Final Report

Protecting Australia’s citrus genetic material

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Auscitrus

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Protecting Australia's citrus genetic material (CT17008)

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Content

Summary

Citrus is one of Australia’s most important horticultural export crops. In 2019/20, the industry produced 767,766 tonnes of citrus fruit worth $924.4 million (Horticulture Innovation 2020). 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 because 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 several graft-transmissible viruses and viroids that can cause stunting, yield loss and even death in some scion and rootstock combinations. The disease-causing agents may also be present in plants without symptoms, but these plants are a source of future infections. Examples of endemic graft-transmissibles include citrus exocortis viroid (CEVd), cachexia (citrus viroid IIb - CVd-IIb) and citrus tatterleaf virus (CTLV). Transmission of these diseases must be prevented by using uninfected budwood and rootstock seed to propagate new plants. Some diseases, such as citrus tristeza virus (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. Budwood from the foundation trees is 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.

This investment continues the work of previous projects funding the long-term repository program. The project supports the maintenance and testing of the foundation trees of publicly owned varieties in the National Citrus Repository and the testing and removal of pathogens from 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 (NSW DPI EMAI) at Menangle. Pathogen testing and elimination is undertaken by NSW DPI. Currently, 124 publicly owned citrus clones are housed in the repository, from Australian and overseas sources. Two of these were new introductions to the repository over the course of the project from August 2018 to July 2021; one imported and one from a local source.

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

ASGV	apple stem grooving virus
CCGaV	citrus concave gum associated virus
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
CiVA	citrus virus A
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
NIASA	Nursery Industry Accreditation Scheme Australia
NSW DPI	New South Wales Department of Primary Industries
NZ MPI	New Zealand Ministry for Primary Industries
PCR	polymerase chain reaction
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
RT-qPCR	reverse transcription quantitative polymerase chain reaction
qPCR	quantitative polymerase chain reaction

Introduction

Biosecurity is a priority for the Australian citrus industry (Citrus Strategic Investment Plan 2017-2021). 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 propagating infected plant material, mechanically on infected cutting tools during grafting, 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 by using 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 several graft-transmissible viruses and viroids that can cause stunting, yield loss and even death in some scion and rootstock combinations. The disease-causing agents may also be present in plants without symptoms, but these plants are a source of future infections. 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. 'Mild strain cross protection' is used successfully in Australia to protect white grapefruit trees against severe stem pitting variants of CTV by inoculation with a mild CTV isolate. Inoculating trees with a viroid that induces mild dwarfing is useful for high density plantings and ease of orchard management. Whilst some viroids and viruses can be non-pathogenic on their own, in combination their effects can be detrimental to the tree. Studies have shown that citrus viroid V (CVd-V) has little impact on trees but co-infection with citrus bent leaf viroid (CBLVd or CVd-I) or citrus dwarfing viroid (CDVd or CVd-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, Water and the Environment (DAWE) 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 post-entry quarantine system managed by DAWE where newly imported citrus varieties undergo pathogen elimination and testing for exotic and endemic plant pathogens before release,
- 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.

The current project continues the work of previous levy investments to support the long-term National Citrus Repository Program. The current project supports 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 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). Hort Innovation funded projects CT14009 'Protecting Australian citrus germplasm through improved diagnostic tools', and the ongoing project CT17007 'Improving diagnostics and biosecurity for graft-transmissible diseases in citrus', 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 projects have been adopted by the National Citrus Repository program and the Auscitrus propagation scheme to test plant material prior to supply to industry. Testing of nucleic acid extracts from repository trees, or local varieties submitted for pathogen testing and elimination, provides further validation of the diagnostic assays.

The work undertaken in this project 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 of existing and new citrus varieties; and
- helping to prepare for an incursion of exotic graft-transmissible disease threats like HLB, the worst disease reported to affect citrus globally.

Methodology

The current phase of 'Protecting Australia's citrus genetic material', active from August 2018 to July 2021, funded the maintenance and testing of public varieties held in the National Citrus Repositories and pathogen elimination of locally selected public varieties.

The maintenance and testing of private varieties in the repository system are covered by a contract agreement between the private variety owner and Auscitrus and is paid for by the variety owner as fee for service.

National Citrus Repositories

The 'National Citrus Repository for High Health Status Clones' contains foundation trees of 124 public citrus clones (Table 1 / Appendix 1). A minimum of 1 tree of each variety is held in screen houses in two secure locations at Dareton and Menangle, NSW. The Dareton repository is situated on the Auscitrus property in the Sunraysia citrus growing region. A second repository is housed at NSW DPI EMAI, on the outskirts of south western Sydney, and is not in a citrus producing area. Repository houses are 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. 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 repository screen houses at EMAI and Dareton to exclude insects, most recently repaired at Dareton in 2020 and replaced at EMAI in June/July 2021. 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. Strict nursery hygiene is observed during all management activities. Trees are drip irrigated and fertigated. Organic fertiliser is also used at EMAI. The repository houses are secure facilities with access only given to essential staff or external auditors, with approval from Auscitrus.

A tree replacement program is ongoing to create new daughter trees from older foundation trees (more than 20 years of age).

Health status

Before a new variety enters the repository system, a foundation tree is propagated and rigorously tested for graft-transmissible citrus pathogens. A range of biological, serological and molecular methods are used to check the health status of the tree. If a pathogen is detected it must be eliminated by shoot tip grafting before the variety can enter the repository system. This ensures the high health status of trees held in the National Citrus Repositories. Imported varieties are tested and undergo pathogen elimination in post-entry quarantine run by DAWE. Auscitrus provides the service of pathogen testing and elimination by shoot tip grafting for new varieties selected in Australia, outsourced to the independent EMAI Citrus Pathology team.

After entering the repository system, foundation trees are re-tested for graft-transmissible pathogens by the EMAI citrus pathology team according to a designated schedule. Trees are tested annually for CTV but are scheduled for testing every 5 years for those pathogens not transmitted by insect vectors. This is because the risk of infection with non-vectored pathogens is low for trees managed under strict biosecurity protocols in the repository.

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.

During the current project, samples were collected from repository trees representing all publicly owned clones. One budstick, with leaves attached, was collected from each quadrant of each tree and grouped to create a sample. Leaf petioles were blotted on nitrocellulose membranes to test for CTV and nucleic acids were extracted from green bark for testing for several endemic and exotic graft-transmissible citrus pathogens. 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.

Table 1 outlines the detection methods used for graft-transmissible citrus pathogens. As new pathogen threats are discovered, diagnostic tools are validated or adopted through project CT17007 prior to adoption by the National Citrus Repository Program, although testing of repository trees serves as part of the validation process.

Table 1: Detection assays used to test trees in the National Citrus Repository

Pathogen	Assay type	Reference
citrus exocortis viroid (CEVd) hop stunt viroid (HSVd or CVd-II) citrus bark cracking viroid (CBCVd or CVd-IV)	multiplex probe-based RT-qPCR	Osman et al. 2017
citrus bent leaf viroid (CBLVd or CVd-I) citrus dwarfing viroid (CDVd or CVd-III) citrus viroid V (CVd-V) citrus viroid VI (CVd-VI)	multiplex SYBR™ RT-qPCR	Vidalakis and Wang, 2013
citrus viroid VII (CVd-VII)	probe-based RT-qPCR	Chambers et al. in press
citrus tristeza virus (CTV)	direct tissue blot immunoassay	Garnsey et al. 1993
	SYBR™ RT-PCR	Cook et al. 2016
citrus leaf blotch virus (CLBV) citrus psorosis virus (CPsV) citrus tatterleaf virus (CTLV)	multiplex probe-based RT-qPCR	Osman et al. 2015 (CLBV, CPsV) Donovan et al. 2018 (CTLV)
citrus virus A (CiVA)	RT-PCR	unpublished
citrus concave gum associated virus (CCGaV)	RT-PCR	Navarro et al. 2018a
'*Candidatus* Liberibacter asiaticus' (CLas)	qPCR	Li et al. 2006
'*Candidatus* Liberibacter africanus' (CLaf)	qPCR	Li et al. 2006
'*Candidatus* Liberibacter americanus' (CLam)	qPCR	Li et al. 2006

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 tree decline and stem pitting.

Every tree in both 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. All repository trees are also tested for CTV using molecular techniques every 5 years (Table 1).

Trees are tested for CTV in either autumn or spring. Trees are 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.

If CTV is not detected in trees in the repository for inoculated clones, they 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 species; CEVd, citrus bent leaf viroid (CBLV or CVd-I), hop stunt viroid (HSVd or CVd-II), citrus dwarfing viroid (CDVd or CVd-III), citrus bark cracking viroid (CBCVd), citrus viroid V (CVd-V), citrus viroid VI (CVd-VI) and the recently discovered citrus viroid VII (CVd-VII). Viroids that are known to be most devastating to citrus production are CEVd and a strain of HSVd that causes cachexia (CVd-IIb). CBCVd is a minor pathogen of citrus. All repository trees are scheduled for testing for citrus viroid infection every 5 years (Table 1).

A probe-based real-time RT-PCR assay to detect CVd-VII has been developed and validated through project CT17007 and the work has been accepted for publication (Chambers et al. in press). Repository trees were tested using this assay as part of the validation process.

Citrus tatterleaf virus (CTLV)

CTLV, known to occur in Australia, causes stunting and chlorosis in infected scions when grafted onto susceptible rootstocks such as *Citrus* (*Poncirus*) *trifoliata*, citrange or Swingle citrumelo. Apple stem grooving virus (ASGV) causes citrus tatterleaf disease but strains of ASGV pathogenic to citrus are more commonly referred to as CTLV. 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 self-indexing. Repository trees on tolerant (symptomless) rootstocks are scheduled for testing for CTLV every 5 years (Table 1).

Citrus leaf blotch virus (CLBV)

CLBV, known to occur in Australia, 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. Trees are scheduled for testing for CLBV every 5 years (Table 1).

Citrus psorosis virus (CPsV)

CPsV is known to occur in Australia and infection leads to tree decline and reduced production in susceptible scions. All repository trees are scheduled for testing for CPsV every 5 years (Table 1).

Citrus concave gum associated virus (CCGaV) and citrus virus A (CiVA)

The international community have long reported on psorosis-like diseases of citrus. Psorosis, ringspot, impietratura, cristacortis and concave gum produced similar chlorotic flecking and oak leaf patterns on the leaves of indicator plants, leading to the conclusion that all diseases were caused by the same causal agent. New technologies led to the recent discovery of CCGaV and CiVA in citrus (Navarro et al. 2018a, b). Repository trees were tested for CCGaV using a published assay (Navarro et al. 2018a) and for CiVA using a new method developed through project CT17007 (Table 1). The work to validate these assays will continue before final recommendations are made.

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' (CLas), 'Ca. L. americanus' (CLam) and 'Ca. L. africanus' (CLaf); 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 (CLas). All repository trees are scheduled for testing for the causal agents of HLB prior to entry to the repository (Table 1).

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 DAWE. Auscitrus provides the service of pathogen testing and elimination by shoot tip grafting for Australian selections using the methods outlined in Table 1, with testing undertaken by NSW DPI at EMAI.

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 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 2020 against the ISO 9001 guidelines. The EMAI repository for high health status citrus clones is also accredited as a New Zealand Ministry of Primary Industries (NZ MPI) offshore quarantine facility for the introduction of new citrus varieties to NZ. A re-accreditation audit was undertaken by NZ MPI in December 2020. The Auscitrus EMAI management committee meets annually at EMAI to tour the facilities and discuss the Auscitrus related work conducted at EMAI.

The Auscitrus nursery facility that houses the Dareton repository is accredited under the Nursery and Garden Industry NIASA program (Nursery Industry Accreditation Scheme Australia), audited annually by independent nursery industry auditors.

Outputs

National Citrus Repositories

Table 2 details the number of publicly owned clones of each citrus type currently housed in the 'National Citrus Repository for High Health Status Clones' that were supported by the current project.

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 2: The number of publicly owned clones of each citrus type housed in the National Citrus Repository for High Health Status Clones (as of July 2021)

Citrus type	No. publicly owned clones
orange	49
mandarin and hybrids	40
grapefruit	9
pomelo	3
lime	2
lemon	10
citron	3
papeda	5
kumquat	2
trifoliate hybrid	1
TOTAL	124

Health status testing

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

All trees in the 'National Repository for Inoculated Citrus Clones' tested positive for CTV each year from 2018 to 2021 using DTBIA, except for Salustiana orange in 2021 (Table 3), although CTV was detected in this tree by RT-PCR.

Some citrus clones are known for being difficult to inoculate with a mild strain of CTV. Table 3 provides a list of citrus clones held in the 'National Repository for Inoculated Citrus Clones' where it has been difficult to detect CTV since 2018. In 2021, CTV was detected in these trees by RT-PCR. However, the molecular assay only weakly detected CTV in 1 tree each of Ray Ruby, Henderson and Flame grapefruit, and these trees tested positive for CTV by DTBIA. This shows the benefit of using 2 assays when the RNA has already been extracted to test for other pathogens.

Table 3: Publicly owned citrus clones where *Citrus tristeza virus* (CTV) has been difficult to detect in inoculated repository trees using direct tissue blot immunoassay

Testing year	Public 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
2019		Orange: Pera (Bianchi), Jenner, Houghton Mandarins: Primosole, Sidi Aissa,
2020		Orange: CSIRO 5, Delta seedless, Natal Lemon: Limoneira Grapefruit: Henderson
2021*	Orange: Salustiana	Orange: Jenner Mandarin: Avana Apireno, Sidi Aissa

* plants tested for CTV using both DTBIA and RT-PCR – all trees tested positive by RT-PCR

No citrus viroids (CEVd, CBLVd, HSVd, CDVd, CBCVd, CVd-V, CVd-VI, CVd-VII) were detected using molecular methods in samples collected from the repository trees of public varieties.

No symptoms consistent with CTLV infection were observed in repository trees of public varieties on susceptible rootstocks. No CTLV, CLBV, CPsV, CCGaV or CiVA 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 repository trees of public varieties using molecular test methods.

New introductions to the repository

Table 4 outlines the new introductions to the 'National Citrus Repository for High Health Status Clones' from August 2018 to June 2021.

Table 4: New introductions of citrus clones to the repository system from July 2018 to June 2021

Accession number	Clone	Year entered repository	Source
A.Q.19.1061	K15 pomelo	2019	local
I.N.20.1068	Shiranui mandarin	2020	United States

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 Cara Cara was sent to South Korea in 2019 from the Dareton repository screen house for high health status clones.

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. Multiple groups have also visited the Auscitrus facility and EMAI and whilst they are not allowed to enter the repository houses, they are given an overview about the program.

<u>Scientific paper</u>

Chambers GA, Bogema DR, Englezou A, Donovan NJ. 2020. First Report of Citrus viroid V and Citrus viroid VI in Australia infecting Citrus. Plant Disease DOI10.1094/PDIS-12-19-2662-PDN

<u>Conference publications</u>

Donovan N, Herrmann T, Hancock N. 2019. On the frontline: preparing for the arrival of HLB in Australia [abstract]. Journal of Citrus Pathology 6(1): 60

Chambers G, Englezou A, Webster J, Bogema D, Donovan N. 2019. Using Next generation sequencing (NGS) to characterize Australia's living pathogen collection [abstract]. Journal of Citrus Pathology 6(1): 4-5

Chambers GA, Donovan NJ, Bodaghi S, Jelinek SM, Vidalakis G. 2018. Citrus viroid VII, a novel citrus viroid found in Lisbon Lemon in Australia. International Conference on Viroids and Viroid-like RNA, Valencia Spain 5-7th July 2018 p 33

<u>Extension material</u>

Donovan N, Sanderson G, Falivene S. 2020. Budwood and graft-transmissible disease. In: Citrus Plant Protection and Management Guide 2020 pp 58. 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. 2020. Diseases and disorders. In: Citrus Plant Protection and Management Guide 2020 pp 43-54. 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. 2020. HLB update. Citrus Connect March 2020

Donovan N, Miles A. 2018. Xylella… a global threat. Australian Citrus News, Winter edition p 22-23

Donovan N, Miles A. 2018. Xylella… a global threat. Citrus Australia newsletter 6th September 2018. https://www.citrusaustralia.com.au/news/latest-news/xylella-a-global-threat

<u>Reports</u>

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

Herrmann, T., Donovan, N, Dando A, Forbes W. 2021. Australian Citrus Propagation Association Incorporated Annual Report. http://www.auscitrus.com.au/wp-content/uploads/2020/09/Auscitrus-Annual-Report-2021-draft.pdf

Herrmann, T., Donovan, N, Forbes W, Dando A. 2020. Australian Citrus Propagation Association Incorporated Annual Report. https://www.auscitrus.com.au/wp-content/uploads/2020/09/Auscitrus-Annual-Report-2020.pdf

Herrmann, T., Donovan, N, Forbes W. 2019. Australian Citrus Propagation Association Incorporated Annual Report. https://www.auscitrus.com.au/wp-content/uploads/2020/08/Auscitrus-Annual-Report-2019.pdf

Donovan N, Chambers G, Englezou A. 2018. Protecting Australian citrus germplasm through improved diagnostic tools (CT14009). Horticulture Innovation Australia ISBN 978 0 7341 4443 0

<u>Presentations</u>

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

Donovan N. 2019. Biggest threats to Australian citrus production. Citrus Technical Forum, Adelaide, Australia 6-7 March 2019

Donovan N, Chambers G, Englezou A. 2019. Protecting Australian citrus germplasm through improved diagnostic tools. Citrus Technical Forum, Adelaide, Australia 6-7 March 2019

Donovan N. 2018. Citrus biosecurity. WA Citrus Industry Day and Citrus Australia Technical Forum. Hervey and Bunbury Western Australia, Australia, 18-19th October 2018

Outcomes

Biosecurity is a priority for the Australian citrus industry (Citrus Strategic Investment Plan 2017-2021). 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.

Project outcomes are outlined below, with reference to desired outcomes from the monitoring and evaluation (M&E) plan.

Desired end of project outcome: maintain a high health status, genetic resource of citrus material of public varieties

This project maintained a high health status, genetic resource of citrus material of 124 public citrus varieties; those of commercial interest and those sought by the community. The Australian citrus industry benefits from increased productivity using 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 (1) or post-entry quarantine (1). The impact of this project extends beyond the national industry, with the EMAI repository also serving as an offshore quarantine facility for the introduction of new citrus varieties to New Zealand.

Desired intermediate outcome: Trees tested routinely for virus, viroids, trueness to type

Foundation trees in the repositories were maintained and managed to reduce the risk of infection by graft-transmissible diseases, including those transmitted by insect vectors. The high health status of foundation trees has been confirmed by regular testing for citrus graft-transmissible pathogens including viroids (8), viruses (6) and bacteria (3).

Desired intermediate outcome: Citrus budwood scheme multiplication trees established from high health true to type foundation budwood

Healthy propagation material has been supplied to Auscitrus for propagation of daughter trees and subsequent supply of healthy budwood to nurseries and growers around Australia.

Desired intermediate outcome: General awareness activities include the importance of high health planting material sourced from the repositories

Extension activities undertaken throughout the project (in conjunction with project CT17007) 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 (5), extension articles (5), presentations at industry forums to growers, nurserymen, service providers and other relevant stakeholders (3). This reduces the incidence and impact of established pathogens and increases the likelihood of early detection and eradication of introduced pathogens.

Outcome (not in M&E plan): Capability for managing citrus biosecurity threats

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.

Access to healthy budwood also reduces the risk of illegal importations of sought-after varieties.

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

Monitoring and evaluation

CT17008 key evaluation questions

Effectiveness

1. To what extent has the project achieved its expected outcomes?

- Has routine testing been carried out?

All scheduled testing of foundation trees of 124 public citrus varieties has been undertaken within the project term.

- Have the trees and insect screened structures been maintained?

Ongoing maintenance of repository houses was undertaken to maintain biosecurity. The Dareton repository was integrated into a new insect screened budwood production facility in 2019/20, while still maintaining a layer of separation from budwood production trees. Insect-proof screen was replaced on the EMAI repository screenhouse in June/July 2021.

- Have extension activities been carried out?

Extension activities were undertaken throughout the project (in conjunction with project CT17007) including reports to industry (5), extension articles (5), and presentations at industry forums to growers, nurserymen, service providers and other relevant stakeholders (3).

Relevance

2. How relevant was the project to the needs of intended beneficiaries?

- Does the foundation repository provide a suitable source of clean material for the purposes of the budwood scheme?

Auscitrus was able to access healthy material for propagation of daughter trees to be used as a healthy budwood source for nurseries and growers around Australia. Approximately 500 daughter trees of Cara Cara, 200 trees of Okitsu satsuma, 200 trees of Calamondin, 200 trees of Nagami cumquat, 50 trees of Arnold Blood, 50 trees of Nielson navel, and 15 trees of Topaz mandarin were established from repository tree budwood, for the purpose of multiplying budwood for supply to nurseries.

Process appropriateness

3. How well have intended beneficiaries been engaged in the project?

- Is the management committee of the budwood scheme satisfied with the resource provided by it?

The Auscitrus Executive Committee, comprised of citrus nurserymen and growers, are provided with updates at each quarterly meeting, as well as via the Auscitrus annual report, and inspect the repository facility at both sites at least annually. No concerns have been raised.

- Is the industry aware of the need to use high health planting material?

Extension activities undertaken throughout the project (in conjunction with project CT17007) have increased biosecurity awareness of the importance of using health-tested and true-to-type budwood due to exotic and established disease threats.

4. To what extent were engagement processes appropriate to the target audience/s of the project?

- Has the project communicated its outputs to industry?

Outputs are communicated to Auscitrus quarterly, and to the larger industry via the Auscitrus annual report. Outputs are also highlighted during presentations at industry forums such as the Citrus Australia Technical Forum or R&D roadshows.

- How has the summary presented in the annual report been communicated?

Auscitrus annual reports can be accessed through the Auscitrus website https://www.auscitrus.com.au/services/

Efficiency

4. What efforts did the project make to improve efficiency?

    - Has the project implemented any initiatives to drive efficiencies for the delivery of outputs?

The work of the National Citrus Repository Program was enhanced by linking with the existing portfolio of the NSW DPI Citrus Pathology Program. Hort Innovation funded projects CT14009 and CT17007 provided the NSW DPI citrus pathology team, in collaboration with Auscitrus, the opportunity to assess current and new procedures for nucleic acid extraction and detection of graft-transmissible citrus pathogens to ensure we are using the most reliable, sensitive and efficient methods available. Testing of nucleic acid extracts from repository trees, or local varieties submitted for pathogen testing and elimination, provides further validation of the diagnostic assays.

Efficiencies include:

- streamlining the nucleic acid extraction process enhancing capability from 24 to 96 samples per batch, although sample collection and preparation for extraction remain time consuming and laborious,
- changing from conventional to real-time PCR assays,
- and multiplexing detection assays allowing for simultaneous detection of multiple pathogens, for example two multiplex assays testing for seven viroids (CEVd, HSVd and CBCVd / CBLVd, CDVd, CVd-V, CVd-VI) and one multiplex assay testing for three viruses (CLBV, CPsV and CTLV).

Diagnostic assays have also been developed and validated with improved specificity and sensitivity (CVd-VII, CTLV and CiVA).

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.

- To improve the health status of germplasm sources of rootstock varieties, all commercial rootstock varieties should undergo pathogen testing and elimination to produce high health status foundation trees.

- 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, supported by the newly developed, voluntary Citrus Secure tree certificate program for nurseries who use Auscitrus material, and the nursery industry's NIASA/Biosecure accreditation programs.

- 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

No refereed scientific papers were published as a direct result of this project work. The following publications were delivered by project team members to disseminate the message about the importance of citrus biosecurity, management of graft-transmissible diseases and the health status of Australian citrus germplasm.

Journal article

Chambers GA, Bogema DR, Englezou A, Donovan NJ. 2020. First Report of Citrus viroid V and Citrus viroid VI in Australia infecting Citrus. Plant Disease DOI10.1094/PDIS-12-19-2662-PDN

Paper in scientific conference proceedings

Donovan N, Herrmann T, Hancock N. 2019. On the frontline: preparing for the arrival of HLB in Australia [abstract]. Journal of Citrus Pathology 6(1): 60

Chambers G, Englezou A, Webster J, Bogema D, Donovan N. 2019. Using Next generation sequencing (NGS) to characterize Australia's living pathogen collection [abstract]. Journal of Citrus Pathology 6(1): 4-5

Chambers GA, Donovan NJ, Bodaghi S, Jelinek SM, Vidalakis G. 2018. Citrus viroid VII, a novel citrus viroid found in Lisbon Lemon in Australia. International Conference on Viroids and Viroid-like RNA, Valencia Spain 5-7th July 2018 p 33

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Cook G, van Vuuren SP, Breytenbach JHJ, Burger JT, Maree HJ. 2016. Expanded strain-specific RT-PCR assay for differential detection of currently known Citrus tristeza virus strain: a useful screening tool. Journal of Phytopathology 164: 847-851. DOI: 10.1111/jph.12454

Donovan N, Chambers G, Englezou A. 2018. Protecting Australian citrus germplasm through improved diagnostic tools – CT14009. Horticulture Innovation, Sydney Australia

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Navarro B, Zicca S, Minutolo M, Saponari M, Alioto D, Di Serio F. 2018b. A negative-stranded RNA virus infecting citrus trees: the second member of a new genus within the Order Bunyavirales. Frontiers in Microbiology 9:2340. doi: 10.3389/fmcib.2018.02340

Osman F, Hodzic E, Kwon S-J, Wang J, Vidalakis G. 2015. Development and validation of a multiplex reverse transcription quantitative PCR (RT-qPCR) assay for the rapid detection of *Citrus tristeza virus*, *Citrus psorosis virus*, and *Citrus leaf blotch virus*. Journal of Virological Methods 220: 64–75

Osman F, Dang T, Bodaghi S, Vidalakis G. 2017. One-step multiplex RT-qPCR detects three citrus viroids from different genera in a wide range of hosts. Journal of Virological Methods 245: 40-52

Serra P, Barbosa CJ, Daros JA, Flores R, Duran-Vila N 2008. Citrus viroid V: Molecular characterisation and synergistic interactions with other members of the genus Apscaviroid. Virology 370: 102-112

Vidalakis G, Wang, J. 2013. Molecular method for universal detection of citrus viroids. US Patent Publication number 20130115591.

Intellectual property, commercialisation and confidentiality

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

There are no project IP, project outputs, commercialisation or confidentiality issues to report.

Acknowledgements

Thank you to Grant Chambers and Anna Englezou (NSW DPI scientists) who worked on developing and validating improved diagnostic tools for pathogen testing of citrus germplasm and provided invaluable technical advice to project team members.

Thank you to Professor Paul Holford (Western Sydney University) for his contribution to the Citrus Pathology team via technical input, mentoring and editorial assistance.

Thank you to citrus growers, nurserymen and representatives of the Australian citrus industry for their valuable feedback and guidance after discussing the project at industry forums.

Appendices

List of Appendices

Appendix 1: Public varieties in the 'National Citrus Repository for High Health Status Clones' and date of most recent pathogen testing for citrus viroids (citrus exocortis viroid, citrus bent leaf viroid, hop stunt viroid, citrus dwarfing viroid, citrus bark cracking viroid, citrus viroid V, citrus viroid VI, citrus viroid VII), citrus viruses (citrus tristeza virus, citrus tatterleaf virus, citrus leaf blotch virus, citrus psorosis virus, citrus concave gum associated virus, citrus virus A) and the causal agents of huanglongbing (HLB) ('*Candidatus* Liberibacter asiaticus', '*Ca*. L. africanus' and '*Ca*. L. americanus').

Appendix 1: Public varieties in the 'National Citrus Repository for High Health Status Clones' and date of most recent pathogen testing for citrus viroids (citrus exocortis viroid, citrus bent leaf viroid, hop stunt viroid, citrus dwarfing viroid, citrus bark cracking viroid, citrus viroid V, citrus viroid VI, citrus viroid VII), citrus viruses (citrus tristeza virus, citrus tatterleaf virus, citrus leaf blotch virus, citrus psorosis virus, citrus concave gum associated virus, citrus virus A) and the causal agents of huanglongbing (HLB) ('*Candidatus* Liberibacter asiaticus', '*Ca*. L. africanus' and '*Ca*. L. americanus').

Accession	**Variety**	**Date of most recent test***	
		Viroids / viruses	**HLB**
Grapefruit			
I.N.91.0736	Flame	4/6/21	2/6/21
I.N.89.0620	Henderson	2/6/21	30/6/19
A.N.73.0068	Marsh (3970 Druitt)	2/6/21	18/2/15
A.N.91.0632	Marsh (3962 Druitt)	2/6/21	18/2/15
I.N.89.0619	Ray Ruby	2/6/21	18/2/15
I.N.89.0708	Rio Red	2/6/21	11/3/15
I.N.89.0709	Star Ruby	2/6/21	18/2/15
A.N.04.0950	Star Ruby (Cant)	2/6/21	11/3/15
A.N.91.0633	Thompson (N Eagle)	2/6/21	18/2/15
Pomelo			
A.Q.19.1061	K15	4/6/21	24/6/19
I.N.01.0925	Namroi	2/6/21	2/6/21
I.N.94.0786	Tambun	2/6/21	29/10/14
Citron			
I.N.01.0926	Bergamot Castagnaro	2/6/21	18/2/15
I.N.94.0904	Buddha's Hand	2/6/21	11/3/15
I.N.09.0979	Etrog	2/6/21	11/3/15
Lemon			
I.N.01.0927	Eureka (Allen)	2/6/21	29/10/14
A.N.75.0034	Eureka (Lambert)	2/6/21	29/10/14
A.N.75.0035	Eureka (Taylor)	2/6/21	29/10/14
I.N.89.0703	Fino	2/6/21	29/10/14
A.Q.93.0785	Lemonade	2/6/21	29/10/14
I.N.00.0918	Lisbon (Limoneira 8A)	2/6/21	29/10/14
I.N.75.0036	Lisbon (Prior)	2/6/21	29/10/14
A.Q.91.0631	Lisbon (Queensland)	2/6/21	29/10/14
A.NT.15.1032	Tropical Meyer	4/6/21	29/10/14
I.N.89.0705	Verna	2/6/21	29/10/14
Lime			
A.N.08.0969	Tahiti	2/6/21	18/2/15
A.N.90.0771	West Indian (Schweppes)	2/6/21	18/2/15
Orange			
A.S.10.0985	Arnold blood	2/6/21	8/12/14
I.N.86.0600	Atwood	2/6/21	11/2/15
A.S.75.5095	B/3010	2/6/21	8/2/14
A.Q.75.4022	Benyenda	2/6/21	8/12/14
A.Q.78.4021	Benyenda - thorny	2/6/21	12/11/14
A.S.94.0782	Berri 3501	2/6/21	8/12/14
I.N.06.0960	Bintangcheng # 2	2/6/21	12/11/14
I.N.08.0973	Bintangcheng Renbin # 5	2/6/21	12/11/14
I.N.97.0924	Cara Cara	2/6/21	18/2/15
A.N.14.0993	Cara cara new	4/6/21	1/5/15
A.V.94.0780	CSIRO 5	2/6/21	3/2/15
I.N.94.0902	Delta seedless	2/6/21	8/12/14
I.N.86.0597	Fisher	2/6/21	11/2/15
I.N.99.0912	Fukumoto	2/6/21	29/10/14
I.N.86.0548	Hamlin	2/6/21	29/10/14
A.S.75.5077	Hockney	2/6/21	12/11/14
A.N.73.0073	Houghton	2/6/21	11/2/15
A.S.92.0772	Hutton	2/6/21	11/2/15
I.N.02.0930	Jaffa	4/6/21	27/3/18
I.N.06.0959	Jincheng 447	2/6/21	29/10/14
A.V.93.0774	Jenner 4439	2/6/21	3/2/15
A.N.75.0032	Lanes Late 3976	2/6/21	12/11/14
A.N.73.0072	Leng	2/6/21	11/2/15

Appendix 1 cont.

Accession	Variety	Date of most recent test*	
		Viroids / viruses	HLB
I.N.92.0901	Lima 156 (acidless orange)	2/6/21	29/10/14
A.V.94.0781	Lloyd/3 Leng	2/6/21	12/11/14
I.N.94.0903	Midknight	2/6/21	8/12/14
I.N.92.0900	Natal	2/6/21	12/11/14
I.N.86.0550	Navelate	2/6/21	12/11/14
I.N.87.0546	Navelina Spain 7.5	2/6/21	12/11/14
I.N.93.0899	Navelina 315 ex Italy	2/6/21	12/11/14
A.S.92.0773	Neilson	2/6/21	12/11/14
A.N.75.0029	Newton – Keenan 3125	2/6/21	3/6/19
A.N.75.0030	Newton – Keenan 3247	2/6/21	2/6/21
I.N.86.0598	Newhall California	2/6/21	29/10/14
I.N.87.0551	Newhall 55-1 Spanish	2/6/21	12/11/14
I.N.10.0984	Palmer 1051	2/6/21	11/2/15
I.N.86.0549	Parson Brown	2/6/21	18/2/15
I.N.90.0739	Pera Bianchi	2/6/21	21/4/15
I.N.90.0741	Pera Olympia	2/6/21	11/2/15
I.N.90.0742	Pera Limeira	2/6/21	18/2/15
I.N.87.0547	Pineapple	2/6/21	11/2/15
A.S.17.1043	Poorman's orange	4/6/21	30/4/18
I.N.93.0860	Salustiana	2/6/21	18/2/15
I.N.98.0921	Sanguine	2/6/21	29/10/14
A.Q.78.4020	Smith - Joppa	2/6/21	12/11/14
I.N.08.0968	Tarocco Ippolito	2/6/21	11/2/15
I.N.07.0965	Tarocco Meli C8158	2/6/21	8/12/14
I.N.07.0966	Tarocco Rosso C4977	2/6/21	12/11/14
A.S.75.5074	Thomson	2/6/21	11/2/15
Mandarin and hybrids			
I.N.99.0909	Afourer	2/6/21	20/1/15
I.N.99.0913	Avana Tardivo	2/6/21	20/1/15
I.N.99.0914	Avana Apireno	2/6/21	20/1/15
I.N.98.0920	Clementine (Caffin)	2/6/21	20/1/15]
I.N.89.0704	Clementine (Clementard)	2/6/21	20/1/15
I.N.99.0910	Clementine (Corsica 1)	2/6/21	3/2/15
I.N.99.0911	Clementine (Corsica 2)	2/6/21	20/1/15
I.N.87.0544	Clementine (Fina)	2/6/21	3/2/15
I.N.87.0552	Clementine (Marisol)	2/6/21	20/1/15
I.N.05.0957	Clementine (Nour)	2/6/21	8/12/14
I.N.87.0543	Clementine (Nules)	2/6/21	20/1/15
I.N.04.0955	Clementine (Orogrande)	2/6/21	3/2/15
I.N.87.0545	Clementine (Oroval)	2/6/21	8/12/14
I.N.04.0953	Clementine (Sidi Aissa)	2/6/21	20/1/15
I.N.91.0733	Daisy	2/6/21	3/2/15
A.N.75.0090	Ellendale (Herps)	2/6/21	11/2/15
	Ellendale / EM3	2/6/21	11/2/15
I.N.90.0736	Encore	2/6/21	20/1/15
I.N.08.0974	Etna	2/6/21	20/1/15
I.N.89.0707	Fallglo (VI 484)	2/6/21	20/1/15
I.N.90.0695	Fallglo (S-837-4-2)	2/6/21	20/1/15
I.N.93.0859	Fortune	2/6/21	9/7/19
A.Q.94.0787	Fremont	2/6/21	3/2/15
A.N.75.0041	Hickson	2/6/21	3/2/15
A.N.75.0043	Imperial 0043/2	2/6/21	3/2/19
A.Q.04.0952	Murcott tangor (Benham)	2/6/21	2/6/21
A.Q.90.4149	Murcott tangor (Turner)	2/6/21	29/3/18
A.Q.94.0778	Nova (Trott)	2/6/21	8/12/14
I.N.91.0734	Nova (Spain)	2/6/21	8/12/14
I.N.04.0951	Parsons Special /2	2/6/21	20/1/15
I.N.86.0599	Pixie	2/6/21	8/12/14
I.N.04.0954	Primosole	2/6/21	3/2/15
A.N.75.0065	Satsuma (Silverhill)	2/6/21	30/6/19
I.N.89.0706	Satsuma (Clausellina)	2/6/21	3/2/15
I.N.91.0852	Satsuma (Okitsu Wase)	2/6/21	3/2/15

Appendix 1 cont.

Accession	Variety	Date of most recent test*	
		Viroids / viruses	HLB
I.N.91.0853	Satsuma (Miho Wase)	2/6/21	11/2/15
I.N.20.1068	Shiranui	4/6/21	2/6/21
A.Q.94.0886	Sunburst	2/6/21	8/12/14
I.N.90.0818	Topaz tangor	2/6/21	11/2/15
A.NT.15.1034	Tropical Emperor	4/6/21	27/3/18
Papeda			
I.N.94.0776	Kaffir lime (Malaysia 4669)	2/6/21	18/2/15
A.D.97.0907	Kaffir lime (Nathanael)	2/6/21	18/2/15
I.N.00.0916	Kaffir lime (Eyles)	2/6/21	18/2/15
I.N.15.1020	Sudachi	4/6/21	27/3/18
A.N.13.0991	Yuzu	2/6/21	1/5/15
Kumquat			
A.N.15.1033	Calamondin	4/6/21	27/3/18
I.N.04.0956	Nagami	2/6/21	29/10/14
Rootstock			
A.N.18.1054	Benton citrange	4/6/21	29/5/18

* Date is the date of nucleic acid extraction (the same or close to sampling date), not the date of individual pathogen assays.