Hort Innovation

Final Report

Protecting Australia's Citrus Genetic Material

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Content

Summary

Citrus is one of Australia's most important horticultural export crops. In 2016/17, the industry produced 708,121 tonnes of citrus fruit worth $724 million. Diseases can destroy an industry therefore it is vital that we protect the health status of the national citrus industry by ensuring that disease-free, true-to-type propagation material is available to prevent incurable diseases from entering citrus nurseries and orchards. Use of healthy planting material will avoid potential yield loss and the costly exercise of replanting infected blocks.

Graft-transmissible diseases, spread by infected plant material, are of most concern as they can kill trees and there is no cure. Major graft-transmissible citrus diseases, such as huanglongbing (HLB) and citrus variegated chlorosis (CVC), are not known to occur in Australia. However, within our country, there are a number of graft-transmissible viruses and viroids that can cause stunting, yield loss and even death in some scion and rootstock combinations, yet other varieties may be symptomless carriers. Examples of endemic graft-transmissibles include citrus exocortis viroid (CEVd), cachexia (citrus viroid IIb - CVd-IIb) and *Citrus tristeza virus* (CTV). Transmission of these diseases must be prevented through the use of uninfected propagation material. Some diseases, such as CTV, HLB and CVC, can also be transmitted via insect vectors. It is vital that industry protects the high health status of Australian citrus through the National Citrus Repository Program.

The National Citrus Repository Program maintains high health status foundation trees as a source of budwood for industry. Trees may be disease-free or inoculated with a mild isolate of CTV to protect against severe isolates of CTV. Small quantities of budwood from the foundation trees are used by the Australian Citrus Propagation Association, trading as Auscitrus, to create daughter trees and multiply large numbers of buds for industry. New varieties can enter the program if no known diseases are detected after pathogen testing and elimination.

Project CT15005 'Protecting Australia's citrus genetic material' continues the work of previous projects funding the long-term repository program. Funds support the maintenance and disease testing of the foundation trees of publicly owned varieties in the National Citrus Repository and the disease testing of new Australian citrus selections. Repository houses are located on the Auscitrus property at Dareton and at the NSW Department of Primary Industries' Elizabeth Macarthur Agricultural Institute at Menangle. Disease testing is undertaken by NSW DPI. Currently 122 publicly owned citrus clones are housed in the repository, from Australian and overseas sources. Five of these were new introductions to the repository over the course of the project from 2015 to 2018, all from local sources.

The National Citrus Repository is an industry asset that serves as an insurance policy for the future health and economic viability of the Australian citrus industry.

Keywords

Citrus; biosecurity; germplasm; repository; budwood; true-to-type; graft-transmissible disease

List of acronyms

CEVd	Citrus exocortis viroid
CLBV	*Citrus leaf blotch virus*
CPsV	*Citrus psorosis virus*
CTLV	*Citrus tatterleaf virus*
CTV	*Citrus tristeza virus*
CVC	Citrus variegated chlorosis
CVd-I	Citrus viroid I or Citrus bent leaf viroid
CVd-IIa	Citrus viroid IIa
CVd-IIb	Citrus viroid IIb or cachexia
CVd-III	Citrus viroid III or Citrus dwarfing viroid
CVd-IV	Citrus viroid IV or Citrus bark cracking viroid
CVd-V	Citrus viroid V
CVd-VI	Citrus viroid VI
CVd-VII	Citrus viroid VII
DAWR	Department of Agriculture and Water Resources
DNA	Deoxyribonucleic acid
DTBIA	Direct tissue blot immunoassay
EMAI	Elizabeth Macarthur Agricultural Institute
HLB	Huanglongbing
HSVd	Hop Stunt viroid or Citrus viroid II
NAQS	Northern Australian Quarantine Strategy
NSW DPI	New South Wales Department of Primary Industries
OSP	Orange stem pitting
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
RT-qPCR	Reverse transcription quantitative polymerase chain reaction

Introduction

Biosecurity is a priority for the Australian citrus industry (Citrus Strategic Investment Plan 2017-2022). It is important to maintain the high health status of Australian citrus to maximize orchard productivity and maintain market access. Graft-transmissible diseases are spread by grafting or the use of infected plant material, mechanically on infected cutting tools during pruning and hedging, and in some cases by aphids or other insect vectors. There is no cure for these diseases hence they must be prevented through the use of uninfected propagation material.

Major graft-transmissible citrus diseases, such as huanglongbing (HLB) and citrus variegated chlorosis (CVC), are not known to occur in Australia. However, within our country, there are a number of graft-transmissible viruses and viroids that can cause stunting, yield loss and even death in some scion and rootstock combinations, yet other varieties may be symptomless carriers. Examples of endemic graft-transmissible diseases include citrus exocortis viroid (CEVd), cachexia (CVd-IIb) and *Citrus tristeza virus* (CTV). Studies recorded yield loss in an orchard infected by CEVd was nearly 50% on citrange and 65% on trifoliata rootstock during the first 9 years of production (Bevington and Bacon 1977). For most graft-transmissible diseases, symptoms will not be seen in nursery trees, the signs will appear a few years later in the orchard. By that time, the disease is likely to have spread to surrounding trees. Nothing can be done to save infected trees.

Viroids and viruses are not all pathogenic, some can be beneficial. Inoculating trees with a viroid that induces mild dwarfing is useful for high density plantings and ease of orchard management. 'Mild strain cross protection' is used successfully in Australia to protect white grapefruit trees against severe stem pitting isolates of *Citrus tristeza virus* (CTV) by inoculation with a mild CTV isolate. Whilst some viroids and viruses can be non-pathogenic on their own, in combination their effects can be detrimental to the tree. For example, studies have shown that citrus viroid V (CVd-V) has little impact on trees but co-infection with Citrus viroid I or III (CVd-I or III) results in severe dwarfing (Serra et al. 2008). This highlights the importance of knowing what organisms are present in propagation material before its use.

Australian quarantine managed by the Federal Department of Agriculture and Water Resources (DAWR) significantly reduces the risk of entry of graft-transmissible diseases into Australia. Graft-transmissible citrus diseases are managed within Australia by surveillance programs for early detection to increase the chance of eradication, the DAWR post-entry quarantine system where newly imported citrus varieties are tested for exotic and endemic plant pathogens before release from quarantine, the National Citrus Repository Program where foundation trees of commercial citrus varieties are maintained in biosecure repositories and tested for citrus pathogens, and the Auscitrus propagation scheme, managed by a non-profit industry organisation (Australian Citrus Propagation Association) that supplies high health status, true-to-type budwood and rootstock seed to nurseries for tree production.

Project CT15005 'Protecting Australia's citrus genetic material' continues the work of previous projects funding the long-term National Citrus Repository Program. Funds support the pathogen testing of new Australian citrus selections and the maintenance and pathogen testing of the foundation trees of publicly owned varieties in the National Citrus Repository. Trees may be pathogen-free or inoculated with a mild isolate of CTV to protect against severe CTV isolates. Small quantities of budwood from the foundation trees are used by the Australian Citrus Propagation Association, trading as Auscitrus, to create daughter trees and multiply large numbers of buds for industry. New Australian varieties are tested for graft-transmissible pathogens and pathogens are removed by shoot-tip grafting. These varieties can enter the program if no known diseases are detected in a tree; this becomes the foundation tree for that variety.

Independent testing of the repository trees is provided by the NSW Department of Primary Industries (NSW DPI) citrus pathology team based at Elizabeth Macarthur Agricultural Institute (EMAI). This work is part of the NSW DPI Citrus Pathology Program which aims to protect the health status of the Australian citrus industry by expanding our knowledge and capability on disease threats and maintaining the resources to respond to new threats.

Detecting graft-transmissible pathogens can be difficult because the pathogen particles may be present below detectable levels or unevenly distributed within the tree. It is important that diagnostic tests are specific to the target organism, sensitive (i.e. will detect even at low levels), and efficient (time and cost). A support project was funded by Hort Innovation from 2014-2018, CT14009 'Protecting Australian citrus germplasm using improved diagnostic tools', to assess current and new diagnostic methods for graft-transmissible pathogens of citrus and ensure we are using the most robust, sensitive and efficient methods available. Some of these methods have been adopted and used to test trees in the National Citrus Repository Program.

The work undertaken in project CT15005 'Protecting Australian's citrus genetic material' enhanced the ability of Auscitrus and the NSW DPI team to deliver for industry and government by:

- ensuring the availability of healthy planting material to industry; and
- helping to prepare for an incursion of exotic disease threats like HLB, the worst disease reported to affect citrus globally.

Methodology

Project CT15005 'Protecting Australia's citrus genetic material' from July 2015 to June 2018 funds the maintenance and testing of public varieties held in the National Citrus Repositories and pathogen elimination of locally selected public varieties. All work outlined in this report refers to the maintenance and testing of public varieties.

The maintenance and testing of private varieties in the repository system is covered by a contract agreement between the private variety owner and Auscitrus and is paid for by the variety owner as fee for service. There are currently foundation trees of 110 private varieties in the 'National Citrus Repository for High Health Status Clones'.

National Citrus Repositories

The 'National Citrus Repository for High Health Status Clones' maintains foundation trees of 122 public citrus clones (Table 1 / Appendix 1). A minimum of 1 tree of each variety is held in screen houses in 2 secure locations at Dareton and Menangle, NSW. The Dareton repository is situated in the Sunraysia citrus growing region on the Auscitrus property. Menangle, on the outskirts of south western Sydney, is not in a citrus producing area and the repository is sited at the NSW DPI EMAI. Repository houses are located in two different regions to provide a level of redundancy should a catastrophic event such as fire, storm, vandalism, or disease incursion occur at one of the sites.

The 'National Repository for Inoculated Citrus Clones' contains high health status mother trees that have been inoculated with a mild isolate of CTV. This mild isolate serves to protect against more severe isolates of the virus that may be introduced to trees in the field by aphid vectors. This management tool is called mild strain cross protection and the inoculation of trees is often referred to as 'pre-immunisation' in the international citrus literature. The inoculated trees are housed in a controlled environment greenhouse at EMAI.

Plant maintenance

The foundation trees are maintained using industry best practice. Quarantine mesh is used on the screen houses at EMAI and Dareton containing the high health status foundation trees. The inoculated clones are maintained in a controlled environment greenhouse. All houses have double entry doorways. The trees are regularly inspected for pest infestation, disease symptoms and off-type shoots. Fruit on foundation trees are observed and photographed and the images are maintained in a database. Trees are drip irrigated and fertigated. Strict nursery hygiene is observed during all management activities. Organic fertiliser is also used at EMAI. The repository houses are secure facilities with access only granted to essential staff or external auditors, with approval from Auscitrus.

A tree replacement program is ongoing to create daughter trees from foundation trees; with the oldest foundation trees (over 20 years old) replaced over the course of the project.

Health status testing

Trees in the citrus repositories are tested regularly for citrus graft-transmissible pathogens by the NSW DPI citrus pathology team at EMAI. It is important to note that the high health status of repository trees means that no graft-transmissible pathogens have been detected in the trees. These trees have a high health status but pathogens may be detected in these trees through improved test methods or the discovery of new pathogens.

Hort Innovation funded project CT14009 'Protecting Australian citrus germplasm through improved diagnostic tools' provided the NSW DPI citrus pathology team, in collaboration with Auscitrus, the opportunity to assess current and new diagnostic tests for graft-transmissible citrus pathogens to ensure we are using the most reliable, sensitive and efficient methods available. Improved diagnostic tools developed and validated through the project have been adopted by the National Citrus Repository program and the Auscitrus propagation scheme to test plant material prior to supply to industry.

Samples were collected from the end of October 2014 to early March 2015 for molecular testing of repository trees representing all publicly owned clones. Leaf samples were collected from each quadrant of each tree and grouped. Nucleic acids were extracted from leaf midribs for testing; ribonucleic acid (RNA) was used to test for

citrus viruses and viroids and deoxyribonucleic acid (DNA) was used to test for the causal agents of HLB. Extractions and PCR assays were performed in duplicate.

***Citrus tristeza virus* (CTV)**

CTV is the most devastating viral disease affecting citrus globally and is endemic throughout Australia. There are many isolates of the virus from mild to severe causing a range of disease symptoms. The most economically significant symptoms include decline and stem pitting.

Every tree in the citrus repositories is tested annually for the presence of CTV using a serological test called direct tissue blot immunoassay (DTBIA) (Garnsey et al. 1993). This test is used to confirm that the virus is not present in the high health status clones and to confirm that the virus is present in every inoculated tree.

Each year, trees in the EMAI and Dareton repositories were tested for CTV in either autumn or spring. Trees were not tested in winter or summer because viral titre (i.e. the levels of viral particles present in the plant) are affected by temperature and therefore fluctuate across the seasons.

Trees in the repository for inoculated clones that initially test positive for CTV after inoculation but which test negative for CTV during the annual testing, are tagged and re-tested. It is likely that the virus is still present in these plants but at such a low level that it escaped detection. If the tree is still found to be negative upon re-testing the tree is either re-inoculated or discarded. Budwood is only sourced from inoculated trees that have tested positive for CTV during the past year (their most recent test). Each bud stick is blotted before dispatch and tested to confirm the presence of CTV.

Citrus viroids

Eight viroids are known to infect citrus around the world and there are different viroid strains within each of those viroid types. Viroids that are known to be most devastating to citrus production are CEVd and a strain of hop stunt viroid that causes cachexia. Citrus bark cracking viroid (CBCVd) is a minor pathogen of citrus.

All repository trees are scheduled for testing for citrus viroid infection every 3 years.

RNA extracts were tested for CEVd and citrus cachexia viroid (CVd-IIb) using a probe-based quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay (Lin et al. 2015) or using a multiplex conventional RT-PCR for CEVd, CVd I, II and III (Wang et al 2009; Wildman 2013). RNA extracts were also tested for citrus apscaviroids (CVd-I, III, V, VI and the tentatively named VII) using a patented, real-time, SYBR™ RT-qPCR assay (Vidalakis and Wang 2013). A number of repository trees were tested for CBCVd using RT-qPCR (Osman et al. 2017).

***Citrus tatterleaf virus* (CTLV)**

CTLV is known to occur in Australia. Infection causes stunting and chlorosis in infected scions when grafted onto susceptible rootstocks such as *Citrus* (*Poncirus*) *trifoliata*, citrange or Swingle citrumelo. A yellow ring is seen at the bud union of symptomatic trees and may be mistaken for horticultural incompatibility. Repository trees on susceptible rootstocks would show symptoms of stunting, chlorosis and a yellow ring if infected with CTLV and therefore are considered to be self-indexing. Repository trees on tolerant (symptomless) rootstocks are tested for CTLV every 9-12 years.

Repository trees on tolerant rootstocks were tested for CTLV in 2010 using biological and molecular methods therefore testing was not due within this project term. No symptoms consistent with CTLV infection were observed on repository tees on susceptible rootstocks.

***Citrus leaf blotch virus* (CLBV)**

CLBV causes a bud union disorder of susceptible scions (such as Nagami kumquat and calamondin) on trifoliate type rootstocks and is known to occur in Australia. Until recently, Australian citrus selections were not tested for CLBV prior to entering the repository program as test methods had not been developed.

During the term of the current project, RNA extracts were tested for CLBV using a probe based RT-PCR assay

(Osman et al. 2015).

Huanglongbing (HLB)

HLB is one of the most destructive diseases of citrus in the world and one of the major factors limiting citrus production in South East Asia, Florida and Brazil. Three forms of HLB have been described in association with phloem limited bacteria '*Candidatus* Liberibacter asiaticus', '*Ca*. L. americanus' and '*Ca*. L. africanus'; none of which have been recorded in Australia. The pathogen of most concern to Australia, and the one reported to be closest to our shores is the Asian strain ('*Ca*. L. asiaticus').

Australian citrus selections were not tested for HLB prior to entering the repository program because HLB is not known to occur in Australia. Imported varieties were only tested in post-entry quarantine for HLB by biological indexing until recently when a molecular test was added to the post-entry quarantine requirements. The importance of testing the repository trees for HLB using sensitive molecular techniques was recognised due to their role in supplying high health status material to the Australian citrus industry.

During the course of this project, and partner project CT14009, trees representing all of the public varieties held in the National Citrus Repository were tested for '*Ca*. L. asiaticus', '*Ca*. L. americanus' and '*Ca*. L. africanus' using real-time PCR (Li et al. 2006; Coy et al. 2014), the most sensitive and robust technique currently available in our laboratory for detecting these organisms.

New introductions to the repository

Graft-transmissible pathogens can be removed from infected mother trees by heat treatment and shoot tip grafting. Successful shoot tip grafted plants then require testing to determine if all known pathogens have been eliminated. Imported varieties are tested and undergo pathogen elimination in post-entry quarantine run by the Federal Department of Agriculture. Auscitrus provides the service of pathogen testing and elimination by shoot tip grafting for Australian selections but these are only tested for pathogens known to occur in Australia.

When an imported or locally selected variety is 'released', the high health status mother tree is placed in one of the repository houses at Menangle or Dareton and a daughter tree is propagated for placement in the other repository house.

Quality assurance

The Auscitrus nursery is accredited by NIASA (Nursery Industry Accreditation Scheme Australia) and is audited by the NSW Nursery Industry Development Officer annually. The nursery and laboratory work conducted by the NSW DPI citrus pathology team has been certified since 2005 and was most recently externally audited in June 2018 against the ISO 9001:2015 guidelines. The EMAI repository for high health status citrus clones is also accredited as a New Zealand Ministry of Primary Industries (NZ MPI) off-shore quarantine facility for the introduction of new citrus varieties to NZ. A re-accreditation audit was undertaken by NZ MPI in April 2018. The Auscitrus EMAI management committee meets at least annually at EMAI to tour the facilities and discuss the Auscitrus related work conducted at EMAI.

Outputs

National Citrus Repositories

Table 1 details the number of publicly owned clones of each citrus type currently housed in the 'National Citrus Repository for High Health Status Clones'. Appendix 1 provides a full list of public varieties, also published each year in the Auscitrus Annual Report. The annual report will also list the total distribution of budwood from the daughter trees grown from repository material, indicating the widespread benefit of this project.

Table 1: The number of publicly owned clones of each citrus type housed in the National Citrus Repository for High Health Status Clones (as of June 2018)

Citrus type	No. publicly owned clones
Orange	49
Mandarin	34
Tangor / tangelo	5
Grapefruit	9
Pumelo	2
Lime	2
Lemon	10
Citron	3
Papeda	5
Kumquat	2
Trifoliate orange	1

Health status testing

No CTV was detected in foundation trees in the 'National Citrus Repository for High Health Status Citrus Clones' using DTBIA.

All trees but one (see Table 2) in the 'National Repository for Inoculated Citrus Clones' tested positive for CTV each year from 2015 to 2018 using DTBIA.

A number of citrus clones are known for being difficult to inoculate with a mild strain of CTV. Table 2 provides a list of citrus clones held in the 'National Repository for Inoculated Citrus Clones' where it has been difficult to detect CTV since 2015. Note that all inoculated trees tested positive in 2015 and 2017.

Table 2: Citrus clones where *Citrus tristeza virus* (CTV) has been difficult to detect in inoculated repository trees

Testing year	Citrus clones inoculated with a mild strain of CTV where:	
	CTV was not detected in at least 1 inoculated tree	CTV was detected at a low level in at least 1 inoculated plant
2016	Berri Valencia	Keenan 3125, Lima 156 acidless orange, CSIRO 5 Valencia, Hutton navel, Salustiana common orange
2018		Clementard and Caffin Clementine mandarins

No viroids (CEVd, CVd I, II, III, IV, V, VI, VII) were detected using molecular methods in samples collected from the repository trees of public varieties. Note that new detection methods validated in Hort Innovation project CT14009 'Protecting Australian citrus germplasm through improved diagnostic tools', have detected viroids in some trees of privately owned varieties which provides greater confidence in the results for the publicly owned varieties.

No symptoms consistent with CTLV infection were observed in repository trees of public varieties on susceptible rootstocks.

No CLBV was detected in repository trees of public varieties using molecular test methods.

The putative causal agents of HLB; '*Ca*. Liberibacter asiaticus', '*Ca*. L. africanus' and '*Ca*. L. americanus' were not detected in the 122 public varieties tested.

New introductions to the repository

Table 3 outlines the new introductions to the 'National Citrus Repository for High Health Status Clones' from July 2015 to June 2018.

Table 3: New introductions of citrus clones to the repository system from July 2015 to June 2018

Accession number	Clone	Year entered repository	Source
A.NT.15.1032	Tropical Meyer	2015	Local
A.N.15.1033	Calamondin	2015	Local
A.NT.15.1034	Tropical Emperor	2015	Local
A.S.17.1043	Poorman's orange	2017	Local
A.N.18.1054	Benton citrange	2018	Local

Budwood exports

Budwood was sourced from both EMAI and Dareton repository trees over the course of the project, to establish new budwood multiplication trees for subsequent distribution of budwood to the wider citrus and nursery industry.

Budwood of public varieties was sent to South Korea and the USA in 2017 from the Dareton repository screen

house for high health status clones, as part of informal ad-hoc variety exchange agreements.

Communication

Communication activities listed below were delivered by project team members to disseminate the message about the importance of citrus biosecurity, graft-transmissible diseases and the health status of Australian citrus germplasm.

Scientific paper

Chambers GA, Donovan NJ, Bodaghi S, Jelinek SM, Vidalakis G. 2018. A novel citrus viroid found in Australia, tentatively named citrus viroid VII. Archives of Virology 163(1), 215-218

Conference publications

Herrmann T. Citrus Nursery Scene in Australia. XI Congress of the International Society of Citrus Nurserymen. Mildura Australia, 24-28th July 2017

Donovan N, Herrmann T, Jelinek SM, Chambers GA, Englezou A. Supply of healthy propagating material to Australian citrus nurseries. XI Congress of the International Society of Citrus Nurserymen, Mildura Australia 24-28th July 2017

Donovan N, Englezou A, Chambers G, Jelinek S, Tan M, Chapman T, Holford P. 2017. Protecting Australian citrus germplasm through improved diagnostic tools. Citrus Technical Forum and Field Day, Mildura Australia 1-2 March 2017

Chambers GA, Donovan NJ, Jelinek SM, Vidalakis G. A novel citrus viroid found in Australia, tentatively named citrus viroid VII. 20th International Organisation of Citrus Virologists Conference, Chongqing China 10-15th April 2016

Donovan NJ, Englezou A, Chambers GA, Jelinek SM, Tan MK, Chapman TA, Holford P. Protecting Australian citrus germplasm through improved diagnostic tools. International Citrus Congress, Foz Do Iguacu, Brazil 18-23 September 2016

Extension material

Donovan N, Holford P, Chambers G. 2018. Citrus Viruses in Australia. Auscitrus fact sheet

Donovan N, Holford P, Chambers G. 2018. *Citrus tristeza virus* in Australia. Auscitrus fact sheet

Donovan N, Chambers G, Holford P. 2018. Viroids in Australian Citrus. Auscitrus fact sheet

Donovan N, Sanderson G, Falivene S. 2017. Budwood and graft-transmissible disease. In: Citrus Plant Protection and Management Guide 2017 pp 52-53. Eds: Falivene S, Creek A. State of New South Wales through NSW Department of Industry. ISSN – 2208-5963 (print) ISSN – 2208-5971 (online)

Donovan N, Creek A. 2017. Diseases and disorders. In: Citrus Plant Protection and Management Guide 2017 pp 35-46. Eds: Falivene S, Creek A. State of New South Wales through NSW Department of Industry. ISSN – 2208-5963 (print) ISSN – 2208-5971 (online)

Ong R. 2017. Congress key message: don't risk disease. Australian Citrus News Spring 2017 p 33 (N.B. content and editing provided by N. Donovan)

Donovan N. 2016. Huanglongbing (HLB) worldwide update. Citrus Connect December 2016

Plant Biosecurity and Product Integrity. 2016. Exotic Plant Pests and Diseases of Citrus. NSW DPI booklet

Donovan N. 2015. Untested budwood can cost you thousands. Citrus Connect December 2015

Donovan N, Herrmann T. 2015. Preventing disease at the source. Australian Citrus News Summer 2015/16 p24

Reports

In addition to contracted milestone reports, project activities were outlined in the following reports.

Herrmann, T., Donovan, N., Jelinek, S. 2018. Australian Citrus Propagation Association Incorporated Annual Report.

Herrmann, T., Donovan, N., Jelinek, S. 2017. Australian Citrus Propagation Association Incorporated Annual Report.

Herrmann, T., Donovan, N., Jelinek, S. 2016. Australian Citrus Propagation Association Incorporated Annual Report.

Herrmann, T., Donovan, N., Jelinek, S. 2015. Australian Citrus Propagation Association Incorporated Annual Report.

Donovan N. 2017. International Research Conference on Huanglongbing, Florida - March 2017. Report submitted to industry

Donovan N, Sanderson G. 2016. International Citriculture Congress, Brazil – September 2016. Report submitted to industry

Donovan N, Chambers G. 2016. International Organisation of Citrus Virologists, China – April 2016. Report submitted to industry

Presentations

The importance of the National Citrus Repository Program to the health status of the Australian Citrus industry was highlighted in the following presentations:

Donovan N. NSW DPI Citrus R&D Roadshows. Perth WA 12/9/17, Griffith NSW 16/10/17, Mildura Vic 18/10/17, Loxton SA 19/10/17

Donovan N. The dangers of using untested budwood. Advances in Disease Management and Detection Workshop, Citrus Technical Forum and Field Day. Mildura Vic 1-2 March, 2017

Donovan N. Citrus Pathology Program – Protecting Australian Citrus Germplasm through Improved Diagnostic Tools. Citrus Strategic Investment Advisory Panel, Horticulture Innovation Australia, Central Coast Primary Industries Institute, Ourimbah, NSW Australia 1st February 2017

Donovan N. Management of citrus diseases and disorders of concern to industry. Leeton and Griffith NSW, 9/3/16

Herrmann T. Securing our future budwood supply. Preparing the Australian citrus nursery industry for Huanglongbing / Asian Citrus Psyllid Workshop. Dareton NSW 19/8/15

Herrmann T. Nursery structures for ACP/HLB exclusion in the USA and South Africa. Preparing the Australian citrus nursery industry for Huanglongbing / Asian Citrus Psyllid Workshop. Dareton NSW 19/8/15

Donovan N. The dangers of using untested budwood. Preparing the Australian citrus nursery industry for Huanglongbing / Asian Citrus Psyllid Workshop. Dareton NSW 19/8/15

Donovan N. Yellow Dragon: A worldwide citrus epidemic – our greatest threat. Preparing the Australian citrus nursery industry for Huanglongbing / Asian Citrus Psyllid Workshop. Dareton NSW 19/8/15

Donovan N. The Citrus Repository and Indexing Program. Preparing the Australian citrus nursery industry for Huanglongbing / Asian Citrus Psyllid Workshop. Dareton NSW 19/8/15

Outcomes

This project maintained a high health status, genetic resource of citrus material of public citrus varieties; those of commercial interest and those sought by the community. Foundation trees in the repositories were maintained and managed to reduce the risk of infection by graft-transmissible diseases and their high health status has been confirmed by regular testing. Healthy propagation material has been supplied to Auscitrus for propagation of daughter trees and supply of healthy budwood to nurseries and growers around Australia. The Australian citrus industry benefits from increased productivity through the use of disease-free planting material, along with greater control of biosecurity and a reduced risk of spreading significant graft-transmitted citrus diseases. Access to healthy budwood also reduces the risk of illegal importations of sought after varieties.

New varieties have been introduced to the repository after release from the pathogen elimination and testing program at EMAI. The impact of this project extends beyond the national industry, with the EMAI repository also serving as an off-shore quarantine facility for introduction of new citrus varieties to New Zealand.

The National Citrus Repository will prove invaluable in the event of a disease outbreak such as HLB, as it will provide a disease-free source of genetic material protected from insects associated with disease transmission. The maintenance of citrus repository facilities in two locations has provided functional redundancy to the system. The foundation trees will serve as a readily available source of health-tested, true-to-type propagation material to rebuild the industry in the event of an incursion of an emergency plant pest. The repository is an insurance policy for the Australian industry.

Extension activities undertaken throughout the project (in conjunction with projects CT14009) have increased biosecurity awareness of the importance of using health-tested and true-to-type budwood due to exotic and established disease threats. This was achieved through reports to industry and presentations at industry forums to growers, nurserymen, service providers and other relevant stakeholders. This reduces the incidence and impact of established pathogens and increases the likelihood of early detection and eradication of introduced pathogens.

Internal and external collaboration by the project team has enhanced the citrus biosecurity skill base available to industry and government.

Monitoring and evaluation

Biosecurity is a priority for the Australian citrus industry (Citrus Strategic Investment Plan 2017-2022). It is critical to the longevity of the Australian citrus industry that biosecurity strategies focus on disease exclusion, particularly given the number of diseases for which there is no cure. Therefore it is essential that industry and the community have access to healthy propagation material to enhance productivity and reduce disease incidence and impact. Access to healthy budwood reduces the risk of illegal importations of sought after varieties.

The work of the National Citrus Repository Program was enhanced by linking with the existing portfolio of the NSW DPI Citrus Pathology Program. Of particular benefit was a partner project funded by Hort Innovation from 2014-2018 CT14009 'Protecting Australian citrus germplasm through improved diagnostic tools'. Diseases can threaten industry survival and the ability to effectively detect these diseases is important for reducing their impact. The diagnostic tools project delivered improved knowledge and diagnostic tools for graft-transmissible citrus pathogens. Improved tools were then used to test Australian citrus germplasm held in the National Citrus Repository. To support diagnostic tool development, the NSW DPI citrus pathogen collection was catalogued and expanded providing a valuable resource. The team evaluated published methods or developed new tests to improve our capability to detect 15 endemic and 10 exotic graft-transmissible citrus pathogens; including the causal agents of HLB, the biggest threat to global citrus. New viroid detections were made using improved diagnostic tools. Efficiency of testing was improved by changing from conventional to real-time PCR for most assays, and by multiplexing assays (testing for more than one pathogen in the same assay). Eight viroid assays were successfully combined into three assays, and four viral assays into three. Within the scope of the project, multiplex assays were adopted by Auscitrus and the repository program, improving the efficiency of testing and confidence in diagnostic results.

Project progress was reported to Auscitrus via quarterly updates throughout the project term. The Auscitrus Executive Committee provided industry perspective and advice, helping to keep the project on track. Project updates were also given annually at the Auscitrus General meetings to the entire Auscitrus Board and visiting industry representatives (e.g. Citrus Australia), and in February 2017 to the Hort Innovation Strategic Investment Advisory Panel.

Recommendations

- The National Citrus Repository Program should be considered an ongoing commitment, with a long term view to the maintenance of this industry resource.
- The value of the citrus repositories should be recognized in industry biosecurity plans as a resource of high health status material available in the event of an incursion of an exotic graft-transmissible disease.
- To maximise the return on this investment, the use of high health status propagation material supplied from daughter trees of this repository via the Auscitrus budwood scheme should be supported by the citrus industry and actively promoted to growers.
- The National Citrus Repository Program is one component of an integrated biosecurity program. The biosecurity risk to the Australian industry from graft-transmissible citrus diseases would be further reduced by:
    - the introduction of a mandatory certification scheme across all states and territories governing the use of health tested propagation material; and
    - a nursery registration system to allow the tracking of 'at risk' commodities like citrus and orange jasmine; given orange jasmine is the preferred host of the insect vector associated with transmission of HLB.

These initiatives will only be successful if supported by industry and, in conjunction with existing biosecurity strategies, will help to maintain the high health status of Australian citrus germplasm and allow industry and government to respond efficiently to disease threats.

Refereed scientific publications

N/A

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Intellectual property, commercialisation and confidentiality

There is shared IP with Hort Innovation associated with project reports, extension articles and other publications.

There are no commercialisation or confidentiality issues to report.

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Appendices

Appendix 1: Clones of public varieties in the 'National Citrus Repository for High Health Status Clones' as of June 2018

Accession number	Citrus clone
Grapefruit	
I.N.91.0736	Flame
I.N.89.0620	Henderson
A.N.73.0068	Marsh (3970 Druitt)
A.N.91.0632	Marsh (3962 Druitt)
I.N.89.0619	Ray Ruby
I.N.89.0708	Rio Red
I.N.89.0709	Star Ruby
A.N.04.0950	Star Ruby (Cant)
A.N.91.0633	Thompson (N Eagle)
Pummelo	
I.N.01.0925	Namroi
I.N.94.0786	Tambun
Citron	
I.N.01.0926	Bergamia Bergamot Castagnaro
I.N.94.0904	Buddha's Hand
I.N.09.0979	Etrog
Lemon	
I.N.01.0927	Eureka (Allen)
A.N.75.0034	Eureka (Lambert)
A.N.75.0035	Eureka (Taylor)
I.N.89.0703	Fino
A.Q.93.0785	Lemonade
I.N.00.0918	Lisbon (Limoneira 8A)
I.N.75.0036	Lisbon (Prior)
A.Q.91.0631	Lisbon (Queensland)
A.NT.15.1032	Tropical Meyer
I.N.89.0705	Verna
Lime	
A.N.08.0969	Tahiti lime
A.N.90.0771	West Indian lime (Schweppes)
Orange	
Navel	
I.N.86.0600	Atwood
A.Q.78.4021	Benyenda - thorny
A.N.14.0993	Cara cara new
I.N.86.0597	Fisher
I.N.99.0912	Fukumoto
A.S.75.5077	Hockney
A.N.73.0073	Houghton
A.S.92.0772	Hutton
I.N.02.0930	Jaffa
A.N.75.0032	Lanes Late 3976
A.N.73.0072	Leng
A.V.94.0781	Lloyd/3 Leng
I.N.86.0550	Navelate
I.N.87.0546	Navelina Spain 7.5
I.N.93.0899	Navelina 315 ex Italy
A.S.92.0773	Neilson
I.N.86.0598	Newhall California
I.N.87.0551	Newhall 55-1 Spanish
I.N.10.0984	Palmer 1051
A.S.75.5074	Thomson
Valencia	
A.S.75.5095	B/3010
A.Q.75.4022	Benyenda
A.S.94.0782	Berri 3501
A.V.94.0780	CSIRO 5
A.V.93.0774	Jenner 4439
A.N.75.0029	Newton – Keenan 3125

Accession number	Citrus clone
A.N.75.0030	Newton – Keenan 3247
Other oranges	
I.N.92.0901	Lima 156 (acidless)
A.S.10.0985	Blood orange (Arnold)
I.N.98.0921	Blood orange (Sanguine)
I.N.08.0968	Blood orange (Tarocco Ippolito)
I.N.07.0965	Blood orange (Tarocco Meli C8158)
I.N.07.0966	Blood orange (Tarocco Rosso C4977)
I.N.06.0960	Common orange (Bintangcheng # 2)
I.N.08.0973	Common orange (Bintangcheng Renbin # 5)
I.N.94.0902	Common orange (Delta seedless)
I.N.86.0548	Common orange (Hamlin)
I.N.02.0930	Common orange (Jaffa)
I.N.06.0959	Common orange (Jincheng 447)
I.N.94.0903	Common orange (Midknight)
I.N.92.0900	Common orange (Natal)
I.N.86.0549	Common orange (Parson Brown)
I.N.90.0741	Common orange (Pera Olympia)
I.N.90.0742	Common orange (Pera Limeira)
I.N.87.0547	Common orange (Pineapple)
I.N.93.0860	Common orange (Salustiana)
A.Q.78.4020	Common orange (Smith - Joppa)
I.N.97.0924	Pigmented navel (Cara Cara)
A.S.17.1043	Poorman's Orange
Mandarin	
I.N.99.0909	Afourer
I.N.99.0913	Avana Tardivo
I.N.99.0914	Avana Apireno
I.N.98.0920	Clementine (Caffin)
I.N.89.0704	Clementine (Clementard)
I.N.99.0910	Clementine (Corsica 1)
I.N.99.0911	Clementine (Corsica 2)
I.N.87.0544	Clementine (Fina)
I.N.87.0552	Clementine (Marisol)
I.N.05.0957	Clementine (Nour)
I.N.87.0543	Clementine (Nules)
I.N.04.0955	Clementine (Orogrande)
I.N.87.0545	Clementine (Oroval)
I.N.04.0953	Clementine (Sidi Aissa)
I.N.91.0733	Daisy
I.N.90.0736	Encore
I.N.08.0974	Etna
I.N.89.0707	Fallglo (VI 484)
I.N.90.0695	Fallglo (S-837-4-2)
I.N.93.0859	Fortune
A.Q.94.0787	Fremont
A.N.75.0041	Hickson
A.N.75.0043	Imperial 0043/2
A.Q.94.0778	Nova (Trott)
I.N.91.0734	Nova (Spain)
I.N.04.0951	Parsons Special /2
I.N.86.0599	Pixie
I.N.04.0954	Primosole
A.N.75.0065	Satsuma (Silverhill)
I.N.89.0706	Satsuma (Clausellina)
I.N.91.0852	Satsuma (Okitsu Wase)
I.N.91.0853	Satsuma (Miho Wase)
A.Q.94.0886	Sunburst
A.NT.15.1034	Tropical Emperor
Tangor/elo	
A.N.75.0090	Ellendale (Herps)
	Ellendale / EM3
A.Q.04.0952	Murcott tangor (Benham)
A.Q.90.4149	Murcott tangor (Turner)
I.N.90.0818	Topaz tangor

Accession number	Citrus clone
Papeda	
I.N.94.0776	Kaffir lime (Malaysia 4669)
A.D.97.0907	Kaffir lime (Nathanael)
I.N.00.0916	Kaffir lime (Eyles)
I.N.15.1020	Sudachi
A.N.13.0991	Yuzu
Kumquat	
A.N.15.1033	Calamondin
I.N.04.0956	Nagami
Rootstock	
A.N.18.1054	Benton citrange