Horticulture Innovation Australia

Final Report

Monash Remediation

Ross Skinner
Almond Board of Australia (ABA)

Project Number: AL12011

AL12011

This project has been funded by Horticulture Innovation Australia Limited using funds from the almond industry and funds from the Australian Government.

ISBN 0 7341 3896 2

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

Almonds (Prunus dulcis) are susceptible to a variety of endemic and exotic bacteria, phytoplasmas, viruses, viroids and fungi which if introduced to an orchard at an early age are likely to reduce orchard health and ultimately limit production. The almond industry has been a strong advocate of high health material that dates back to the 1970's where Brenton Baker and Dr van Velson implemented an almond plant improvement program.

Following the closure of the site developed in the 1970's, another almond high health material site was developed in the early 1990's by the Australian Almond Improvement Society at Monash, S.A. The site was leased from the Riverland Vine Improvement Committee (RVIC) and was co-located with wine grapes, citrus and apricots. The almond planting is now approximately 1 hectare and consists of 64 cultivars/clones and 4 rootstocks, and serves two major purposes:

1. Budwood supply of the common commercial cultivars, clones and rootstocks to the nursery industry

2. Germplasm of local and imported varieties used in the Australian almond breeding program (AL08000)

In recent years, the production and management of the high-health pathogen-tested almond material has been conducted by the Almond Board of Australia (ABA) on behalf of the Almond Plant Improvement Committee (APIC) and the Australian almond industry.

In September 2011, some indifferent visual symptoms were observed on numerous budwood trees. An immediate site visit, expert assistance and industry support was requested from Dr Michelle Wirthensohn (Australian almond breeding project leader, University of Adelaide), Dr Brendan Rodoni (Senior Virologist, DPI Victoria); and past and current almond plant improvement advocates Andrew Lacey, Tony Spiers, Brendan Sidhu, Tim Orr and Daryl Winter.

Dr Wirthensohn and Dr Rodoni sampled leaves from a selection of 25 trees and took the samples back to their respective laboratories to assess the virus status and cross-check results. Nine (36%) of the trees were assessed positive for Prunus Necrotic Ringspot Virus (PNRSV), which combined with the visual symptoms being observed, lead to a cessation of budwood supply from the entire site in 2011/12.

This project will research, develop and implement new high-health pathogen tested material for planting in a new almond budwood repository. The outputs and outcomes from the project will ensure the Australian almond industry has access to high health material and orchard productivity will be optimised.

Keywords

Almond, *Prunus dulcis*, budwood, PCR, germplasm, virus, Prunus Necrotic Ringspot Virus (PNRSV), Prune Dwarf Virus (PDV), Apple Chlorotic Leafspot Virus (ACLSV), Apple Mosaic Virus (ApMV), Ilarvirus.

Introduction

Almonds (*Prunus dulcis*) are susceptible to a variety of endemic and exotic bacteria, phytoplasmas, viruses, viroids and fungi which if introduced to an orchard at an early age are likely to reduce orchard health and ultimately limit production. The almond industry has been a strong advocate of high health material that dates back to the 1970's where Brenton Baker and Dr van Velson implemented an almond plant improvement program with grower membership on the Improvement Committee elected from the S.A. Almond Co-operative. The program was located at the Horticultural Research Unit, Northfield S.A. and initially consisted of imported material from California.

Following closure of Northfield, another high health material site was developed in the early 1990's by the Australian Almond Improvement Society at Monash, S.A. The site was leased from the Riverland Vine Improvement Committee (RVIC) and was co-located with wine grapes, citrus and apricots. The almond planting is now approximately 1 hectare and consists of 64 cultivars/clones and 4 rootstocks, and serves two major purposes:

1. Budwood supply of the common commercial cultivars, clones and rootstocks to the nursery industry
2. Germplasm of local and imported varieties used in the Australian almond breeding program (AL08000)

In recent years, the production and management of the high-health pathogen-tested almond material has been conducted by the Almond Board of Australia (ABA) on behalf of the Almond Plant Improvement Committee (APIC) and the Australian almond industry. The site has undergone strict management strategies appropriate for budwood repository blocks which includes annual: heavy pruning; flower removal; pest and disease monitoring and control; virus testing (initially achieved using ELISA, but with more recent advances in molecular and diagnostic technology PCR has been used); and sterilisation of pruning equipment between individual trees. The routine virus testing was conducted by Dr Michelle Wirthensohn, University of Adelaide.

In September 2011, some indifferent visual symptoms were observed on numerous budwood trees. An immediate site visit, expert assistance and industry support was requested from Dr Michelle Wirthensohn (Australian almond breeding project leader, University of Adelaide), Dr Brendan Rodoni (Senior Virologist, DPI Victoria); and past and current almond plant improvement advocates Andrew Lacey, Tony Spiers, Brendan Sidhu, Tim Orr and Daryl Winter.

Dr Wirthensohn and Dr Rodoni sampled leaves from a selection of 25 trees and took the samples back to their respective laboratories to assess the virus status and cross-check results. Nine (36%) of the trees were assessed positive for Prunus Necrotic Ringspot Virus (PNRSV), which combined with the visual symptoms being observed, lead to a cessation of budwood supply from the entire site in 2011/12.

This project will research, develop and implement new high-health pathogen-tested material for a new almond budwood repository. The outputs and outcomes from this project will ensure the Australian almond industry has access to high health material and orchard productivity will be optimised. In addition to this project, a new project lead by DPI Victoria is also being considered by the almond industry to review and develop molecular diagnostic tools to detect endemic and exotic pathogens of almonds in Australia.

Methodology

The project will focus on two objectives:

1. Detailed virus assessment of all the important cultivars, clones and rootstocks planted at the Monash budwood repository using PCR technology and biological indexing.
2. Production of high-health pathogen-tested material for future almond budwood supply.

1. Detailed virus assessment of all the important cultivars, clones and rootstocks planted at the Monash budwood repository using PCR technology and biological indexing.

It will be necessary to develop a new set of Foundation Trees (FT) that will be stored in an insect proof repository so that Mother Trees (MT) can be propagated and developed without risking the longevity of the FT. To develop the FT, all important cultivars, clones and rootstocks will undergo a PCR virus assessment for four of the major endemic viruses known to affect almonds: Prunus Necrotic Ringspot Virus (PNRSV); Prune Dwarf Virus (PDV); Apple Chlorotic Leafspot Virus (ACLSV); Apple Mosaic Virus (ApMV); and an additional assessment of other "general" Ilarviruses using a generic Ilar primer.

Monash trees that test virus negative

Those trees that test virus negative in the first assessment will continue with more detailed assessment which involved three seasons of biological indexing using woody and herbaceous indicators. The indicators will undergo visual assessment for virus symptoms (e.g. chlorotic leaves, erratic branch patterns, leaf distortions, etc.) in addition to further PCR virus assessment. Those trees that test virus negative at the end of the three-year period will be propagated onto rootstocks of equivalent health status and kept in DPI Victoria's high-health screen house at Bundoora, Victoria and become the almond industry's new FT.

Monash trees that test virus positive

Those trees that test virus positive in the first assessment will undergo heat treatment or thermotherapy combined with meristem tip culture to eliminate the virus. Plants will be placed in a heat treatment chamber at 37 degrees Celsius under a 16-hour light 8-hour dark regime for 8 to 10 weeks. Growing tips will be removed from heat treated plants and each bud meristem will be dissected to establish tissue cultures. The cultures will then be grown in tissue culture conditions, planted out and grown under glass house conditions. Pathogen testing will then commence using PCR to detect the presence of virus isolates. Those that test virus negative will undergo a further three years of biological indexing and PCR tests as described above. Those trees that test virus positive will undergo further heat treatment until PCR tests indicate virus negative.

Those trees that are assessed as true-to-type will be propagated onto rootstocks of equivalent health status and kept in DPI Victoria's high-health screen house at Bundoora, Victoria and become the almond industry's new FT.

2. Production of high-health pathogen-tested material for future almond budwood supply.

Following the successful development of FTs, MTs will be propagated onto rootstocks of equivalent health status and bulked up for planting and growing on for budwood supply to the Australian almond

nursery industry. The MTs will be planted in a new budwood repository at quantities suitable for budwood supply and planted on a rolling and regular basis of younger MTs where they will be kept for the optimum amount of time for budwood supply and minimise virus infection risk.

Outputs

1. Development of two FTs per cultivar, clone and rootstock for all the important plant material to the Australian almond industry.
2. Production of MTs for each of the important cultivars, clones and rootstocks for all the important plant material to the Australian almond industry.

Outcomes

The expected outcomes from this project are:

1. Enhanced biosecurity of the Australian almond industry at the orchard level.
2. Efficient and productive Australian almond orchards through the availability and supply of high-health pathogen-tested planting material.

Evaluation and Discussion

Candidate lines from a total of 45 almond varieties (Appendix 1) were sampled from the Australian almond industry's budwood repository at Monash, South Australia and delivered to Agriculture Victoria-Crop Hygiene (formerly DEJDTR) for assessment of virus associated diseases.

Two methods were employed to detect virus symptoms and the health status of the 45 varieties: 1) Biological (woody) Indexing and 2) Reverse Transcription Polymerase Chain Reaction (RT-PCR).

Biological (woody) Indexing
Biological indexing takes advantage of a sensitive plant response to the presence of pathogens. Sensitive indicator plants, such as GF305, are inoculated with material or may be grafted onto material of another source and are observed for symptoms characteristic of virus infection. Viruses of almond that may be associated with symptoms on GF305 include *Prune Dwarf Virus* (PDV), *Prunus Necrotic Ringspot Virus* (PNRSV), *Apple Chlorotic Leafspot Virus* (ACLSV) and *Apple Mosaic Virus* (ApMV). This method may also detect other virus species. The indicators have been observed for symptoms over a period of three years. Only two of the 45 varieties have shown positive visual symptoms; these are Avalon 1B-9 (faint lesions/ring pattern) and Tardy NP 6B-2 (leaf distortion and lumps).

RT-PCR Testing
Reverse Transcription Polymerase Chain Reaction (RT-PCR) is a sensitive and specific method that detects the RNA of the target of interest. The grafted plants for biological indexing described above were also tested by RT-PCR methods for the specific detection of PDV, PNRSV, ACLSV and ApMV in spring 2014 to determine if virus transmission to the grafted indicator has occurred. They have been tested at least twice by molecular methods that were specific for ACLSV, ApMV, PDV and PNRSV. They were also tested using a generic "Ilarvirus test" that can detect other PNRSV, PDV or ApMV strains that might be missed with the specific tests. This test may also confirm a positive result, with the specific tests, but due to the variability of these viruses it can also miss strains that the specific tests can detect.

Using molecular methods ApMV or PNRSV were detected in 11 of the varieties. Nonpareil 12 10A-20 produced inconsistent and weak positive result for PNRSV. A suspect positive result was obtained for Marcona 1B-15 with the Ilarvirus test; the identity of the virus that was detected is not known. It can be observed that specific symptoms were not always present on indicators in which viruses were detected or suspected to be present using molecular methods. Although these strains that were detected may be symptomless on the indicator, virus infection may still have an impact on quality and yield in the cultivar. The results of the PCR tests during woody indexing are shown in appendix 3.

The original 45 candidate varieties in the Monash Repository have also been undergoing PCR testing to determine their health status. The results of the last round of testing is shown in appendix 4. Thirteen varieties have tested positive to the presence of a virus, either a specific virus test or the general Ilarvirus test. Four of the thirteen varieties have two trees which are undergoing PCR testing of which only one tree is positive and the other is negative. The other 11 varieties will undergo heat therapy if they are to be retained as virus free trees.

Foundation Trees

One of the primary outputs of this project was the production and storage of foundations trees in a secure environment. At the onset of the project the methodology to be implemented was to retain those trees grafted onto GF305 that tested negative to virus symptoms as the core foundation trees. Upon reviewing the condition of the trees at the end of the three-year woody indexing period, in consultation with Brendan Rodoni and Fiona Constable, it was decided that the trees were not of sufficient health to be maintained as foundation trees. Instead propagation material would be sourced from the original virus free trees at the Monash Repository and grafted to the most appropriate rootstock to ensure the best possible long-term health of the foundation trees. Whilst it will take longer than planned to secure the required foundation trees, the experience has helped greatly in the securing of high health foundation material for the Australian Almond Breeding project (AL12015). Seventeen current selections that are in the secondary evaluation phase have already secured two foundation trees each. As new selections are chosen after primary evaluation and are to move onto secondary commercial yield and agronomic evaluation, extra trees are propagated to be used are foundation trees.

Monash Budwood Repository re-planting program

Approximately one third of the Monash Budwood Repository has been replanted to the following varieties:

- Nonpareil – 54 trees (2013
- Price – 27 trees (2013)
- Carmel – 27 trees (2013)
- Wood Colony – 54 trees (2014)
- Almond 1 – 27 trees (2014)
- Almond 2 – 27 trees (2014)
- Almond 3 – 27 trees (2014)
- Almond 5 – 27 trees (2014
- Almond 7 – 27 trees (2014)

The remaining two thirds of the repository will be planted in July 2016. These trees were propagated in January 2015 and will be planted as two-year-old trees. This decision was made to enable stronger tree growth in the first growing season in the repository and bring the trees into budwood production sooner. This is especially important given the current high demand for propagation material during the current boom the almond industry is experiencing. The repository will also be treated with a nematicide to further enhance early tree growth and long-term tree health. After replanting is finished, the repository will again be one hectare in size but with a better varietal mix to suit current industry requirements.

Recommendations

The primary recommendations of this project are:

- Continue the ongoing PCR testing of budwood trees to ensure the availability of high health propagation material to meet industry needs.
- Incorporate the findings of MT12005 (Development of molecular diagnostic tools to detect endemic and exotic pathogens of Prunus species for Australia) into future virus testing.
- Continue to proactively secure Foundation Trees from the Australian Almond Breeding project (AL12015) as soon as candidate selections pass primary evaluation.
- Finalise the heat therapy of the varieties that have tested positive to woody indexing and PCR testing.

Scientific Refereed Publications

None to report.

Intellectual Property/Commercialisation

No commercial IP generated.

Appendices

Appendix 1 – Almond cultivars under assessment

Appendix 2 – List of abbreviations

Appendix 3 – PCR results during Woody Indexing

Appendix 4 – PCR results (Monash Repository, 2015)

Appendix 1 – Almond cultivars under assessment

Variety	Number of samples	Code
12-350	1	7B-6
Adafuel	1	2B-4
Ai (R269)	1	1B-20
Alnem88 mutant	1	4B-2
Antoneta	1	7B-22
Avalon	1	1B-9
Butte 353288 (1F?)	1	1A-14
Carmel (mature)	2	18A-5,17
Chellaston	1	6B-9
Constanti (22-120)	1	7B-2
Desmayo Largueta (F)	1	7B-26
Ferradual (R485)	1	1B-25
Ferragnes (R486)	1	5B-3
Fritz	1	6B-14
GF557	1	2B-6
GF677	1	2B-5
GF749	1	5B-4
H184	1	2B-3
Keanes	1	6B-20
Lauranne (R916)	1	1B-5
Livingston (F?)	1	1A-8
Marcona (R185)	1	1B-15
Masbovera	1	7B-20
Mission	1	15A-14
Monterey 362193 (F?)	4	1A-20,21,22,3B 12-16
Ne Plus 37272 (F?)	1	1A-10
Nonpareil-04 (mature)	2	2A-13,21
Nonpareil-05	2	3A-9,22
Nonpareil-12 (mature)	2	10A-8,20
Nonpareil-15 (mature)	2	12A-5; 13A-15
Padre 329175 (F?)	1	1A-17
Parkinson	1	6B-7
Peerless (NFC)	2	19A-8,16
Price (mature)	1	14A-6
R1049	1	5B-7
R1065	1	5B-24
R1066	1	4B-23
R1148	1	4B-20
R887	1	5B-13
Somerton	1	6B-11
Sonora 315170 (F?)	1	1A-18
Tardy NP	1	6B-2
Tarraco (21-169)	1	7B-8
Vairo (21-323)	1	6B-23
Wood Colony (F?)	6	1A-7; 3B-22,23,24,25,26
Total	**45**	

Appendix 2 – List of abbreviations

- **ACLSV**= Apple chlorotic leafspot virus
- **ApMV** = Apple mosaic virus
- **PDV** = Prune dwarf virus
- **PNRSV** = Prunus necrotic ringspot virus
- **Ilarvirus** = Generic test that detects viruses in the Ilarvirus family including ApMV, PDV, PNRSV and others

Appendix 3 – PCR results during Woody Indexing

Variety	ACLSV	ApMV	PDV	PNRSV	Ilarvirus	Code
12-350	Negative	Negative	Negative	Negative	Negative	7B-6
Adafuel	Negative	Negative	Negative	Positive	Suspect	2B-4
Ai (R269)	Negative	Negative	Negative	Negative	Negative	1B-20
Alnem88 mutant	Negative	Negative	Negative	Negative	Negative	4B-2
Antoneta	Negative	Negative	Negative	Negative	Negative	7B-22
Avalon	Negative	Negative	Negative	Positive	Positive	1B-9
Butte 353288 (1F?)	Negative	Negative	Negative	Negative	Negative	1A-14
Carmel (mature)	Negative	Negative	Negative	Negative	Negative	18A-5,17
Chellaston	Negative	Negative	Negative	Positive	Negative	6B-9
Constanti (22-120)	Negative	Negative	Negative	Negative	Negative	7B-2
Desmayo Largueta (F)	Negative	Negative	Negative	Negative	Negative	7B-26
Ferradual (R485)	Negative	Negative	Negative	Negative	Negative	1B-25
Ferragnes (R486)	Negative	Negative	Negative	Negative	Negative	5B-3
Fritz	Negative	Negative	Negative	Negative	Negative	6B-14
GF557	Negative	Negative	Negative	Positive	Positive	2B-6
GF677	Negative	Negative	Negative	Negative	Negative	2B-5
GF749	Negative	Negative	Negative	Negative	Negative	5B-4
H184	Negative	Negative	Negative	Positive	Negative	2B-3
Keanes	Negative	Negative	Negative	Negative	Negative	6B-20
Lauranne (R916)	Negative	Negative	Negative	Negative	Negative	1B-5
Livingston (F?)	Negative	Negative	Negative	Negative	Negative	1A-8
Marcona (R185)	Negative	Negative	Negative	Negative	Suspect	1B-15
Masbovera	Negative	Negative	Negative	Negative	Negative	7B-20
Mission	Negative	Negative	Negative	Negative	Negative	15A-14
Monterey 362193 (F?)	Negative	Negative	Negative	Positive	Negative	1A-20,21,22,3B 12-16
Ne Plus 37272 (F?)	Negative	Negative	Negative	Negative	Negative	1A-10
Nonpareil-04 (mature)	Negative	Negative	Negative	Negative	Negative	2A-13,21
Nonpareil-05	Negative	Positive	Negative	Negative	Negative	3A-9,22
Nonpareil-12 (mature)	Negative	Negative	Negative	Suspect	Negative	10A-8,20
Nonpareil-15 (mature)	Negative	Negative	Negative	Positive	Negative	12A-5; 13A-15
Padre 329175 (F?)	Negative	Positive	Negative	Negative	Negative	1A-17
Parkinson	Negative	Negative	Negative	Positive	Suspect	6B-7
Peerless (NFC)	Negative	Negative	Negative	Negative	Negative	19A-8,16
Price (mature)	Negative	Negative	Negative	Negative	Negative	14A-6
R1049	Negative	Negative	Negative	Negative	Negative	5B-7
R1065	Negative	Negative	Negative	Negative	Negative	5B-24
R1066	Negative	Negative	Negative	Negative	Negative	4B-23
R1148	Negative	Negative	Negative	Negative	Negative	4B-20
R887	Negative	Negative	Negative	Negative	Negative	5B-13
Somerton	Negative	Negative	Negative	Negative	Negative	6B-11
Sonora 315170 (F?)	Negative	Negative	Negative	Negative	Negative	1A-18
Tardy NP	Negative	Negative	Negative	Negative	Negative	6B-2
Tarraco (21-169)	Negative	Negative	Negative	Negative	Negative	7B-8
Vairo (21-323)	Negative	Negative	Negative	Negative	Negative	6B-23
Wood Colony (F?)	Negative	Negative	Negative	Negative	Negative	1A-7; 3B-22,23,24,25,26
Total	**45**					

Appendix 4 – PCR results (Monash Repository, 2015)

Variety	ACLSV	ApMV	PDV	PNRSV	Ilarvirus	Code
12-350	Negative	Negative	Negative	Negative	Negative	7B-6
Adafuel	Negative	Negative	Negative	Positive	Positive	2B-4
Ai (R269)	Negative	Negative	Negative	Negative	Negative	1B-20
Alnem88 mutant	Negative	Negative	Negative	Positive	Negative	4B-2
Antoneta	Negative	Negative	Negative	Negative	Negative	7B-22
Avalon	Negative	Negative	Negative	Positive	Negative	1B-9
Butte 353288 (1F?)	Negative	Negative	Negative	Negative	Negative	1A-14
Carmel (mature)	Negative	Negative	Negative	Positive	Positive	18A-5,17
Chellaston	Negative	Negative	Negative	Negative	Negative	6B-9
Constanti (22-120)	Negative	Negative	Negative	Negative	Negative	7B-2
Desmayo Largueta (F)	Negative	Negative	Negative	Negative	Negative	7B-26
Ferradual (R485)	Negative	Negative	Negative	Negative	Negative	1B-25
Ferragnes (R486)	Negative	Negative	Negative	Negative	Negative	5B-3
Fritz	Negative	Negative	Negative	Negative	Negative	6B-14
GF557	Negative	Negative	Negative	Positive	Negative	2B-6
GF677	Negative	Negative	Negative	Positive	Negative	2B-5
GF749	Negative	Negative	Negative	Negative	Negative	5B-4
H184	Negative	Negative	Negative	Positive	Negative	2B-3
Keanes	Negative	Negative	Negative	Negative	Negative	6B-20
Lauranne (R916)	Negative	Negative	Negative	Positive	Negative	1B-5
Livingston (F?)	Negative	Negative	Negative	Negative	Negative	1A-8
Marcona (R185)	Negative	Negative	Negative	Negative	Negative	1B-15
Masbovera	Negative	Negative	Negative	Negative	Negative	7B-20
Mission	Negative	Negative	Negative	Negative	Negative	15A-14
Monterey 362193 (F?)	Negative	Negative	Negative	Negative	Negative	1A-20,21,22,3B 12-16
Ne Plus 37272 (F?)	Negative	Negative	Negative	Negative	Negative	1A-10
Nonpareil-04 (mature)	Negative	Negative	Negative	Negative	Negative	2A-13,21
Nonpareil-05	Negative	Negative	Negative	Positive	Negative	3A-9,22
Nonpareil-12 (mature)	Negative	Negative	Negative	Positive	Negative	10A-8,20
Nonpareil-15 (mature)	Negative	Negative	Negative	Negative	Negative	12A-5; 13A-15
Padre 329175 (F?)	Negative	Negative	Negative	Negative	Positive	1A-17
Parkinson	Negative	Negative	Negative	Negative	Negative	6B-7
Peerless (NFC)	Negative	Negative	Negative	Positive	Positive	19A-8,16
Price (mature)	Negative	Negative	Negative	Positive	Negative	14A-6
R1049	Negative	Negative	Negative	Negative	Negative	5B-7
R1065	Negative	Negative	Negative	Negative	Negative	5B-24
R1066	Negative	Negative	Negative	Negative	Negative	4B-23
R1148	Negative	Negative	Negative	Negative	Negative	4B-20
R887	Negative	Negative	Negative	Negative	Negative	5B-13
Somerton	Negative	Negative	Negative	Negative	Negative	6B-11
Sonora 315170 (F?)	Negative	Negative	Negative	Negative	Negative	1A-18
Tardy NP	Negative	Negative	Negative	Negative	Negative	6B-2
Tarraco (21-169)	Negative	Negative	Negative	Negative	Negative	7B-8
Vairo (21-323)	Negative	Negative	Negative	Negative	Negative	6B-23
Wood Colony (F?)	Negative	Negative	Negative	Negative	Negative	1A-7; 3B-22,23,24,25,26
Total	**45**					