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# ALL ABOUT ALMONDS

ORCHARD MANAGEMENT

# VIRUSES IN AUSTRALIAN ALMONDS

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## Introduction

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It is believed by many people that viruses are an inevitable factor of an almond orchard's life and in almost all cases it is. But exactly to what extent are they in effect? Is everything affected? Why should we put effort into producing high health trees if they are going to just get the virus at a later stage in life anyway?

Agriculture Victoria have produced the following information on the relevant bacteria, viruses and viroids in the Australian almond industry. This fact sheet uses information from Agriculture Victoria to portray a grower story and notes why it is important to use high health material at the early stage of life.

It is recognised and promoted by the ABA's Plant Improvement Committee that a virus free will enable the best chance of strong growth in the early stages of its life, enabling the possibility of higher yields. A high health tree will also reduce stress on the tree reducing the chance of non-infectious bud failure.

Image 1: Uninfected almond tree cv. Padre (left) and declining almond trees infected with two different strains of Candidatus Phytoplasma pruni (middle and right), which are present in the USA but are exotic to Australia. (Photo: Fiona Constable)

*The following text utilises the information produced by Agriculture Victoria with notes relating to growing added by ABA Industry Development staff where noted.*

## Background in biosecurity

***Agriculture Victoria - A world-class diagnostic capanility to support the biosecurity of the Australian almond industry***

The biosecurity of the Australian almond and summerfruit industries are maintained at the border by the Department of Agriculture (DA) in post entry quarantine (PEQ) facilities and internally through schemes that supply high-health planting material throughout Australia. Newly imported material is tested in PEQ for significant exotic pathogens to prevent their introduction into Australia.

Industry-based certification programs, including the program managed by the Almond Board of Australia (ABA), underpin onshore biosecurity of the almond and summerfruit industries through the production of high-health pathogen-tested budwood for growers. The distribution and use of this material minimizes the risk of introduction and subsequent spread of endemic pathogens that can reduce yield and quality of fruit and nut trees and the sustainability and longevity of orchards.

The success of PEQ and budwood programs depends on accurate and sensitive diagnostic tests for the detection of important exotic and endemic pathogens.

In 2016, Agriculture Victoria successfully completed a Hort Innovation funded project: (MT12005) Development of molecular diagnostic tools to detect endemic and exotic pathogens of Prunus species for Australia (www.horticulture.com.au/MT12005).

An important outcome of the project is an improved diagnostic capability for the detection of economically significant endemic and exotic bacteria, virus and virus-like pathogens to support biosecurity of the Australian almond and summerfruit industries. A diagnostic manual for endemic and exotic pathogens was developed and the molecular diagnostic tools have been implemented by several Australian laboratories, including Agriculture Victoria's Crop Health Services. During the verification of the diagnostic tools, several viruses that were previously not known to occur in Australia were identified in Australian grown Prunus species. This information has been used to update test requirements for all Prunus species during post entry quarantine and to underpin the standard to produce pathogen-tested almond and summerfruit propagation material in Australia.

# Developing a world-class diagnostics capability

## Review of significant pathogens of almonds

A review of literature identified 60 significant pathogens of Prunus species occurring worldwide and 48 of those pathogens were exotic to Australia. Of the 48 exotic pathogens infecting Prunus, three bacteria, 13 fungi, eight phytoplasmas, seven viruses and one viroid are known to infect almonds (Table 1). Pathogens that occur in Australia and could infect Australian grown almonds include three bacteria, seven viruses and two viroids. Except for cucumber mosaic virus (CMV), most of these should be monitored visually or by laboratory testing in a certification program that produces high-health planting material (Table 2). A summary of the biology, associated diseases and diagnostic symptoms, geographic distribution, host range, economic impact pathway for entry and spread and available diagnostic tests was collated for each endemic and exotic bacterium, phytoplasma, virus and viroid. This information was used to identify optimal pest and disease management strategies for maintaining high-health almond and summerfruit budwood repository blocks.

## Molecular diagnostic tools for bacteria, phytoplasmas, viruses and viroids

Molecular diagnostic tools were developed for the detection of exotic and endemic bacteria, phytoplasmas, viruses and viroids for almonds and summerfruit. The primary molecular diagnostic tools that have been developed use polymerase chain reaction (PCR) based 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, which causes almond leaf scorch) some simpler tests, which are potentially adaptable for rapid field-based diagnostics or smart pathogen surveillance (e.g. Loop Mediated Isothermal Amplification; LAMP), were assessed. In addition, a high-throughput sequencing (HTS) capability was developed to investigate the genetic diversity of Prunus infecting viruses.

**Image 2: Leaf scorching in almond associated with Xylella fastidiosa. Photo Fiona Constable**

## A national survey

The molecular tests were assessed during a national survey, in which 100 Prunus trees, comprising of 33 almonds, 54 summerfruit species and 13 cherries, were tested for the presence of five bacteria, 10 phytoplasmas or phytoplasma groups, 34 viruses and three viroids. It was vital that these surveys were conducted prior to the use of these protocols for certification in a commercial or quarantine situation to ensure they were fit for purpose and easy to interpret. As a result:

- The presence or absence of each pathogen in Australian almonds (Table 2) and summerfruit was updated.
- The test protocols were shown to be reliable under Australian conditions.
- A diagnostic manual incorporating the endemic and exotic protocols was developed and made available to other laboratories.

In this survey, bacteria, viruses and viroids were detected in 70 per cent (70/100) of samples. The viruses and viroids that are known to occur in Australia and that were detected in samples from this survey included apple chlorotic leafspot virus (ACLSV), apricot pseudo-chlorotic leaf spot virus (APCLSV), apple mosaic virus (ApMV), apple stem grooving virus (ASGV), cherry virus A (CVA), prune dwarf virus (PDV), prunus necrotic ring spot (PNRSV), hop stunt viroid (HSVd), little cherry virus 2 (LChV2) and peach latent mosaic viroid (PLMVd). ASGV occurs in other crops in Australia and was detected in one plum tree. PNRSV was detected in 51 per cent (51/100) of the Prunus trees that were tested and in 17/33 almond trees tested. ApMV, HSVd and the bacterium Agrobacterium tumefaciens were each detected in one almond tree.

Viruses previously considered exotic to Australia, including cherry green ring mottle virus (CGRMV), cherry necrotic rusty mottle virus (CNRMV) and plum bark necrosis stem pitting associated virus (PBNSPaV) were detected in summerfruit species and cherries. Only A. tumefaciens, ApMV, PNRSV and HLVd were detected in almonds. The results indicated that for some viruses, such as ApMV, PDV and PNRSV there is genetic diversity that affects reliable detection using a single species-specific test or a single generic-based test for detection of multiple species within a genus, therefore a combination of tests may be required.

**Industry Development Officer's Note**

This survey found that 48% of almond trees planted throughout the orchard are virus free. As a grower, you would want this number to be as high as possible to minimise the effect of viruses in the orchard.

Therefore, to have the highest chance of increasing this percentage it makes sense to use high-health material. It is worth the investment when you consider viruses have been reported to cause up to 60% yield loss. (Page 4 - The most common viruses in almonds)

## Genetic diversity of viruses

HTS was used for an in-depth study of the genetic variability of endemic Prunus viruses. Amplicon sequencing is a targeted HTS approach to analyse the depth of diversity in targeted genome regions of a specific virus species or genus within a sample and amongst samples. This approach was used to study the genetic diversity of ApMV, PDV, PNRSV and other related Ilarvirus species. Metagenomic sequencing was used to assemble and analyse whole genomes of these viruses to confirm their presence in some samples and to also analyse their genetic diversity. It is important to understand this variability because it can affect the reliability of any diagnostic test and may also affect the biology and epidemiology of the pathogen, including the virulence of the pathogen.

The results of amplicon HTS reflected those of PCR testing and indicated that there is significant diversity amongst Australian strains of ApMV, PDV and PNRSV, even within the same sample. They supported the recommendation that both species specific PCR tests for ApMV, PDV and PNRSV should be combined with a generic RT-PCR test for virus species in the genus Ilarvirus to ensure accurate diagnostic testing. Amplicon HTS also identified the presence of American plum line pattern virus (APLPV), another Ilarvirus species which was tested but not detected during the survey.  Using metagenomic sequencing APLPV was confirmed in the infected samples. Metagenomic sequencing was also used to confirm the presence of CVA, CNRMV, CGRMV, LChV2 and PBNSPaV in summerfruit and cherry samples. During sequencing, little cherry virus 1 (LChV1) was also detected in a cherry sample that was not tested as part of the national survey but included to confirm PCR results from another laboratory for this virus and others. Metagenomic sequencing produced the first full Australian genomes of ACLSV, ApMV, APCLSV, PDV, and PNRSV, which were previously reported in Australia by other methods.

Image 3: Dr. Cliff Kinoti running real time RT-PCR for virus detection at AgriBio, Centre for AgriBiosciences

**Table 1.** A list of pathogens that are known to infect almonds, that occur in Australia and may require testing at the certification level.

| Pathogen | Pathogen known to infect almonds | Disease |
|---|---|---|
| Bacteria | *Agrobacterium tumefaciens* | *Crown gall, root knot and canker* |
| | *Pseudomonas syringae pv. syringae* | *Bacterial leaf spot, shot-hole and black spot* |
| | *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)* | *Almond mosaic and line pattern* |
| | *Little cherry virus 1* | *Specific disease is unknown* |
| | *Prune dwarf Ilarvirus (PDV)* | *Almond mosaic* |
| | *Prunus necrotic ringspot Ilarvirus (PNRSV)* | *Necrotic shock, bud failure and calico* |
| | *Plum bark necrosis stem pitting-associated ampleovirus (PBNSPaV)* | *Bark necrosis and stem pitting* |
| | *Cucumber mosaic cucumovirus (CMV) – not known on Prunus in Australia* | *Specific disease is unknown* |
| Fungi | *Botryosphaeria dothidea* | *Canker* |
| | *Calosphaeria pulchella* | *Canker* |
| | *Diplodia seriata* | *Canker* |
| | *Eutypa lata* | *Canker* |
| | *Neofusicoccum australe* | *Canker* |
| | *Neofusicoccum parvum* | *Canker* |
| Viroids | *Hop stunt viroid (Australian strains)* | *May be symptomless in almonds* |
| | *Peach latent mosaic viroid* | *May be symptomless in almonds* |



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# Impact of Bacteria, Viruses and viroids in almonds

## THE MOST COMMON VIRUSES IN ALMONDS

**Prunus Necrotic Ringspot Virus (PNRSV), Prunus Dwarf Virus (PDV) and Apple Mosaic Virus (ApMV)** are important viruses of almonds in Australia. In almonds, PNRSV has been associated with necrotic shock, bud failure, calico and chlorotic mottling (Figure 1). It may be symptomless in some almond cultivars. When PNRSV occurs in mixed infection with other viruses, such as 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 per cent 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, whereas ApMV is only transmitted vegetatively. 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).

Figure 2: Crown gall symptoms that are associated with Agrobacterium tumefaciens in cherry (OSU, USA).

**Industry Development Officers Note**

It has been observed through planting surveys that Carmel plantings within the almond industry are still significant. This is because Carmel is a high consistent yielder and the market for the kernel is well established. The variety comes with a major risk of non-infectious bud failure. A hypothesis is that if you can get through the first six seasons without getting bud failure then the block will be a strong producer, even if bud failure takes hold and needs management. This is due canopy growth on a three year old being a greater percentage of the canopy, then that of a six year old.

Therefore, to reduce the risk of incidence within that time frame it is important to utilise high-health nursery material. It will provide a much better probability of getting through to that sixth year and beyond.

Adding to this, some viruses transfer through pollen. There are added benefits to reducing flowers in the second year of tree life, potentially extending the period before significant infection occurs.

Figure 1: Chlorotic symptoms that are associated with prunus necrotic ring spot (PNRSV) in almonds (UCS, USA)

## OTHER BACTERIA, VIRUSES AND VIROIDS OF NOTE

**Plum bark necrosis stem pitting associated virus (PBNSPaV)** is transmitted in propagation material. No vector is reported, although natural spread occurs. Management of PBNSPaV is through removal of infected trees.

**Hop stunt viroid (HSVd)**, which was detected in one almond tree, 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 not known to be associated with symptoms in almond. HSVd is transmitted by infected propagation material through grafting and mechanically on pruning equipment and harvest equipment.

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.

A. **tumefaciens**, which was detected in one almond tree, causes large tumour-like growths on affected roots and at the collar just above the soil (Figure 2). 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. tumefaciens is soil-borne, where it can last for several years in the absence of a host. It can be transmitted through planting material and may be transmitted from plant to plant on pruning equipment.

**Plum bark necrosis stem pitting associated virus (PBNSPaV)** is associated with bark necrosis and stem pitting disease in susceptible almond, plum, prune, peach, cherry, and apricot varieties. In sensitive cultivars it can also cause gummosis and graft union failure . Symptomless infections occur. PBSPaV was not detected in almonds but was found in other Prunus species during the national survey. It can have significant impacts on almonds yields in the case of mixed infections with other viruses and/or abiotic stress such as nutrient deficiencies.



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**Table 2.** 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 | Pathogen known to infect almonds | Disease |
|---|---|---|
| Bacteria | *Erwinia amylovora* | *Symptomless* |
| | *Pseudomonas amygdali* | *Hyperplasic canker* |
| | *Xylella fastidiosa* | *Almond leaf scorch* |
| Phytoplasmas | *Candidatus Phytoplasma prunorum* | *Almond leaf scorch* |
| | *X-Disease phytoplasma* | *European stone fruit yellows* |
| | *Candidatus Phytoplasma pyri* | *Decline, Almond brown line and kernel shrivel* |
| | *Candidatus Phytoplasma phoenicium* | *Almond witches' broom* |
| | *Peanut witches' broom group phytoplasmas (16SrII - Candidatus Phytoplasma aurantifolia related strains)* | *Almond little leaf, Shoot prolifer-ation* |
| | *Clover proliferation group phytoplasmas (16SrVI - Ca. P. trifolii related strains)* | *Yellowing or little leaf* |
| | *Stolbur (16SrXII-A) group phytoplasmas* | *Yellowing or little leaf* |
| Viruses | *Cherry twisted leaf virus* | *Almond is an experimental host* |
| | *Peach mosaic virus trichovirus (PcMV)* | *Symptomless* |
| | *Peach rosette mosaic virus (PRMV)* | *Specific disease is unknown* |
| | *Plum pox potyvirus (PPV)* | *No or inconspicuous leaf symp-toms* |
| | *Raspberry ringspot nepovirus (RpRSV)* | *Chlorosis, mosaic, decline.* |
| | *Tomato black ring nepovirus* | *Specific disease is unknown* |
| | *Tomato ringspot nepovirus* | *Yellow bud mosaic* |
| Viroids | *Hop stunt viroid** | *Stunting, chlorosis, mosaic* |

*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.

## Conclusions

The survey results have assisted the development of management strategies and biosecurity plans for the almond industry. Based on the results of this survey, ApMV, PDV and PNRSV, HSVd and A. tumefaciens remain important to the production of high-health almond plantings in Australia. Testing for these endemic pathogens should be undertaken within certification and high-health programs. Although PBNSPaV was not detected, testing of almond budwood remains important because spread may occur. Bioinformatic analyses for all 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 endemic and exotic pathogens that were verified by Agriculture Victoria have been incorporated into a national pathogen-testing manual that can be used by pathologists and industry in Australia to support biosecurity. They are used to support the provision of certified high-health plant material by the ABA, who undertake annual virus testing of trees held in the almond budwood program.


## Acknowledgments

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## USEFUL RESOURCES

***Information sourced from***
Original Fact Sheet Location?

***MT12005 - Project Final Report***
Development of molecular diagnostic tools to detect endemic and exotic pathogens of Prunus species for Australia

www.horticulture.com.au/MT12005

## MORE INFORMATION


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## PROJECT CODE

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***AL16001 - Australian almond industry innovation and adoption program***


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