to successfully eradicate ASGV from infected varieties.
References: Clover et al 2003, Constable et al 2007, Fuchs and Grntzig 1994, Hassan et al 2006,Hilf 2008, Ito et al 2002, James 1999, James 2008, Kinard et al 1996, Marinho et al 1998, Massart et al 2008, Negi et al 2010, Nickel et al 2004, Roy et al 2005, Takahashi et al 1990, Yoshikawa et al 1992, Yoshikawa et al 1996.

Apricot pseudochlorotic leaf spot *virus* **(APCLSV) Genus *Trichovirus*, Family *Betaflexiviridae***
Host range: *P. ameniaca (*Apricot) *P. domestica* (plum), *P. salicina* (Japanese plum) and *P. persica* (peach).
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting.
Disease: decline, stem-grooving, butteratura (pockmark) in apricot.
It has been associated with various symptoms including decline of plum, stem-grooving symptoms in plum, butteratura symptoms on apricot fruit. It is also associated with symptoms similar to those caused by ACLSV and symptoms of apricot ringpox disease.
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of virus disease. Symptoms appear from early summer and may only be present on a few leaves.

Tree: Decline.
Trunk, limbs, branches: Stem grooving
Buds (leaf and flower): Bud failure
Fruit: Deformation of apricot associated with depressions in the fruit
Leaves: Leaf chlorotic mottling, calico, necrosis – shot hole, line pattern. . In combination with PDV rosetting of the shoots may be observed.

Preferred location of the repository block: Isolated from other orchards.
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.
Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove infected trees.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Heat therapy: Heat therapy following by meristem tip culture can be used to successfully eradicate APCLSV from infected varieties.
References: Barone et al 2006, Barone et al 2008, Liberti et al 2005, Sarec et al 2003.

Little cherry *virus* ***2*** (LChV-2) **Genus *Ampelovirus*, Family *Closteroviridae***
Host range: *P. avium, P. serrulata, P emarginata. Plum (*the *Prunus* species was not identified but could be any one of *P. domestica, P. salicina, P. bokhariensis,* and *P. munsoniana)*

Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting, including root grafting. Infectious mealybug.
Disease: Little cherry disease of cherry. Disease in plum is not described.
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of virus disease. Symptoms may only be evident on cherry fruit in some cultivars. Symptoms in plum have not been reported.

Tree: Reduced tree vigour, less production

Fruit: Late ripening, lack of flavour, sweetness, size and colour may be angular and pointed.

Leaves: Premature interveinal reddening or bronzing in late summer and autumn

Preferred location of the repository block: Isolated from other orchards.
Control: Some of the symptoms described may be confused with symptoms associated with other pathogens and accurate diagnosis should inform control strategies.
Diseased trees should not be used for propagation material.
Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove infected trees.
Vectors: Monitor for mealybug and scale. Although the known vectors (apple mealybug (*Phenacoccus aceris*) and grape mealybug (*Pseudococcus maritimus))* of this virus are not known to occur in Australia the vector status of other mealy bug and scale is not known.
Disinfect pruning equipment: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees.
Heat therapy: Heat therapy following by meristem tip culture can be used to successfully eradicate LChV-2 from infected varieties.
References: Bajet et al 2008, Eastwell and Bernardy 2001, Isogai et al 2004, Jelkmann et al 1997, Kiem-Konrad and Jelkmann 1996, Matic et al 2010, Osman et la 2012, Raine et al 1986, Rott and Jelkamnn 2005, Yorston et al 1981.

Peach latent mosaic viroid (PLMVd) **Genus *Pelamoviroid*, Family *Avsunviroidae***
Host range: Many hosts including *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry), *P. domestica* (plum) *and P. persica* (peach).
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting. Mechanically transmitted on pruning equipment and harvest equipment. Transmitted n
Disease: plum spotted fruit, peach mosaic, peach yellow mosaic, peach calico. Latent infections occur that may be associated with strain variation and/or host. In peach infection may be latent for many years
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of disease.

Tree: decline and reduced longevity of the plants.

Buds: bud necrosis, delayed bud-burst

Flowers: Delayed flowering and,

Leaves: mosaic, calico, chlorosis

Fruit: discoloured and deformed fruits with cracked sutures and enlarged pits, delayed fruit ripening

Preferred location of the repository block: Isolated from other orchards.
Control:

Infected trees should not be used for propagation material.
Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove infected trees.
Disinfect: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees. Wear gloves. Wash hands thoroughly after handling infected material. Do not handle infected material before handling un-infected material.
Movement: Do not use equipment from a contaminated area in an uninfected area.
References: Barba et al 2007, Boubouakas et al 2009, Desvignes 1986, Di Serio et al 1999, Flores et al 2006, Hadid et al 1997. Hassen et al 2006, Hassen et al 2009, Hernandez and Flores 1992, Luigi and Faggioli 2011, Parisi et al 2011, Ragozzino et al 2004,

Hop stunt viroid (HSVd) **Genus *Hostuviroid*, Family *Pospoviroidae***
Host range: Many hosts including *P. dulcis* (almond), *P. armeniaca* (apricot), *P. avium* (sweet cherry), *P. domestica* (plum) *and P. persica* (peach).
Pathway: Transmitted on infected propagation material and plants. Transmitted by grafting. Mechanically transmitted on pruning equipment and harvest equipment. Transmitted n
Disease: dapple fruit of plum and peach. Latent infections occur that may be associated with strain variation and/or host. In peach infection may be latent for many years
Many genetic variants exist. Latent infections occur that may be associated with strain variation and/or host.

Leaves: mosaic, calico, chlorosis
Fruit: discoloured and deformed fruit, delayed fruit ripening, especially in apricot.
Surveillance: Active virus testing using RT-PCR. Trees should be inspected regularly for symptoms of disease. Strains occur worldwide, including Australia in grapevine and citrus. Not known to occur in *Prunus* species in Australia.

Preferred location of the repository block: Isolated from other orchards.
Control:
Infected trees should not be used for propagation material.
Choose pathogen tested material from an accredited certification/high health scheme.
Minimise stress: provide adequate water and nutrients. Minimise other pest and disease.
Removed diseased material: Remove infected trees.
Disinfect: Treat secateurs and saws with 20% bleach or other appropriate disinfectant between trees. Wear gloves. Wash hands thoroughly after handling infected material. Do not handle infected material before handling un-infected material.
Movement: Do not use equipment from a contaminated area in an uninfected area.
References: Amari et al 2007, Astruc et al 1996, Biosecurity Australia 2010, Cañizares et al 1999, Gillings et al 1988, Hadidi et al 1992, Hadidi et al 2003, Kofalvi et al 1997,Koltunow et al 1988, Pallas et al 2002, Pallas et al 2003, Pehtybridge et al 2008, Ragozzino et al 2004, Sano et al 1989, Sano 2003, Zhou et al 2006

Appendix 7: References

Abou Ghanem-Sabanadzovic, N., Mahboubi, M., Di Terlizzi, B., Sabanadzovic, S., Savino, V., Uyemoto, J.K. and Matelli, G.P. 2001. Molecular detection of a closterovirus associated with apricot stem pitting in southern Italy. Journal of Plant Pathology. 83, 125-132.

Abou-Jawdah, Y., Dakhil, H., El-Mehtar, S. and Lee, I.M. 2003. 'Candidatus Phytoplasma phoenicium' sp. nov., a novel phytoplasma associated with an emerging lethal disease of almond trees in Lebanon and Iran. International Journal of Systematic and Evolutionary Microbiology. 53, 833-838.

Abou-Jawdah, Y., Karakashian, A., Sobh, H., Martini, M. and Lee, I.M. 2002. PCR procedure for detection and identification of European stone-fruit yellows (ESFY) phytoplasma on trees of Prunus species. Bulgarian Journal of Agricultural Science. 8, 19-22.

Abou-Jawdah, Y., Sobh, H. and Akkary, M. 2009a. Characterization of phytoplasmas associated with almond diseases in Iran. Journal of Phytopathology. 157, 736-741.

Abou-Jawdah, Y., Sobh, H. and Akkary, M. 2009b. First report of Almond witches' broom phytoplasma ('Candidatus Phytoplasma phoenicium') causing a severe disease on nectarine and peach trees in Lebanon. Bulletin OEPP/EPPO Bulletin. 39, 94-98.

Abou Kubaa, R., Digiaro, M., Bottalico, G. and Elbeaino, T. 2014. Plum hosts apricot vein clearing associated virus. Journal of Plant Pathology. 96, 603-611.

Accotto, G.P., Riccioni, L., Barba, M. and Boccardo, G. 1997. Comparison of some molecular properties of ourmia melon and epirus cherry viruses, two representatives of a proposed new virus group. Journal of Plant Pathology. 78, 87-91.

Adams Jr., G.C., Surve-Iyer, R.S., and Iezzoni, A.F. 2002. Ribosomal DNA sequence divergence and group I introns within the Leucostoma species L. cinctum, L. persoonii, and L parapersoonii sp. nov., Ascomycetes that cause Cytospora canker of fruit trees. Mycologia. 94, 947-967.

Adaskaveg, J.E., Forster, H. and Connell, J.H. 1999. First report of fruit rot and associated branch dieback of almond in California caused by a Phomopsis species tentatively identified as P. amygdali. Plant Disease. 83, 1073.

Adaskaveg, J.E. and Ogawa, J.M. 1990. Wood decay pathology of fruit and nut trees in California. Plant Disease. 74, 341-352.

Adhikari, R.S. 1989. Some new host records of fungi from India. Indian Phytopathology. 42, 480-481.

Ahmed, A.A. and Fath-Allah, M.M. 2012. Double Infections with Cucumber Mosaic Virus and Plum Pox Virus (Sharka) in Apricot Trees. International Journal of Virology. 8, 50-60.

Alayasa, N., Al-Rwahnih, M., Myrta, A., Herranz, M.C., Minafra, A., Boscia, D., Castellano, M.A. and Pallas, V. 2004. Monitoring American plum line pattern virus in plum by ELISA and dot-blot hybridisation throughout the year. Journal of Plant Pathology. 86, 167-169.

Albrechtova, L., Polak, J. and Kudela, V. 1980. Occurrence of tomato bushy stunt virus in Prunus sp. in Czechoslovakia. Acta Phytopathologica Academiae Scientiarum Hungaricae. 15, 319-322.

Al-Chaabi, S. and Darwesh, A.R. 2008. A survey for transmission of Prune dwarf virus, Prunus necrotic ring spot virus and Apple mosaic virus in rootstock seedlings of stone fruits in Syria. Arab Journal of Plant Protection. 26, 20-26.

Allen, W.R. and Davidson, T.R. 1967. Tomato bushy stunt virus from Prunus avium L. I. Field studies and virus characterization. Canadian Journal of Botany. 45, 2375-2383.

Allen, W.R., Schagen, J.G.V. and Ebsary, B.A. 1984. Comparative transmission of the peach rosette mosaic virus by Ontario populations of Longidorus diadecturus and Xiphinema americanum (Nematoda: Longidoridae). Canadian Journal of Plant Pathology. 6, 29-32.

Allen, W.R. 1988. Transmission of raspberry ringspot, tomato black ring, and peach rosette mosaic viruses by an Ontario population of Longidorus elongatus. Canadian Journal of Plant Pathology. 10, 1-5.

Al-Rwahnih, M., Myrta, A., Herranz, M.C. and Pallas, V. 2005. Simultaneous detection of six stone fruit viruses by non-isotopic molecular hybridization using a unique riboprobe or 'polyprobe'. Journal of Virological Methods. 124, 49-55.

Al Rwahnih, M., Uyemoto, J.K., Falk, B.W. and Rowhani, A. 2007. Molecular characterization and detection of Plum bark necrosis stem pitting-associated virus. Archives of Virology. 152, 2197-2206.

Al-Rwahnih, M., Turturo, C., Minafra, A., Saldarelli, P., Myrta, A., Pallas, V. and Savino, V. 2004. Molecular variability of apple chlorotic leaf spot virus in different hosts and geographical regions. Journal of Plant Pathology. 86, 117-122.

Alvarez, M.G. 1976. Primer catalogo de enfermedades de plantas Mexicanas. Fitofilo. 71, 1-169.

Amano, K. (Hirata) 1986. Host range and geographical distribution of the powdery mildew fungi. Japan Science Society Press, Tokyo, 741 pages.

Amari, K., Ruiz, D., Gomez, G., Sanchez-Pina, A., Pallás, V. and Egea, J. 2007. An important new apricot disease in Spain is associated with Hop stunt viroid infection. European Journal of Plant Pathology. 118, 173-181.

Amari,K., Burgos, L., Pallas, V. and Sanchez-Pina, N.M. 2009. Vertical transmission of Prunus necrotic ringspot virus: hitch-hiking from gametes to seedling. Journal of General Virology. 90, 1767-1774.

Ambros, S., Hernandez, C., Desvignes, J. and Flores, R. 1998. Genomic structure of three phenotypically different isolates of Peach latent mosaic viroid: implications of the existence of constraints limiting the heterogeneity of viroid quasispecies. Journal of Virology. 72, 7397-7406.

Amenduni, T., Bazzoni, A., Minafra, A., Attolico, A. and Savino, V. 2004. Stem pitting in apricots. (Speciale: Albicocco). Rivista di Frutticoltura e di Ortofloricoltura. 66, 55-57.

Amenduni, T., Hobeika, C., Minafra, A., Boscia, D., Castellano, M.A. and Savino, V. 2005. Plum bark necrosis stem pitting-associated virus in different stone fruit species in Italy. Journal of Plant Pathology. 87, 131-134.

Amenduni, T., Hobeika, C., Minafra, A. and Savino, V. 2004. Detection of Plum bark necrosis stem pitting-associated virus (PBNSPaV) from different stone fruit species and optimisation of diagnostic tools. Acta Horticulturae. 657, 93-97.

Anonymous. 1960. Index of Plant Diseases in the United States. U.S.D.A. Agriculture Handbook. 165, 1-531.

Anonymous. 2005. Pseudomonas syringae pv. persicae. EPPO Bulletin. 35, 285-287.

Anonymous. 2006. Xanthomonas arboricola pv. pruni. EPPO Bulletin 36, 129-133.

Aparicio, F., Myrta, A., Terlizzi, B.D. and Pallas, V. 1999. Molecular variability among isolates of Prunus necrotic ringspot virus from different Prunus spp. Phytopathology. 89, 991-999.

Arif, M., Marek, S.M., Ochoa-Corona, F.M., Young, C., and Garzon, C.D. 2010. PCR detection and identification of Phymatotrichopsis omnivora. Phytopathology. 100(Supplement), S7.

Arzanlou, M. and Dokhanchi, H. 2013. Calsophaeria canker of almond caused by Calsophaeria pulchella in Iran. Archives of Phytopathology and Plant Protection. 46, 215-226.

Asef, M.R. and Goltapeh, E.M. 2002. New hosts for Armillaria species in Iran. Iranian Journal of Plant Pathology. 38, 278-279.

Ashkan, M. and Assadi, P. 1974. Red blotch, Polystigma ochraceum, of almonds in Iran. [Persian]. Iranian Journal of Plant Pathology. 10, 25-26, 49-63. Secondary Journal: (Abstracts on Tropical Agriculture 2, 10748).

Astruc, N., Marcos, J.F., Macquaire, G., Candresse, T. and Pallas, V. 1996. Studies on the diagnosis of hop stunt viroid in fruit trees: identification of new hosts and application of a nucleic acid extraction procedure based on non-organic solvents. European Journal of Plant Pathology. 102, 837-46.

Atanasova, I., Kabadjova, P., Bogatzevska, N., and Moncheva P. 2005. New host plants of Erwinia amylovora in Bulgaria. Zeitschrift für Naturforschung. 60, 893-8.

Auger, J., Leal, G., Magunacelaya, J.C. and Esterio, M. 2009. Xiphinema rivesi from Chile transmits Tomato ringspot virus to cucumber. Plant Disease. 93, 971.

Avgelis, A., Barba, M. and Rumbos, I. 1989. Epirus cherry virus, an unusual virus isolated from cherry with rasp-leaf symptoms in Greece. Journal of Phytopathology. 126, 51-58.

Azery, T. and Cycek, Y. 1997. Detection of virus diseases affecting almond nursery trees in western Anatolia (Turkey). Bulletin OEPP. 27, 547-550.

Babovic, M.V., Perisic, M. and Matijevic, M. 1980. Cherry mottle leaf. Acta Phytopathologica Academiae Scientiarum Hungaricae. 15, 129-133.

Bagheri, A.N., Salehi, M., Faghihi, M.M., Samavi, S. and Sadeghi, A. 2009. Transmission of Candidatus Phytoplasma aurantifolia to Mexican lime by the leafhopper Hishimonus phycitis in Iran. Journal of Plant Pathology. 91.4(Supplement), S4.105.

Bajet, N.B., Unruh, T.R., Druffel, K.L. and Eastwell, K.C. 2008. Occurrence of two little cherry viruses in sweet cherry in Washington State. Plant Disease. 92, 234-238.

Ballard, E.L., Dietzgen, R., Sly, L., Gouk, C., Horlock, C. and Fegan, M. Development of a Bio-PCR protocol for the detection of Xanthomonas arboricola pv. pruni. Plant Disease. 95, 1109-1115.

Banihashemi, Z. 1990. Biological control of Polystigma ochraceum, the cause of almond red leaf blotch. Plant Pathology. 39, 309-315.

Barba, M. 1986. Detection of apple mosaic and Prunus necrotic ringspot viruses in almond by ELISA. Archiv fur Phytopathologie und Pflanzenschutz. 22, 279-282.

Barba, M., Sanctis, F.D. and Cupidi, A. 1985. Distribution of almond viruses in Central Italy. Phytopathologia Mediterranea. 24, 267-269.

Barba, M., Pasquini, G. and Quacquarelli, A. 1986. Role of seeds in the epidemiology of two almond viruses. Acta Horticulturae. 193, 127-130.

Barba, M., Ragozzino, E. and Faggioli, F. 2007. Pollen transmission of Peach latent mosaic viroid. Journal of Plant Pathology. 89, 287-289.

Barba, M., Czosnek, H. and Hadidi, A. 2014. Historical perspective, development and applications of next-generation sequencing in plant virology. Viruses. 6, 106-136.

Barone, M., Alioto, D., Ragozzino, A. and Candresse, T. 2008. Investigation on occurrence of Tricho-, Fovea- and Capilloviruses in ancient fruit tree cultivars in Campania. Acta Horticulturae. 781, 53-58.

Barone, M., Alioto, D., Marais, A., Candresse, T. and Ragozzino, A. 2006. First report and high prevalence in noncherry host of Cherry virus A in Italy. Plant Disease. 90, 1459.

Bashir, N.S., Kalhor, M.R. and Zarghani, S.N. 2006. Detection, differentiation and phylogenetic analysis of cucumber mosaic virus isolates from cucurbits in the northwest region of Iran. Virus Genes. 32, 277-88.

Batlle, A., Altabella, N., Sabate, J. and Lavina, A. 2008. Study of the transmission of stolbur phytoplasma to different crop species, by Macrosteles quadripunctulatus. Annals of Applied Biology. 152, 235-242.

Baumgartner, K., Bhat, R. and Fujiyoshi, P. 2010. A rapid infection assay for Armillaria and real-time PCR quantitation of the fungal biomass in planta. Fungal Biology. 114, 107–119.

Behl, M.K., Khurana, S.M.P. and Parakh, D.B. 1998. Bumpy fruit and other viroid and viroid-like diseases of apple in HP, India. Acta Horticulturae. 472, 627-630.

Belli, G., Fortusini, A. and Vegetti, G. 1980. Properties of a strain of strawberry latent ringspot virus, associated with a rosetting disease of peach in northern Italy. Acta Phytopathologica Academiae Scientiarum Hungaricae. 15, 113-117.

Bennett, C.W. and Costa, A.S. 1961. Sowbane mosaic caused by a seed transmitted virus. Phytopathology. 51, 546-550.

Berger F., Cronfeld P., Lex S., Vermeulen M. 2000. Fire blight on plum (Prunus domestica) and roses (Rosa rugosa). [in German] Erwerbsobstbau. 42, 207–210.

Berges, R., Rott, M. and Seemüller, E. 2000. Range of phytoplasma concentration in various hosts as determined by competitive polymerase chain reaction. Phytopathology. 90, 1145–1152.

Bercks, R. and Mischke, W. 1964. Proof of Tomato black ring virus in leaves of Sweet Cherry (P. avium). [not specified]. Phytopathologische Zeitschrift. 51, 437-441.

Berniak, H., Malinowski, T. and Kaminska, M. 2010. Characterization of polyclonal antibodies raised against two isolates of cucumber mosaic virus. Journal of Plant Pathology. 92, 231-234.

Bertolini, E., Olmos, A., Lopez, M.M. and Cambra, M. 2003. Multiplex nested reverse transcription-polymerase chain reaction in a single tube for sensitive and simultaneous detection of four RNA viruses and Pseudomonas savastanoi pv. Savastanoi in olive trees. Phytopathology. 93, 286-292.

Bertozzi, T., Alberts, E. and Sedgley, M. 2002. Detection of Prunus necrotic ringspot virus in almond: effect of sampling time on the efficiency of serological and biological indexing methodologies. Australian Journal of Experimental Agriculture. 42, 207-210.

Biosecurity Australia Review of policy: importation of hop (Humulus species) propagative material into Australia. 2010.
http://www.daff.gov.au/__data/assets/pdf_file/0003/1880571/Final_Hop_Review.pdf

Blattny, C. and Janeckova, M. 1980. Apricot bare twig and unfruitfulness. Acta Phytopathologica Academiae Scientiarum Hungaricae. 15, 383-390.

Bliefernicht, K., and Krczal, G. 1995. Epidemiological studies on apple proliferation disease in southern Germany. Acta Horticulturae. 386, 444-447.

Boari, A., Boscia, D., Yurtmen, M., Potere, O., Turturo, C. and Savino, V. 1997. Production of monoclonal antibodies to prune dwarf ilarvirus and their use in the serological characterization of almond virus isolates. Bulletin OEPP. 27, 555-556.

Blomquist, C.L. and Kirkpatrick, B.C. 2002. Frequency and seasonal distribution of pear psylla infected with the pear decline phytoplasma in California pear orchards. Phytopathology. 92, 1218-1226.

Bobine, E.W., Newton, J.H. and Kreutzer, W.A. 1942. Four new virus diseases of stone fruits found in Peach mosaic study in Colorado. Farm Bulletin. Colorado Agricultural Experiment Station. 4, 6-10.

Bodine, E.W. and Durrell, L.W. 1941. Host range of Peach-mosaic virus in western Colorado. Phytopathology. 31, 322-333.

Boscia, D. and Myrta, A. 1998. Serological detection of viruses included in certification protocols for stone fruits. In: Option Méditerranéennes. Sér. B/n°19 - Stone fruit viruses and certification in the Mediterranean countries: problems and prospects (Di Terlizzi, B., Myrta, A. and Savino, V., Eds).- Bari, Italy. 171-190.

Bouani, A., Minafra, A., Alami, I., Zemzami, M. and Myrta, A. 2004. First report of plum bark necrosis stem pitting-associated virus in Morocco. Journal of Plant Pathology. 86, 91.

Boubourakas, I.N., Fukuta, S. and Kyriakopoulou, P.E. 2009. Sensitive and rapid detection of peach latent mosaic viroid by the reverse transcription loop-mediated isothermal amplification. Journal of Virological Methods. 160, 63-68.

Boulila, M. 2002. Prune dwarf ilarvirus in Tunisia: detection by biological, serological and electron microscopic methods and biological characterization of an almond isolate using a range of herbaceous hosts. Bulletin OEPP. 32, 515-519.

Boulila, M. 2002. Characterization of an almond isolate of Prunus necrotic ringspot ilarvirus. Advances in Horticultural Science. 16, 30-37.

Boulila, M. 2009. Molecular characterization of an almond isolate of Prune dwarf virus in Tunisia: putative recombination breakpoints in the partial sequences of the coat protein-encoding gene in isolates from different geographic origin. Phytopathologia Mediterranea. 48, 411-421.

Boulila, M. and Marrakchi, M. 2001. Detection and characterization of stone fruit virus diseases in Tunisia. Phytopathologia Mediterranea. 40, 125-136.

Boulila, M. and Marrakchi, M. 2001. Reverse transcription-polymerase chain reaction-based assay (RT-PCR) and restriction fragment length polymorphism analysis (RFLP) of some isolates of Prunus necrotic ringspot ilarvirus. Bulletin OEPP. 31, 173-178.

Bove, J.M., Danet, J.L., Bananej, K., Hassanzadeh, N., Taghizadeh, M., Salehi, M. and Garnier, M. 1999. Witches' broom disease of lime (WBDL) in Iran. In Proceedings of the 14th Conference of the International Organization of Citrus Virologists (IOCV), pp. 207–212. Edited by J. V. da Grac¸a, R. F. Lee and R. K. Yokomi. Riverside, CA: IOCV.

Braun, U. 1995. Miscellaneous notes on phytopathogenic hyphomycetes (II). Mycotaxon. 55, 223-241.

Bressan, A., Semetey, O., Nusillard, B., Clair, D. and Boudon-Padieu, E. 2008. Insect vectors (Hemiptera: Cixiidae) and pathogens associated with the disease syndrome "basses richesses" of sugar beet in France. Plant Disease. 92, 113-119.

Brown, D.J.F. and Trudgill, D.L. 1984. The spread of carnation ringspot virus in soil with or without nematodes. Nematologica. 30, 102-105.

Brown, D.J.F., Halbrendt, J.M., Jones, A.T., Vrain, T.C., Robbins, R.T., 1994b. Transmission of three North American nepoviruses by population of four distinct species of the Xiphinema americanum group. Phytopathology. 84, 646–649.

Brown, D.J.F., Robertson,W.M. and Trudgill, D.L., 1995. Transmission of viruses by plant nematodes. Annual Review of Phytopathology. 33, 223–249.

Brown, D.J.F., Halbrendt, J.M., Jones, A.T., Vrain, T.C. and Robbins, R.T. 1995. The transmission of three nepoviruses by populations of four Xiphinema americanum-group species. Acta Horticulturae. 385, 105-109.

Brown, D.J.F., Zheng, J. and Zhou, X. 2004. Virus vectors. Nematology: advances and perspectives. Volume 2: Nematode management and utilization. 717-770.

Bühlmann, A., Pothier, J.F, Rezzonico, F., Smits, T.H.M., Andreou, M., Boonham, N., Duffy, B. and Frey, J.E. 2013. Erwinia amylovora loop-mediated isothermal amplification (LAMP) assay for rapid pathogen detection and on-site diagnosis of fire blight. Journal of Microbiological Methods. 92, 332-339.

Burgyan, J., Beczner, L. and Nemeth, M. 1980. Relationship among some tobamoviruses II. Serological characterization of a tobacco mosaic virus isolated from plum. Acta Phytopathologica Academiae Scientiarum Hungaricae. 15, 339-348.

Büttner, C., Jacobi, V. and Koenig, R. 1987. Isolation of Carnation Italian Ringspot Virus from a Creek in a Forested Area South West of Bonn. Journal of Phytopathology. 118, 131-134.

Cabrera-la Rosa, J.C., Johnson, M.W., Civerolo, E.L., Chen, J.C. and Groves, R.L. 2008. Seasonal population dynamics of Draeculacephala minerva (Hemiptera: Cicadellidae) and transmission of Xylella fastidiosa. Journal of Economic Entomology. 101, 1105-1113.

Cambra, M., Olmos, A., Capote, N., Africander, N.L., Levy, L., Lenardon S.L., Clover G. and Wright, D. 2010. Draft annex to ISPM 27: Plum pox virus.

Candresse, T., Lanneau, M., Revers, F., Macquaire, G., German, S., Dunez, J., Grasseau, N. and Malinowski, T. 1995. An immunocapture PCR assay adapted to the detection and the analysis of the molecular variability of Apple chlorotic leaf spot virus. Acta Horticulturae. 386, 136-147.

Candresse, T. and Cambra, M. 2006. Causal agent of sharka disease: historical perspective and current status of Plum pox virus strains. Bulletin OEPP/EPPO Bulletin. 36, 239–246.

Candresse, T., Delbos, R.P., Gall, O.l., Desvignes, J.C. and Dunez, J. 1998. Characterization of stocky prune virus, a new nepovirus detected in French prunes. Acta Horticulturae. 472, 175-181.

Candresse T., Martinez-Gomez P. and Rubio M. 2015. RNA-seq transcriptome and ESTs analysis provides evidence of the existence of several peach-infecting Marafiviruses. In: Book of Abstracts, ICVF. 23rd International Conference on Virus and Other Graft Transmissible Diseases of Fruit Crops. June 8-12, 2015, Aiina Hall, Morioka, Iwata, Japan.

Candresse, T., Svanella-Dumas, L. and Gall, O.l. 2006. Characterization and partial genome sequence of Stocky prune virus, a new member of the genus Cheravirus. Archives of Virology. 151, 1179-1188.

Canizares, M.C., Marcos, J.F. and Pallas, V. 1998. Studies on the incidence of hop stunt viroid in apricot trees (Prunus armeniaca) by using an easy and short extraction method to analyse a large number of samples. Acta Horticulturae. 472, 581-585.

Cannon, P.F. 1996. Systematics and diversity of the Phyllachoraceae associated with Rosaceae, with a monograph of Polystigma. Mycological Research. 100, 1409-1427.

Cao, T., Connell, J.H., Wilhelm, M. and Kirkpatrick, B.C. 2011. Influence of inoculation date on the colonization of Xylella fastidiosa and the persistence of almond leaf scorch disease among almond cultivars. Plant Disease. 95, 158-165.

Carraro, L., Osler, R., Loi, N., Ermacora, P and Refatti, E. 1998. Transmission of European stone fruit yellows phytoplasma by Cacopsylla pruni. Journal of Plant Pathology. 80, 233-239.

Carraro, L., Osler, R., Loi, N., Ermacora, P and Refatti, E. 2001. Fruit tree phytoplasma diseases diffused in nature by psyllids. Acta Horticulturae. 550, 345-350.

Carraro, L., Loi, N. and Ermacora, P. 2001. The 'life cycle' of pear decline phytoplasma in the vector Cacopsylla pyri. Journal of Plant Pathology. 83, 87-90.

Carraro, L., Ferrini, F., Ermacora, P., and Loi, N. 2008. Infectivity of Cacopsylla picta (syn. Cacopsylla costalis), vector of ‘Candidatus Phytoplasma mali’ in north east Italy. Acta Horticulturae. 781, 403-407.

Carraro, L., Ferrini, F., Ermacora, P. and Loi, N. 2004a. Transmission of European stone fruit yellows phytoplasma to Prunus species by using vector and graft transmission. Acta Horticulturae. 657, 449-453.

Carraro, L., Ferrini, F., Labonne, G., Ermacora, P. and Loi, N. 2004b. Seasonal infectivity of Cacopsylla pruni, vector of European stone fruit yellows phytoplasma. Annals of Applied Biology. 144, 191-195.

Castelain, C., Chastellière, M.G., Jullian, J.P., Morvan, G. and Lemaire, J.M. 1997. La prémunition contre l'enroulement chlorotique de l'abricotier. Bilan de dix annés d'observations sur huit vergers. Phytoma. 493, 39-44.

Chi, P., Jiang, Z. and Xiang, M. 2007. Flora Fungorum Sinicorum. 34: Phomopsis**.** Science Press, Beijing. Vol 34.

Chang, C.J., Donaldson, R., Brannen, P., Krewer, G. and Boland, R .2009. Bacterial leaf scorch, a new blueberry disease caused by *Xylella fastidiosa*. Hortscience. 44, 413–417.

Chib, H.S., Andotra, P.S., Teng, R.K. and Qureshi, A.S. 1989. A probe into Cercospora defoliation of almonds in Kashmir. Research and Development Reporter. 6, 116-118.

Chillali, M., Idder-Ighili, H., Guilaumin, J.J., Mohammed, C., Lung Escarmant, B. and Botton, B. 1998. Variation in the ITS and IGS regions of ribosomal DNA among the biological species of European Armillaria. Mycologicla Research. 102, 533–540.

Christensen, N.M., Nicolaisen, M., Hansen, M. and Schulz, A. 2004. Distribution of phytoplasmas in infected plants as revealed by Real-Time PCR and bioimaging, Molecular Plant Microbe Interactions. 17 , 1175-1184.

Choueiri, E., Jreijiri, F., Issa, S., Verdin, E., Bove, J. and Garnier, M. 2000. Detection of phytoplasmas from Iran using polymerase chain reaction. Iranian Journal of Plant Pathology. 36, 106-107.

Chohan, J.S., Kang, I.S. and Rattan, G.S. 1984. Control of root rot and sap-wood rot of peaches (Flordasun cultivar) caused by Polyporus palustrus, Ganoderma lucidum associated with Schizophyllum commune. International Journal of Tropical Plant Diseases. 2, 49-54.

Cicarone, A. 1958. [Notes on the pathology of almond with particular regard to Sicilia.] Te cni ca Agri cola. 10, (in Italian).

Cieslinska, M. and Morgas, H. 2010. Occurrence and detection of lesser known viruses and phytoplasmas in stone fruit orchards in Poland. Folia Horticulturae. 22, 51-57.

Cieslinska, M., and Morgas, H. 2011. Detection and identification of 'Candidatus Phytoplasma prunorum'. 'Candidatus Phytoplasma mali', and 'Candidatus Phytoplasma pyri' in stone fruit trees in Poland. Journal of Phytopathology. 159, 217-222.

Cieslinska, M., Morgas, H. and Jakubowski, T. 2004. Phytoplasma diseases of stone fruit trees in Poland. Acta Horticulturae. 657, 523-526.

Clark, M.F. and Adams, A.N. 1977. Characteristics of a microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. Journal of General Virology. 34, 475-483.

Clarke, B., Urquhart, J. and Moran, J. 1998. Proposed post-entry quarantine protocols for pome fruit. Final Report for project No. AP 627 “An evaluation of the post-entry quarantine protocols for pome fruit”. Department of Natural Resources and Environment, Victoria.

Cmen, I. and Ertugrul, B.B. 2007. Determination of mycoflora in almond plantations under drought conditions in southeastern Anatolia project region, Turkey. Plant Pathology Journal (Faisalabad). 6, 82-86.

Cochran, L.C. and Hutchins, L.M. 1938. Further studies on host relationships of Peach mosaic in southern California. Phytopathology. 28, 890-892.

Cole, A., Mink, G.I. and Regev, S. 1982. Location of Prunus necrotic ringspot virus on pollen grains from infected almond and cherry trees. Phytopathology. 72, 1542-1545.

Conci, C., Rapisarda, C. and Tamanini, L. 1992. Annotated catalogue of Italian Psylloideae. Attidell Accademia Roveretana degli Agiati II-B (ser. VII), Rovereto, Italy, 104-107.

Constable, F.E. 2009a. Diagnostic protocols for the detection of European stone fruit yellows phytoplasma. Prepared for the Subcommittee on Plant Health Diagnostic Standards, Department of Agriculture, Fisheries and Forestry, Australia.

Constable, F.E. 2009b. Diagnostic protocols for the detection of Western-X phytoplasma. Prepared for the Subcommittee on Plant Health Diagnostic Standards, Department of Agriculture, Fisheries and Forestry, Australia.

Constable, F., Gibb, K. and Symons, R. 2003. Seasonal distribution of phytoplasmas in Australian grapevines. Plant Pathology. 52, 267-276.

Constable F.E., Joyce, P.A. and Rodoni, B.C. 2007. Surveying key pome fruit growing districts in Australia for exotic and endemic pathogens**.** Australasian Plant Pathology. 36, 165-172.

Constable, F., Rodoni, B. and Joyce, P. 2006. Improving diagnostic protocols to ensure rapid and safe access to imported pome fruit propagating germplasm. Final report for Horticulture Australia project AP01030.

Cook, R.P., and Dubé, A.J. 1989. Host-pathogen index of plant diseases in South Australia. South Australian Department of Agriculture, 142 pages.

Cox, K.D. and Scherm, H. 2006. Interaction dynamics between saprobic lignicolous fungi and Armillaria in controlled environments: exploring the potential for competitive exclusion of Armillaria on peach. Biological Control. 37, 291-300.

Cropley, R. 1964. Further studies on European rasp-leaf and leaf-roll diseases of Cherry trees. Annals of Applied Biology. 53, 333-341.

Cropley, R. 1961. Viruses isolated from cherry trees with rasp-leaf and leaf-roll diseases. Annals of Applied Biology. 49, 384.

Crosslin, J.M. and Mink, G.I. 1992. Biophysical differences among Prunus necrotic ringspot ilarviruses. Phytopathology. 82, 200-206.

Crosslin, J.M., Eastwell, K.C., Davitt, C.M. and Abad, J.A. 2010. First report of seedborne Cherry leaf roll virus in wild potato, Solanum acaule, from South America. Plant Disease. 94, 782-783.

Crous, P.W., Slippers, B. , Wingfield, M.J., Rheeder, J. , Marasas, W.F.O., Philips, A.J.L., Alves, A., Burgess, T., Barber, P. , and Groenewald, J.Z. 2006. Phylogenetic lineages in the Botryosphaeriaceae. Studies in Mycology. 55, 235-253.

Cubero, J., Lastra, B., Salcedo, C.I., Piquer, J. and López, M.M. 2006. Systemic movement of Agrobacterium tumefaciens in several plant species. Journal of Applied Microbiology. 101, 412-421.

Cubero, J., Martinez, M.C., Llop, P. and Lopez, M.M. 1999. A simple and efficient PCR method for the detection of Agrobacterium tumefaciens in plant tumours. Journal of Applied Microbiology. 86, 591-602.

Cunnington, J. 2003. Pathogenic fungi on introduced plants in Victoria. A host list and literature guide for their identification. Department of Primary Industries, Victoria.

Dakhil, H.A., Abou-Fakhr, H.E., El-Mohtar, C. and, Abou-Jawdah, Y. 2011. Survey of leafhopper species in almond orchards infected with almond witches'-broom phytoplasma in Lebanon. Journal of Insect Science. 11(1), 60. (insectscience.org/11.60).

Damsteegt, V.D., Waterworth, H.E., Mink, G.I., Howell, W.E. and Levy, L. 1997. Prunus tomentosa as a diagnostic host for detection of plum pox virus and other Prunus viruses. Plant Disease. 81, 329-332.

Damsteegt, V.D., Scorza, R., Stone, A.L., Schneider, W.L., Webb, K., Demuth, M. and Gildow, F.E. 2007. Prunus host range of Plum pox virus (PPV) in the United States by aphid and graft inoculation. Plant Disease. 91, 18-23.

Davidson, R.W. and Campbell, W.A. 1943. Decay in merchantable Black Cherry in the Allegheny National Forest. Phytopathology. 33, 965-985.

Deng, S.J. and Hiruki, C. 1991. Amplification of 16S rRNA genes from culturable and non-culturable Mollicutes. Journal of Microbiological Methods. 14, 53-61.

Desvignes, J.C. 1990. Stocky prune. In : Les virus des arbres fruitiers (Desvignes, J. C., Ed). CTIFL, 51-52.

Desvignes, J.C. 1999. Virus Diseases of Fruit Trees. Éditions Centre technique interprofessionnel des fruit et légumes. Paris.

Dhanvantari, B.N. 1982. Relative importance of Leucostoma cincta and L. persoonii in perennial canker of peach in southwestern Ontario. Canadian Journal of Plant Pathology. 4, 221-225.

Diekmann, M., and Putter, C.A.J. 1996. Stone fruits In: FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm (FAO/IPGRI), no. 16 / FAO, Rome (Italy). Plant Production and Protection Div.; International Plant Genetic Resources Inst., Rome (Italy) , 110.

Digiaro, M. and Savino, V. 1992. Role of pollen and seeds in the spread of ilarviruses in almond. Advances in Horticultural Science. 6, 134-136.

Digiaro, M. and Savino, V. 1993. Response of different Prunus species to infection by three ilarviruses. Phytopathologia Mediterranea. 32, 55-57.

Digiaro, M., Savino, V. and Di Terlizzi, B. 1992. Ilarviruses in apricot and plum pollen. Acta Horticulturae. 309, 93-98.

Digiaro, M., Savino, V., Terlizzi, B.D. and Martelli, G.P. 1992. The relationship of ilarviruses to almond mosaic. Advances in Horticultural Science. 6, 161-166.

Digiaro, M., Elbeaino, T. and Martelli, G.P. 2007. Development of degenerate and species specific primers for the differential and simultaneous RT-PCR detection of grapevine-infecting nepoviruses of subgroups A, B and C. Journal of Virological Methods. 141, 34–40.

Diogo, E., Santos, J. and Phillips, A. 2010. Phylogeny, morphology and pathogenicity of Diaporthe and Phomopsis species on almond in Portugal. Fungal Diversity. 44, 107-115.

Di Serio, F., Malfitano, M., Flores, R. and Randles, J.W. 1999. Detection of peach latent mosaic viroid in Australia. Australasia Plant Pathology. 28, 80–81

Di Terlizzi, B. 1998. Biological diagnosis of virus and viruslike disease: a special reference to stone fruits certification, pp. 151-170. In: Option Méditerranéennes. Sér. B/n°19 - Stone fruit viruses and certification in the Mediterranean countries: problems and prospects (Di Terlizzi, B., Myrta, A. and Savino, V., Eds).- Bari, Italy.

Di Terlizzi B. and Savino V., 1995. A stem pitting of apricot. Acta Horticulturae. 386, 115-117.

Doddapaneni, H., Francis, M., Yao, J.Q., Lin, H. and Civerolo, E.L. 2007. Genome-wide analysis of Xylella fastidiosa: implications for detection and strain relationships. African Journal of Biotechnology. 6, 55-66.

Domenichini, G. 1967. Psylla melanoneura (Homoptera) in northern Italy. [not specified]. Bollettino di Zoologia Agraria e di Bachicoltura. 8, 169-80.

Dosba, F., Maison, P., Lansac, M. and Massonié, G. 1987. Experimental transmission of plum pox virus (PPV) to Prunus mahaleb and Prunus avium. Journal of Phytopathology. 120, 199-204.

Dovas, C. and Katis, N. 2003a. A spot nested RT-PCR method for the simultaneous detection of members of the Vitivirus and Foveavirus genera in grapevine. Journal of Virological Methods. 107, 99-106.

Dovas, C. and Katis, N. 2003b. A spot multiplex nested RT-PCR for the simultaneous and generic detection of viruses involved in the aetiology of grapevine leafroll and rugose wood of grapevine. Journal of Virological Methods. 109, 217-226.

Duduk, B., Ivanovic, M., Paltrinieri, S. and Bertaccini, A. 2008. Phytoplasmas infecting fruit trees in Serbia. Acta Horticulturae. 781, 351-358.

Dunez, J., Delbos, R., Desvignes, J.C., Marenaud, C., Kuszala, J. and Vuittenez, A. 1971. Mise en evidence d'un virus de type ring spot sur Prunus cerasifera. Annales de Phytopathologie Hors Serie. 117–128.

Dunez, J. and Delbos, R. 1976. Myrobalan latent ring spot, a bipartite genome virus and a strain of tomato black ring virus. Mitteilungen der Biologischen Bundesanstalt fur Land- und Forstwirtschaft. 170, 23–27.

Dunez, J. and Marenaud, C. 1969. Contribution to the study of the properties of chlorotic leaf spot virus. Compte Rendu Hebdomadaire des Seances de l'Academie d'Agriculture de France. 55, 642-651.

Eastwell, K.C. and Howell, W.E. 2010. Characterization of Cherry leafroll virus in sweet cherry in Washington State. Plant Disease. 94, 1067.

Eastwell, K., Villamor, D., McKinney, C. and Druffel, K. 2010. Characterization of an isolate of Sowbane mosaic virus. Archives of Virology. 155, 2065-2067.

Eastwell, K.C. and Bernardy, M.G. 2001. Partial characterization of a closterovirus associated with apple mealybug-transmitted little cherry disease in North America. Phytopathology. 91, 268-273.

EFSA PLH Panel (EFSA Panel on Plant Health). 2014. Scientific Opinion on the pest categorisation of Pseudomonas syringae pv. persicae (Prunier et al.) Young et al. EFSA Journal 12, 3855, 26 pp. doi:10.2903/j.efsa.2014.3855.

EFSA PLH Panel (EFSA Panel on Plant Health). 2014. Scientific Opinion on pest categorisation of Xanthomonas campestris pv. pruni (Smith) Dye. EFSA Journal 12, 3857, 25 pp. doi:10.2903/j.efsa.2014.3857.

EFSA PLH Panel (EFSA Panel on Plant Health). 2015. Scientific Opinion on the risks to plant health posed by Xylella fastidiosa in the EU territory, with the identification and evaluation of risk reduction options. EFSA Journal 13, 3989, 262 pp., doi:10.2903/j.efsa.2015.3989.

Elbeaino, T., Choueiri, E., Jreijiri, F. and Digiaro, M. 2007. First report of Strawberry latent ringspot virus in Lebanese cherry orchards. Journal of Plant Pathology. 89.3(Supplement), S74.

Elbeaino, T., Giampetruzzi, A., De Stradis, A. and Digiaro, M. 2014. Deep-sequencing analysis of an apricot tree with vein clearing symptoms reveals the presence of a novel betaflexivirus. Virus Research. 181, 1-5.

El-Maghraby, I., Matic, S., Fahmy, H. and Myrta, A. 2007. Viruses and viroids of stone fruits in Egypt. Journal of Plant Pathology. 89, 427-430.

English, H., Davis, J.R. and DeVay, J.E. 1975. Relationship of Botryosphaeria dothidea and Hendersonula toruloidea to a canker disease of almond. Phytopathology. 65, 114-122.

English, H., Lownsbery, B.F., Schick, F.J. and Burlando, T. 1982. Effect of ring and pin nematodes on the development of bacterial canker and Cytospora canker in young French prune trees. Plant Disease. 66, 114-116.

EPPO. 2004. Diagnostic protocol for regulated pests: Plum pox potyvirus. Bulletin OEPP/EPPOBulletin. 34, 247-256.

EPPO. 1995. Raspebrry Ringspot virus http://www.eppo.org/QUARANTINE/virus/Raspberry_ringspot_virus/RPRSV0_ds.pdf

EPPO. 1995. Tomato Ring spot virus www.eppo.org/.../virus/Tomato_ringspot_virus/TORSV0_ds.pdf

EPPO. 1995. Citrus vein enation 'virus' http://www.eppo.org/QUARANTINE/virus/Citrus_vein_enation_virus/CVEV00_ds.pdf

EPPO. 2013. PM 7/20 (2) Erwinia amylovora. EPPO Bulletin, 43: 21–45. doi: 10.1111/epp.12019.

Ermacora P., Loi, N., Ferrini, F., Loschi, A., Martini, M., Osler, R. and Carraro, L. 2009. Hypo and hyper-virulence in apricot trees infected by European stone fruit yellows. Proceedings of the

21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops, Neustadt. 2009: 48.

Ercolani, G.L. and Ghaffer, A. 1985. Outbreaks and new records. Afghanistan. Bacterial canker and gummosis of stone fruit. FAO Plant Protection Bulletin. 33, 37-39.

Ershad, D. and Barkhordary, M. 1974. Host range and vectors of Nematospora coryli Peglion in Kerman of Iran. Iranian Journal of Plant Pathology. 10, 34-39.

Everett, K.R., Milne, K.S. and Forster, R.L.S. 1994. A new host record: strawberry latent ringspot virus isolated from flowering cherry. Australasian Plant Pathology. 23, 11-15.

Faggioli, F. and Ragozzino, E. 2002. Detection of pome fruit viroids by RT-PCR using a single primer pair. Journal of Plant Pathology. 84, 125-128.

Faggioli, F., Ferretti, L., Pasquini, G. and Barba, M. 2002. Detection of Strawberry latent ring spot virus in leaves of olive trees in Italy using a one-step RT-PCR. Journal of Phytopathology. 150, 636-639.

Farr, D.F., Elliott, M., Rossman, A.Y., and Edmonds, R.L. 2005. Fusicoccum arbuti sp. nov. causing cankers on Pacific madrone in western North America with notes on Fusicoccum dimidiatum, the correct name for Scytalidium dimidiatum and Nattrassia mangiferae. Mycologia. 97, 730-741.

Fernando, W.G.D., Zhang, J.X., Chen, C.Q., Remphrey, W.R., Schurko, A. and Klassen, G.R. 2002. Apiosporina morbosa. [Distribution map]. Distribution Maps of Plant Diseases. (Edition 4), Map 48.

Fernando, W.G.D., Zhang, J.X., Chen, C.Q., Remphrey, W.R., Schurko, A., and Klassen, G.R. 2005. Molecular and morphological characteristics of Apiosporina morbosa, the causal agent of blackknot in Prunus spp. Canadian Journal of Plant Pathology. 27, 364-375.

Festic, H. (1978) Investigation of new sharka virus hosts. Acta Horticulturae. No. 74, 233-240.

Flores, R., Delgado, S., Rodio, M.E., Ambros, S., Hernandez, C. and Serio, F.D. 2006. Peach latent mosaic viroid: not so latent. Molecular Plant Pathology. 7, 209-221.

Foissac, X., Svanella-Dumas, L., Dulucq, M. and Gentit, P. 2001. Polyvalent detection of fruit tree tricho, capillo and foveaviruses by nested RT-PCR using degenerated and inosine containing primers (PDO RT-PCR). Acta Horticulturae. 550, 37-44.

Foissac, X., Svanella-Dumas, L., Gentit, P., Dulucq, M.J., Marais, A. and Candresse, T. 2005. Polyvalent degenerate oligonucleotides reverse transcription-polymerase chain reaction: a polyvalent detection and characterization tool for trichoviruses, capilloviruses, and foveaviruses. Phytopathology. 95, 617-625.

Fonseca, F., Neto, J.D., Martins, V. and Nolasco, G. 2005. Genomic variability of prune dwarf virus as affected by agricultural practice. Archives of Virology. 150, 1607-1619.

Fotouhifar, K.B., Hedjaroude, G.A. and Leuchtmann, A. 2010. ITS rDNA phylogeny of Iranian strains of Cytospora and associated teleomorphs. Mycologia. 102, 1369-1382.

Francis, M., Civerolo, E.L. and Bruening, G. 2008. Improved bioassay of Xylella fastidiosa using Nicotiana tabacum cultivar SR1. Plant Disease. 92, 14-20.

Fraser, L.R. and Broadbent, P. 1979. Virus and virus-related diseases of citrus in New South Wales. Vein enation-woody gall. In Dept. Agr. NSW publication, p. 50-51. Surrey Beatty and Sons, Chipping Norton, NSW 2170, Australia.

Fridlund, P.R. 1963. Stone fruit viruses as latent infections in indigenous plants in British Columbia. Stone fruit viruses as latent infections in indigenous plants in British Columbia. 3, 199-202.

Fridlund, P.R. 1966. Transmission and lack of transmission of seven viruses through Primus seed. Plant Disease Reporter. 50, 902-904.

Fuchs, E. 1980. Serological detection of apple chlorotic leaf spot virus (CLSV) and apple stem grooving virus (SGV) in apple trees. A c t a Pkytopatkologica Acadenziae S c i e n t i a n m Hungaricae. 15, 69-73.

Fuchs, E. 1982. Studies of the development of concentration of apple chlorotic leaf spot virus (CLSV) and apple stem grooving virus (SGV) in apple trees. Acta Pkytopathologica Academiae Scientiarum Hungaricae. 17, 23-27.

Fuchs, E. and Gruntzig, M. 1994. Investigations on the distribution of viruses in woody plant hosts. Kuhn-Archiv. 88, 40-53.

Fuchs, M., Abawi, G.S., Marsella-Herrick, P., Cox, R., Cox, K.D., Carroll, J.E. and Martin, R.R. 2010. Occurrence of Tomato ringspot virus and Tobacco ringspot virus in highbush blueberry in New York State. Journal of Plant Pathology. 92, 451-459.

Fulton, R.W. 1984. American plum line pattern virus. CMI/AAB Descriptions of Plant Viruses No. 280, Association of Applied Biologists, Wellesbourne, UK.

Fulton, R.W. 1983, Ilarvirus Group. CMI/AAB Descriptions of Plant Viruses No. 213, Kew, Surrey, UK.

Fulton, R.W. 1972, Apple mosaic virus. CMI/AAB Descriptions of Plant Viruses No. 83, Kew, Surrey, UK.

Fry, P.R. and Wood, G.A. 1973. Further viruses of Prunus in New Zealand. New Zealand Journal of Agricultural Research. 16, 131-142.

Gramaje, D., Agustí-Brisach, C., Pérez-Sierra, A., Moralejo, E., Olmo, D., Mostert, L. and Damm, U. Armengol, J. 2012. Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). Persoonia. 28, 1-13.

Gallitelli, D., Piazzolla, P., Savino, V., Quacquarelli, A. and Martelli, G.P. 1981. A comparison of myrobalan latent ringspot virus with other nepoviruses. Journal of General Virology. 53, 57-65.

Gambino, G. and Gribaudo, I. 2006. Simultaneous detection of nine grapevine viruses by multiplex reverse transcription-polymerase chain reaction with coamplification of a plant RNA as internal control. Phytopathology. 96, 1223-1229.

Garau, R., Barba, M., Cugus, M.I., Laurett, F.I., Prota, U. and Piredda, A. 1989. [Notes on some virological aspects of almond in Sardegna.] Quaderno Istituto Agronomico Mediterraneo, Valenzano (Bari). 1, 109- 119.

García, J.A., Glasa, M., Cambra, M. and Candresse, T. 2014. Plum pox virus and sharka: a model potyvirus and a major disease. Molecular Plant Pathology. 15, 226–241. doi: 10.1111/mpp.12083

Garcia-Ibarra, A., Martinez-Gomez, P., Rubio, M., Dicenta, F., Soler, A., Pallas, V. and Sanchez-Navarro, J.A. 2010. First report of Apricot latent virus and Plum bark necrosis stem pitting-associated virus in apricot from Spain. Plant Disease. 92, 275.

Garcia-Ibarra, A., Rubio, M., Martinez-Gomez, P., Dicenta, F., Soler, A. and Sanchez-Navarro, J.A. 2010. Pollen and seed transmission of Apple chlorotic leaf spot virus (ACLSV) in apricot. Acta Horticulturae. 862, 483-486.

Gatineau, F., Larrue, J., Clair, D., Lorton, F., Richard-Molard, M. and Boudon-Padieu, E. 2001. A new natural planthopper vector of stolbur phytoplasma in the genus Pentastiridius (Hemiptera: Cixiidae). European Journal of Plant Pathology. 107, 263-271.

Gentit, P., Delbos, R.P., Candresse, T. and Dunez, J. 2001. Characterization of a new nepovirus infecting apricot in Southeastern France: apricot latent ringspot virus. European Journal of Plant Pathology. 107, 485-494.

Gentit, P., Foissac, X., Svanella-Dumas, L., Peypelut, M. and Candresse, T. 2001. Characterization of two different apricot latent virus variants associated with peach asteroid spot and peach sooty ringspot diseases. Archives of Virology. 146, 1453-1464.

German, S., Candresse, T., Lanneau, M., Huet, J.C., Pernollet, J.C. and Dunez J. 1990. Nucleotide sequence and genomic organization of Apple chlorotic leaf spot closterovirus. Virology. 179, 104-112.

German, S., Delbos, R.P., Candresse, T., Lanneau, M. and Dunez, J. 1997. Complete nucleotide sequence of the genome of a severe cherry isolate of Apple chlorotic leaf spot trichovirus (ACLSV). Archives of Virology. 142, 833-841.

Ghanem-Sabanadzovic, N.A., Mahboubi, M., Terlizzi, B.D., Sabanadzovic, S., Savino, V., Uyemoto, J.K. and Martelli, G.P. 2001. Molecular detection of a closterovirus associated with apricot stem pitting in Southern Italy. Journal of Plant Pathology. 83, 125-132.

Ghanem-Sabanadzovic, N.A., Abbadi, H., Al-Rwahnih, M., Castellano, M.A. and Myrta, A. 2005. Identification and partial characterization of an isolate of apricot latent virus from Palestine. Journal of Plant Pathology. 87, 37-41.

Gibson, P.G., Reighard, G.L., Scott, S.W. and Zimmerman, M.T. 2001. Identification of graft-transmissible agents from 'Ta Tao 5' peach and their effects on 'Coronet' peach. Acta Horticulturae. 550, 309-314.

Gibson, P.G., Reighard, G.L., Scott, S.W. and Marini, D. 2008. Phenotypical variation in peach trees inoculated with defined mixtures of viruses and Peach latent mosaic viroid. Acta Horticulturae. 781, 541-546.

Gillings, M.R., Broadbent, P., Gollnow, B.I. and Lakeland, C. 1988. Viroids in Australian citrus. Citriculture. Proceedings of the Sixth International Citrus Congress, Middle-East, Tel Aviv, Israel, 6-11 March 1988. Volume 2. Cultural practices, diseases and nematodes. 881-895.

Gilmer, R.M. and Wilks, J.M. 1967. Apple chlorotic leaf spot and Tobacco mosaic viruses in Cherry. Plant Disease Reporter. 51, 823-25.

Gilmer, R.M. and Blodgett, E.C. 1976. X-disease. In: Virus diseases and non-infectious disorders of stone fruits in North America. USDA Agriculture Handbook No. 437, pp. 145-155. United States Department of Agriculture, USA.

Gilmer, R.M., Palmiter, D.H., Schaefers, G.A. and McEwen, F.L. 1966. Leafhopper transmission of X-disease virus of stone fruits in New York. New York State Agriculture Experimental Station Bulletin 813, 1-22.

Ginns, J.H. 1986. Compendium of plant disease and decay fungi in Canada 1960-1980. Nordic Journal of Botany. 7(5), 576.

Gispert, C., Perring, T.M. and Creamer, R. 1998. Purification and characterization of peach mosaic virus. Plant Disease. 82: 8, 905-908.

Gottsberger, R. 2010. Development and evaluation of a real-time PCR assay targeting chromosomal DNA of Erwinia amylovora. Letters in Applied Microbiology. 51, 285-292.

Gramaje, D., Agustí-Brisach, C., Pérez-Sierra, A., Moralejo, E., Olmo, D., Mostert, L., Damm, U. and Armengol, J. 2012. Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). Persoonia: Molecular Phylogeny and Evolution of Fungi. 28, 1–13. doi:10.3767/003158512X626155

Graya, A.J., Digiaro, M., Savino, V., and Martelli, G.P. 1993. A survey of sweet cherry viruses in Apulia. Advances in Horticultural Science. 7, 27-31.

Greber, R.S., Teakle, D.S. and Mink, G.I. 1992. Thrips-facilitated transmission of prune dwarf and Prunus necrotic ringspot viruses from cherry pollen to cucumber. Plant Disease. 76, 1039-1041.

Green M.J., Thompson, D.A. and MacKenzie, D.J. 1999. Easy and efficient DNA extraction from woody plants for the detection of phytoplasmas by polymerase chain reaction. Plant Disease. 83, 482-485.

Griesbach, J.A. 1995. Detection of tomato ringspot virus by polymerase chain reaction. Plant Disease. 79, 1054-1056.

Guerra, L. J. 1997. *Biological and molecular characterization of phytoplasmas infecting fruit and nut trees in California*. PhD thesis, University of California, CA, USA.

Guerra, L.J., and Eastwell, K.C. 2006. Phytoplasma in Deciduous fruit and nut trees. In: Harrison NA, Rao, GP and Marcone, C (eds) Characterisation and identification of Phytoplasmas. Chapter 5, pp 93-136, Studiom Press, Texas, USA.

Guerra, L.J. and Eastwell, K.C. 2008. Phytoplasmas in deciduous fruit and nut trees. (Plant Pathogens Series - 5). Characterization, diagnosis and management of phytoplasmas. 93-136.

Guillaumin, J.J., Anderson, J.B., Legrand, P., Ghahari, S. and Berthelay, S. 1996. A comparison of different methods for the identification of genets of Armillaria spp. New Phytologist. 133, 333–343.

Guillaumin, J.J., Pierson, J. and Grassely, C. 1991. The susceptibility to Armillaria mellea of different Prunus species used as stone fruit rootstocks. Scientia Horticulturae. 46, 43-54.

Guillaumin, J.J., Mercier, S. and Dubos, B. 1987. Peach trees and root rot (Armillaria mellea), performance of various rootstocks. [French]. Arboriculture Fruitiere. 34, 41-44.

Gumus, M., Al-Rwahnih, M. and Myrta, A. 2004. First report of apricot latent virus in Turkey. Journal of Plant Pathology. 86, 92.

Gumus, M., Paylan, I.C., Matic, S., Myrta, A., Sipahioglu, H.M. and Erkan, S. 2007. Occurrence and distribution of stone fruit viruses and viroids in commercial plantings of Prunus species in western Anatolia, Turkey. Journal of Plant Pathology. 89, 265-268.

Gundogdu, M. and Demir, G. 1991. Detection and characterization of plasmids in Pseudomonas amygdali. Phytopathologia Mediterranea. 30, 112-115.

Gustafson, W.A. and Morrissey, T.M. 2003. G03-1518 Chip Budding: An Old Grafting Technique for Woody Plants With Rediscovered Advantages for Nebraska. Historical Materials from University of Nebraska-Lincoln Extension. Paper 1736. http://digitalcommons.unl.edu/extensionhist/1736

Hadfield, J. and Eldridge, M.D. 2014. Multi-genome alignment for quality control and contamination screening of next-generation sequencing data. Frontiers in genetics. 5, 31. doi: 10.3389/fgene.2014.00031

Hadidi, A., Flores, R., Randles, J.W. and Semancik, J.S. 2003. Viroids. Collingwood, Australia: CSIRO Publishing.

Hadidi, A., Terai, Y., Powell, C.A., Scott, S.W., Desvignes, J.C., Ibrahim, L.M. and Levy, L., 1992. Enzymatic cDNA amplification of hop stunt viroid variants from naturally infected fruit crops. Acta Horticulturae. **309**, 339-344.

Hadidi A, Levy L and Podleckis EV. 1995. Polymerase chain reaction technology in plant pathology. In Molecular Methods in Plant Pathology, In: Molecular Methods in Plant Pathology, (R.P. Singh and U.S. Singh, Eds.) CRC Press, Florida, USA. Pp. 167-187.

Hadidi, A., Giunchedi, L., Shamloul, A.M., Poggi Pollini, C., and Amer, A.M. 1997. Occurrence of peach latent mosaic viroid in stone fruits and its transmission with contaminated blades. Plant Disease. 81, 154-158.

Hadidi, A., Hansen, A.J., Parish, C.L. and Yang, X. 1991. Scar skin and dapple apple viroids are seed-borne and persistent in infected apple trees. Research in Virology. 142(4), 289-296.

Hadidi, A., Huang, C., Hammond, R.W. and Hashimoto, J. 1990. Homology of the agent associated with dapple apple disease to apple scar skin viroid and molecular detection of these viroids. Phytopathology. 80(3), 263-268.

Handa, A., Thakur, P.D. and Bhardwaj, S.V. 1998. Status of dapple apple viroid in India. Acta Horticulturae. 472, 631-633.

Hansen, A.J. 1975. Differences between twisted leaf and tomato bushy stunt virus in sweet cherry. Acta Horticulturae. 44, 55-57.

Hansen, A.J. 1978. Cherry virus and virus-like diseases. Goodfruit Grower. 29(10), 1, 24.

Hansen, A.J., and Cheney, P.W. 1976. Cherry twisted leaf. Pages 222- 225 in: Virus Diseases and Noninfectious Disorders of Stone Fruits in North America. Gilmer, R.M., Moore, J.D., Nyland, G., Welsh, M.F. and Pine, T.S. eds. U.S. Dep. Agric., Agric. Handb. No. 437.

Hansen, A.J., Nyland, G., McElroy, F.D. and Stace-Smith, R. 1975. Nematode transmitted viruses in British Columbia, Canada. Nematode vectors of plant viruses. 287-288.

Hari, V., Abdel-Ghaffar, M.H., Levy, L. and Hadidi, A., 1995. Asian Prunus latent virus: an unusual Potyvirus detected in germplasm from east asia. Acta Horticulturae. 386, 78–85.

Harper, S.J., Ward, L.I. and Clover, G.R.G. 2010. Development of LAMP and real-time PCR methods for the rapid detection of Xylella fastidiosa for quarantine and field applications. Phytopathology. 100(12), 1282-1288.

Harper, S.J., Delmiglio, C., Ward, L.I. and Clover, G.R.G. Detection of Tomato black ring virus by real-time one-step RT-PCR. Journal of Virological Methods. 2011. 171(1), 190-194.

Harrington, T.C. and Wingfield, B.D. 1995. A PCR-based identification method for species of Armillaria. Mycologia. 87, 280–288.

Harrison, B.D. and Murant, A.F. 1977. Nematode transmissibility of pseudo-recombinant isolates of tomato black ring virus. Annals of Applied Biology. 86(2), 209-212.

Hassan, M., Zouhar, M. and Rysanek, P. 2006. Variants of Peach latent mosaic viroid inducing peach calico: uneven distribution in infected plants and requirements of the insertion containing the pathogenicity determinant. Journal of General Virology. 87(1), 231-240.

Hassen, I.F., Roussel, S., Kummert, J., Fakhfakh, H., Marrakchi, M. and Jijakli, M.H. 2006. Development of a rapid RT-PCR test for the detection of peach latent mosaic viroid, pear blister canker viroid, hop stunt viroid and apple scar skin viroid in fruit trees from Tunisia. Journal of Phytopathology. 154(4), 217-223.

Hayova, V.P. and Minter, D.W. 1998. Leucostoma persoonii. [Descriptions of Fungi and Bacteria]. IMI Descriptions of Fungi and Bacteria. 137, Sheet 1363.

Heaton, J.B. and Dullahide, S.R. 1993. White root rot of apple. In Desley DM (ed) Diseases of Fruit Crops. Department of Primary Industries, Brisbane. pp 12-24.

Heleguera, P.R., Taborda, R., Docampo, D.M. and Ducasse, D.A. 2001. Immunocapture reverse transcription-polymerase chain reaction combined with nested PCR greatly increases the detection of Prunus necrotic ring spot virus in the peach. Journal of Virological Methods. 95, 93-100.

Hernandez, C. and Flores, R. 1999. Detection of peach latent mosaic viroid in Australia. Australasian Plant Pathology. 28(1), 80-81.

Hernandez-Martinez, R., Costa, H.S., Dumenyo, C.K. and Cooksey, D.A. 2006a. Differentiation of strains of Xylella fastidiosa infecting grape, almonds, and oleander using a multiprimer PCR assay. Plant Disease. 90(11), 1382-1388.

Hernandez-Martinez, R., Pinckard, T.R., Costa, H.S., Cooksey, D.A. and Wong, F.P. 2006b. Discovery and characterization of Xylella fastidiosa strains in Southern California causing mulberry leaf scorch. Plant Disease. 90(9), 1143-1149.

Herranz, M.C., Al-Rwahnih, M., Sanchez-Navarro, J.A., Elena, S.F., Choueiri, E., Myrta, A. and Pallas, V. 2008. Low genetic variability in the coat and movement proteins of American plum line pattern virus isolates from different geographic origins. Archives of Virology. 153(2), 367-373.

Herranz, M.C., Sanchez-Navarro, J.A., Aparicio, F. and Pallas, V. 2005. Simultaneous detection and identification of eight stone fruit viruses by one-step RT-PCR. European Journal of Plant Pathology. 111(1), 77-84.

Herrera, M.G. and Madariaga, V. M. 2001. Presence and incidence of grapevine viruses in the central zone of Chile. [Spanish]. Agricultura Tecnica. 61(4), 393-400.

Hilf, M.E. 2008. An immunocapture RT-PCR procedure using Apple stem grooving virus antibodies facilitates analysis of Citrus tatter leaf virus from the original Meyer lemon host. Plant Disease. 92, 746-750.

Hiratsuka, N. 1952. On the white rust of Peach. [Japanese]. Shokubutsu Kenkyu Zasshi (Journal of Japanese Botany). 27(8), 229-238.

Hiruki, C. and Wang, K. 2004. Clover proliferation phytoplasma: 'Candidatus Phytoplasma trifolii'. International Journal of Systematic and Evolutionary Microbiology. 54(4), 1349-1353.

Hollings, M., and Stone, O. M. 1975. Serological and Immunoelectrophoretic relationships among viruses in Tombusvirus group. Annals of Applied Biology. 80, 37-48.

Hong, M., Zhang, C., Li, Z., Zhang, J., Zhao, Z., Song, J. and Wu, Y. 2011. Identification of elm yellows phytoplasma in plum trees in China. Journal of Phytopathology. 159(1), 57-59.

Hood, I.A., Redfern, D.B. and Kile, G.A. (1991) Armillaria in planted hosts. In: Armillaria Root Disease Agriculture Handbook No. 691 (Shaw, C.G., III and Subterranean pathosystem of Armillaria Kile, G.A., eds), pp. 122–149. Washington, DC: United States Department of Agriculture Forest Service.

Howell, W.E. and Mink, G.I. 1988. Natural spread of cherry rugose mosaic disease and two Prunus necrotic ringspot virus biotypes in a central Washington sweet cherry orchard. Plant Disease. 72, 636-640.

Hubschen, J., Kling, L., Ipach, U., Zinkernagel, V., Brown, D.J.F. and Neilson, R. 2004. Development and validation of species-specific primers that provide a molecular diagnostic for virus-vector longidorid nematodes and related species in German viticulture. European Journal of Plant Pathology. 110(9), 883-891.

Hull, R. and Fargette, D. 2005. Genus Sobemovirus, p885-890. In C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball (ed.), Virus taxonomy, 8th report of the ICTV. Elsevier/Academic Press, London, United Kingdom.

Huguet, J.G., Castelnaud, G., Marenaud, C. and Giraudon, J. 1977. Influence of the presence of virus on mineral absorption and growth of the almond tree. Comptes Rendus des Seances de l'Academie d'Agriculture de France. 63(16), 1123-1134.

Imed, A., Boscia, D., Boari, A., Saldarelli, P., Digiaro, M. and Savino, V. 1997. A comparison of apple mosaic virus isolates from Prunus trees and production of specific monoclonal antibodies. Bulletin OEPP. 27(4), 563-564.

Inderbitzin, P., Bostock, R.M., Trouillas, F.P., and Michailides, T.J. 2010. A six locus phylogeny reveals high species diversity in Botryosphaeriaceae from California almond. Mycologia. 102, 1350-1368.

Iqbal, S.H., Afshan, N.S., Khalid, A.N., Niazi, A.R. and Sultan, A. 2009. Additions to the rust fungi of Fairy Meadows, the Northern Areas of Pakistan. Mycotaxon. 109, 1-7.

IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma taxonomy group 'Candidatus Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects International Journal of Systematic and Evolutionary Microbiology. 54, 1243-1255.

Isogai, M., Aoyagi, J., Nakagawa, M., Kubodera, Y., Satoh, K., Katoh, T., Inamori, M., Yamashita, K. and Yoshikawa, N. 2004. Molecular detection of five cherry viruses from sweet cherry trees in Japan. Journal of General Plant Pathology. 70(5), 288-291.

Ito, T., Ieki, H. and Ozaki, K. 2002. Simultaneous detection of six citrus viroids and Apple stem grooving virus from citrus plants by multiplex reverse transcription polymerase chain reaction. Journal of Virological Methods. 106, 235-239.

Jacob, H. 1974. Investigations on heat therapy of stone fruit virus diseases. [German]. Acta Phytomedica. 2, 56.

Jacobi, V., Bachand, G.D., Hamelin, R.C., Castello, J.D. 1998. Development of a multiplex immunocapture RT-PCR assay for detection and differentiation of tomato and tobacco mosaic tobamoviruses. Journal of Virological Methods. 74, 167-178.

James, D. 2011. Cherry mottle leaf virus.In: Virus and Virus-like Diseases of Pome and Stone Fruits. (Eds. Hadidi, A., Candresse, T., Jelkmann, W., and Barba, M). American Phytopathological Society (APS) Press. (In press)

James, D. 2011. Cherry twisted leaf disease and its associated virus.In: Virus and Virus-like Diseases of Pome and Stone Fruits. (Eds. Hadidi, A., Candresse, T., Jelkmann, W., and Barba, M). American Phytopathological Society (APS) Press. (In press)

James, D. 1999. A simple and reliable protocol for the detection of apple stem grooving virus by RT-PCR and in a multiplex PCR assay. Journal of Virological Methods. 83(1/2), 1-9.

James, D. 2008. Apple stem grooving virus. (Plant Pathogens Series - 2). Characterization, diagnosis and management of plant viruses. Volume 2: horticultural crops. 37-53.

James, D. and Glasa, M. 2006. Causal agent of sharka disease: New and emerging events associated with Plum pox virus characterization. Bulletin OEPP/EPPO Bulletin, 36, 247–250.

James, D. and Howell, W.E. 1998. Isolation and partial characterization of a filamentous virus associated with peach mosaic disease. Plant Disease. 82(8), 909-913. 2

James, D. and Mukerji, S. 1996. Comparison of ELISA and immunoblotting techniques for the detection of cherry mottle leaf virus. Annals of Applied Biology. 129(1), 13-23. 2

James, D. and Mukerji, S. 1993. Mechanical transmission, identification, and characterization of a virus associated with mottle leaf in cherry. Plant Disease. 77(3), 271-275. 2

James, D. and Upton, C. 2001. Detection and differentiation of Cherry mottle leaf virus and Peach mosaic virus. Acta Horticulturae. 550(1), 185-189. 1

James, D. and Upton, C. 1999. Single primer pair designs that facilitate simultaneous detection and differentiation of peach mosaic virus and cherry mottle leaf virus. Journal of Virological Methods. 1999. 83(1/2), 103-111.

James, D. and Upton, C. 2001. Cherry rasp leaf virus. [Distribution map]. Distribution Maps of Plant Diseases. October (Edition 4), Map 303.

James, D. and Upton, C. 2005. Genome segment RNA-1 of a flat apple isolate of Cherry rasp leaf virus: nucleotide sequence analysis and RT-PCR detection. Archives of Virology. 150(7), 1469-1476. 2

James, D., Jelkmann, W. and Upton, C. 1999. Specific detection of Cherry mottle leaf virus using digoxigenin-labeled cDNA probes and RT-PCR. Plant Disease. 83(3), 235- 239.

James, D., Jelkmann, W. and Upton, C. 2000. Nucleotide sequence and genome organisation of cherry mottle leaf virus and its relationship to members of the Trichovirus genus. Archives of Virology. 145(5), 995-1007. 3

James, D., Howell, W.E. and Martin, R.R. 1995. Identification of a flexuous virus associated with cherry twisted leaf disease. Acta Horticulturae. 386, 86-91. 1

James, D., Howell, W.E. and Mink, G.I. 2001. Molecular evidence of the relationship between a virus associated with flat apple disease and Cherry rasp leaf virus as determined by RT-PCR. Plant Disease. 85(1), 47-52. 2

James, D., Varga, A., Croft, H., Rast, H., Thompson, D. and Hayes, S. 2006. Molecular characterization, phylogenetic relationships, and specific detection of Peach mosaic virus. Phytopathology. 96(2), 137- 144.

James, D., Varga, A. and Croft, H. Analysis of the complete genome of peach chlorotic mottle virus: identification of non-AUG start codons, in vitro coat protein expression, and elucidation of serological cross-reactions. Archives of Virology. 2007. 152: 12, 2207-2215.

Janse, J.D. 2010. Diagnostic methods for phytopathogenic bacteria of stone fruits and nuts in COST 873. Bulletin OEPP/EPPO Bulletin. 40(1), 68-85.

Janse, J.D. and Obradovic, A. 2010. Xylella fastidiosa: Its biology, diagnosis, control and risks. Journal of Plant Pathology. 92.1(Supplement), S1.35-S1.48 Edizioni ETS Pisa, S1.35.

Jarosova, J. and Kundu, J.K. 2010. Detection of Prune dwarf virus by one-step RT-PCR and its quantitation by real-time PCR**.** Journal of Virological Methods. 164(1), 139-144.

Jarausch, W., Eyquard, J.P., Lansac, M., Mohns, M. and Dosba, F. 2000. Susceptibility and tolerance of new french Prunus domestica cultivars to European stone fruit yellows phytoplasmas. Journal of Phytopathology. 148, 489-493.

Jarausch, W., Fuchs, A. and Jarausch, B., 2009. Establishment of a quantitative real-time PCR assay for the specific quantification of Candidatus Phytoplasma prunorum in plants and insects. Proceedings of the 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops, Neustadt 2009: 80-81.

Jarausch, W., Lancas, M. and Dosba, F. 1999. Seasonal colonization pattern of European stone fruit yellows phytoplasmas in different Prunus species detected by specific PCR. Journal of Phytopathology. 147, 47–54.

Jarausch, W., Lansac, M., Saillard, C., Broquaire, J.M. and Dosba, F. 1998. PCR assay for specific detection of European stone fruit yellows phytoplasmas and its use for epidemiological studies in France. European Journal of Plant Pathology. 104, 17-27.

Jarrar, S., Choueiri, E., Sanchez-Navarro, J.A., Myrta, A., El-Zammar, S., Savino, V. and Pallas, V. 2007. First report of Apricot latent virus in Lebanon. Journal of Plant Pathology. 89(2), 303.

Jelkmann, W., 2011. Cherry Detrimental Canker In: Virus and Virus-like Diseases of Pome and Stone Fruits. (Eds. Hadidi, A., Candresse, T., Jelkmann, W., and Barba, M). American Phytopathological Society (APS) Press. (In press).

Jelkmann J., 1996. The nucleotide sequence of a strain of Apple chlorotic leaf spot virus (ACLSV) responsible for plum pseudopox and its relation to an apple and plum bark split strain. Phytopathology. 86, 101.

Jelkmann, W. 1995. Cherry virus A: cDNA cloning of dsRNA, nucleotide sequence analysis and serology reveal a new plant capillovirus in sweet cherry. Journal of General Virology. 76(8), 2015-2024.

Jelkmann, W., Fechtner, B. and Agranovsky, A.A. 1997. Complete genome structure and phylogenetic analysis of little cherry virus, a mealybug-transmissible closterovirus. Journal of General Virology. 78(8), 2067-2071.

Jelkmann, W., Leible, S. and Rott, M. 2008. Little cherry closteroviruses-1 and -2, their genetic variability and detection by real time-pcr. Acta Horticulturae. 781, 321-329.

Jensen, D.D., Griggs, W.H., Gonzales, C.Q. and Schneider, H. 1964. Pear decline virus transmission by pear psylla. Phytopathology. 54, 1346-1351.

Jenser, G., Gyorgy, K. and Seljahudin, A. 1984. The transmission of Arabis mosaic virus to peach (Persica vulgaris Mill.) and apricot (Armeniaca vulgaris Lam.) seedlings by using the nematode Xiphinema diversicaudatum (Micoletzky) Thorne. Acta Phytopathologica Academiae Scientiarum Hungaricae. 19(3/4), 291-293.

Jindal, K.K. and Sharma, R.C. 1987. Outbreaks and new records.Almond leaf scorch - a new disease from India. FAO Plant Protection Bulletin. 35, 64-65.

Johnson, K.B., Sawyer, T.L. and Temple, T.N. 2006. Rates of epiphytic growth of Erwinia amylovora on flowers common in the landscape. Plant Disease. 90, 1331-1336.

Johnstone, G.R., Munro, D., Brown, G.S. and Skotland, C.B. 1995. Serological detection, occurrence and spread of Ilarviruses in temperate fruit crops, hops and roses in Tasmania. Acta Horticulturae. 386, 132-135.

Jones, A.T. 1985. Cherry leaf roll virus. CMI/AAB Descriptions of Plant Viruses No. 306. Association of Applied Biologists, Wellesbourne, UK.

Jones, P., Arocha, Y., Antesana, O., Montilliano, E. and Franco, P. 2005. First report of an isolate of 'Candidatus Phytoplasma australiense' associated with a yellow leaf roll disease of peach (Prunus persicae) in Bolivia. Plant Pathology. 54(4), 558.

Jones, A.T., Brown, D.J.F., McGavin, W.J., Rüdel, M. and Altmayer, B. 1994. Properties of an unusual isolate of raspberry ringspot virus from grapevine in Germany and evidence for its possible transmission by Paralongidorus maximus. Annals of Applied Biology. 124, 283-300.

Jovic, J., Cvrkovic, T., Mitrovic, M., Krnjajic, S., Petrovic, A., Redinbaugh, M.G., Pratt, R.C., Hogenhout, S.A. and Tosevski, I. 2009. Stolbur phytoplasma transmission to maize by Reptalus panzeri and the disease cycle of maize redness in Serbia. Phytopathology. 99(9), 1053-1061.

Jung, H.Y., Sawayanagi, T., Kakizawa, S., Nishigawa, H., Wei, W., et al., 2003. 'Candidatus Phytoplasma ziziphi', a novel phytoplasma taxon associated with jujube witches'-broom disease. International Journal of Systematic and Evolutionary Microbiology. 53, 1037-41.

Jovic, J., Cvrkovic, T., Mitrovic, M., Petrovic, A., Krstic, O., Krnjajic, S. and Tosevski, I. 2011. Multigene sequence data and genetic diversity among 'Candidatus Phytoplasma ulmi' strains infecting Ulmus spp. in Serbia. Plant Pathology. 60(2), 356-368.

Kanaan-Atallah, Z.H., Abou-Jawdah, Y. and Saad, A. 2000. Virus diseases infecting almond germplasm in Lebanon. Phytopathologia Mediterranea. 39(3), 417-422.

Kanematsu, S., Minaka, N., Kobayashi, T., Kudo, A. and Ohtsu, Y. 2002. Effect of fungicides, application timing, and canker removal on incidence and severity of constriction canker of peach. Plant Disease. 86(7), 721-728.

Kaponi, M.S., Luigi, M., Barba, M. and Kyriakopoulou P.E. 2010. Pospiviroidae viroids in naturally infected stone and pome fruits in Greece Julius-Kühn-Archiv, Nr. 427.

Karthikeyan, M., Radhika, K., Bhaskaran, R. Mathiyazhagan, S. and Velazhahan, R. 2009. Rapid detection of Ganoderma lucidum and assessment of inhibition effect of various control measures by immunoassay and PCR. African Journal of Biotechnology. 8(10), 2202-2208.

Kaszonyi, S. 1966. Life cycle of Blumeriella jaapii (Rehm) v. Arx infecting stone-fruits. . Acta Phytopathologica Academiae Scientiarum Hungaricae. 1(1-2), 93-100.

Kaul, T.N. 1967. Diseases of stone fruits in Kashmir. Horticulturist. 2, 52-58.

Keane, P.W.L. and May, J. 1963. Natural root grafting in Cherry, and spread of Cherry twisted leaf virus. Canadian Plant Disease Survey. 43(2), 54-60.

Kegler, G. and Kegler, H. 1980. Research on natural transmission of tomato bushy stunt virus in fruit trees. Tagungsbericht der Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik. 297-302.

Kegler, H., Kegler, G. and Kleinhempel, H. 1983. Epidemiological investigations on carnation ringspot virus and tomato bushy stunt virus in fruit orchards. Zeszyty Problemowe Postepow Nauk Rolniczych. 291, 155-162.

Kegler, G. and Schimanski, H.H. 1982. Investigations on the spread and seed transmissibility of tomato bushy stunt virus in pome and stone fruit in the GDR. Archiv fur Phytopathologie und Pflanzenschutz. 18(2), 105-109.

Kegler, H., Proll, E., Schmidt, H.B. and Opel, H. 1969. Evidence of Tobacco necrosis virus in fruit trees. Phytopathologische Zeitschrift. 65(1), 21-42.

Kegler, H., Spaar, D. and Otto, H. 1972. The effect of viruses on yield and growth of the sour cherry cultivar Schattenmorelle. [German]. Archiv fur Gartenbau. 20(6), 479-487.

Kehoe, M.A., Coutts, B.A., Buirchell, B.J. and Jones, R.A.C. 2014. Plant Virology and Next Generation Sequencing: Experiences with a Potyvirus. PLoS ONE 9(8): e104580. doi:10.1371/journal.pone.0104580

Keldysh, M.A., Papko, I.O., Vozna, L.I., Pomazkov, Y.I. and Chervyakova, O.N. 2005. Conditions for realizing the infection potential of viruses in soil. Russian Agricultural Sciences. 3, 15-20.

Keldish, M., Pomazkov, Y., Arushanova, E. and Chervyakova, O. 1998. Vectors as epidemiological factors in widening the host range for viruses of fruit trees and small fruit crops. Acta Horticulturae. 472, 147-152.

Kelley, R.D. and Cameron, H.R. 1986. Location of prune dwarf and Prunus necrotic ringspot viruses associated with sweet cherry pollen and seed. Phytopathology. 76(3), 317-322.

Kheder, A.A., Ibrahim, I.A.M. and Mazyad, H.M. 2004. Isolation and characterization of Peach rosette mosaic virus (PRMV)in Egypt. Egyptian Journal of Virology. 1, 259-272.

Keim-Konrad, R. and Jelkmann, W. 1996. Genome analysis of the 3¢-terminal part of the little cherry disease associated dsRNA reveals a monopartite clostero-like virus. Archives of Virology. 141, 1437-1451.

Kleinhempel, H., Gruber, G. and Kegler, H. 1980. Investigations on carnation ringspot virus in fruit trees. Acta Phytopathologica Academiae Scientiarum Hungaricae. 15, 107-111.

Kinard, G.R., Scott, S.W. and Barnett, O.W. 1996. Detection of Apple chlorotic leaf spot and Apple stem grooving viruses using RT-PCR. Plant Disease. 80, 616-621.

Kison, H., Kirkpatrick, B. C. and Seemüller, E. 1997. Genetic comparison of the peach yellow leaf roll agent with European fruit tree phytoplasmas of the apple proliferation group. Plant Pathology. 46, 538–544.

Kison, H. and Seemüller, E. 2001. Differences in strain virulence of the European stone fruit yellows phytoplasma and susceptibility of stone fruit trees on various rootstocks to this pathogen. Journal of Phytopathology. 149, 533-541.

Kirkpatrick, B.C. 1991. Mycoplasma like organisms – plant and invertebrate pathogens, Chapter 229, p. 4050-4067. In: Balows A, Truper HG, Dworkin M, Harder W and Schleifer KH (eds.) The prokaryotes, Volume IV, 2nd Ed.. Springer-Verlag, New York.

Kirkpatrick, B.C., Uyemoto, J.K. and Purcell, A.H. 1995. X-disease. In: Compendium of stone fruit diseases. American Phytopathological Society, St. Paul, USA.

Kirkpatrick, H.C. and Fulton, R.W. 1976. Virus diseases of plum and prune. Plum line pattern. In: Virus diseases and noninfectious disorders of stone fruits in North America. Agriculture Handbook, Agricultural Research Service, US Department of Agriculture No. 437, pp. 166-175.

Kishi, K., Takanashi, K. and Abiko, K. 1973. Studies on virus diseases of stone fruits. VI. The effect of seven stone fruit viruses on the growth of peach and Japanese apricot trees. Bulletin of the Horticultural Research Station, A (Hiratsuka). 12, 217-226.

Koenig, R. and Kunze, L. 1982. Identification of tombusvirus isolates from cherry in southern Germany as petunia asteroid mosaic virus. Phytopathologische Zeitschrift. 103(4), 361-368.

Koenig, R., and Lesemann, D. E. 1985. Plant viruses in German rivers and lakes. Phytopathology. 112, 105-116.

Koenig, R., Verhoeven, J. T. J., Fribourg, C. E., Pfeilstetter, E. and Lesemann, D. E. 2004. Evaluation of various species demarcation criteria in attempts to classify ten new tombusvirus isolates. Archives of Virology. 149, 1733-1744.

Kommineni, K.V. and Ramsdell, D.C. 1997. An Anatomical Study of Prune Brown Line Disease and Immuno-localization of Tomato Ringspot Virus in Plum Bark. Plant Disease. 81(8), 855-861.

Kofalvi, S.A., Marcos, J.F., Canizares, M.C., Pallas, V. and Candresse, T. 1997. Hop stunt viroid (HSVd) sequence variants from Prunus species: evidence for recombination between HSVd isolates. Journal of General Virology. 78(12), 3177-3186.

Koltunow, A.M., Krake, L.R. and Rezaian, M.A. 1988. Hop stunt viroid in Australian grapevine cultivars: potential for hop infection. Australasian Plant Pathology. 17(1), 7-10.

Korba, J. and Sillerova, S. 2010. First occurence of fire blight infection on apricot (Prunus armeniaca) in Chech Republic. 12th International Workshop on Fire Blight. 16-20 August, 2010. Warsaw. Abstracts, 107.

Krczal, G., Krczal, H., and Kunze, L. 1988. Fieberiella florii (Stal), a vector of apple proliferation agent. Acta Horticulturae. 235, 99-106.

Kurbetli, I. and Hancoglu, O. 2008. Fungal diseases on almond in Isparta province. [Turkish]. Bitki Koruma Bulteni. 48(3), 43-55.

Kurcman, S. 1977. Research on virus diseases of cherry noted in Afyon province. Bitki Koruma Bulteni. 17(2/4), 113-149.

Kumari, S. 2009. Detection of Cherry leaf roll virus and Strawberry latent ring spot virus by one-step RT-PCR. Plant Protection Science. 45(4), 140-143.

Kunz, P. 1998. The cherry rosette disease and its vector nematode Longidorus arthensis. Obst- und Weinbau. 134(9), 248-250.

Kunz, P. and Bertschinger, L. 1998. Mit Luftbildern und Bodenanalysen der Rosettenkrankheit auf der Spur. Schweiz. Z. Obst-Weinbau. 23, 588-591.

Kunze, L. and Krczal, H. 1971. Transmission of sharka virus by aphids. In: Proceedings of the 8th European Symposium on Fruit Tree Virus Diseases, pp. 255-260. INRA, Paris, France.

Kurihara, J., Tomaru, K., Otsubo, T., Arimoto, Y., Sakakibara, M., Natsuaki, K.T., Tsuda, S. and Kirita, M. 1998. A new disease of Prunus mume (Japanese apricot) caused by multiple infection with cucumber mosaic cucumovirus and Prunus necrotic ringspot related ilarvirus. Acta Horticulturae. 472, 183-193.

Kyriakopoulou, P.E. and Hadidi, A. 1998. Natural infection of wild and cultivated pears with apple scar skin viroid in Greece. Acta Horticulturae. 472, 617-625.

Lalancette, N. and Polk, D.F. 2001. Species of Phomopsis and a Libertella sp. occurring on grapevines with specific reference to South Africa: morphological, cultural, molecular and pathological characterization. Mycologia. 93(1), 146-167.

Lalancette, N. and Robison, D.M. 2000. Molecular phylogenetic analysis of ribosomal DNA internal transcribed spacer regions and comparison of fertility in Phomopsis isolates from fruit trees. Journal of General Plant Pathology. 66(3), 191-201.

Lamberti, F., Landriscina, S., Ciancio, A. and Catalano, L. 1993. Control of Xiphinema diversicaudatum, nematode vector of SLRV on peach in Piedmont. [Italian]. Informatore Fitopatologico. 43(5), 57-59.

Lamberti, F., Roca, F., Landriscina, S. and Ciancio, A. 1986. Seasonal transmissibility of strawberry latent ringspot virus by Xiphinema diversicaudatum. Nematologia Mediterranea. 14(2), 173-179.

Landi, F., Prandini, A., Paltrinieri, S., Mori, N. and Bertaccini, A. 2007. Detection of different types of phytoplasmas in stone fruit orchards in northern Italy. Bulletin of Insectology. 60(2), 163-164.

Lansac, M., Detienne, G., Bernhard, R. and Dunez, J. 1980. Analysis of nine isolates of almond mosaic. Acta Phytopathologica Academiae Scientiarum Hungaricae. 15, 359-366.

Larsen, H.J., Hatch, A.H. and Yu, K.S. 1998. Expression of peach mosaic symptoms in nectarine and peach cultivars. Acta Horticulturae. 472, 281-284.

Laurence, M., Hatzis, C. and Brash D.E. 2014. Common Contaminants in Next-Generation Sequencing That Hinder Discovery of Low-Abundance Microbes. PLoS ONE 9(5): e97876. doi:10.1371/journal.pone.0097876

Lebas, B. and Ward, L. 2012. Vitis (Grapevine) Post-Entry Quarantine Testing Manual. Ministry for Primary Industries, Auckland, New Zealand.

Leclant, F. 1973. Aspect sérologique de la transmission de la sharka (plum pox) dans le Sud-Est de la France. Mise en évidence de nouvelles espèces d'aphides. Annales de Phytopathologie. 4, 431-439.

Ledbetter, C.A. and Rogers, E.E. 2009. Differential susceptibility of Prunus germplasm (subgenus Amygdalus) to a California isolate of Xylella fastidiosa. HortScience. 44(7), 1928-1931.

Lee, I.M., Gundersen, D.E., Hammond, R.W. and Davis RE. 1994. Use of mycoplasmalike organism (MLO) group-specific oligonucleotide primers for nested-PCR assays to detect mixed-MLO infections in a single host plant. Phytopathology. 84, 559-566.

Lee, I.M., Hammond, R.W., Davis, R.E. and Gundersen, D.E. 1993. Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasma-like organisms. Phytopathology. 83, 834-842.

Lee, I.M., Bertaccini, A., Vibio, M., Gundersen, D.E., Davis, R.E., Mittempergher, L., Conti, M. and Gennari, F. 1995. Detection and characterization of phytoplasmas associated with diseases in Ulmus and Rubus in northern and central Italy. Phytopathologia Mediterranea. 34(3), 174-183.

Lee, I.M., Gunderson-Rindal, D.E., Davis, R.E. and Bartoszyk, I.M. 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16SrRNA and ribosomal protein gene sequences. International Journal of Systematic Bacteriology. 48, 1153-1169.

Lee, I.M., Martini, M., Marcone, C., Zhu, S.F. 2004. Classification of phytoplasma strains in the elm yellows group (16SrV) and proposal of 'Candidatus Phytoplasma ulmi' for the phytoplasma associated with elm yellows. International Journal of Systematic and Evolutionary Microbiology. 54, 337–47.

Lee, J., Park, J., Lee, D., Uhm, J., Ghim, S. and Lee, J. 2001. Occurrence of Apple scar skin viroid-Korean strain (ASSVd-K) in apples cultivated in Korea. Plant Pathology Journal. 17(5), 300-304.

Le Gall, O., Lanneau, M., Candresse, T. and Dunez, J. 1995. The nucleotide sequence of the RNA-2 of an isolate of the English serotype of Tomato black ring virus: RNA recombination in the history of nepoviruses. Journal of General Virology. 76, 1279-1283.

Le Gall, O., Sanfaçon, H., Ikegami, M., Iwanami, T., Jones, T., Karasev, A., Lehto, K., Wellink, J., Wetzel, T. and Yoshikawa, N. 2007. Cheravirus and Sadwaviru: two unassigned genera of plant positive-sense single-stranded RNA viruses formerly considered atypical members of the genus Nepovirus (family Comoviridae). Archives of Virology. 152(9), 1767-1774.

Lesemann, D. E., Kunze, L., Krischke, G., and Koenig, R. 1989. Natural occurrence of carnation Italian ringspot virus in a cherry tree. Journal of Phytopathology. 124, 171-174.

Letschert, B., Gunter, A., Dietrich-Eckhardt, L., Willingmann, P. and Heinze, C. 2002. Detection and differentiation of serologically cross-reacting tobamoviruses of economical importance by RT-PCR and RT-PCR-RFLP. Journal of Virological Methods. 106, 1-10.

Levy, L., Damsteegt, V., Scorza, R. and Kolber, M. 2000. Plum pox - potyvirus disease of stone fruits. The American Phytopathological Society (http://www.apsnet.org/online/feature/PlumPox/Top.html)

Li, R.H. and Mock, R. 2005. An improved reverse transcription-polymerase chain reaction (RT-PCR) assay for the detection of two cherry flexiviruses in Prunus spp. Journal of Virological Methods. 129, 162-169.

Li, R.H., Mock, R., Fuchs, M., Halbrendt, J., Howell, B. and Liu, Z.R. 2011. Characterization of the partial RNA1 and RNA2 3' Untranslated region of Tomato ringspot virus isolates from North America. Canadian Journal of Plant Pathology. 33(1), 94-99.

Liberato, J.R. 2006. Cotton root rot (*Phymatotrichopsis omnivora*). Pest and Diseases Image Library . Available online: PaDIL - http://www.padil.gov.au

Liberato, J.R. 2007. White root rot (Rosellinia necatrix). Pest and Diseases Image Library. Available online: PaDIL - http://www.padil.gov.au

Liberti, D., Marais, A., Svanella-Dumas, L., Dulucq, M.J., Alioto, D., Ragozzino, A., Rodoni, B. and Candresse, T. 2005. Characterization of Apricot pseudo-chlorotic leaf spot virus, a novel trichovirus isolated from stone fruit trees. Phytopathology. 95(4), 420-426.

Liberti, D., Marais, A., Svanella-Dumas, L., Ragozzino, A. and Candresse, T. 2005. Partial genome sequence of an apricot isolate of Cherry green ring mottle virus (CGRMV). Archives of Virology. 150(1), 185-188.

Liberti, D., Ragozzino, A., Gentit, P., Marais, A., Svanella-Dumas, L. and Candresse, T. 2004. Biological properties and partial molecular characterization of an apricot strain of CGRMV. Acta Horticulturae. 657, 103-108.

Liberti, D., Ragozzino, A., Marais, L., Svanella-Dumas, L., and Candresse, T. 2004. Identification of a new Trichovirus in some fruit trees infected by Apple chlorotic leaf spot virus (ACLSV). Acta Horticulturae. 657, 81-85.

Lim, P.O. and Sears, B.B. 1992. Evolutionary relationships of a plant-pathogenic mycoplasmalike organism and Acholeplasma laidlawii deduced from two ribosomal protein gene sequences. Journal of Bacteriology. 174, 2606-2611.

Lister, R.M. 1964. Strawberry latent ringspot: a new nematode-borne virus. Annals of Applied Biology. 54(2), 167-176.

Little, S**.** 1987. Cercospora circumscissa. CMI Descriptions of pathogenic fungi and bacteria. 911, 1-2.

Liu, K.C. and Allen, T.C. 1965. Serological reaction of virus isolates from Cherry with Eola rasp leaf. Phytopathology. 55(11), 1280-1281.

Llacer, G., Cambra, M., Camarasa, E. and Gorris, M.T. 1997. Viruses infecting almond trees in the region of Valencia (Spain). Bulletin OEPP. 27(4), 551-553.

Lochman, J., Sery, O. and Mikes, V. 2004. The rapid identification of European Armillaria species from soil samples by nested PCR. FEMS Microbiology Letters. 237(1), 105-110.

Lochman, J., Sery, O., Jankovsky, L. and Mikes, V. 2004. Variations in rDNA ITS of Czech Armillaria species determined by PCR and HPLC. Mycological Research. 108(10), 1153-1161. 2

Loi, N., Ferrini, F., Loschi, A., Martini, M. and Carraro, L. 2008. Fenomeni di recovery in albicocchi infetti da European stone fruit yellows. Petria. 18, 377-379.

Lorenz, K.H., Dosba, F., Poggi Pollini, C., Llacer, G. and Seemüller, E. 1994. Phytoplasma diseases of Prunus species in Europe are caused by genetically similar organisms. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. 101, 567-575.

Lorenz, K.H., Schneider, B., Ahrens, U. and Seemuller, E. 1995. Detection of the apple proliferation and pear decline phytoplasmas of ribosomal and nonribosomal DNA. Phytopathology. 85, 771-776.

Luck, J., Mann, R., van Rijswijk, B., Moran, J. and Meriman, P. 2002. Pierce's disease draft diagnostic manual. Institute for Horticultural Development, Department of Natural Resources and Environment.

Luigi, M. and Faggioli, F. 2011. Development of quantitative real-time RT-PCR for the detection and quantification of Peach latent mosaic viroid. European Journal of Plant Pathology. 130(1), 109-116.

Lukens, R.J., Miller, P.M., Walton, G.S. and Hitchcock, S.W. 1971. Incidence of X-disease of peach and eradication of chokecherry. Plant Disease Reporter. 55, 645-647.

MacDiarmid, R., Rodoni, B., Melcher, U., Ochoa-Corona, F., and Roossinck, M. 2013. Biosecurity Implications of New Technology and Discovery in Plant Virus Research. PLoS Pathogens, 9(8), e1003337. doi:10.1371/journal.ppat.1003337

Mackenzie, D.J., McLean, M.A., Mukerji S. and Green, M. 1997. Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription-polymerase chain reaction. Plant Disease. 81(2), 222-226.

MacNish, G.C. 1963. Diseases recorded on native plants, weeds, field and fibre crops in Western Australia. [not specified]. Journal of Agriculture of Western Australia. 4(6), 401-404, 407-408.

Maharaj, S.B. and da Graca, J.V. 1989. Transmission of citrus vein enation virus by Toxqtera citricidus. Phytophylactica. 21, 81-82.

Mali, V.R., Chaudhuri, K.G. and Rane, S.D. 1976. The vein-enation virus disease of citrus in India. Indian Phytopathology. 29, 43-45.

Maliogka, V., Dovas, C.I., Efthimiou, K. and Katis, N.I. 2004. Detection and differentiation of Comoviridae species using a semi-nested RT-PCR and a phylogenetic analysis based on the polymerase protein. Journal of Phytopathology. 152(7), 404-409.

Ma, Y.X., Li, J.J., Li, G.F. and Zhu, S.F. 2014a. First Report of Cherry mottle leaf virus Infecting Cherry in China. Plant Disease. 98, 1161.

Ma, Y.X., Li, J.J., Li, G.F. and Zhu, S.F. 2014b. First Report of Cherry rasp leaf virus Infecting Cherry in Shandong province, China. Journal of Plant Pathology. 96.4(Supplement), S4.113-S4.131.

Maliogka, V.I., Charou, A., Efthimiou, K., Katsiani, A.T., Chatzivassiliou, E.K. and Katis, N.I. 2010 Identification of Ilarviruses in almond and cherry fruit trees using nested PCR assays. 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops. Julius-Kühn-Archiv. 427, 281.

Maliogka, V.I., Dovas, C.I, and Katis, N.I. 2007. Demarcation of ilarviruses based on the phylogeny of RNA2-encoded RdRp and a generic ramped annealing RT-PCR. Archives of Virology. 152, 1687-1698.

Maliogka, V., Dovas, C. and Katis, N. 2008. Generic and species-specific detection of viruses belonging to an evolutionary distinct lineage within the Ampelovirus genus. Journal of Virological Methods. 154 (1-2), 41-47.

Mandic, B., Matic, S., Al-Rwahnih, M., Jelkmann, W. and Myrta, A. 2007. Viruses of sweet and sour cherry in Serbia. Journal of Plant Pathology. 89(1), 103-108.

Mandic, B., Matic, S., Jelkmann, W. and Myrta, A. 2005. First report of three filamentous viruses from cherry in Serbia. Journal of Plant Pathology. 87(3), 246.

Marais, A., Svanella-Dumas, L., Foissac, X., Gentit, P. and Candresse, T. 2006. Asian prunus viruses: new related members of the family Flexiviridae in Prunus germplasm of Asian origin. Virus Research. 120, 176-183.

Marais, A., Svanella-Dumas, L., Candresse, T., Barone, M., Ragozzino, A. and Gentit, P. 2008. Genomic diversity of Cherry capillovirus A (CVA) and suitability of various assays for its detection. Acta Horticulturae. 781, 37-45.

Marais, A., Faure, C., Gentit, P., Candresse, T., 2009. Detection of a divergent variant of Plum bark necrosis and stem pitting associated virus (PBNSPaV) in Prunus domestica with peach red marbling disease symptoms. Proceedings of the 21st International Conference on Virus and other Graft Transmissible Diseases of Fruit Crops, Neustadt. 2009, 57.

Marais, A., Svanella-Dumas, L., Barone, M., Gentit, P., Faure, C., Charlot, G., Ragozzino, A. and Candresse, T. 2012. Development of a polyvalent RT-PCR detection assay covering the genetic

diversity of Cherry capillovirus A. Plant Pathology. 61, 195–204. doi: 10.1111/j.1365-3059.2011.02488.x

Marais, A., Faure, M., Mustafayev, E., Barone, M., Alioto, D. and Candresse, T. 2015. Characterization by Deep Sequencing of Prunus virus T, a novel Tepovirus Infecting Prunus Species Phytopathology. 105, 135-140. doi: http://dx.doi.org/10.1094/PHYTO-04-14-0125-R

Marais, A., Faure, M., Mustafayev, E., Barone, M., Alioto, D., Navratil, M., Safarova, D. and Candresse, T. 2015. Tepovirus and Prunevirus: Betaflexiviridae genera with novel Prunus-infecting agents. In: Book of Abstracts, ICVF. 23rd International Conference on Virus and Other Graft Transmissible Diseases of Fruit Crops. June 8-12, 2015, Aiina Hall, Morioka, Iwata, Japan.

Marcone, C., Ragazzino, A. and Seemüller, E. 1996. European stone fruit yellows phytoplasma as the cause of peach vein enlargement and other decline diseases of stone fruits in southern Italy. Journal of Phytopathology. 144, 559-564.

Marcone, C., Ragozzino, A., Del Serrone, P., Aloj, B., Barba, M. and Seemüller, E. 1996**.** Detection of apple proliferation and pear decline in southern Italy. Petria. 6(2), 149-157.

Marcone, C., Jarausch, B. and Jarausch, W. 2010. Candidatus Phytoplasma prunorum, the causal agent of European stone fruit yellows: an overview. Journal of Plant Pathology. 92(1), 19-34.

Marenaud, C. and Lansac, M. 1977. Degenerative diseases of almond. Virological analysis of a collection. In Arti Ill Riunione del GREMPA, Valenzano (Bari) 1997, pp. 371-380 (in Italian).

Marinho, V..LA., Kummert, J., Rufflard, G., Colinet, D. and Lepoivre, P. 1998. Detection of apple stem grooving virus in dormant apple trees with crude extracts as templates for one-step RT-PCR. Plant Disease. 82(7), 785-790.

Marini, D.B., Gibson, P.G. and Scott, S.W. 2009. The complete nucleotide sequence of an isolate of Asian Prunus virus 1 from peach [Prunus persica (L) Batch]. Archives of Virology. 154(8), 1375-1377.

Marini, D.B., Gibson, P.G. and Scott, S.W. 2008. The complete nucleotide sequence of an isolate of Apple chlorotic leaf spot virus from peach (Prunus persica (L.) Batch). Archives of Virology. 153, 1003–1005.

Marini, D.B., Zhang, Y.P., Rowhani, A. and Uyemoto, J.K. 2002. Etiology and host range of a Closterovirus associated with plum bark necrosis-stem pitting disease. Plant Disease. 86(4), 415-417.

Martelli, G.P. 1993. Graft-transmissible Diseases of Grapevines, Handbook for Detection and Diagnosis. Rome, Italy: FAO.

Martelli, G.P. and Savino, V. 1997. Infectious diseases of almond with special reference to the Mediterranean area. Bulletin OEPP. 27(4), 525-534.

Martelli, G.P., Abou Ghanem-Sabanadzovic, N., Agranovsky, A.A., Al Rwahnih, M., Dolja, V.V., Dovas, C.I., Fuchs, M., Gugerli, P., Hu, J.S., Jelkmann, W., Katis, N.I., Maliogka, V.I., Melzer, M.J., Menzel, W., Minafra, A., Rott, M.E., Rowhani, A., Sabanadzovic, S., and Saldarelli, P. 2012. Taxonomic revision of the family Closteroviridae with special reference to the grapevine leafroll-associated members of the genus Ampelovirus and the putative species unassigned to the family. Journal of Plant Pathology. 94, 7-19.

Martin, R.R., Pinkerton, J.N. and Kraus, J. 2009. The use of collagenase to improve the detection of plant viruses in vector nematodes by RT-PCR. Journal of Virological Methods. 155(1), 91-95.

Marzachi, C. 2004. Molecular diagnosis of phytoplasmas. Phytopathologia Mediterranea. 43, 228-231.

Massart, S., Brostaux, Y., Barbarossa, L., Csar, V., Cieslinska, M., Dutrecq, O., Fonseca, F., Guillem, R., Lavina, A., Olmos, A., Steyer, S., Wetzel, T., Kummert, J. and Jijakli, M.H. 2008. Inter-laboratory evaluation of a duplex RT-PCR method using crude extracts for the simultaneous detection of Prune dwarf virus and Prunus necrotic ringspot virus. European Journal of Plant Pathology. 22(4), 539-547.

Massart, S., Olmos, A., Jijakli, H. and Candresse, T. 2014. Current impact and future directions of high throughput sequencing in plant virus diagnostics. Virus Research. 188, 90–96. 10.1016/j.virusres.2014.03.029

Matic, S., Minafra, A., Boscia, D., Cunha, A.T.P.D. and Martelli, G.P. 2009. Production of antibodies to Little cherry virus 1 coat protein by DNA prime and protein boost immunization. Journal of Virological Methods. 155(1), 72-76.

Matic, S., Minafra, A., Sanchez-Navarro, J.A., Pallas, V., Myrta, A. and Martelli, G.P. 2009. 'Kwanzan Stunting' syndrome: detection and molecular characterization of an Italian isolate of Little cherry virus 1. Virus Research. 143(1), 61-67.

Matic, S., Myrta, A. and Minafra, A. First report of little cherry virus 1 in cherry, plum, almond and peach in Italy. Journal of Plant Pathology. 2007. 89.3(Supplement), S75.

Matic, S., Myrta, A. and Minafra, A. 2010. Detection of three closteroviruses in stone fruit trees by multiplex assays. Journal of Plant Pathology. 92(1), 57-63.

Mattedi, L., Forno, F., Cainelli, C., Grando, M.S., and Jarausch, W. 2008. Research on Candidatus Phytoplasma mali transmission by insect vectors in Trentino. Acta Horticulturae. 781, 369-374.

Mayer, C.J., Jarausch, B., Jarausch, W., Jeklmann, W., Vilcinskas, A., and Gross, J. 2009. Cacopsylla melanoneura has no relevance as vector of apple proliferation in Germany. Phytopathology. 99, 729-738.

McClure, M.S. 1980. Role of wild host plants in the feeding, oviposition, and dispersal of Scaphytopius acutus (Homoptera: Cicadellidae), a vector of peach X-disease. Environmental Entomology. 9, 283-292.

McAlpine, D. 1902. Cyphella marginate McAlpine, Fungus Diseases of Stone-fruit Trees in Australia: 120.

McCoy, R.E. 1984. Mycoplasma-like organisms of Plants and invertebrates. In: Krieg NR and Holt JG (eds). Bergey's manual of systematic bacteriology. Volume 1, 792-793. William and Wilkins, Baltimore/London.

Mehle, N., Brzin, J., Boben, J., Hren, M., Frank, J., Petrovic, N., Gruden, K., Dreo, T., Zezlina, I.,Seljak, G., and Ravnikar, M. 2006. First report of 'Candidatus Phytoplasma mali' in Prunus avium, P.armeniaca, and P. domestica. New Disease Reports. 14, 42.

Mehle, N., Ambrožič Turk, B., Brzin, J., Nikolič, P., Dermastia, M., Boben, J. and Ravnikar, M. 2010. Diagnostics of fruit trees phytoplasmas - the importance of latent infections. 21st International

Conference on Virus and other Graft Transmissible Diseases of Fruit Crops. Julius-Kühn-Archiv. 427, 412.

Mekuria, T.A., Druffel, K.L., Susaimuthu, J., and Eastwell, K.C. 2013. Complete nucleotide sequence of a strain of cherry mottle leaf virus associated with peach wart disease in peach. Archives of Virology. 158(10), 2201–2203. doi:10.1007/s00705-013-1698-3

Mekuria, G., Ramesh, S.A., Alberts, E., Bertozzi, T., Wirthensohn, M., Collins, G. and Sedgley, M. 2005. Comparison of ELISA and RT-PCR for the detection of PNRSV and PDV in Australian almond trees. Options Mediterraneennes. Serie A, Seminaires Mediterraneens. 63, 193-196.

Mekuria, G., Ramesh, S.A., Alberts, E., Bertozzi, T., Wirthensohn, M., Collins, G. and Sedgley, M. 2003. Comparison of ELISA and RT-PCR for the detection of Prunus necrotic ring spot virus and prune dwarf virus in almond (Prunus dulcis). Journal of Virological Methods. 114(1), 65-69.

Menard, M., Sutra, L., Luisetti, J., Prunier, J.P. and Gardan, L. 2003. Pseudomonas syringae pv. avii (pv. nov.), the causal agent of bacterial canker of wild cherries (Prunus avium) in France. European Journal of Plant Pathology. 109(6), 565-576.

Menzel, W., Jelkmann, W. and Maiss, E. 2002. Detection of four apple viruses by multiplex RT-PCR assays with co-amplification of plant mRNA as internal control. Journal of Virological Methods. 99, 81-92.

Milne, J.R. and Walter, G.H. 2003. The coincidence of thrips and dispersed pollen in PNRSV-infected stonefruit orchards - a precondition for thrips-mediated transmission via infected pollen. Annals of Applied Biology. 142, 291–298. doi: 10.1111/j.1744-7348.2003.tb00253.x

Minsavage, G., Thompson, C., Hopkins, D., Leite, R. and Stall, R. 1994. Development of a polymerase chain reaction protocol for detection of Xylella fastidiosa in plant tissue. Phytopathology. 84(5), 456-461.

Minoiu, N. 1974. Biology, ecology and control of sweet cherry and morello cherry anthracnose - Coccomyces hiemalis Higg. [Romanian]. Revista de Horticultura si Viticultura. 23(3), 65-73.

Minoiu, N., 1975. New investigations on Plum pox (sharka) virus. Acta für Phytopathologie und Pflanzenschutz. 11, 389–397.

Mink, G.I., Howell, W.E., Cole, A. and Regev, S. 1987. Three serotypes of Prunus necrotic ringspot virus isolated from rugose mosaic-diseased sweet cherry trees in Washington. Plant Disease. 71(1), 91-93.

Mircetich, S.M. and Moller, W.J. 1977. Prunus Stem Pitting. EPPO Bulletin 7(1), 29-36.

Mircetich, S.M., Lowe, S.K., Moller, W.J. and Nyland, G. 1976. Etiology of almond leaf scorch disease and transmission of the causal agent. *Phytopathology.* 66, 17-24.

Mischke, W. and Bercks, R. 1965. Brief note on an occurrence of Tomato black ring virus on Almond trees (P. amygdalus). [not specified]. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes. 17(12), 186-187.

Mischke, W. and Bercks, R. 1963. Further studies on the cause of a virus shoot stunting of Peach and its identification as a strain of Tomato black ring virus. [not specified]. Phytopathologische Zeitschrift. 1963. 49(2), 147-155.

Mitrofanova, O.V. and Teslenko, A.V. 1982. Diagnosis of virus diseases of peach in the Crimea. Vrediteli i Bolezni Plodovykh i Dekorativnykh Kul'tur Kryma. 1982, 89-99.

Mmbaga, M.T. 2000. Winter survival and source of primary inoculum of powdery mildew of dogwood in Tennessee. Plant Disease. 84, 574-579.

Mohan, S.K. 2007. Natural incidence of shoot blight in Pluot® caused by Erwinia amylovora. 11th International Workshop on Fire Blight. Portland, Oregon. Book of Abstracts, 64.

Mohan, S.K. and Bijman, V.P. 1999. Susceptibility of Prunus species to Erwinia amylovora. Acta Horticulturae. 489, 145-147.

Mohan, S.K. and Thomson, S.V. 1996. An outbreak of fire blight in plums. Acta Horticulturae. 411, 73-76.

Mohan, S.K., Bijman, V.P. and Fallahi, E. 2001. Field evaluation of Prunus species for susceptibility to Erwinia amylovora by artifical inoculation. The 9th Int.Workshop on Fire Blight, Abstract. Volume P, 53.

Moini, A.A. 2010. Identification of Tomato ringspot virus (ToRSV) on apple in Iran. Australasian Plant Disease Notes. 5(1), 105-106.

Moncalvo, J.M., Wang, H.F., and Hseu, R.S. 1995. Gene phylogeny of the Ganoderma lucidum **c**omplex based on ribosomal DNA sequences. Comparison with traditional taxonomic characters. Mycological Research. 99, 1489-1499.

Mostert, L., Crous, P.W., Kang, J. and Phillips, A.J.L. 2001. Seasonal availability of inoculum for constriction canker of peach in New Jersey. Phytopathology. 91(11), 1109-1115.

Motohashi, K., Inaba, S., Anzai, K., Takamatsu, S., and Nakashima, C**.** 2009. Phylogenetic analyses of Japanese species of Phyllosticta sensu stricto. Mycoscience. 50, 291-302.

Mouchacca, J. 2004. Novel fungal taxa from the arid Middle East (1940-2000): omissions from previous notes. Cryptogamie Mycologie. 25, 149-171.

Mougou-Hamdane, A., Giresse, X., Dutech, C. and Desprez-Loustau, M.L. 2010. Spatial distribution of lineages of oak powdery mildew fungi in France, using quick molecular detection methods. Annals of Forest Science. 67(2),212.

Moury, B.T., Cardin, L.C., Onesto, J.P., Candresse, T. and Poupet, A. 2000. Enzyme-Linked Immunosorbent Assay Testing of Shoots Grown In Vitro and the Use of Immunocapture-Reverse Transcription-Polymerase Chain Reaction Improve the Detection of Prunus necrotic ringspot virus in Rose. Phytopathology. 90(5), 522-528.

Munro, D. 1987. Viruses infecting hop, Humulus lupulus, in Australia. Australian Journal of Agricultural Research. 38(1), 83-90.

Murant, A.F. 1983. Seed and pollen transmission of nematode-borne viruses. Seed Science and Technology. 11, 973-987.

Nassuth, A., Pollari, E., Helmeczy, K., Stewart, S., Kofalvi, S.A. 2000. Improved RNA extraction and one-tube RT-PCR assay for simultaneous detection of control plant RNA plus several viruses in plant extracts. Journal of Virological Methods. 90**,** 37-49.

Navratil, M., Valova, P., Fialova, R., Petrova, K., Franova, J., Nebesarova, J., Poncarova-Vorackova, Z. and Karesova, R. 2002. An epidemic of almond witches'-broom in Lebanon: classification and phylogenetic relationships of the associated phytoplasma. Plant Disease. 86(5), 477-484.

Navratil, M., Valova, P., Fialova, R., Petrova, K., Franova, J., Nebesarova, J., Poncarova-Vorackova, Z. and Karesova, R. 2001. Survey for stone fruit phytoplasmas in the Czech Republic. Acta Horticulturae. 550(2), 377-382.

Necas, T. and Krska, B. 2006. Selection of woody indicators and the optimum plant material and sampling time for phytoplasma ESFY detection. Acta Horticulturae. 717, 101-105.

Necas, T., Maskova, V. and Krska, B. 2008. The possibility of ESFY phytoplasma transmission: through flowers and seeds. Acta Horticulturae. 781, 443-447.

Negi, A., Rana, T., Kumar, Y., Ram, R., Hallan, V. and Zaidi, A.A. 2010. Analysis of the coat protein gene of Indian strain of Apple stem grooving virus. Journal of Plant Biochemistry and Biotechnology. 19(1), 91-94.

Nemchinov, L. and Hadidi, A. 1998. Apricot latent virus: a novel stone fruit pathogen and its relationship to apple stem pitting virus. Acta Horticulturae. 472, 159-173.

Nemchinov, L.G., Shamloul, A.M., Zemtchik, E.Z., Verderevskaya, T.D. and Hadidi, A. 2000. Apricot latent virus: a new species in the genus Foveavirus. Archives of Virology. 145(9), 1801-1813.

Nemeth, M. 1986. *Virus, Mycoplasma and Rickettsia Diseases of Fruit Trees.* Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Nemeth, M. 1980. Effect of peach viruses occurring in Hungary on the growth of GF 305 peach seedlings with mixed infections. Effect of the viruses on unions on bud grafting and on vegetative development of the trees of two peach cultivars in the nursery. Tagungsbericht der Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik. 1980, 403-408.

Nickel, O., Targon, M.L.P.N., Fajardo, T.V.M., Machado, M.A. and Trivilin, A.P. 2004. Polyclonal antibodies to the coat protein of Apple stem grooving virus expressed in Escherichia coli: production and use in immunodiagnosis. Fitopatologia Brasileira. 29, 558-562.

Nicol, J.M., Stirling, G.R., Rose, B.J., May, P. and Heeswijck, R.V. 1999. Impact of nematodes on grapevine growth and productivity: current knowledge and future directions, with special reference to Australian viticulture. Australian Journal of Grape and Wine Research. 5(3), 109-127.

Nicolotti, G., Gonthier, P. , Guglielmo, F, and Garbelotto, M.M. 2009. A Biomolecular Method for the Detection of Wood Decay Fungi: A Focus on Tree Stability Assessment. Journal of Arboriculture and Urban Forestry. 35(1), 14–19.

Niu, Y.B., Qing, L., Yao, M., Wang, D.F., Liu, J.D. and Wang, J.S. 2009. First Report of Tobacco mosaic virus in Abutilon theophrasti in China. Plant Disease. 93(11), 1221-1221.

Noorani, M.S., Awasthi, P., Singh, R.M., Raja, R., Sharma, M.P., Singh, S.R., Ahmed, N., Hallan, V. and Zaidi, A.A. 2010. Complete nucleotide sequence of cherry virus A (CVA) infecting sweet cherry in India. Archives of Virology. 155(12), 2079-2082.

Novak, J.B. and Lanzova, J. 1977. Identification of tomato bushy stunt virus in cherry and plum trees showing fruit pitting symptoms. Biologia Plantarum. 19(3), 234-237.

Novak, J.B. and Lanzova, J. 1980. Some diseases of fruit trees in which the tomato bushy stunt virus occurs and new natural hosts of this virus. Acta Phytopathologica Academiae Scientiarum Hungaricae. 15, 323-327.

Nyland, G. 1974. Cherry rasp leaf. In: Virus diseases and non-infectious disorders of stone fruits in North America. Agriculture Handbook. No. 437, pp. 219-221. US Department of Agriculture, USA.

Nyland, G., Lownsbery, B.F., Lowe, S.K. and Mitchell, J.F. 1969. The transmission of cherry rasp leaf virus by Xiphinema americanum. [not specified]. Phytopathology. 59(8), 1111-1112.

Ochoa-Corona, F.M., Lebas, B.S.M., Tang, J.Z., Bootten, T.J., Stewart, F.J., Harris, R., Elliott, D.R. and Alexander, B.J.R. 2006. RT-PCR detection and strain typing of Raspberry ringspot virus. Proceedings of the XXth International Symposium on Virus and Virus-like Diseases of Temperate Fruit Crops and XIth International Symposium on Small Fruit Virus Diseases, Antalya, Turkey, May 22-26, 2006.

Oldfield, G.N., Creamer, R., Gispert, C., Osorio, F., Rodriguez, R. and Perring, T.M. 1995. Incidence and distribution of peach mosaic and its vector, Eriophyes insidiosus (Acari:Eriophyidae) in Mexico. Plant Disease. 79(2), 186-189.

Olmos, A., Bertolini, E. and Cambra, M. 2002. Simultaneous and co-operational amplification (Co-PCR): a new concept for detection of plant viruses. Journal of Virological Methods. 106, 51-59.

Olmos, A., Bertolini, E., Gil, M. and Cambra, M. 2005. Real-time assay for quantitative detection of non-persistently transmitted Plum pox virus RNA targets in single aphids. Journal of Virological Methods. 128, 151-155.

Osaki, H., Kudo, A. and Ohtsu, Y. 1996. Japanese pear fruit dimple disease caused by apple scar skin viroid (ASSVd). Annals of the Phytopathological Society of Japan. 62(4), 379-385.

Osman, F., Al-Rwahnih, M., Golino, D., Pitman, T., Cordero, F., Preece, J.E. and Rowhani, A. 2012. Evaluation of the phytosanitary status of the Prunus species in the National Clonal Germplasm Repository in California: survey of viruses and viroids. Journal of Plant Pathology. 94(1), 249-253.

Ossiannilsson, F. 1992. *The Psylloidea (Homoptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinava*, **Vol. 26**. Leiden, the Netherlands: E. J. Brill.

Pagani, M.C. 2004. An ABC transporter protein and molecular diagnoses of Xanthomonas arboricola pv. pruni causing bacterial spot of stone fruits. Ph.D. Thesis, North Carolina State University, Raleigh, NC, USA.

Pallas, V., Gomez, G., Amari, K., Canizares, M.C. and Candresse, T. 2003. Hop stunt viroid in apricot and almond. Viroids. 168-170.

Paltrinieri, S. and Bertaccini, A. 2008. Phytoplasmas of peach and cherry. (Plant Pathogens Series - 5). Characterization, diagnosis and management of phytoplasmas. 59-71.

Paltrinieri, S., Botti, S., Bertaccini, A., Molin, F.D., Mori, N. and Fiore, N. 2006. Are phytoplasmas involved in a severe peach decline? Acta Horticulturae. 713, 421-426.

Parakh, D.R., Shamloul, A.M., Hadidi, A., Waterworth, H.E., Scott, S.W., Howell, H.E. and Mink, G.I. 1995. Detection of Prune dwarf ilarvirus from infected stone fruits using reverse transcription-polymerase chain reaction. Acta Horticulturae. 386, 421-430.

Parisi, O., Lepoivre, P. and Jijakli, M.H. 2011. Development of a quick quantitative real-time PCR for the in vivo detection and quantification of Peach latent mosaic viroid. Plant Disease. 95(2), 137-142.

Park, S., Lee, Y., Koh, Y., Hur, J., and Jung, J. 2010. Detection of Xanthomonas arboricola pv. pruni by PCR using primers based on DNA sequences related to the hrp genes. Journal of microbiology. 48(5), 554-558.

Parker, K.G., Fridland, P.R. and Gilmer, G.M. 1976. Green ring mottle. In Virus Diseases and Noninfectious Diseases of Stone Fruit in North America, pp. 193-199. US Department of Agriculture, Agriculture Handbook No. 437.

Paulechova, K. 1983. Characteristics of tobacco necrosis virus isolated from plum. Prace Ustavu Experimentalnej Fytopatologie a Entomologie. 2, 81-87.

Paulechova, K. and Baumgartnerova, H. 1980. Some properties of tobacco necrosis virus isolated from plums. Acta Phytopathologica Academiae Scientiarum Hungaricae. 15: 119-122.

Paulsen, A.Q. and Fulton, R.W. 1968. Hosts and properties of a plum line pattern virus. Phytopathology. 58, 766-772.

Pérez-Sierra, A., Whitehead, D.S. and Whitehead, M.P. 1999. Investigation of a PCR-based method for the routine identification of BritishArmillaria species. Mycological Research. 103(12), 1631-1636.

Petrzik, K. and Lenz, O. 2002. Remarkable variability of apple mosaic virus capsid protein gene after nucleotide position 141. Archives of Virology. 147(7), 1275-1285.

Petrzik, K. and Svoboda, P. 1997. Screening of Apple mosaic virus in hop cultivars in the Czech Republic by reverse transcription-polymerase chain reaction. Acta Virologica. 41(2), 101-103.

Pethybridge, S.J., Hay, F.S., Barbara, D.J., Eastwell, K.C., and Wilson, C.R. 2008. Viruses and viroids infecting hop: Significance, epidemiology and management. Plant Disease. 92, 324-338.

Pfeilstetter, E., Zinkernagel, V., and Kunze, L. 1992. Occurence of petunia asteroid mosaic virus (PAMV) and carnation Italian ringspot (CIRV) viruses in cherry orchards in northern Bavaria. Acta Horticulturae. 309, 345-352.

Pfeilstetter, E., Kunze, L., and Zinkernagel, V. 1996. Viral twig necrosis of sweet cherry. modes of transmission and spread of petunia asteroid mosaic virus (PeAMV). Annals of Applied Biology. 128, 285-301.

Phillips, A.J.L., Oudemans, P.V., Correia, A., and Alves, A. 2006. Characterisation and epitypification of **Botryosphaeria corticis**, the cause of blueberry cane canker. Fungal Diversity. 21, 141-155.

Pignatta, D., Poggi Pollini, C., Giunchedi, L., Ratti, C., Reggiani, N., Forno, F., Mattedi, L., Gobber, M., Miorelli, P. and Ropelato, E. 2008. A real-time PCR assay for the detection of European stone fruit yellows phytoplasma (ESFYP) in plant propagation material. Acta Horticulturae. 781, 499-504.

Pine, T.S. 1965. Host range and strains of Peach mosaic virus. Phytopathology. 55(10), 1151-1153.

Pirc, M., Ravnikar, M., Tomlinson, J. and Dreo, T. 2009. Improved fire blight diagnostics using quantitative real-time PCR detection of Erwinia amylovora chromosomal DNA. Plant Pathology. 58, 872‐881.

Pitt, W.M., Huang, R., Steel, C.C. and Savocchia, S. 2010a. Management of Botryosphaeria canker of grapevines. Australian Viticulture - Practical Vineyard Management. 14(2), 52-56.

Pitt, W.M., Sosnowski, M.R., Taylor, A., Huang, R., Quirk, L., Hackett, S., Somers, A., Steel, C.C. and Savocchia, S. 2010b. Lasiodiplodia theobromae. [Distribution map]. Distribution Maps of Plant Diseases. October, Map 561 (Edition 2).

Plant Health Australia (2009) Industry Biosecurity Plan for the Nut Industry (Version 2.0). 2009. Plant Health Australia. Canberra, ACT.

Poggi Pollini, C., Zelger, R., Wolf, M., Bissani, R. and Giunchedi, L. 2002. Indagine sulla presenza di psillidi infetti dal fitoplasma degli scopazzi del melo (AP = apple proliferation) in provincia di Bolzano. ATTI Giornate Fitopatologiche. **2**, 607-12.

Polak, J., Chaloupkova, M. and Jokes, M. 2004. Biological and serological procedures to detect three nepoviruses in fruit trees. Plant Protection Science. 40(4), 121-127.

Posnette, A.F. and Cropley, R. 1954. 1955. Leaf roll : a virus disease of cherry. Annual Report East Malling Research Station. A38, 126-127.

Pothier, J., Vorholter, F., Blom, J., Goesmann, A., Pühler, A., Smits, T. and Duffy, B. 2011. The ubiquitous plasmid pXap41 in the invasive phytopathogen Xanthomonas arboricola pv. Pruni: complete sequence and comparative genomic analysis. FEMS Microbiology Letters. 323, 52-60.

Powney, R., Plummer, K., Luck, J.E., Beer, S. and Rodoni, B. 2007. Evaluation of PCR-based Protocols for the Detection of Erwiniaamylovora. Acta Horticulturae. 793, 505-511.

Powney, R., Beer, S., Plummer, K., Luck, J. and Rodoni, B. 2011. The specificity of PCR-based protocols for detection of Erwinia amylovora. Australasian Plant Pathology. 40(1), 87-97.

Proffer, T.J., Berardi, R., Ma, Z., Nugent, J.E., Ehret, G.R., McManus, P.S., Jones, A.L. and Sundin, G.W. 2006. Occurrence, Distribution, and Polymerase Chain Reaction-Based Detection of Resistance to Sterol Demethylation Inhibitor Fungicides in Populations of Blumeriella jaapii in Michigan. Phytopathology. 96(7), 709-717.

Prunier, J.P., Luisetti, J. and Gardan, L. 1970. Etudes sur les bacte_rioses des arbres fruitiers[II Caracte_risation d|un Pseudomonas non fluorescent agent d'une bacteriose nouvelle chez le Pecher. Annales de Phytopathologie. 57, 8.

Psallidas, P.G. 1997. Hyperplastic canker - a perennial disease of almond caused by Pseudomonas amygdali. Bulletin OEPP. 27(4), 511-517.

Pulawska, J., Willems, A. and Sobiczewski, P. 2006. Rapid and specific identification of four Agrobacterium species and biovars using multiplex PCR. Systematic and Applied Microbiology. 29, 470-479.

Puttoo, B.L. and Razdan, V.K. 1988. Fungal diseases of almond in India. International Journal of Tropical Plant Diseases. 6(2), 207-211.

Quader, M., Riley, I.T. and Walker, G.E. 2003. Spatial and temporal distribution patterns of dagger (Xiphinema spp.) and root lesion (Pratylenchus spp.) nematodes in a South Australian vineyard. Australasian Plant Pathology. 32(1), 81-86.

Qi, H.A. 2007. Natural occurrence of Xylella fastidiosa in a commercial nursery in Maryland. Canadian Journal of Plant Pathology. 29(3), 299-303.

Qiu, Y., Steel, C.C., Ash, G.J. and Savocchia, S. 2011. Survey of Botryosphaeriaceae associated with grapevine decline in the Hunter Valley and Mudgee grape growing regions of New South Wales. Australasian Plant Pathology. 40(1), 1-11.

Ragozzino, E., Faggioli, F. and Barba, M. 2004. Development of a PCR method of Peach latent mosaic viroid and Hop stunt viroid detection for certification of planting material. Acta Horticulturae. 657, 391-395.

Raine, J., McMullen, R.D., and Forbes, R.D. 1986. Transmission of the agent causing little cherry disease be the apple mealybug Phenacoccus aceris and the dodder Cuscuta lupuliformis. Canadian Journal of Plant Pathology. 8, 6-11.

Ramsdell, D.C. and Gillett, J.M. 1981. Peach rosette mosaic virus in highbush blueberry. Plant Disease. 65(9), 757-758.

Ramsdell, D.C. and Myers, R.L. 1974. Peach rosette mosaic virus, symptomatology and nematodes associated with grapevine 'degeneration' in Michigan. Phytopathology. 64(9), 1174-1178.

Rana, T., Chandel, V., Hallan, V. and Zaidi, A.A. 2009. Molecular evidence for the presence of Apple chlorotic leaf spot virus in infected peach trees in India. Scientia Horticulturae. 120(2), 296-299.

Rana, T., Chandel, V., Hallan, V. and Zaidi, A.A. 2008. Characterisation of Apple chlorotic leaf spot virus infecting almonds in India. Australasian Plant Disease Notes. 2008. 3(1), 65-67.

Rana, T., Chandel, V., Hallan, V. and Zaidi, A.A. Himalayan wild cherry (Prunus cerasoides D. Don): a new host of Apple chlorotic leaf spot virus. Forest Pathology. 2008. 38: 2, 73-77.

Rana, T., Chandel, V., Hallan, V. and Zaidi, A. 2011. Expression of recombinant Apple chlorotic leaf spot virus coat protein in heterologous system: production and use in immunodiagnosis. Journal of Plant Biochemistry and Biotechnology. 20(1), 138-141.

Randles, J.W. and Franco, R.I.B. 1965. Some properties of a Tobacco ringspot virus isolate from South Australia. [not specified]. Australian Journal of Biological Sciences. 18(5), 979-986.

Rao, W.L., Zhang, Z.K. and Li, R. First report of Cherry virus A in sweet cherry trees in China. Plant Disease. 2009. 93(4), 425.

Rastgou, M., Habibi, M.K., Izadpanah, K., Masenga, V., Milne, R.G., Wolf, Y.I., Koonin, E.V. and Turina, M. 2009. Molecular characterization of the plant virus genus Ourmiavirus and evidence of inter-kingdom reassortment of viral genome segments as its possible route of origin. Journal of General Virology. 90(10), 2525-2535.

Ray, J.D., Burgess, T. and Lanoiselet, V.M. 2010. First record of Neoscytalidium dimidiatum and N. novaehollandiae on Mangifera indica and N. dimidiatum on Ficus carica in Australia. Australasian Plant Disease Notes. 5(1), 48-50.

Rebenstorf, K., Candresse, T., Dulucq, M.J., Buttner, C. and Obermeier, C. 2006. Host species-dependent population structure of a pollen-borne plant virus, Cherry leaf roll virus. Journal of Virology. 80(5), 2453-2462.

Reynolds, B. and Teakle, D.S. 1976. Viruses infecting commercial gladiolus cultivars in Queensland. APPS Newsletter. 5(2), 22-23.

Richter, J. and Kegler, H. 1967. Isolation of Strawberry latent ring spot virus from stunted Peach trees. Phytopathologische Zeitschrift. 58(3), 298-301.

Rodrigues, J.L.M., Silva-Stenico, M.E., Gomes, J.E., Lopes, J.R.S. and Tsai, S.M., 2003. Detection and diversity assessment. Journal of Plant Pathology. 92.1(Supplement), S1.35-S1.48.

Rosenberger, D.A .and Jones, A.L. 1978. Leafhopper vectors of the peach X-disease pathogen and seasonal transmission from chokecherry. Phytopathology. 68, 782-790.

Rott, M.E. and Jelkmann, W. 2001. Detection and partial characterization of a second closterovirus associated with little cherry disease, Little cherry virus-2. Phytopathology. 91(3), 261-267.

Rott, M.E. and Jelkmann, W. 2005. Little cherry virus-2: sequence and genomic organization of an unusual member of the Closteroviridae. Archives of Virology. 150(1), 107-123.

Rott, M.E. and Jelkmann, W. 2001. Characterization and detection of several filamentous viruses of cherry: adaptation of an alternative cloning method (DOP-PCR), and modification of an RNA extraction protocol. European Journal of Plant Pathology. 107(4), 411-420.

Rott, M.E. and Jelkmann, W. 2001. Detection and partial characterization of a second closterovirus associated with little cherry disease, little cherry virus-2. Virology. 91(3), 261-267.

Rott, M.E. and Jelkmann, W. 2001. Complete nucleotide sequence of cherry necrotic rusty mottle virus. Acta Horticulturae. 550, 207-212.

Roy, A., Fayad, A., Barthe, G., and Brlansky, R. H. 2005. A multi-plex polymerase chain reaction method for reliable, sensitive and simultaneous detection of multiple viruses in citrus trees. Journal of Virological Methods. 129, 47-55.

Rubino, L., Burgyan, J., and Russo, M. 1995. Molecular cloning and complete nucleotide sequence of carnation Italian ringspot tombusvirus genomic and defective interfering RNAs. Archives of Virology. 140, 2027-2039.

Rubio, M., Rodríguez-Moreno, L., Ballester, A.R., de Moura, M.C., Bonghi, C., Candresse, T. and Martínez-Gómez, P. 2015. Analysis of gene expression changes in peach leaves in response to Plum pox virus infection using RNA-Seq. Molecular Plant Pathology. 16, 164–176. doi: 10.1111/mpp.12169

Russo, M., Vovlas, C., Rubino, L., Grieco, F. and Martelli, G. 2002. Molecular characterization of a tombusvirus isolated from diseased pear trees in southern Italy. Journal of Plant Pathology. 84(3), 161-166.

Saad, A.T. and Masannat, K. 1997. Economic importance and cycle of Polystigma ochraceum, causing red leaf blotch disease of almond, in Lebanon. Bulletin OEPP. 27(4), 481-485.

Saade, M., Aparicio, F., Sanchez-Navarro, J.A., Herranz, M.C., Myrta, A., Terlizzi, B.d. and Pallas, V. 2000. Simultaneous detection of the three ilarviruses affecting stone fruit trees by nonisotopic

molecular hybridization and multiplex reverse-transcription polymerase chain reaction. Phytopathology. 90(12), 1330-1336.

Spiegel, S., Thompson, D., Varga, A. and James, D. 2005. An apple chlorotic leaf spot virus isolate from ornamental dwarf flowering almond (Prunus glandulosa 'Sinensis'): detection and characterization. HortScience. 40(5), 1401-1404.

Sabanadzovic, S., Ghanem-Sabanadzovic, N.A., Rowhani, A., Grant, J.A. and Uyemoto, J.K. 2005. Detection of Cherry virus A, cherry necrotic rusty mottle virus and Little cherry virus 1 in California orchards. Journal of Plant Pathology. 87(3), 173-177.

Sakalidis, M.L., Hardy, G.E.S., and Burgess, T.I. 2011. Endophytes as potential pathogens of the baobab species Adansonia gregorii**:** a focus on the Botryosphaeriaceae. Fungal Ecology. 4, 1-14.

Salehi, M., Heydarnejad, J. and Izadpanah, K. 2005. Molecular characterization and grouping of 35 phytoplasmas from central and southern provinces in Iran. Iranian Journal of Plant Pathology. 41(1), 62-65.

Salehi, M., Izadpanah, K. and Heydarnejad, J. 2006. Phytoplasma diseases of fruit trees in germplasm and commercial orchards in Turkey. Journal of Plant Pathology. 88(2), 179-185.

Salem, N., Mansour, A., Al-Musa, A. and Al-Nsour, A. 2003. Seasonal variation of Prunus necrotic ringspot virus concentration in almond, peach, and plum cultivars. Phytopathologia Mediterranea. 42(2), 155-160.

Salmon, M., Vendrame, M., Kummert, J. and Lepoivre, P. 2002. Detection of apple chlorotic leaf spot virus using a 5'nuclease assay with a fluorescent 3' minor groove binder-DNA probe. Journal of Virological Methods, 104, 99-106.

Sampson, P.J., and Walker, J. 1982. An Annotated List of Plant Diseases in Tasmania. Department of Agriculture Tasmania, 121 pages.

Sanfaçon, H., Wellink, S., Le Gall, O., Karasev, A., Van der Vlugt, R.A.A., and Wetzel, T. 2009. Secoviridae: a proposed familyof plant viruses within the order Picornavirales that combines the families Sequiviridae and Comoviridae, the unassigned genera Cheravirus and Sadwavirus, and the proposed genus Torradovirus. Archives of Virology. 154, 899-907.

Sanchez-Navarro, J., Aparicio, F., Herranz, M., Minafra, A., Myrta, A. and Pallas, V. 2005. Simultaneous detection and identification of eight stone fruit viruses by one-step RT-PCR. European Journal of Plant Pathology. 111, 77-84.

Sano, T. 2003. Hop stunt viroid in plum and peach. Viroids. 165-167.

Sano, T., Mimura, R. and Oshsima, K. 2001. Phylogenetic analysis of hop and grapevine isolates of Hop stunt viroid supports a grapevine origin for hop stunt disease. Virus Genes. 22(1), 53-59.

Sano, T., Hataya, T., Terai, Y. and Shikata, E. 1989. Hop stunt viroid strains from dapple fruit disease of plum and peach in Japan. Journal of General Virology. 70, 1311-9.

Santi, F., Russell, K., Menard, M. and Dufour, J. 2004. Screening wild cherry (Prunus avium) for resistance to bacterial canker by laboratory and field tests. Forest Pathology. 34(6), 349-362.

Sarec, R., Moran, J., and Rodoni, B. 2003. An association of Apple chlorotic leaf spot virus strains with tree decline in plum. Abstract. 7.81, 8th International Congress of Plant Pathology, Christchurch, NZ.

Saric, A. and Velagic, Z. 1980. Viruses and virus-like diseases of maraska sour cherry (Cerasus acida ssp. Maraska). Acta Phytopathologica Academiae Scientiarum Hungaricae. 15, 367-369.

Sato, K., Yoshikawa, N. and Takahashi, T., 1993. Complete nucleotide sequence of the genome of an apple isolate of Apple chlorotic leaf spot virus. Journal of General Virology. 69, 1503-1507.

Savage, E.F. et al. 1953. Report on the Agricultural Experiment Stations 1953. Report on the Agricultural Experiment Stations 1953-1954. 177 pp.

Savino, V., Martelli, G.P., Digiaro, M. and Terlizzi, B.D. 1994. Virus and virus-like diseases of almond in south east Italy. Acta Horticulturae. 373, 299-308.

Shabi, E. 1997. Disease management of the almond pathogens Glomerella cingulata, Polystigma ochraceum and Tranzschelia pruni-spinosae. Bulletin OEPP. 27(4), 479-480.

Schaper, U. and Seemuller, E. 1982. Condition of the phloem and the persistence of mycoplasmalike organisms associated with apple proliferation and pear decline. Phytopathology. 72, 736-742.

Schaub, L. and Monneron, A. 2003. Phenology of Cacopsylla pruni, vector of apricot fruit yellows. [French]. Revue Suisse de Viticulture, Arboriculture et Horticulture. 35(2), 123-126.

Schena, L. and Ippolito, A. 2003. Rapid and sensitive detection of Rosellinia necatrix in roots and soils by real time Scorpion-PCR. Journal of Plant Pathology. 85, 15-25.

Schena, L., Nigro, F. and Ippolito, A. 2002. Identification and detection of Rosellinia necatrix by conventional and real-time Scorpion-PCR. European Journal of Plant Pathology. 108, 355-366.

Schena, L., Nigro, F. and Ippolito, A. 2004. Real-time PCR detection and quantification of soilborne fungal pathogens: the case of Rosellinia necatrix, Phytophthora nicotianae, P. citrophthora, and Verticillium dahliae. Phytopathologia Mediterranea. 43, 273-280.

Schimanski, H.H., Schmelzer, K. and Albrecht, H.J. 1975. The late flowering black cherry (Prunus serotina Ehrh.) as natural host of cherry leaf roll virus. [German]. Archiv fur Phytopathologie und Pflanzenschutz. 11(5), 329-334.

Schimanski, H.H., Schmelzer, K. and Albrecht, H.J. 1976. Seed transmission of cherry leaf roll virus in black cherry (Prunus serotina Ehrh.). [German]. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, II. 131(2), 117-119.

Schnabel, G., Ash, J.S. and Bryson, P.K. 2005. Identification and characterization of Armillaria tabescens from the southeastern United States. Mycological Research. 109(11), 1208-1222.

Schneider, H. 1946. The anatomy of peach and cherry phloem. Bulletin of the Torrey Botanical Club. 72, 137-156.

Schneider, B., Seemüller, E., Smart, C.D. and Kirkpatrick, B.C. 1995. Phylogenetic classification of plant pathogenic Mycoplasma like organisms or phytoplasmas. In: Razin S and TullyJG (eds). Molecular and Diagnostic Procedures in Mycoplasmology, Vol. 1, pp369-380. Academic press, San Diego.

Schneider, W.L., Sherman, D.J., Stone, A.L., Damsteegt, V.D. and Frederick, R.D. 2004. Specific detection and quantification of Plum pox virus by real-time fluorescent reverse transcription-PCR. Journal of Virological Methods. 120, 97-105.

Schubert, K., Ritschel, A., and Braun, U. 2003. A monograph of Fusicladium s. lat. (hyphomycetes). Schlechtendalia. 9, 1-132.

Scott, S.W. and Zimmerman, M.T. 2002. Distribution Maps of Plant Diseases.

Schulze, S., Bahnweg, G., Tesche, M. and Sandermann, H. 1995. Identification of European Armillaria species by restriction-fragment length polymorphisms of ribosomal DNA. European Journal of Forest Pathology. 25, 214-223.

Scott, S.W. and Zimmerman, M.T. 2001. American plum line pattern virus is a distinct ilarvirus. Acta Horticulturae. 550(1), 221-227.

Scott, S.W. and Zimmerman, M.T. 2001. Peach rosette, little peach, and red suture are diseases induced by a phytoplasma closely related to western X-disease. Acta Horticulturae. 550(2), 351-354.

Scott, S.W. and Zimmerman, M.T. 2008. Production of full length copies of the genomic RNAs of Prunus necrotic ringspot virus. Acta Horticulturae. 781, 47-51.

Scott, S.W., Zimmerman, M.T., Yilmaz, S., Zehr, E.I. and Bachman, E. 2001. The interaction between Prunus necrotic ringspot virus and prune dwarf virus in peach stunt disease. Acta Horticulturae. 550(1), 229-236.

Scotto La Massese, C., Marenaud, C. and Dunez, J. 1973. Analysis of a decline phenomenon of peach trees in the Eyrieux valley. Comptes Rendus des Seances de l'Academie d'Agriculture de France. 1973. 59(5), 327-339.

Seemüller, E., Foster, J.A. 1995. European stone fruit yellows. In: Ogawa, J.M., Zehr, E.I., Bird, G.W., Ritchie, D.F., Uriu, K., Uyemoto, J.K., eds. Compendium of stone fruit diseases. St. Paul, MN, USA: American Phytopathological Society, 59-60.

Seemüller, E. and Schneider, B. 2004. Taxonomic description of ‘Candidatus Phytoplasma mali’ sp. nov., ‘Candidatus Phytoplasma pyri’ sp. nov. and ‘Candidatus Phytoplasma prunorum’ sp. nov., the causal agents of apple proliferation, pear decline and European stone fruit yellows, respectively. International Journal of Systematic and Evolutionary Microbiology. 54, 1217-1226.

Seemuller, E., Schaper, U., and Zimbelmann, F. 1984. Seasonal variation in the colonization patterns of mycoplasmalike organisms associated with apple proliferation and pear decline. Zeitschrift fur Pflanzenkrankheiten und Pflanzenchutz (Journal of Plant Disease and Protection). 91(4): 371-382.

Seemüller, E., Marcone, E., Lauer, U., Ragozzino, A. and Göschl, M. 1998. Current status of molecular classifiation of the phytoplasmas. Journal of Plant Pathology. 80, 3-26.

Serrone, P.D., Starza, S.l., Krystai, L., Kolber, M. and Barba, M. 1998. Occurrence of apple proliferation and pear decline phytoplasmas in diseased pear trees in Hungary. Journal of Plant Pathology. 80(1), 53-58.

Sertkaya, G., Martini, M., Ermacora, P., Musetti, R. and Osler, R. 2005. Detection and characterization of phytoplasmas in diseased stone fruits and pear by PCR-RFLP analysis in Turkey. Phytoparasitica. 33, 380-390.

Sforza, R., Clair, D., Daire, X., Larrue, J. and Boudon-Padieu, E. 1998. The role of Hyalesthes obsoletus (Hemiptera: Cixiidae) in the occurrence of bois noir of grapevines in France. Journal of Phytopathology. 146(11/12), 549-556.

Shamloul, A., Hadidi, A., Minafra, A., Giunchedi, L., Waterworth, H. and Allam, E. 1995. Peach latent mosaic viroid: nucleotide sequencing of an Italian isolate, sensitive detection by RT-PCR and cRNA probe, and geographical distribution. Acta Horticulturae. 386, 522-530.

Shaw, C.G**.** 1973. Host fungus index for the Pacific Northwest - I. Hosts. Washington State University. Bulletin, Washington Agricultural Experimental Station. 765, 1-121.

Shaw III, C.G. and Kile, G.A. 1991 Armillaria Root Disease. Agriculture Handbook No. 691, Forest Service, United States Department of Agriculture, Washington, D.C. pp 233.

Shaw, M.E., Kirkpatrick, B.C. and Golino, D.A. 1993. The beet leafhopper-transmitted virescence agent causes tomato big bud disease in California. Plant Disease. 77, 290-295.

Shiller, J.B., Lebas, B.S.M., Horner, M., Pearson, M.N. and Clover, G.R.G. 2010. Sensitive detection of viruses in pollen using conventional and real-time reverse transcription-polymerase chain reaction. Journal of Phytopathology. 158(11/12), 758-763.

Shi, A., Kantartzi, S., Mmbaga, M. and Chen P. 2010. PCR-RFLP is a useful tool to distinguish two powdery mildew pathogens of flowering dogwood (Cornus florida). Agriculture and Biology journal of North America. 1(3), 208-212.

Shivas, R.G**.** 1989. Fungal and bacterial diseases of plants in Western Australia (review). Journal of the Royal Society of Western Australia. 72, 1-62.

Shivas, R.G., Smith, M.W., Marney, T.S., Newman, T.K., Hammelswang, D.L., Cooke, A.W., Pegg, K.G. and Pascoe, I.G. 2005. *First record of Nematospora coryli in Australia and its association with dry rot of Citrus.* Australian Journal of Agricultural Research. 34(1), 99-101.

Sicoli, G., Fatehi, J. and Stenlid, J. 2003. Development of species-specific PCR primers on rDNA for the identification of European Armillaria species. Forest Pathology. 33(5), 287-297.

Simmonds, J.H. 1966. Host index of plant diseases in Queensland. Queensland Department of Primary Industries, Brisbane, 111.

Sipahioglu, H.M., Ocak, M. and Usta, M. 2007. Comparison of three conventional extraction methods for the detection of plant virus/viroid RNAs from heat-dried high-phenolic host leaves. Asian Journal of Plant Sciences. 6(1), 102-107.

Sipahioglu, H.M., Usta, M. and Ocak, M. 2008. Occurrence of Cherry green ring mottle virus in Turkey. Plant Pathology. 57(2), 392.

Sipahioglu, H.M., Tekin, Z. and Usta, M. 2011. Detection and partial characterization of two distinct walnut isolates of cherry leaf roll virus (CLRV). African Journal of Biotechnology. 10(14), 2579-2587.

Sit, T.L., Haikal, P.R., Callaway, A.S. and Lommel, S.A. 2001. A single amino acid mutation in the Carnation ringspot virus capsid protein allows virion formation but prevents systemic infection. Journal of Virology. 75(19), 9538-9542.

Smith, P.R., Stubbs, L.L. 1976. Transmission of prune dwarf virus by peach pollen and latent infection in peach trees. Australian Journal of Agricultural Research**.** 27, 839-843.

Spiegel, S., Scott, S.W., Bowmanvance, V., Tam, Y., Galiakparov, N.N. and Rosner, A. 1996. Improved detection of Prunus necrotic ringspot virus by the polymerase chain reaction. European Journal of Plant Pathology. 102, 681-685.

Stace-Smith, R. 1985. Tobacco ringspot virus. CMI/AAB Descriptions of Plant Viruses No. 309 (No. 17 revised). Association of Applied Biologists, Wellesbourne, UK.

Stace-Smith, R. and Hansen, A.J. 1974. Occurrence of tobacco ringspot virus in sweet cherry. Canadian Journal of Botany. 52(7), 1647-1651.

Stobbe, A.H., Schneider, W.L., Hoyt, P.R. and Melcher, U. 2014. Screening Metagenomic Data for Viruses Using the E-Probe Diagnostic Nucleic Acid Assay. Phytopathology. 104(10), 1125-1129.

Suslow, K.G. and Purcell, A.H. 1982. Seasonal transmission of the X-disease agent from cherry by the leafhopper vector Colladonus montanus. Plant Disease. 66, 28-31.

Sutic, D. and Juretic, N. 1976. Occurrence of *sowbane mosaic virus* in plum tree. Acta Horticulturae. 67, 328.

Sutic, D.D., Ford, R.E., Tosic, M.T. 1999. Virus diseases of fruit trees. In: Handbook of Plant Virus Diseases. Boca Raton, Florida: CRC Press, 345-347.

Sweet, J.B. 1976. Prunus necrotic ringspot virus (PNRV) in ornamental and indigenous Prunus spp. Plant Pathology. 25(1), 55.

Sweet, J.B. 1980. Fruit tree virus infections of ornamental rosaceous trees and shrubs. Journal of Horticultural Science. 55(2), 103-111.

Sztejnberg, A. 1986. Etiology and control of cherry leaf spot disease in Israel caused by Cercospora circumscissa**.** Plant Disease. 70, 349-351.

Taubenhaus, J.J., and Ezekiel, W.N. 1936. A rating of plants with reference to their relative resistance or susceptibility to Phymatotrichum root rot. Texas Agricultural Experiment Station Bulletin. 527, 1-52.

Takahashi, T., Saito, N., Goto, M., Kawai, A., Namba, S. and Yamashita, S. 1990. Apple stem grooving virus isolated from Japanese apricot (Prunus mume) imported from China. Research Bulletin of the Plant Protection Service, Japan. 26, 15-21.

Takemoto, S., Nakamura, H., Sasaki, A. and Shimane, T. 2011. Species-specific PCRs differentiate Rosellinia necatrix from R. compacta as the prevalent cause of white root rot in Japan. Journal of General Plant Pathology. 77, 107-111.

Taylor, R., Guilford, P., Clark, R., Hale, C. and Forster, S. 2001. Detection of Erwinia amylovora in plant material using novel polymerase chain reaction (PCR) primers. New Zealand Journal of Crop and Horticultural Science. 29(1), 35-43.

Tedeschi, R. and Alma, A. 2004. Transmission of apple proliferation phytoplasma by Cacopsylla melanoneura (Homoptera: Psyllidae). Journal of Economic Entomology. 91(1): 8-13.

Tedeschi, R., and Alma, A. 2006. Fieberiella florii (Homoptera: Auchenorrhyncha) as a vector of "Candidatus Phytoplasma mali". Plant Disease. 90, 284-290.

Tedeschi, R., Bosco, D. and Alma, A. 2002. Population dynamics of *Cacopsylla melanoneura* (Homoptera: Psyllidae), a vector of apple proliferation in northwestern Italy. Journal of Economic Entomology. **95**, 544–51.

Tedeschi, R., Ferrato, V., Rossi, J. and Alma A. 2006. Possible phytoplasma transovarial transmission in the psyllids Cacopsylla melanoneura and Cacopsylla pruni. Plant Pathology. 55, 18-24.

Terlizzi, B.D., Digiaro, M. and Savino, V. 1994. Preliminary studies on virus like diseases of almond. Acta Horticulturae. 373, 293-298.

Uyemoto, J.K., Grant, J.A., Kruegar, W.H., Olson, W.H., Osgood, J.W., Sibbett, G.S., Viveros, M. and Weakley, C.V. 1989. Survey detects viruses in almonds, prune, and sweet cherry orchards. California Agriculture. 43(5), 14-15.

Thakur, P.D., Handa, A., Chowfla, S.C., Krczal, G. 1998. Outbreak of a phytoplasma disease of peach in the Northwestern Himalayas of India. Acta Horticulturae. 472, 737-742.

Thakur, P.D., Ito, T. and Sharma, J.N. 1995. Natural occurrence of a viroid disease of apple in India. Indian Journal of Virology. 11(2), 73-75.

Thébaud, G. 2005. Etude du développement spatio-temporel d'une maladie transmise par vecteur en intégrant modélisation statistique et expérimentation: cas de l'ESFY (European stone fruit yellows). 176 pages, PhD Thesis, SupAgro, Montpellier, France.

Thébaud G., Yvon M., Alary R., Sauvion N. and Labonne, G. 2009. Efficient transmission of 'Candidatus Phytoplasma prunorum' is delayed by eight months due to a long latency in its host-alternating vector. Phytopathology. 99, 265-273.

Theilmann, J., Mozafari, J., Reade, R., Wu, Z., Xie, W., Jesperson, G., Bernardy, M., Eastwell, K.C. and Rochon, D. 2002. Partial nucleotide sequence and genome organization of a Canadian isolate of Little cherry virus and development of an enzyme-linked immunosorbent assay-based diagnostic test. Phytopathology. 92(1), 87-98.

Thompson, J.R., Wetzel, S., Klerks, M.M., Vaskova, D., Schoen, C.D., Spak J. and Jelkmann, W. 2003. Multiplex RT-PCR detection of four aphid-borne strawberry viruses in Fragaria spp. in combination with a plant mRNA specific internal control. Journal of Virological Methods. 111, 85-93.

Thompson, J.R., Perry, K.L. and Jong, W.D. 2004. Nucleotide sequence analysis and detection of Cherry rasp leaf virus. Acta Horticulturae. 657, 99-101.

Tomlinson, J.A. and Faithfull, E.M. 1984. Studies on the occurrence of tomato bushy stunt virus in English rivers. Annals of Applied Biology. 104, 485-495.

Topchiiska, M., Marcone, C. and Seemuller, E. 2001. First report of a Phytoplasma disease of almond (Prunus amygdalus) in Lebanon. Plant Disease. 85(7), 802.

Topchiiska, M. and Sakalieva, D. 2003. Almond witches' broom phytoplasma: a potential threat to almond, peach, and nectarine. Canadian Journal of Plant Pathology. 25(1), 28-32.

Topchiiska, M. and Topchiiski, I. 1976. Isolation of cucumber mosaic virus from almond (Prunus amygdalus Stokes) in Bulgaria. Gradinarska i Lozarska Nauka. 13(3), 44-54.

Tremaine, J. H. 1970. The composition of tomato bushy stunt virus from Prunus arium. Phytopathology. 60, 454-456.

Tremaine, J.H. and Ronald, W.P. 1986. Highly basic cyanogen bromide peptides from the proteins of turnip rosette virus and the Prunus strain of tomato bushy stunt virus. Canadian Journal of Botany. 64(12), 3043-3046.

Trudgill, D.L., Brown, D.J.F. and McNamara, D.G. 1983. Methods and criteria for assessing the transmission of plant viruses by longidorid nematodes. Revue de Ne´matologie. 6, 133–141.

Tsopelas, P. 1999. Distribution and ecology of Armillaria species in Greece. European Journal of Forest Pathology. 29(2), 103-116.

Tsopelas, P. and Tjamos, E.C. 1997. Occurrence and pathogenicity of Armillaria tabescens on almond in Greece. Bulletin OEPP. 27(4), 455-461.

Tuset, J.J., Hinarejos, C. and Portilla, M.T. 1999. First report of fruit rot and associated branch dieback of almond in California caused by a Phomopsis species tentatively identified as P. amygdali. Plant Disease. 83(11), 1073.

Ulubas, C. and Ertunc, F. 2005. Apple chlorotic leaf spot virus (ACLSV) status in Turkey and sensitive detection using advanced techniques. Turkish Journal of Agriculture and Forestry. 2005. 29(4), 251-257.

Ulubaş Serçe, Ç., Candresse, T., Svanella-Dumas, L., Krizbai, L., Gazel, M. and Çağlayan, K. 2009. Further characterization of a new recombinant group of Plum pox virus isolates, PPV-T, found in the Ankara province of Turkey. Virus Research. 142, 121–126.

Ulubaş Serçe, Ç., Ertunç, F. and ÖZtürk, A. 2009. Identification and Genomic Variability of Prune dwarf virus Variants Infecting Stone Fruit Trees in Turkey. Journal of Phytopathology. 157(5), 298-305.

Uppalapati, S.R., Young, C.A., Marek, S.M. and Mysore, K.S. 2010. Phymatotrichum (cotton) root rot caused by Phymatotrichopsis omnivora: retrospects and prospects. Molecular Plant Pathology 11(3), 325-334.

Usta, M., Sipahioglu, H.M., Ocak, M. and Myrta, A. 2007. Detection of Apricot latent virus and Plum bark necrosis stem pitting-associated virus by RT-PCR in Eastern Anatolia (Turkey). Bulletin OEPP/EPPO Bulletin. 37(1), 181-185.

Uyemoto JK. 1989. Union aberration of sweet cherry on Prunus mahaleb rootstock associated with X disease. Plant Disease. 73, 899-902.

Uyemoto, J.K. 1997. Infectious diseases of almond with special reference to the Mediterranean area. Bulletin OEPP. 27(4), 525-534.

Uyemoto, J.K. 1997. Union disorders of almond trees on plum rootstock. Bulletin OEPP. 27(4), 565-568.

Uyemoto, J.K. and Scott, S.W. 1992. Important diseases of Prunus caused by viruses and other graft-transmissible pathogens in California and South Carolina. Plant Disease 76(1), 5–11.

Uyemoto, J.K. and Teviotdale, B.L. 1996. Graft-transmission of the causal agent of a bark necrosis-stem pitting disease of Black Beaut plum (Prunus salicina). (Abstract) Phytopathology. 86, 111–112.

Uyemoto, J.K., Asai, W.K. and Kirkpatrick, B.C. 2000. Detection of pear decline and European stone fruit yellows in Bulgaria. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz. 107(6), 658-663.

Uyemoto, J.K., Beede, R.H., Connell, J.H., Vivero, M. and Yoshikawa, F.T. 1993. Reduced incidence of ilarviruses in almond and peach in California. Plant Disease. 77(7), 756.

Uyemoto, J.K., Connell, J.H. and Greer, C.A. 1998. Yellow canopy disorders of almond trees in California. Acta Horticulturae. 470, 534-538.

Uyemoto, J.K. and Gilmer, R.R. 1972. Properties of Tobaccco nerosis virus strains isolated from apple. Phytopathology. 62,478-481.

Uyemoto, J.K., Rowhani, A. and Luhn, C.F. 1996. Mechanical transmission of Prunus necrotic ringspot virus to young trees of Nemaguard peach and Nanking cherry. Plant Disease. 80(1), 104.

Uyemoto, J.K., Welsh, M.F. and Williams, E. 1977. Pathogenicity of tobacco ringspot virus in cherry. Phytopathology. 67(4), 439-441.

Valiunas, D., Davis, R.E. and Jomantiene, R. 2009. Establishment of a new Phytoplasma subgroup, 16SRI-Q, to accommodate a previously undescribed Phytoplasma found in diseased cherry in Lithuania. Journal of Plant Pathology. 91, 71-75.

Vanev, S.G., Sameva, E.F., and Bakalova, G.G. 1997. Order Sphaeropsidales. Fungi Bulgaricae. 3, 1-335.

VanGuilder, H.D., Vrana, K.E. and Freeman, W.M. 2008. Twenty-five years of quantitative PCR for gene expression analysis. BioTechniques. 44,619-626. doi 10.2144/000112776

Vanneste, J.L., Lex, S., Vermeulen, M., Berger, F. 2002. Isolation of Erwinia amylovora from blighted plums (Prunus domestica) and potato roses (Rosa rugosa). Acta Horticulturae. 590, 89-94.

Van Oosten, H.J. 1970. Herbaceous host plants for the sharka (plum pox) virus. Netherlands Journal of Plant Pathology. 76, 253-260.

Varveri, C., Holeva, R. and Bem, F.P. 1997. Effect of sampling time and plant part on the detection of two viruses in apricot and one in almond by ELISA. Annales de l'Institut Phytopathologique Benaki. 18(1), 25-33.

Vaskova, D., Petrzik, K., ˇSpak, J. 2000. Molecular variability of the capsid protein of the prune dwarf virus. European Journal of Plant Pathology. 106, 573–580.

Végh, A., Némethy, Z.S., Hajagos L, Palkovics L (2012). First Report of Erwinia amylovora Causing Fire Blight on Plum (Prunus domestica) in Hungary. Plant Disease 96(5):759.

Verdin, E., Salar, P., Danet, P., Danet, J.L., , Gelie, B., Bove, J.M., Garnier, M., Choueiri, E., Jreijiri, F. and El-Zammar, S. 2004. Phylogenetical characterization and PCR detection of a new

phytoplasma in almond (Prunus amygdalus) and peach (Prunus persicae) in the Mediterranean area. Acta Horticulturae. 657, 527-532.

Villamor, D.E.V., and Eastwell, K.C. 2013. Viruses associated with rusty mottle and twisted leaf diseases of sweet cherry are distinct species. Phytopathology. 103, 1287-1295.

Villamor, D.E.V., Ward, K.F., Collman, S.J., and Eastwell, K.C. 2014. First report of infection of cherry rusty mottle associated virus in Portuguese laurel (Prunus lusitanica) in Washington State. Plant Disease. 98 (5), 699.

Villamor, D.V., Druffel, K.L., and Eastwell, K.C. 2013. Complete nucleotide sequence of a virus associated with rusty mottle disease of sweet cherry (Prunus avium). Archives of Virology. 158, 1805-1810.

Villamor, D.E.V., Susaimuthu, J., and Eastwell, K.C. 2015. Genomic analyses of cherry rusty mottle group and cherry twisted leaf-associated viruses reveal a possible new genus within the family Betaflexiviridae. Phytopathology. 105, 399-408.

Vitushkina, M., Fechtner, B., Agranovsky, A. and Jelkmann, W. 1997. Development of an RT-PCR for the detection of little cherry virus and characterization of some isolates occurring in Europe. European Journal of Plant Pathology. 103(9), 803-808.

Vuittenez, A. and Kuszala, J. 1971. Arabis mosaic virus associated with an enation disease in kirsch Cherry (Prunus avium L.) in the Vosges. Annales of Phytopathologie. 1971. 3(4), 485-491.

Wagnon, H.K., Traylor, J., Williams, H.E. and Weiner, A.C. 1969. The transmission of Cherry rasp leaf virus by Xiphinema americanum. Phytopathology. 59(8), 1111-1112.

Walia, Y., Dhir, S., Bhadoria, S., Hallan, V. and Zaidi, A. A. 2012. Molecular characterization of Apple scar skin viroid from Himalayan wild cherry. Forest Pathology. 42, 84–87. doi: 10.1111/j.1439-0329.2011.00723.x

Walker, G.E. 2004. New Australian record for Xiphinema vuittenezi on Vitis vinifera. Australasian Plant Pathology. 33(1), 131-132.

Wallace, J.M. and Drake, R.J. 1960. Woody galls on citrus associated with vein-enation virus infection. Plant Disease Reporter. 44, 580-584.

Wang, L.P., Hong, N., Wang, G.P., Michelutti, R. and Zhang, B.L. 2009. First report of Cherry green ring mottle virus in plum (Prunus domestica) in North America. Plant Disease. 93(10), 1073.

Waterworth, H.E. and Kaper, J.M. 1980. Cucumber mosaic virus from Prunus domestica: some diseases incited in herbaceous species in the presence and absence of a small replicating RNA. Acta Phytopathologica Academiae Scientiarum Hungaricae. 15, 123-127.

Waterworth, H.E. and Fulton, R.W., 1964. Variation among isolates of necrotic ringspot and prune dwarf viruses isolated from sour cherry. Phytopathology. 54, 1155–1160.

Watson, M.A. 1949. Some notes on plant virus diseases in South Australia. [not specified]. Journal of the Australian Institute of Agricultural Science. 15, 76-81.

Wei, T., Lu, G. and Clover, G. 2008. Novel approaches to mitigate primer interaction and eliminate inhibitors in multiplex PCR, demonstrated using an assay for detection of three strawberry viruses. Journal of Virological Methods. 151(1), 132-139.

Weisberg, W.G., Barns, S., Pelletier, D.A. and Lane, D.J. 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology. 173, 697-703.

Werner, R., Mühlbach, H. and Büttner, C. 1997. Detection of cherry leaf roll nepovirus (CLRV) in birch, beech and petunia by immune-capture RT-PCR using a conserved primer pair. European Journal of Forest Pathology. 27, 309-318.

Westhuizen, G.C.A.V.D. 1975. Laetiporus sulphureus. [Descriptions of Fungi and Bacteria]. IMI Descriptions of Fungi and Bacteria. 45, Sheet 441.

Wetzel, T., Candresse, T., Macquaire, G., Ravelonandro, M. and Dunez, J. 1992. A highly sensitive immunocapture polymerase chain reaction method for plum pox potyvirus detection. Journal of Virological Methods. 39, 27-37.

Wetzel, T., Candresse, T., Ravelonandro, M. and Dunez, J. 1991. A polymerase chain reaction assay adapted to plum pox potyvirus detection. Journal of Virological Methods. 33, 355-365.

Wetzel, T., Jardak, R., Meunier, L., Ghorbel, A., Reustle, G.M. and Krczal, G. 2002. Simultaneous RT/PCR detection and differentiation of arabis mosaic and grapevine fanleaf nepoviruses in grapevines with a single pair of primers. Journal of Virological Methods. 101(1/2), 63-69.

Wei, T. and Clover, G. 2008. Use of primers with 5_ non-complementary sequences in RT-PCR for the detection of nepovirus subgroups A and B. Journal of Virological Methods. 153, 16-21.

Wheeler, J.E. and Hine, R.B. 1972. Influence of soil temperature and moisture on survival and growth of strands of Phymatotrichum omnivorum. Phytopathology. 62, 828-832.

Wilks, J.M. and Welsh, M.F. 1961. Host range studies of the little Cherry disease virus. Canadian Journal of Plant Science. 41(3), 544-548.

Wilkinson, R.E. 1952. Woody plant hosts of the tobacco ringspot virus. Phytopathology. 42(9), 478.

Williams, M.A.J. 1987. Cercospora rubro-tincta**.** C.M.I. Descriptions of Pathogenic Fungi and Bacteria. 937, 1-2.

Williams, H.E., Jones, R.W., Traylor, J.A. and Wagnon, H.K. 1970. Passage of necrotic ringspot virus through Almond seeds. Plant Disease Reporter. 54(10), 822-824.

Wood, G.A. and Fry, P.R. 1970. Little Cherry virus found in New Zealand Cherries. Orchardist of New Zealand. 43(7), 233-234.

Wylie, S., Wilson, C.R., Jones, R.A.C. and Jones, M.G.K. 1993. A Polymerase chain reaction assay for Cucumber mosaic virus in lupin seeds. Australian Journal of Agricultural Research. 44, 41-51.

Yang, W., Zheng, Y., Chen, Z., Zhang, G. and Wu, S. 2007. Detection of Tobacco ringspot virus by RT-Realtime PCR. Acta Phytophylacica Sinica. 34(2), 157-160.

Yoshikawa, N., Matsuda, H., Oda, Y., Isogai, M., Takahashi, T., Ito, T., and Yoshida, K. 2001. Genome heterogeneity of apple stem pitting virus in apple trees. Acta Horticulturae. 550, 285-290.

Young, J.M. 1988. Pseudomonas syringae pv. persicae from nectarine, peach, and Japanese plum in New Zealand. Bulletin OEPP/EPPO Bulletin 18, 141-151.

Youssef, S.A. and Shalaby, A.A. 2009. Single-step multiplex reverse transcription-polymerase chain reaction (m-RT-PCR) for simultaneous detection of five RNA viruses affecting stone fruit trees. International Journal of Virology. 5(2), 100-108.

Youssef, A.S., Shalaby, A.A., Mazyad, H.M. and Hadidi, A. 2002. Detection and identification of Prune dwarf virus and Plum pox virus by standard and multiplex RT-PCR probe capture hybridization (RT-PCR-ELISA). Journal of Plant Pathology. 84, 113-119.

Yorston, J.M., McMullen, R.D., Slykhuis, J.T. and Welsh, M.F. Little cherry disease in British Columbia. Publication, British Columbia Ministry of Agriculture and Food. 1981. 81-4.

Yvon, M., Thébaud, G., Alary, R. and Labonne, G. 2009. Specific detection and quantification of the phytopathogenic agent 'Candidatus Phytoplasma prunorum'. Molecular and Cellular Probes. 23, 227-234.

Zawadzka, B. 1986. Raspberry ringspot virus infection in plum trees. Acta Horticulturae. 193, 49-50.

Zeller, S.M. and J.A. Milbrath. 1947. Mild rusty mottle of sweet cherry (Prunus avium). Phytopathology. 37, 77-84.

Zemtchik, E.Z. and Verderevskaya, T.D. 1993. Latent virus on apricot unknown under Moldavian conditions. Selskohozeaystvennaya Biologia (Russian Agricultural Biology). 3, 130-133.

Zemtchik, E.Z., Verderevskaya, T.D. and Kalashian, Y.A. 1998. Apricot latent virus: transmission, purification and serology. Acta Horticulturae. 472, 153-158.

Zhang, Y. P., Mink, G. I., Tiffany, M. G., and Howell, W. E. 1992. Isolation of viruses associated with cherry twisted leaf, apricot ring pox, and apricot pit pox diseases and their relationship to apple stem pitting virus. Phytopathology. 82,1149.

Zhang, Y.P., Kirkpatrick, B.C., Smart, C.D. and Uyemoto, J.K. 1998. cDNA cloning and molecular characterization of cherry green ring mottle virus. Journal of General Virology. 79(9), 2275-2281.

Zhang, Y.P., Kirkpatrick, B.C., Terlizzi, B.D. and Uyemoto, J.K. 2000. Comparison of cherry green ring mottle virus strains using RT-PCR and coat protein sequence phylogeny. Journal of Plant Pathology. 82(1), 49-53.

Zhang, J.X., Fernando, W.G.D. and Remphrey, W.R. 2005a.Genetic diversity and structure of the Apiosporina morbosa populations on Prunus spp. Phytopathology. 95(8), 859-866.

Zhang, J. X., Fernando, W. G. D., and Remphrey, W. R. 2005b. Molecular detection of Apiosporina morbosa, causal agent of black knot in Prunus virginiana. Plant Disease. 89, 815-821.

Zhao, Y. and Niu, J. 2006. Cloning and sequencing of Sinkiang isolate of apple scar skin viroid (ASSVd). [Chinese]. Journal of Fruit Science. 23(6), 896-898.

Zhao, Y. and Niu, J. 2007. Detection of apple scar skin viroid in pear cultivars by RT-PCR and spot hybridization in Xinjiang area. [Chinese]. Journal of Fruit Science. 24(6), 761-764.

Zhang, J.X., Fernando, W.G.D. and Remphrey, W.R. 2005. Genetic diversity and structure of the Apiosporina morbosa populations on Prunus spp. Phytopathology. 95(8), 859-866.

Zhou, Y., Guo, R., Cheng, Z., Sano, T. and Li, S.F. 2006. First report of Hop stunt viroid from Prunus persica with dapple fruit symptoms in China. Plant Pathology. 55(4), 564.

Zhou, J., Wang, G., Kuang, R., Wang, L. and Hong, N. 2011. First report of Cherry green ring mottle virus on cherry and peach grown in China. Plant Disease. 95(10), 1319. DOI: 10.1094/PDIS-04-11-0326

Zhou, J. F., Wang, G. P., Qu, L. N., Deng, C. L., Wang, Y., Wang, L. P., and Hong, N. 2013. First Report of Cherry necrotic rusty mottle virus on Stone Fruit Trees in China. Plant Disease. 97, 290-290.

Zhu, S.F., Hadidi, A., Yang, X., Hammond, R.W. and Hansen, A.J. 1995. Nucleotide sequence and secondary structure of pome fruit viroids from dapple apple diseased apples, pear rusty skin diseased pears and apple scar skin symptomless pears. Acta Horticulturae. 386, 554-559.

Zirak, L., Bahar, M. and Ahoonmanesh, A. 2010. Molecular characterization of phytoplasmas associated with peach diseases in Iran. Journal of Phytopathology. 158(2), 105-110.

Zirak, L., Bahar, M. and Ahoonmanesh, A. 2009. Psyllids (Hemiptera, Psylloidea) captured in commercial apple and stone fruit orchards in southwest Germany, eastern France and northwest Switzerland. Mitteilungen der Schweizerischen Entomologischen Gesellschaft. 82(3/4), 205-215.

Zirak, L., Bahar, M. and Ahoonmanesh, A. 2009. Molecular characterization of phytoplasmas related to peanut witches' broom and stolbur groups infecting plum in Iran. Journal of Plant Pathology. 91(3), 713-716.

Zitikaite, I. and Staniulis, J. 2006. The use RT-PCR for detection of viruses infecting cucumber. Agronomy Research. 4, 471-474.

Zitikaite, I., Staniulis, J., Navalinskiene, M. and Kaseta, V. 2005. Detection of Tobacco necrosis virus (TNV) in Phaseolus vulgaris plants. Phytopathologia Polonica. 35, 141-145.

Appendix 8: Conference abstracts, papers and industry articles

XXIX International Horticultural Congress: IHC2014

The partial characterization of Ilarviruses infecting *Prunus* species in Australia.

W. Kinoti, F. Constable,N. Nancarrow and B. Rodoni
AgriBio, 5 Ring Road
Bundoora, Victoria 3086
Australia

K. Plummer
Department of Botany
Latrobe University
Bundoora, Victoria 3086
Australia

Keywords: *Ilarvirus*, *Prunus*, PNRSV, ApMV, PDV, RT-PCR, variation, diversity

Abstract

Ilarviruses infect *Prunus* species with significant economic impact on commercial *Prunus* industries in Australia. Important *Ilarvirus* species of *Prunus* species in Australia are *Apple mosaic virus* (ApMV), *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus (*PDV) and their diversity and incidence in Australia is not well understood. To understand their strain variation, 178 *Prunus* tree samples were tested using species-specific *Ilarvirus* RT-PCR tests targeting the coat protein gene of PNRSV, ApMV and PDV and genus-specific RT-PCR test that targets the RdRP gene of Ilarviruses. Variation in the detection of ilarviruses between the species-specific and genus-specific RT-PCR tests indicated genetic variation of ilarviruses in the *Prunus* trees. Selected samples were inoculated on cucumber (*Cucumis sativus*) indicators and a variation in symptom expression and detection of ilarviruses using the species-specific and genus-specific RT-PCR tests was observed. The PCR products from the *Prunus* tree and cucumber indicators were sequenced and phylogenetic analysis of the coat protein and RdRP sequences showed clustering of cucumber *Ilarvirus* isolates away from the *Prunus* tree isolates suggesting that the cucumber indicators were selecting for specific sequence variants. Further sequence analysis indicated presence of genetic variation amongst *Ilarvirus* variants in *Prunus* tree and the cucumber indicators were selecting for these sequence variants.

INTRODUCTION

Ilarviruses can infect a large number of cultivated fruit trees including *Prunus* species such as stone fruits and almonds (Uyemoto & Scott, 1992) with considerable economic impact on commercial *Prunus* industries. *Ilarvirus* species are a group of isometric labile viruses that are characterized by a tripartite positive sense RNA genome similar to all members of the family *Bromoviridae* (Van Regenmortel et al 2013).

The three most common and economically important *Ilarvirus* species of *Prunus* species in Australia are *Apple mosaic virus* (ApMV), *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus (*PDV). These three viruses can occur as single infections but they also frequently occur as mixed infections that further increase their damage to *Prunus* species (Nemeth et al 1986; Uyemoto & Scott, 1992). These three viruses are transmitted by grafting, pollen and seed, and this has contributed to their worldwide distribution, including Australia (Mink, 1993; Uyemoto & Scott, 1992).

The incidence and diversity of ilarviruses affecting *Prunus* has not been well documented in many parts of the world including Australia with an exception of a wide geographic field survey

carried out in Europe and the Mediterranean region (Gümüs et al 2007; Myrta et al 2003). This survey demonstrated a high incidence (23.5%) of *Ilarvirus* infection with mixed infections recorded in 76.4% of the 24,000 trees sampled. This high incidence of *Ilarvirus* infection coupled with reported high yield losses of up to 70% depending on *Prunus species* affected (Çağlayan et al 2011; Cembali et al 2003; Uyemoto & Scott, 1992) underpins the importance of understanding their incidence and diversity in Australia *Prunus* industries.

The aim of this study is to use advanced molecular technologies to characterize isolates of PNRSV, PDV and ApMV and related ilarviruses from different hosts and different regions of Australia for development of molecular diagnostic tools to survey their incidence.

MATERIALS AND METHODS

Plant Material

Prunus tree samples consisting of 178 stone fruits and almonds that were showing symptoms or were symptomless were collected from Knox, Mildura and Gippsland and Queensland.

Nucleic Acid Extraction

RNA was extracted from 0.3g of plant material from each sample using the RNeasy® Plant Mini Kit (QIAGEN Pty Ltd, Doncaster, VIC Australia) as described previously (MacKenzie et al 1997).

Virus Detection RT-PCR

Species-specific RT-PCR primers for PNRSV, ApMV and PDV were used to amplify 455 bp, 261 bp and 172 bp product of the coat protein gene respectively (MacKenzie et al 1997; Parakh et al 1994; Petrzik & Svoboda, 1997). The samples were also tested using a *Ilarvirus* genus-specific RT-PCR test that amplifies 371 bp product of the RNA-dependent RNA-polymerase (RdRp) respectively (Maliogka et al 2007).

After amplification, 10 µL of each RT-PCR reaction was run on a 2% agarose gel using 0.5 × TBE (0.045 M Tris-borate, 1 mM EDTA, pH 8.0) running buffer at 100 V, stained with SYBR® Safe (Invitrogen ™ Life Technologies) incorporated in the gel and visualised on a UV transilluminator. A 1 Kb DNA Ladder (Invitrogen ™ Life Technologies) was used alongside the samples to determine the size of the resulting PCR products.

The results of the species-specific and genus-specific RT-PCR tests were then used to generate *Prunus* tree RT-PCR profiles.

Inoculations

Prunus tree samples from Mildura and Queensland that previously tested positive for ilarviruses were used to inoculate cucumber (*Cucumis sativus*) indicators grown under normal glasshouse conditions. Briefly, 1g of plant tissue was ground in 5ml of 0.1M phosphate buffer (pH 7.0) containing 0.1% sodium sulphite and 0.01M 2- mercaptoethanol and the homogenate was gently rubbed on leaves of 10 day old cucumber indicators that were lightly dusted with carborundum powder. Symptom development was observed during 15 days, after which total RNA was extracted and tested using the *Ilarvirus* species-specific and genus-specific RT-PCR tests as described above. The symptom observations and the RT-PCR test results were used to generate a biological/PCR profile of ilarviruses present in the inoculated cucumber plants.

Sequencing and Analysis.

Selected species-specific RT-PCR products from the *Prunus* trees and the cucumber indicators were directly sequenced. The genus-specific RT-PCR products were cloned using the pGEM-T Easy Vector system according to the manufacturer's protocol (Promega). Five clones from

each sample were selected for plasmid purification using a small-scale alkaline plasmid preparation (Birnboim & Doly, 1979) and sequenced once in each direction using SP6 and T7 primers.

Species-specific and genus-specific nucleotide sequences and amino acid similarity were computed. A maximum likelihood phylogeny tree for species specific and genus specific nucleotide sequences was inferred using MEGA (version 5.1) (Tamura et al 2011).

RESULTS AND DISCUSSION

PNRSV was detected more frequently than ApMV and PDV using the species-specific primers. The occurrence of the three different PCR profiles (1, 2 and 3; Table 1) generated from the *Prunus* trees, which were based on the results of the *Ilarvirus* species-specific and genus-specific RT-PCR tests results indicated genetic variation in the *Ilarvirus* species that were present.

Only PNRSV was successfully inoculated onto the cucumber indicators and some of these inoculated plants showed symptoms while others were symptomless despite testing positive with either the PNRSV specific-test or the genus-specific tests. This indicates that herbaceous indexing is not reliable for PNRSV detection. The six symptom/RT-PCR profiles observed on the cucumber indicators also provided evidence of genetic variation amongst the ilarviruses that were detected (Table 2).

Analysis of the coat protein and RNA-dependent RNA-polymerase (RdRp) sequences confirmed the occurrence of genetic variability amongst PNRSV isolates with nucleotide identity of between 86 to 100% between the PNRSV isolates observed. Phylogenetic analysis of the PNRSV coat protein and RdRp nucleotide sequences (data not shown) showed clustering of cucumber indicators isolates, which were located in a different clade to the rest of the *Prunus* tree isolates suggesting that inoculation to cucumber selects for particular PNRSV isolates.

Computed nucleotide identities between sequences from the cucumber-inoculated *Ilarvirus* and the original *Prunus* tree showed 2 to 3% differences for the coat protein (Table 3). Comparison of the RdRp nucleotide sequences from cucumber indicated four groupings of PNRSV isolates from cucumber, sequences in two groups shared 100% similarity while sequences in the remaining two groups shared only 90% similarity (Table 4). Sequence variation observed amongst the cucumber-inoculated *Ilarvirus* could however not be related to the different symptoms observed and this may be due to the partial *Ilarvirus* genome regions this study focused on.

CONCLUSIONS

The variation observed in the *Prunus* tree RT-PCR profiles and cucumber/RT-PCR profiles have been confirmed with the nucleotide analysis indicating presence of sequence variants in the *Prunus* trees that elicit different symptoms when inoculated on to cucumber plants. This sequence variation between the *Prunus* tree and cucumber indicators shows that there is a diversity of *Ilarvirus* populations in *Prunus* trees and that the cucumber indicators were selecting or filtering certain sequence variants.

Literature Cited

Birnboim HC, Doly J (1979) A rapid alkaline extractionprocedure for screening recombinant plasmid DNA. *Nucleic Acids Research* **7:** 1513–1523

Çağlayan K, Ulubas-Serce C, Gazel M, Varveri C (2011) *Prune dwarf virus*. *Virus and Virus-Like Diseases of Pome and Stone Fruits***:** 199-205

Cembali T, Folwell RJ, Wandschneider P, Eastwell KC, Howell WE (2003) Economic implications of a virus prevention program in deciduous tree fruits in the US. *Crop Protection* **22:** 1149-1156

Gümüs M, Paylan I, Matic S, Myrta A, Sipahioglu H, Erkan S (2007) Occurrence and distribution of stone fruit viruses and viroids in commercial plantings of *Prunus* species in western Anatolia, Turkey. *Journal of Plant Pathology* **89:** 265-268

MacKenzie DJ, McLean MA, Mukerji S, Green M (1997) Improved RNA Extraction from Woody Plants for the Detection of Viral Pathogens by Reverse Transcription-Polymerase Chain Reaction. *Plant Disease Reporter* **81** 222-226

Maliogka V, Dovas C, Katis N (2007) Demarcation of ilarviruses based on the phylogeny of RNA2-encoded RdRp and a generic ramped annealing RT-PCR. *Archives of virology* **152:** 1687-1698

Mink G (1993) Pollen and seed-transmitted viruses and viroids. *Annual Review of Phytopathology* **31:** 375-402

Myrta A, Di Terlizzi B, Savino V, Martelli G (2003) Virus diseases affecting the Mediterranean stone fruit industry: a decade of surveys. *Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region Bari: CIHEAM (Centre International de Hautes Etudes Agronomiques Méditerranéennes) Options Méditerranéennes, Série B***:** 15-23

Nemeth M, Szalay-Marzsó L, Posnette A (1986) *Virus, mycoplasma and rickettsia diseases of fruit trees*: Akademiai Kiado Budapest.

Parakh D, Shamloul A, Hadidi A, Waterworth H, Scott S, Howell H, Mink G (1994) Detection of prune dwarf *Ilarvirus* from infected stone fruits using reverse transcription-polymerase chain reaction. In *XVI International Symposium on Fruit Tree Virus diseases 386*, pp 421-430.

Petrzik K, Svoboda P (1997) Screening of *Apple mosaic virus* in hop cultivars in the Czech Republic by reverse transcription-polymerase chain reaction. *Acta virologica* **41:** 101-103

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution* **28:** 2731-2739

Uyemoto J, Scott S (1992) Important diseases of *Prunus* caused by viruses and other graft-transmissible pathogens in California and South Carolina. *Plant Disease* **76:** 5-11

Van Regenmortel MH, Ackermann H-W, Calisher CH, Dietzgen RG, Horzinek MC, Keil GM, Mahy BW, Martelli GP, Murphy FA, Pringle C (2013) Virus species polemics: 14 senior virologists oppose a proposed change to the ICTV definition of virus species. *Archives of virology* **158:** 1115-1119

Table 1. PCR profiles of ilarviruses generated from *Prunus* samples using species and genus specific *Ilarvirus* RT-PCR tests and the total number of samples in each profile.

Species/genus specific RT-PCR profile:			RT-PCR results			
Profiles	Species/Genus	Total :	PNRSV	ApMV	PDV	*Ilarvirus* genus
✓ Profile 1:	+/+	56/168 :	39	8	9	56
✓ Profile 2:	+/-	18/168 :	11	6	1	-
✓ Profile 3:	-/+	6/168 :	-	-	-	6
✓ Profile 4:	-/-	88/168 :	-	-	-	-

Table 2. Cucumber/biological-PCR profiles of transmitted ilarviruses, based on symptom expression on inoculated cucumbers and PNRSV and *Ilarvirus* genus specific RT-PCR tests and the total number of samples in each profile.

Profiles	Symptoms	PCR profile	Total samples
✓ 1	Symptomatic	PNRSV +ve/Genus -ve	7
✓ 2	Symptomatic	PNRSV +ve/Genus +ve	2
✓ 3	Symptomatic	Genus +ve/PNRSV -ve	3
✓ 4	Symptomless	PNRSV +ve/Genus +ve	5
✓ 5	Symptomless	Genus +ve/PNRSV -ve	3

Table 3. Coat protein nucleotide sequence identity of PNRSV isolates detected in selected samples of *Prunus* tree and cucumber indicators. Column in blue indicates two PNRSV isolates with the lowest sequence similarity compared to the rest of PNRSV isolates sequenced. The red, green and yellow column highlights the sequence similarities between selected cucumber indicators and their corresponding *Prunus* tree isolates used for inoculation.

	Knox64	Knox74	CMildura5	CQLD2	CQLD16	Mildura5	QLD2	QLD16
Knox64	100							
Knox74	100	100						
CMildura5	86.3	86.3	100					
CQLD2	86.3	86.3	100	100				
CQLD16	85.8	85.8	99.5	99.5	100			
Mildura5	86.6	86.6	98.5	98.5	98	100		
QLD2	86.6	86.6	98.3	98.3	97.8	98.8	100	
QLD16	86.1	86.1	98	98	97.5	98.5	98.8	100

Table 4. RNA-dependent RNA-polymerase (RdRp) nucleotide sequence identity of *Ilarvirus* isolates from selected PNRSV positive *Prunus* tree and cucumber indicator plants. Column in blue indicates clones with the lowest sequence similarity compared to the rest of PNRSV isolates sequenced. Red and orange indicates cucumber isolates grouping that were 100% similar within each grouping while green and yellow indicates the comparison of nucleotide identities between these two groupings.

	K64-1	K64-5a	CM12-4a	CM20-4a	M11-3a	M33-1a	CM5-2a	CM5-3a
K64-1	100							
K64-5a	99.4	100						
CM12-4a	87.5	87.5	100	100				
CM20-4a	87.5	87.5	100	100				
M11-3a	89.8	89.2	90.7	90.7	100			
M33-1a	89.2	89.2	91.2	91.2	98.9	100		
CM5-2a	89	88.4	90.4	90.4	98.6	99.2	100	100
CM5-3a	89	88.4	90.4	90.4	98.6	99.2	100	100

Australian Nutgrower 2013

Development of molecular diagnostic tools to detect endemic and exotic pathogens of *Prunus* species for Australia

Fiona Constable[1], Wycliff Kinoti[1,2,] Narelle Nancarrow[1] and Brendan Rodoni[1,2]

[1]AgriBio, Department of Environment and Primary Industries, 5 ring Road, Bundoora, Victoria 3083

[2]La Trobe University, 5 ring Road, Bundoora, Victoria 3083

The biosecurity of the Australian almond and summerfruit industries are maintained at the border by the Department of Agriculture Fisheries and Forestry (DAFF; formerly Australian Quarantine Inspection Service, AQIS) in post entry quarantine (PEQ) facilities and internally through schemes that supply high-health planting material throughout Australia. Over the last 5 years approximately 250 accessions of *Prunus* species, including summerfruit varieties and almonds, have been screened for quarantine pests and pathogens and released from Australian PEQ stations. Currently DAFF recognises two bacteria, six phytoplasmas or phytoplasma-like diseases, 13 viruses, one viroid and 70 fungi, to be of quarantine significance for *Prunus* species. DAFF also recognises that there are many diseases of *Prunus* species of unknown aetiology.

On arrival into Australian PEQ, imported *Prunus* propagation material, including almond, is inspected for insect pests and disease symptoms. If there are no obvious disease symptoms, the budwood is fumigated with methyl bromide (32gms/m2 for 2.5hrs) and then dipped in 1% sodium hypochlorite for 2 minutes to kill any epiphytic organisms. If disease symptoms are detected, the budwood is stored until the causal agent is identified. The fumigation and dipping procedures kill most fungal pathogens on the propagation material. However, all *Prunus* viruses, viroids, phytoplasmas and bacteria and some fungi can be transmitted internally in propagation material and fumigation and dipping will not kill these pathogens. Many *Prunus* pathogens do not cause symptoms on the stems of their host and may be missed during visual inspection of the propagation material. Consequently, *Prunus* germplasm, including almonds, imported into Australia requires a minimum of two years post entry quarantine testing using a range of diagnostic techniques for detection of diseases and pathogens of quarantine significance.

Plants grown in PEQ are visually inspected for disease symptoms associated with pathogens that might be transmitted through propagation material. Light microscopy and culturing are used for confirmation if fungal or bacterial pathogens are suspected. Biological indexing is mandatory and is specifically used for the detection of viruses, viroids and graft transmissible diseases of unknown aetiology. The imported variety is inoculated by grafting onto sensitive *Prunus* varieties and by rubbing a plant extract onto the leaves of sensitive herbaceous plants such as cucumber or *Nicotiana* species. These plants are observed for characteristic symptom development. Enzyme linked immunosorbent assay (ELISA) is mandatory for *Plum pox virus* (Figure 1) detection and may be used for confirmation of the presence of other viruses. Molecular testing using polymerase chain reaction (PCR) can be used to detect the genetic material of various pathogens. PCR testing is required for material originating from host countries (Taiwan, Turkey, North America, Central America and South America) of *Xylella fastidiosa*, which causes almond leaf scorch (Figure 1), and phytoplasmas which are associated with various diseases depending on which species is present (Figure 2; Table 1). For

all other pathogens this method is optional and dependent on the availability of a validated diagnostic test.

Recent advances in molecular and diagnostic technology have allowed characterisation of new and emerging pathogens, new strains of known pathogens of *Prunus* species and clarification of pathogens associated with diseases of *Prunus* species which have an unknown aetiology. As a consequence there is a requirement to review and update the list of pathogens and pathogens significant at the quarantine and certification levels for *Prunus* species in Australia. New and/or improved molecular tools for the rapid and sensitive detection of many of these pathogens have also been developed, which can be used to better support pathogen detection during PEQ, certification programs and to improve stone fruit biosecurity in Australia.

The biosecurity of the almond industry is also supported at the local level via an industry based certification programs for the production of high health almond budwood, which is run by the Almond Board of Australia. In Australia high-health pathogen-tested almond trees are routinely tested for several endemic viruses that can significantly affect quality and yield, including *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple chlorotic leafspot virus* (ACLSV) and *Apple mosaic virus* (ApMV).

A Department of Environment and Primary Industries and Horticulture Australia Ltd. funded project is underway to develop new tools to detect both endemic and exotic pathogens of almonds and summerfruit for Australia. The research activities of this three year project will centre around five objectives:

1. Update the PEQ list for *Prunus* species to include recently reported pathogens and review the latest information of known pathogens
2. Identify, develop and validate molecular diagnostic tools for the detection of endemic and exotic pathogens of *Prunus* species under Australian conditions
3. Develop a post entry quarantine diagnostic manual that is specific for *Prunus* species, including almonds
4. Investigate strain variation of major endemic viruses of *Prunus* species in Australia
5. Identify optimal pest and disease management strategies for maintaining *Prunus* budwood repository blocks.

The exotic bacteria, phytoplasmas, viruses and viroids that are currently known to infect almond which are significant to quarantine have been identified (Table 1). The quarantine list includes several pathogens that have not been listed previously, some of which are associated with diseases for which the cause was previously unknown. This information will be used to update the current list of pathogens that may require testing for in almonds during PEQ. Although some pathogens may not be associated with disease or are associated with mild symptoms in almond, infected plants could act as a reservoir for other susceptible plant hosts.

In addition to PNRSV, PDV, ACLSV and ApMV, the endemic pathogens of almonds that may be significant to Australian almond certification programs include *Cucumber mosaic virus (*CMV), *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd) (Table 2). The incidence of CMV, PLMVd, and HSVd in Australian *Prunus* species, including almond, is not known and they are not tested for within the certification program. The effect of CMV on almonds is unknown but it is associated with

diseases in other *Prunus* species, and might also have significant impact on quality and yield. PLMVd and HSVd can also be associated with loss in quality and yield in some *Prunus* species.

Molecular tools to detect exotic and endemic bacteria, phytoplasmas, viruses and viroids have been identified and their development and validation under Australian conditions is underway. To complete the validation of the diagnostic test, an Australia-wide survey will be conducted in the final two years of the project. Approximately 200 trees of different commercial and ornamental *Prunus* species will be collected from major growing districts throughout Australia and sampled and the tested using the protocols selected and developed in this project. It is vitally important that these surveys are conducted prior to the use of these protocols for certification in a quarantine or commercial situation. The purpose of the survey is two-fold: (i) to update the disease status for each pathogen in Australia and (ii) to test protocols under "local" conditions and identify any potential "false positives" or organisms that can make interpretation of results difficult.

Acknowledgements

This project has been funded by HIA Limited using the Almond and Summerfruit levies and matched funds from the Australian Government.

Table 1. A list of pathogens that are known to infect almonds, which do not occur in Australia and may be significant at the quarantine level.

Pathogen group	Pathogens known to infect almond	Disease
Bacteria	*Erwinia amylovora*	Symptomless
	Pseudomonas amygdali	Hyperplasic canker
	Xylella fastidiosa	
Phytoplasmas and phytoplasma groups	*Candidatus* Phytoplasma prunorum	Almond leaf scorch
	X-Disease phytoplasma	European stone fruit yellows
	Candidatus Phytoplasma pyri	Decline, Almond brown line and Almond kernel shrivel Almond witches' broom
	Candidatus Phytoplasma phoenicium'	Almond little leaf
	Peanut witches' broom group phytoplasmas *(*16SrII - *Candidatus* Phytoplasma aurantifolia related strains)	Shoot proliferation Yellowing or little leaf
	Clover proliferation group phytoplasmas (16SrVI - *Ca.* P. trifolii related strains)	
	Stolbur (16SrXII-A) group phytoplasmas	
Viruses	*Cherry necrotic rusty mottle foveavirus*	Almond is an experimental host
	Cherry twisted leaf virus – tentative *foveavirus*	Almond is an experimental host
	Little cherry virus 1 (unassigned genus, LChV-1)	Symptomless
	Peach mosaic virus trichovirus (PcMV)	Symptomless
	Peach rosette mosaic nepovirus (PRMV)	Unknown
	Plum bark necrosis stem pitting-associated ampleovirus (PBNSPaV)	Bark necrosis and stem pitting
	Plum pox potyvirus (PPV)	

		Sharka disease – mild chlorosis
	Raspberry ringspot nepovirus (RpRSV)	of leaves on almond
		Chlorosis, mosaic, decline. Death
	Tomato black ring nepovirus	Unknown in almond
	Tomato ringspot nepovirus	Yellow bud mosaic
Viroids	*Hop stunt viroid**	

Hop stunt viroid is noted in the PEQ conditions for almonds as being present in crops in Australia and therefore not of quarantine significance. However hop strains are not reported on hops in Australia and are considered quarantine pathogens.

Table 2. A list of pathogens that are known to infect almonds, which occur in Australia and may be significant at the certification level.

Pathogen group	Pathogens known to infect almond	Disease
Bacteria	*Agrobacterium tumefaciens*	Crown gall, root knot and hairy root
	Pseudomonas syringae pv. syringae	Bacterial canker and blast
	Xanthomonas arboricola pv. pruni	Bacterial leaf spot, shot-hole and black spot
Viruses	*Apple chlorotic leaf spot trichovirus* (ACLSV)	Chlorotic leafroll of almond in combination with PDV
	Apple mosaic virus Ilarvirus (ApMV)	Mosaic and/or line pattern of almond
	Prune dwarf Ilarvirus (PDV)	Almond mosaic
	Prunus necrotic ringspot Ilarvirus (PNRSV)	Necrotic shock, bud failure, calico and chlorotic mottling of almond
	Cucumber mosaic cucumovirus (CMV) – not known on *Prunus* in Australia	Specific disease is unknown – may have a significant impact in combination with other viruses
Viroids	*Hop stunt viroid* (Australian strains)	May be symptomless in almonds
	Peach latent mosaic viroid	May be symptomless in almonds
Fungi	*Botryosphaeria dothidea*	Canker
	Calosphaeria pulchella – not known on almonds in Australia	Canker
	Diplodia seriata	Canker
	Eutypa lata	Cankers, associated with pruning wounds
	Neofusicoccum australe	Canker
	Neofusicoccum parvum	Canker

Figure 1 An almond tree infected with *Xylella fastidiosa* and exhibiting almond leaf scorch disease

Figure 2 An X-disease phytoplasma infected almond tree cv. Padre (front, left) compared to an uninfected tree (back) and PD/PYLRV2 (pear decline phytoplasma, 16Srgroup X) infected tree (right).

Australian Nutgrower, 2015

Update: Development of molecular diagnostic tools to detect endemic and exotic pathogens of almonds and summerfruit for Australia

Fiona Constable[1], Narelle Nancarrow[1] Wycliff Kinoti[1,2,] and Brendan Rodoni[1,2]

[1]AgriBio, Department of Environment and Primary Industries, 5 ring Road, Bundoora, Victoria 3083

[2]La Trobe University, 5 ring Road, Bundoora, Victoria 3083

The plant microbiology group of the BioSciences Research Division, Department of Economic Development, Jobs, Transport and Resources (formerly DEPI) in Victoria have undertaken a research project to develop molecular diagnostic tools to detect endemic and exotic pathogens of almonds and summerfruit for Australia. These diagnostic tools will support biosecurity continuum of the Australian almond and summerfruit industries at the border during post entry quarantine (PEQ) facilities, during an incursion event and internally through schemes that supply high-health planting material.

Diagnostic tools were developed for a total of 48 exotic pathogens of almonds and/or summerfruit including five bacteria, ten phytoplasma species or groups, 31 viruses and two viroids. Molecular tools have also been identified, developed and established for 15 endemic pathogens of almonds and/or summerfruit including four bacteria, nine viruses and two viroids. The primary molecular diagnostic tools that have been developed use polymerase chain reaction (PCR) techniques, which detect and make multiple copies of a small portion of the genome of the targeted pathogen. For a few pathogens, (e.g. *Xylella fastidiosa*, almond leaf scorch) some simpler tests, such as Loop Mediated Isothermal Amplification, have been developed, which are potentially adaptable to field-based diagnostics for smart surveillance.

A national survey was undertaken to validate the molecular diagnostic tools on field collected samples. It is vitally important that these surveys are conducted prior to the use of these protocols for certification in a quarantine or commercial situation. The purpose of the survey is two-fold: (i) to update the disease status for each pathogen in Australia and (ii) to test protocols under "local" conditions and identify any potential "false positives" or organisms that can make interpretation of results difficult.

So far, a total of 80 samples were collected from New South Wales (33), Queensland (16), South Australia (5), Tasmania (18) and Victoria (8) and have undergone testing. The samples included 33 almond, four apricot, 12 cherry, five nectarine, nine peach, two peach/almond hybrids, 14 Plum and one *Prunus cerasifera.*

The following viruses are considered present or endemic in Australia: *Prunus necrotic ring spot* (PNRSV)*, Prune dwarf virus* (PDV), *Apple mosaic virus* (ApMV), *Apple chlorotic leafspot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), *Little cherry virus 2* (LChV2), *Cherry virus A* (CVA) and *Cucumber mosaic virus* (CMV).

ACLSV is an economically important pathogen of almond and summerfruit species worldwide. It has been associated with pseudopox disease of plum and apricot, apricot viruela disease and plum bark split. It may be associated with chlorotic leafroll of almond in combination with PDV. ACLSV is associated with graft incompatibility. ACSLV was detected in two apricot, two cherry one peach and

four plum samples in the survey. Some strains were only detected using a generic test for viruses in the family *Betaflexiviridae* to which this virus species belongs. Conversely some of the ACLSV strains were detected with the specific test but not the generic test. This result and the result of sequencing of some isolates indicates significant diversity amongst ACLSV strains that can impact upon detection using molecular methods. Further work is required to determine the best test to detect ACLSV in *Prunus* species in Australia.

PNRSV, PDV and *ApMV* are members of the genus *Ilarvirus* in the family *Bromoviridae* and are important viruses of almonds and summerfruit in Australia. PNRSV was most frequently detected (43/80 samples) during the survey compared to PDV (2/80 samples) and ApMV (1/80 samples). In Almond PNRSV has been associated with necrotic shock, bud failure, calico and chlorotic mottling. It may be symptomless in some almond cultivars. In other *Prunus* species PNRSV may be associated with more serious diseases such as sweet cherry rugose mosaic, necrotic ringspot and European plum line pattern. When PNRSV occurs in mixed infection with other viruses, such as *Prune dwarf virus* (PDV), the impact of virus infection can be greater causing severe stunting in some species and varieties of *Prunus*. Yield losses of up to 60% have been reported in trees infected with PNRSV and PDV. In *Prunus* species, both PNRSV and PDV are spread in pollen and seed as well as in propagation material. There is some evidence for spread of Ilarviruses, particularly PNRSV and PDV, by vectors including mite (*Aculus fockeui*), nematode (*Longidorus macrosoma*) and thrips (*Frankliniella occidentailis*).

During the survey the detection of PNRSV, PDV and ApMV viruses was often confirmed using a generic test, which can detect all species in the *Ilarvirus* genus. However occasionally PNRSV or PDV strains were detected by the generic test and not with their specific test and vice versa, suggesting genetic diversity amongst *Ilarvirus* species occurring in Australia. Interestingly in some of these trees the generic test confirmed the presence of a virus in the *Ilarvirus* genus, but sequence analysis suggested the presence of another *Ilarvirus* species. The biological significance of this detection is unknown.

As a part of the project next generation sequencing (NGS) methods have been developed so that the sequence of full genomes of endemic *Prunus* viruses can be obtained. This information will give a greater understanding of the genetic variability along the whole genome of a virus and allow better tests to be developed. This methodology will be used to characterise the *Ilarvirus* species that was detected at the molecular level.

CVA is known to infect several *Prunus* sp. overseas including sweet cherry, sour cherry, apricot and plum. It is not associated to disease and is considered a latent virus. CVA was also detected in *Prunus cerasifera* during the development of NGS techniques. This constitutes a first report of this virus in Australia and *P. cerasifera* is a new host for CVA. It has subsequently been detected during the survey in nine of the cherry samples, confirming its presence in Australia.

ASPV, ASGV and CMV have been infrequently detected in *Prunus* species overseas. ASPV is an important pathogen of pome fruits. There is an unconfirmed report of an association between ASPV and yellow vein disease in sweet and sour cherry in India. ASGV naturally infects and is an important pathogen of pome fruits, citrus, lily and kiwifruit. Natural infections of ASGV are also reported from nectarine, plum, apricot and cherry but the economic impact is unknown. CMV has a very broad host range, including almond, apricot, flowering cherry, sour cherry and plum. In combination with other

viruses it is asscoiated with deformed, chlorotic mottled leaves in sweet cherry and pseudopox disease of plum. The economic impact when CMV occurs on its own in *Prunus* species is unknown.

ASPV and CMV were not detected during the survey. ASGV was detected in one 50+ year old plum tree. The low frequency of detection of these viruses in *Prunus* trees in Australia and overseas indicates that they are likely to be minor pathogen of almonds and summerfruit in Australia and low risk to certification programs.

During the survey *Cherry green ring mottle virus* (CGRMV), *Cherry necrotic rusty mottle virus* (CNRMV) and *Plum bark necrosis stem pitting associated virus* (PBNSPaV) were also detected and are now considered present in Australia. CGRMV is associated with green ring mottle disease of flowering and sour cherry and may be associated with cherry rusty mottle and cherry necrotic mottling diseases. A strain of CGRMV has also been associated with apricot ring pox disease. CNRMV is associated with necrotic rusty mottle in cherry. It also naturally infects apricot, peach and plum but the association with disease is unclear. PBNSPaV is associated bark necrosis and stem pitting disease in susceptible almond, plum, prune, peach, cherry, and apricot varieties. In sensitive cultivars can also cause gummosis and graft union failure (Figure 1). All three viruses are transmitted during propagation and grafting. No vector is reported for the three viruses, although natural spread of PBNSPaV has been observed overseas.

LChV2 was detected for the first time in Australia in early 2014 and is now considered to be present. LChV2 was detected in two cherry samples during the survey. Sweet cherry, Japanese cherry and bitter cherry are primary hosts of the virus. There has been one unconfirmed report from plum in the USA. This virus is not known to be a significant pathogen of almonds or summerfruit and may not be important as a part of their certification programs. However, as the name suggests this virus can have a significant impact on the quality and yield of sweet cherry fruit and it may result in decline of infected trees.

Based on the results of this survey PNRSV, PDV, ApMV and ACLSV remain important to the production of high health almond and summerfruit planting in Australia. PBNSPaV is present in Australia and may also have an impact on the production of high health almond and summerfruit material. Testing for PBNSPaV should be undertaken within certification and high health programs. CGRMV and CNRMV are important to the cherry industry, and may have an impact on summerfruit species. Bioinformatic analyses for all of the viruses that were detected indicate that significant genetic variability may occur within some virus species. This information has been used to design more reliable molecular diagnostic tools. The diagnostic protocols for both endemic and exotic pathogens that have been validated have been incorporated into a National pathogen-testing manual that can be used by pathologists and industry in Australia to support biosecurity. These results have also assisted the development of management strategies and biosecurity plans for the summerfruit and almond industries. .

Acknowledgements

We thank the growers, consultants and intestate colleagues who assisted with the sample collection for this survey. This project has been funded by Horticulture Innovation Australia Limited using the almond and summerfruit levies with co-investment from Department of Economic Development, Jobs, Transport and Resources and funds from the Australian Government.

Figure 1. An Australian cherry sample infected with *Plum bark necrosis stem pitting associate virus.* Note the discolouration and failure of the graft union between the rootstock and the scion. The yellowing along the veins of the leaves may be caused by disruption of the vascular issue associated with the graft union failure. (Photo: Ramez Aldaoud, DEDJTR)

Development of molecular diagnostic tools to detect endemic and exotic pathogens of *Prunus* species for Australia

Fiona Constable[1], Wycliff Kinoti[1,2], Narelle Nancarrow[1] and Brendan Rodoni[1,2]

[1]AgriBio, Department of Environment and Primary Industries, 5 ring Road, Bundoora, Victoria 3083

[2]La Trobe University, 5 ring Road, Bundoora, Victoria 3083

The biosecurity of the Australian summerfruit and almond industries are maintained at the border by the Department of Agriculture Fisheries and Forestry (DAFF; formerly Australian Quarantine Inspection Service, AQIS) in post entry quarantine (PEQ) facilities and internally through nurseries or schemes that supply high-health planting material throughout Australia. Over the last 5 years approximately 250 accessions of *Prunus* species, including summerfruit varieties, have been screened for quarantine pests and pathogens and released from Australian PEQ stations. Currently DAFF recognises two bacteria, six phytoplasmas or phytoplasma-like diseases, 13 viruses, one viroid and 70 fungi, to be of quarantine significance for *Prunus* species. DAFF also recognises that there are many diseases of *Prunus* species of unknown aetiology.

On arrival into Australian PEQ, imported *Prunus* propagation material, including summerfruit, is inspected for insect pests and disease symptoms. If there are no obvious disease symptoms, the budwood is fumigated with methyl bromide (32gms/m2 for 2.5hrs) and then dipped in 1% sodium hypochlorite for 2 minutes to kill any epiphytic organisms. If disease symptoms are detected, the budwood is stored until the causal agent is identified. The fumigation and dipping procedures kill most fungal pathogens on the propagation material. However, all *Prunus* viruses, viroids, phytoplasmas and bacteria and some fungi can be transmitted internally in propagation material and fumigation and dipping will not kill these pathogens. Consequently, *Prunus* germplasm imported into Australia requires a minimum of two years post entry quarantine testing using a range of diagnostic techniques for detection of diseases and pathogens of quarantine significance.

Plants grown in PEQ are visually inspected for disease symptoms associated with pathogens that might be transmitted through propagation material. Light microscopy and culturing are used for confirmation if fungal or bacterial pathogens are suspected. Biological indexing is mandatory and is specifically used for the detection of viruses, viroids and graft transmissible diseases of unknown aetiology. The imported variety is inoculated by grafting onto sensitive *Prunus* varieties and by rubbing a plant extract onto the leaves of sensitive herbaceous plants such as cucumber or *Nicotiana* species. These plants are observed for characteristic symptom development. Enzyme linked immunosorbent assay (ELISA) is mandatory for *Plum pox virus* (Figure 1) detection and may be used for confirmation of the presence of other viruses. Molecular testing using polymerase chain reaction (PCR) can be used to detect the genetic material of various pathogens. PCR testing is required for material originating from host countries (Taiwan, Turkey, North America, Central America and South America) of *Xylella fastidiosa*, which causes phony peach disease, plum leaf scald and leaf scorch and phytoplasmas which are associated with European stone fruit yellows disease (Figure 2). For all other pathogens this method is optional and dependent on the availability of a validated diagnostic test.

Recent advances in molecular and diagnostic technology have allowed characterisation of new and emerging pathogens, new strains of known pathogens of *Prunus* species and clarification of pathogens associated with diseases of *Prunus* species which have an unknown aetiology. As a consequence there is a requirement to review and update the list of pathogens and pathogens significant at the quarantine and certification levels for *Prunus* species in Australia. New and/or improved molecular tools for the rapid and sensitive detection of many of these pathogens have also been developed, which can be used to better support pathogen detection during PEQ, certification programs and to improve stone fruit biosecurity in Australia.

Although there is no formal certification program, the biosecurity of the summerfruit industry is also supported at the local level via nurseries who produce of high health budwood. Nurseries may test this material for several endemic viruses that can significantly affect quality and yield, including *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple chlorotic leafspot virus* (ACLSV) and *Apple mosaic virus* (ApMV).

A Department of Environment and Primary Industries and Horticulture Australia Ltd. funded project is underway to develop new tools to detect both endemic and exotic pathogens of summerfruit and almonds for Australia. The research activities of this three year project will centre around five objectives:

1. Update the PEQ list for *Prunus* species to include recently reported pathogens and review the latest information of known pathogens
2. Identify, develop and validate molecular diagnostic tools for the detection of endemic and exotic pathogens of stone fruit under Australian conditions
3. Develop a post entry quarantine diagnostic manual that is specific for *Prunus* species, including almonds
4. Investigate strain variation of major endemic viruses of *Prunus* species in Australia
5. Identify optimal pest and disease management strategies for maintaining *Prunus* budwood repository blocks.

The exotic bacteria, phytoplasmas, viruses and viroids that are currently known to infect summerfruit and which are significant to quarantine have been identified (Table 1). The quarantine list includes several pathogens that have not been listed previously, some of which are associated with diseases for which the cause was previously unknown. This information will be used to update the current list of pathogens that may require testing for in summerfruit during PEQ. Although some pathogens may not be associated with disease or are associated with mild symptoms in summerfruit, infected plants could act as a reservoir for other susceptible plant hosts.

In addition to PNRSV, PDV, ACLSV and ApMV, the endemic pathogens of summerfruit that may be significant to Australian certification programs include *Cucumber mosaic virus (*CMV), *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd) (Table 2). The incidence of CMV, PLMVd, and HSVd in Australian *Prunus* species is not known. CMV is associated with pseudopox disease of plum and chlorotic mottle of cherry, and might also have significant impact on quality and yield. PLMVd and HSVd can also be associated with diseases and loss in quality and yield in some *Prunus* species.

Molecular tools to detect exotic and endemic bacteria, phytoplasmas, viruses and viroids have been identified and their development and validation under Australian conditions is underway. To complete the validation of the diagnostic test, an Australia-wide survey will be conducted in the final

two years of the project. Approximately 200 trees of different commercial and ornamental *Prunus* species will be collected from major growing districts throughout Australia and sampled and the tested using the protocols selected and developed in this project. It is vitally important that these surveys are conducted prior to the use of these protocols for certification in a quarantine or commercial situation. The purpose of the survey is two-fold: (i) to update the disease status for each pathogen in Australia and (ii) to test protocols under "local" conditions and identify any potential "false positives" or organisms that can make interpretation of results difficult.

Acknowledgements

This project has been funded by HIA Limited using the Almond and Summerfruit levies and matched funds from the Australian Government.

Table 1. A list of bacteria, phytoplasmas viruses and viroids that are known to infect summerfruit, which do not occur in Australia and may be significant at the quarantine level.

Pathogen group	Pathogen
Bacteria	*Xylella fastidiosa*
	Erwinia amylovora
	Pseudomonas syringae pv. persicae
	Pseudomonas syringae pv. avii
Phytoplasmas and phytoplasma groups	*Candidatus* Phytoplasma prunorum
	X-Disease phytoplasma
	Candidatus Phytoplasma pyri
	Candidatus Phytoplasma phoenicium
	Peanut witches' broom group phytoplasmas *(*16SrII - *Candidatus* Phytoplasma aurantifolia related strains)
	Clover proliferation group phytoplasmas (16SrVI - *Candidatus* P. trifolii related strains)
	Stolbur (16SrXII-A) group phytoplasmas
	Candidatus Phytoplasma mali
	Elm yellows (16SrV) group phytoplasmas
	Aster yellows group (I-B, I-F, I-Q) phytoplasmas
Viruses	*American plum line pattern Ilarvirus* (APLPV)
	Apricot latent ringspot nepovirus (APRSV)*
	Apricot latent virus foveavirus (ApLV)
	Asian Prunus virus 1 foveavirus (APruV-1)
	Asian Prunus virus 2 foveavirus (APruV-2)
	Asian Prunus virus 3 foveavirus (APruV-3)
	Arabis mosaic nepovirus (ArMV)
	Cherry A capillovirus (CVA)
	*Cherry green ring mottle foveavirus (*syn. Sour cherry green ring mottle virus, CGRMV)
	Cherry leaf roll nepovirus (CLRV)
	Cherry mottle leaf trichovirus (CMLV)
	Cherry necrotic rusty mottle foveavirus (CNRMV)
	Cherry rasp leaf cheravirus (CRLV)
	Cherry twisted leaf virus – tentative Foveavirus
	Little cherry virus 1 (unassigned genus, LChV-1)
	Little cherry virus 2 ampleovirus (LChV-2)
	Myrobalan latent ringspot nepovirus (MLRSV)*
	Peach chlorotic mottle foveavirus (PCMV)
	Peach enation nepovirus (PEV)*
	Peach mosaic virus trichovirus (PcMV)
	Peach rosette mosaic nepovirus (PRMV)
	Petunia asteroid mosaic tombusvirus (PeAMV)
	Plum bark necrosis stem pitting-associated ampleovirus (PBNSPaV)
	Plum pox potyvirus (PPV)
	Raspberry ringspot nepovirus (RpRSV)
	Strawberry latent ringspot virus (SLRSV)
	Stocky prune cheravirus (StPV)*
	Tobacco ringspot nepovirus (TRSV)
	Tomato black ring nepovirus (TBRV)
	Tomato ringspot nepovirus (ToRSV)

Pathogen group	Pathogen
	Tomato bushy stunt tombusvirus (TBSV)
Viroids	*Apple scar skin viroid* (ASSVd)
	Hop stunt viroid (HSVd)[†]

* Pathogens may be minor reports, of no economic impact or have a very limited distribution.

[†]HSVd is noted in the PEQ conditions for *Prunus* as being present in crops in Australia and therefore not of quarantine significance. However hop strains are not reported on hops in Australia and are considered quarantine pathogens.

Table 2. A list of bacteria, phytoplasmas viruses and viroids that are known to infect summerfruit, which occur in Australia and may be significant at the certification level.

Pathogen group	Pathogens known to infect other *Prunus* species
Bacteria	*Agrobacterium tumefaciens*
	Pseudomonas syringae pv. mors-prunorum
	Pseudomonas syringae pv. syringae
	Xanthomonas arboricola pv. pruni
Viruses	*Apple stem pitting associated foveavirus** (ASPV)- not known on *Prunus* in Australia
	Apricot pseudochlorotic leaf spot trichovirus (APCLSV)
	Apple stem grooving virus capillovirus (ASGV)
	Apple chlorotic leaf spot trichovirus (ACLSV)
	Apple mosaic virus Ilarvirus (ApMV)
	Prune dwarf Ilarvirus (PDV)
	Prunus necrotic ringspot Ilarvirus (PNRSV)
	Cucumber mosaic cucumovirus (CMV) – not known on *Prunus* in Australia
	*Carnation ringspot dianthovirus** – not known on *Prunus* in Australia
	Citrus enation - woody gall virus*
	*Sowbane mosaic sobemovirus** – not known on *Prunus* in Australia
	*Tobacco necrosis necrovirus** – not known on *Prunus* in Australia
	*Tobacco mosaic tobamovirus** – not known on *Prunus* in Australia
Viroids	*Hop stunt viroid* (HSVd; Australian strains)
	Peach latent mosaic viroid (PLMVd)

* Pathogens may be minor reports or not economically important and may not need to be included into pathogen testing programs.

Figure 1. A Plum tree (cv. Katinka) leaf chlorosis caused by *Plum pox virus*

Figure 2. A peach tree affected by European stone fruit yellows disease exhibiting decline, sparse foliation, chlorosis and smaller leaves

Update: Development of molecular diagnostic tools to detect endemic and exotic pathogens of summerfruit and almonds for Australia

Fiona Constable[1], Narelle Nancarrow[1] Wycliff Kinoti[1,2,] and Brendan Rodoni[1,2]

[1]AgriBio, Department of Environment and Primary Industries, 5 ring Road, Bundoora, Victoria 3083

[2]La Trobe University, 5 ring Road, Bundoora, Victoria 3083

The plant microbiology group of the BioSciences Research Division, Department of Economic Development, Jobs, Transport and Resources (formerly DEPI) in Victoria have undertaken a research project to develop molecular diagnostic tools to detect endemic and exotic pathogens of almonds and summerfruit for Australia. These diagnostic tools will support biosecurity continuum of the Australian almond and summerfruit industries at the border during post entry quarantine (PEQ) facilities, during an incursion event and internally through schemes that supply high-health planting material.

Diagnostic tools were developed for a total of 48 exotic pathogens of almonds and/or summerfruit including five bacteria, ten phytoplasma species or groups, 31 viruses and two viroids. Molecular tools have also been identified, developed and established for 15 endemic pathogens of almonds and/or summerfruit including four bacteria, nine viruses and two viroids. The primary molecular diagnostic tools that have been developed use polymerase chain reaction (PCR) techniques, which detect and make multiple copies of a small portion of the genome of the targeted pathogen. For a few pathogens, (e.g. *Xylella fastidiosa*, almond leaf scorch) some simpler tests, such as Loop Mediated Isothermal Amplification, have been developed, which are potentially adaptable to field-based diagnostics for smart surveillance.

A national survey was undertaken to validate the molecular diagnostic tools on field collected samples. It is vitally important that these surveys are conducted prior to the use of these protocols for certification in a quarantine or commercial situation. The purpose of the survey is two-fold: (i) to update the disease status for each pathogen in Australia and (ii) to test protocols under "local" conditions and identify any potential "false positives" or organisms that can make interpretation of results difficult.

So far, a total of 80 samples were collected from New South Wales (33), Queensland (16), South Australia (5), Tasmania (18) and Victoria (8) and have undergone testing. The samples included 33 almond, four apricot, 12 cherry, five nectarine, nine peach, two peach/almond hybrids, 14 Plum and one *Prunus cerasifera.*

The following viruses are considered present or endemic in Australia: *Prunus necrotic ring spot* (PNRSV)*, Prune dwarf virus* (PDV), *Apple mosaic virus* (ApMV), *Apple chlorotic leafspot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), *Little cherry virus 2* (LChV2), *Cherry virus A* (CVA) and *Cucumber mosaic virus* (CMV).

ACLSV is an economically important pathogen of almond and summerfruit species worldwide. It has been associated with pseudopox disease of plum and apricot, apricot viruela disease and plum bark split. It may be associated with chlorotic leafroll of almond in combination with PDV. ACLSV is associated with graft incompatibility. ACSLV was detected in two apricot, two cherry one peach and

four plum samples in the survey. Some strains were only detected using a generic test for viruses in the family *Betaflexiviridae* to which this virus species belongs. Conversely some of the ACLSV strains were detected with the specific test but not the generic test. This result and the result of sequencing of some isolates indicates significant diversity amongst ACLSV strains that can impact upon detection using molecular methods. Further work is required to determine the best test to detect ACLSV in *Prunus* species in Australia.

PNRSV, PDV and *ApMV* are members of the genus *Ilarvirus* in the family *Bromoviridae* and are important viruses of almonds and summerfruit in Australia. PNRSV was most frequently detected (43/80 samples) during the survey compared to PDV (2/80 samples) and ApMV (1/80 samples). In Almond PNRSV has been associated with necrotic shock, bud failure, calico and chlorotic mottling. It may be symptomless in some almond cultivars. In other *Prunus* species PNRSV may be associated with more serious diseases such as sweet cherry rugose mosaic, necrotic ringspot and European plum line pattern. When PNRSV occurs in mixed infection with other viruses, such as *Prune dwarf virus* (PDV), the impact of virus infection can be greater causing severe stunting in some species and varieties of *Prunus*. Yield losses of up to 60% have been reported in trees infected with PNRSV and PDV. In *Prunus* species, both PNRSV and PDV are spread in pollen and seed as well as in propagation material. There is some evidence for spread of Ilarviruses, particularly PNRSV and PDV, by vectors including mite (*Aculus fockeui*), nematode (*Longidorus macrosoma*) and thrips (*Frankliniella occidentailis*).

During the survey the detection of PNRSV, PDV and ApMV viruses was often confirmed using a generic test, which can detect all species in the *Ilarvirus* genus. However occasionally PNRSV or PDV strains were detected by the generic test and not with their specific test and vice versa, suggesting genetic diversity amongst *Ilarvirus* species occurring in Australia. Interestingly in some of these trees the generic test confirmed the presence of a virus in the *Ilarvirus* genus, but sequence analysis suggested the presence of another *Ilarvirus* species. The biological significance of this detection is unknown.

As a part of the project next generation sequencing (NGS) methods have been developed so that the sequence of full genomes of endemic *Prunus* viruses can be obtained. This information will give a greater understanding of the genetic variability along the whole genome of a virus and allow better tests to be developed. This methodology will be used to characterise the *Ilarvirus* species that was detected at the molecular level.

CVA is known to infect several *Prunus* sp. overseas including sweet cherry, sour cherry, apricot and plum. It is not associated to disease and is considered a latent virus. CVA was also detected in *Prunus cerasifera* during the development of NGS techniques. This constitutes a first report of this virus in Australia and *P. cerasifera* is a new host for CVA. It has subsequently been detected during the survey in nine of the cherry samples, confirming its presence in Australia.

ASPV, ASGV and CMV have been infrequently detected in *Prunus* species overseas. ASPV is an important pathogen of pome fruits. There is an unconfirmed report of an association between ASPV and yellow vein disease in sweet and sour cherry in India. ASGV naturally infects and is an important pathogen of pome fruits, citrus, lily and kiwifruit. Natural infections of ASGV are also reported from nectarine, plum, apricot and cherry but the economic impact is unknown. CMV has a very broad host range, including almond, apricot, flowering cherry, sour cherry and plum. In combination with other

viruses it is asscoiated with deformed, chlorotic mottled leaves in sweet cherry and pseudopox disease of plum. The economic impact when CMV occurs on its own in *Prunus* species is unknown.

ASPV and CMV were not detected during the survey. ASGV was detected in one 50+ year old plum tree. The low frequency of detection of these viruses in *Prunus* trees in Australia and overseas indicates that they are likely to be minor pathogen of almonds and summerfruit in Australia and low risk to certification programs.

During the survey *Cherry green ring mottle virus* (CGRMV), *Cherry necrotic rusty mottle virus* (CNRMV) and *Plum bark necrosis stem pitting associated virus* (PBNSPaV) were also detected and are now considered present in Australia. CGRMV is associated with green ring mottle disease of flowering and sour cherry and may be associated with cherry rusty mottle and cherry necrotic mottling diseases. A strain of CGRMV has also been associated with apricot ring pox disease. CNRMV is associated with necrotic rusty mottle in cherry. It also naturally infects apricot, peach and plum but the association with disease is unclear. PBNSPaV is associated bark necrosis and stem pitting disease in susceptible almond, plum, prune, peach, cherry, and apricot varieties. In sensitive cultivars can also cause gummosis and graft union failure (Figure 1). All three viruses are transmitted during propagation and grafting. No vector is reported for the three viruses, although natural spread of PBNSPaV has been observed overseas.

LChV2 was detected for the first time in Australia in early 2014 and is now considered to be present. LChV2 was detected in two cherry samples during the survey. Sweet cherry, Japanese cherry and bitter cherry are primary hosts of the virus. There has been one unconfirmed report from plum in the USA. This virus is not known to be a significant pathogen of almonds or summerfruit and may not be important as a part of their certification programs. However, as the name suggests this virus can have a significant impact on the quality and yield of sweet cherry fruit and it may result in decline of infected trees.

Based on the results of this survey PNRSV, PDV, ApMV and ACLSV remain important to the production of high health almond and summerfruit planting in Australia. PBNSPaV is present in Australia and may also have an impact on the production of high health almond and summerfruit material. Testing for PBNSPaV should be undertaken within certification and high health programs. CGRMV and CNRMV are important to the cherry industry, and may have an impact on summerfruit species. Bioinformatic analyses for all of the viruses that were detected indicate that significant genetic variability may occur within some virus species. This information has been used to design more reliable molecular diagnostic tools. The diagnostic protocols for both endemic and exotic pathogens that have been validated have been incorporated into a National pathogen-testing manual that can be used by pathologists and industry in Australia to support biosecurity. These results have also assisted the development of management strategies and biosecurity plans for the summerfruit and almond industries. .

Acknowledgements

We thank the growers, consultants and intestate colleagues who assisted with the sample collection for this survey. This project has been funded by Horticulture Innovation Australia Limited using the almond and summerfruit levies with co-investment from Department of Economic Development, Jobs, Transport and Resources and funds from the Australian Government.

Figure 1. An Australian cherry sample infected with *Plum bark necrosis stem pitting associate virus.* Note the discolouration and failure of the graft union between the rootstock and the scion. The yellowing along the veins of the leaves may be caused by disruption of the vascular issue associated with the graft union failure. (Photo: Ramez Aldaoud, DEDJTR)

Development of molecular diagnostic tools to detect endemic and exotic pathogens of *Prunus* species for Australia

Fiona Constable[1], Wycliff Kinoti[1,2] and Brendan Rodoni[1,2]

[1]AgriBio, Department of Environment and Primary Industries, 5 ring Road, Bundoora, Victoria 3083

[2]La Trobe University, 5 ring Road, Bundoora, Victoria 3083

The biosecurity of the Australian Summerfruit and Almond industries are maintained at the border by DAFF Biosecurity (formerly Australian Quarantine Inspection Service, AQIS) in Post Entry Quarantine (PEQ) facilities and internally through schemes that supply high-health planting material throughout Australia. Over the last 5 years approximately 250 accessions of *Prunus* species, including almonds, have been screened and released from Australian PEQ stations.

Recent advances in molecular and diagnostic technology have allowed characterisation of new and emerging pathogens, new strains of known pathogens of *Prunus* species and clarification of pathogens associated with diseases of *Prunus* species which have an unknown aetiology. As a consequence there is a requirement to review and update the list of quarantine pathogens and pathogens significant at the certification level for *Prunus* species in Australia. New and/or improved molecular tools for the rapid and sensitive detection of many of these pathogens have also been developed, which can be used to better support pathogen detection during PEQ, certification programs and to improve stone fruit biosecurity in Australia. The research activities of this three year project will centre around five objectives:

1. Update the PEQ list for stone fruit to include recently reported pathogens and review the latest information of known pathogens.
2. Identify, develop and validate molecular diagnostic tools for the detection of endemic and exotic pathogens of stone fruit under Australian conditions
3. Develop a post entry quarantine diagnostic manual that is specific for *Prunus* species, including almonds
4. Investigate strain variation of major endemic viruses of *Prunus* species in Australia
5. Identify optimal pest and disease management strategies for maintaining stone fruit budwood repository blocks

The bacteria, phytoplasmas, viruses, viroids and fungi that are currently known to infect almond and other *Prunus* species have been reviewed. The pathogens of almonds that are significant to Australian certification programs (Table 1) and PEQ have been identified (Table 2) and include several pathogens that have not been listed previously.

In February 2013 Wycliff Kinoti was appointed as a PhD student associated to this project. His research will focus on the genetic diversity of ilarviruses in almonds and summer fruit, including *Prunus necrotic ringspot virus*, *Prune dwarf virus* and *Apple mosaic virus,* and the development of improved diagnostic tests for their detection.

Table 3. A list of pathogens that are known to infect almonds, which occur in Australia and may be significant at the certification level.Pathogen group	**Pathogens known to infect almond**
Bacteria	*Agrobacterium tumefaciens* *Pseudomonas syringae pv. syringae* *Xanthomonas arboricola pv. pruni*
Viruses	*Apple chlorotic leaf spot trichovirus* (ACLSV) *Apple mosaic virus Ilarvirus* (ApMV) *Prune dwarf Ilarvirus* (PDV) *Prunus necrotic ringspot Ilarvirus* (PNRSV) *Cucumber mosaic cucumovirus* (CMV) – not known on stone fruit in Australia
Viroids	*Hop stunt viroid* (Australian strains) *Peach latent mosaic viroid*
Fungi	*Botryosphaeria dothidea* *Calosphaeria pulchella* – not known on almonds in Australia *Diplodia seriata* *Eutypa lata* *Neofusicoccum australe* *Neofusicoccum parvum*

Development of molecular diagnostic tools to detect endemic and exotic pathogens of *Prunus* species for Australia

Fiona Constable[1], Wycliff Kinoti[1,2], Narelle Nancarrow[1] and Brendan Rodoni[1,2]

[1]AgriBio, Department of Environment and Primary Industries, 5 ring Road, Bundoora, Victoria 3083

[2]La Trobe University, 5 ring Road, Bundoora, Victoria 3083

Introduction

The biosecurity of the Australian almond and summerfruit industries are maintained at the border by the Department of Agriculture (formerly Australian Quarantine Inspection Service, AQIS) in post entry quarantine (PEQ) facilities and internally through schemes that supply high-health planting material throughout Australia. A research project is underway to develop new tools to detect both endemic and exotic pathogens of almonds and summerfruit for Australia.

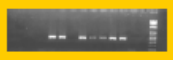
1. **The PEQ list for Prunus species has been updated:**
   - Exotic pathogens of quarantine significance: 4 bacteria, 10 phytoplasmas or phytoplasma groups, 28 viruses and 2 viroids
   - Endemic pathogens of significance to certification: 4 bacteria, 6 viruses and 2 viroids

2. **MOLECULAR DIAGNOSTIC TOOLS** have been developed and are currently being validated on field collected samples as a part of the **NATIONAL SURVEY**. The protocols for both endemic and exotic pathogens will be incorporated into a **NATIONAL PATHOGEN-TESTING MANUAL** that can be used by pathologists and industry in Australia to support biosecurity.

Newer, molecular technologies that are more sensitive and rapid, e.g. **REAL-TIME PCR**, and simpler molecular technologies potentially adaptable to field-based diagnostics for **SMART SURVEILLANCE** e.g. Loop Mediated Isothermal Amplification and recombinase polymerase amplification (RPA) are being trialled. Positive controls are integral for the assays and ensure they are working correctly. As an alternative to biological positive controls, which can be difficult to obtain, a series of **SYNTHETIC POSITIVE CONTROLS** have been designed.

National pathogen testing manual

- Fast and accurate diagnosis underpins the biosecurity of the Australian Almond and Summer Fruit industry.
- The manual will incorporate molecular methods, based on world best practice which improve the sensitivity and efficiency of pathogen testing operations.
- The manual will harmonise diagnostic tests used by organisations involved in screening *Prunus* germplasm for growers in Australia.
  - Organisations may also include offshore approved source laboratories and post entry quarantine.
- Harmonisation of diagnostic protocols for screening of endemic and exotic *Prunus* pathogens will improve biosecurity, increase efficiency and reduce time delays and costs for the Australian Almond and Summerfruit industry.
- The manual will be submitted to the Australian National Plant Biosecurity Diagnostic Network for national endorsement



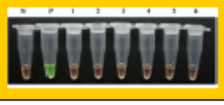
Above: Conventional RT-PCR detection of *Prunus necrotic ringspot virus*

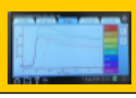
Above: Real time RT-PCR detection of *Cherry leaf roll virus*

SMART surveillance

- Rapid, in-field detection
- Molecular based method that can be field based
- Simple, rapid, specific, cost-effective
- Equipment for real time monitoring of results is available but is not required
- Results can be interpreted by eye if a suitable dye has been added
- E.g. LAMP: Loop Mediated Isothermal Amplification for detection of *Xylella fastidiosa* (Almond leaf scorch)

Clockwise from top left: Almond leaf scorch; LAMP in the field; Detection of *Xylella fastidiosa* by LAMP in real time, Genie® instrument for real time detection by LAMP in the field; The addition of SYBR green produces a colour change that can be seen with naked eyes





National *Prunus* survey

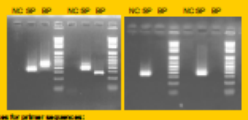
- Material from approximately 200-300 trees of different commercial and ornamental *Prunus* species will be collected from major growing districts throughout Australia.
- Test and validate the diagnostic protocols selected and developed in this project under 'local' Australian conditions and identify the potential for 'false negatives', 'false positives' or banding that can make interpretation of results difficult.
- To determine:
  - The incidence and distribution of endemic pathogens,
  - The presence or absence of exotic pathogens and update the disease status for each pathogen in Australia.

Preliminary results suggest that PNRSV is the most commonly detected endemic virus (37%) occurring in Australian *Prunus* trees

The biosecurity continuum requires rapid validated diagnostic tests for the detection of significant pathogens offshore, at the border and onshore for incursion management and certification

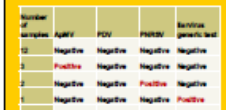
Synthetic positive controls

- Negative and positive controls are required for quality control of molecular tests.
- Biological positive controls are sometimes challenging to obtain, especially for rare, emerging or exotic pathogens
- DNA/RNA fragments can be custom- made:
  - Reliable, cheap, long lasting
  - Preparedness for incursion response
- Below is an example of a synthetic positive control for *Little cherry virus 2* containing the forward and reverse primers for 4 different plant virus assays.



References for primer sequences: Menzies et al., 2012; Gaidwell and Barnady, 2001; Rott and Jelkmann, 2001; Jelkmann, Lebas and Rott, 2006

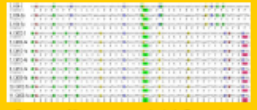
Strain variation occurs within species of major endemic viruses of *Prunus* species in Australia



Number of samples	ApMV	PDV	PNRSV	Ilarvirus genus-specific test
12	Negative	Negative	Negative	Negative
3	Positive	Negative	Negative	Negative
2	Negative	Negative	Positive	Negative
1	Negative	Negative	Negative	Positive
16	Negative	Negative	Positive	Positive
3	Positive	Negative	Positive	Positive

Variation in detection of Ilarviruses in the Prunus samples occurred using the species specific and genus specific RT-PCR assays

Differences in symptom development was observed on cucumber plants inoculated with different isolates of PNRSV. Variation in detection of PNRSV using the specific and genus specific RT-PCR also occurred. Symptomatic (A) and symptomless (B) cucumber indicators that tested positive for PNRSV.



Presence of the sequence variants in Prunus trees and the cucumber indicators were confirmed by sequencing and phylogenetic analysis of the RNA dependent RNA polymerase. Strains from cucumber frequently clustered together (left). Base changes occurred at 2 positions in some samples (above) resulted in amino acid change:

-1st base change = Isoleucine (I) to Veline (V)

-2nd base change = Phenylalanine (F) to leucine (L)

Next generation sequencing is being used to analyse the diversity of viruses infecting Australian *Prunus* species. Analyses suggest that a novel *Ilarvirus* associated with symptoms on the indicator GF305 (above) is most closely related to Blueberry shock virus (86%??)

www.depi.vic.gov.au Summerfruit AUSTRALIA HAL Know-how for Horticulture™ ALMOND BOARD OF AUSTRALIA Department of Environment and Primary Industries State Government Victoria

Identification, development and validation of molecular diagnostic tools for the detection of endemic and exotic pathogens of *Prunus* species under Australian conditions

Narelle Nancarrow[1], Fiona E. Constable[1], Wycliff Kinoti[1,2] & Brendan C. Rodoni[1,2]

[1]AgriBio, Department of Environment and Primary Industries, 5 Ring road, Bundoora Victoria 3086, Australia.

[2]La Trobe University, BundooraVictoria 3086, Australia.
*e-mail: narelle.nancarrow@depi.vic.gov.au

The biosecurity of the Australian almond and summerfruit (apricots, plums, nectarine, peach) industries is maintained at the Australian border by the federal Department of Agriculture, through post entry quarantine facilities and internally through schemes that supply high-health planting material throughout Australia. Within a recent review of post entry quarantine conditions for imports of *Prunus* germplasm, several pathogens were identified as being significant to Australian PEQ for almonds and summerfruit which do not appear on the current *Prunus* species PEQ lists. There are four bacteria, ten phytoplasmas or phytoplasma groups, 28 viruses and two viroids of quarantine significance for almonds and/or summerfruit. Additionally there 12 endemic pathogens within Australia, including four bacteria, six viruses and two viroids, that are important to almond and summerfruit high health programs. A research project is underway to identify, develop and validate molecular diagnostic tools to detect both endemic and exotic pathogens of almonds and summerfruit in Australia.

Molecular tools to detect exotic and endemic bacteria, phytoplasmas, viruses and viroids have been identified and their development underway. These tests include conventional PCR, real time PCR and LAMP (loop mediated isothermal amplification) PCR. If possible generic tests for families or genera rather than species specific tests will be identified from the literature or designed within the project to reduce the cost and time of testing.

To complete the validation of the diagnostic tests, an Australia-wide survey will be conducted in the final two years of the project. Approximately 200 trees of different commercial and ornamental *Prunus* species will be collected from major growing districts throughout Australia and sampled and the tested using the protocols selected and developed in this project. This validation will test protocols under “local” conditions and identify any potential “false positives” or organisms that can make interpretation of results difficult. The survey will also serve to update the disease status for each pathogen in Australia.

The characterisation and development of diagnostic tools for Ilarviruses infecting *Prunus* species in Australia.

By *Wycliff Kinoti*[1,2*], Fiona Constable[1], Kim M. Plummer[2] & Brendan Rodoni[1,2]

[1]AgriBio, Department of Environment and Primary Industries, 5 Ring road, Bundoora Victoria 3086, Australia.

[2]La Trobe University, BundooraVictoria 3086, Australia.

*e-mail: wkinoti@students.latrobe.edu.au

Apple mosaic virus (ApMV), *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus (*PDV) can have significant economic impact on stone fruit and almond production in Australia. These three *Ilarvirus* species can occur as single infections or as mixed infections which further increases their damage to *Prunus* species. All three viruses are transmitted by grafting and PNRSV and PDV are transmitted by pollen or seed, contributing to their worldwide distribution, including Australia.

Within Australia PNRSV, PDV and ApMV are controlled through certification programs that supply high-health planting material. These services require accurate, rapid and validated diagnostic tools. This study was undertaken to gain a better understanding of the diversity of *Prunus* Ilarviruses in Australia with a view to improve molecular detection methods.

Prunus samples were collected from different regions of Australia and tested using ApMV, PDV and PNRSV species specific RT-PCR tests and an *Ilarvirus* genus specific RT-PCR test. PNRSV was the most frequently detected *Ilarvirus* species. Variation in detection of Ilarviruses was observed as viruses were detected in some samples with species specific RT-PCR tests but not with the *Ilarvirus* genus specific test. Occasionally some samples were positive only with the *Ilarvirus* genus specific RT-PCR test.

PNRSV infected tissue was used to inoculate *Cucumus sativis* (cucumber) indicators in an effort to increase virus titre for downstream characterisation of interesting isolates through sequencing of PCR amplicons and next generation sequencing (NGS). Variation in symptom expression on cucumbers was associated with different PNRSV isolates and some cucumber plants were symptomless.

Phylogenetic analysis of the direct sequenced amplicons showed that PNRSV isolates from cucumber cluster together and some of these isolates were different to the *Prunus* tree isolates with which they were inoculated. This suggests that inoculation to cucumber may select for certain *Ilarvirus* strains/variants.

Ilarvirus variants from trees and cucumbers will be characterised using next generation sequencing (NGS) strategies to understand the diversity of *Ilarvirus* affecting Australian *Prunus* trees. This information will be used to improve molecular diagnostic tools for detection of specific *Ilarvirus* species and to develop an *Ilarvirus* genus tests to detect both known and unknown ilarviruses of *Prunus* trees in Australia.

Morioka 23rd International Conference on Virus and Other Graft Transmissible Diseases of Fruit Crops Aiina Center, Morioka, Japan 2015

The characterisation of Ilarviruses infecting *Prunus* species in Australia.

By *Wycliff Kinoti*[1,2*], Fiona Constable[1], Kim M. Plummer[2] & Brendan Rodoni[1,2]

[1]AgriBio, Department of Environment and Primary Industries, 5 Ring road, Bundoora Victoria 3086, Australia.

[2]La Trobe University, Bundoora Victoria 3086, Australia.

*e-mail: wkinoti@students.latrobe.edu.au

Apple mosaic virus (ApMV), *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus (*PDV) can have significant economic impact on stone fruit and almond production in Australia. These three *Ilarvirus* species can occur as single infections or as mixed infections, which further increases their damage to *Prunus* species. All three viruses are transmitted by grafting and PNRSV and PDV are transmitted by pollen or seed, contributing to their worldwide distribution, including Australia.

This study was undertaken to better understand the diversity of *Prunus* Ilarviruses in Australia. *Prunus* samples were collected from different regions of Australia and tested using ApMV, PDV and PNRSV species specific RT-PCR tests and an *Ilarvirus* genus specific RT-PCR test. PNRSV was the most frequently detected *Ilarvirus* species. Variation in detection of Ilarviruses was observed as viruses were detected in some samples with species specific RT-PCR tests but not with the *Ilarvirus* genus specific test. Occasionally some samples were positive only with the *Ilarvirus* genus specific RT-PCR test.

Phylogenetic analysis of the direct sequenced or cloned and sequenced amplicons did not show any clustering according to *Prunus* species, region or identified *Ilarvirus* phylogroups. This may be due to the limited amplicon sequence information of the targeted regions. Therefore we are developing next generation sequencing (NGS) strategies to sequence the full genome of Australian *Ilarvirus* isolates. Several plant virus sample preparation methods for NGS were compared to find a suitable alternative that is fast and yields a higher proportion of virus reads compared to current NGS sample preparation methods.