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Pest Risk Assessments: Citrus spp. nursery stock

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Growing and Protecting New Zealand

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Pest Risk Assessments: *Citrus* spp. nursery stock

Version 1.0

23 September 2016

Approved for general release

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Version information

Version number	Comments	Date of release
1.0	Peer-reviewed and current at date of release	23 September 2016

New Zealand is a member of the World Trade Organisation and a signatory to the Agreement on the Application of Sanitary and Phytosanitary Measures ("The Agreement"). Under the Agreement, countries must base their measures on an International Standard or an assessment of the biological risks to plant, animal or human health.

This document provides a scientific analysis of the risks associated with eight micro-organisms (viruses, viroids, phytoplasmas, and bacteria) on the *Citrus* nursery stock pathway. It assesses the likelihood of entry, exposure, establishment and spread of these eight micro-organisms in relation to imported nursery stock of *Citrus* spp. from all countries and assesses the potential impacts of those organisms should they enter and establish in New Zealand. The document has been internally and externally peer reviewed and is now released publically. Any significant new science information received that may alter the level of assessed risk will be included in a review, and an updated version released.

Contributors to these pest risk assessments

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Contents

Executive summary

A review is being carried out of the Import Health Standard 155.02.06: Importation of Nursery Stock, specific *Citrus* schedule, which is out of date. As inputs to this review, pest risk assessments are required for eight organisms that have been identified as pests of *Citrus* but do not currently have a requirement for specific tests.

These pest risk assessments examine the risks posed by eight micro-organisms (viruses, viroids, phytoplasmas, and bacteria) associated with the importation of *Citrus* nursery stock from all countries.

All eight species are found to be non-negligible risks associated with *Citrus* nursery stock.

The findings of the risk assessments are summarised in Table 1 below:

Table 1. Summary of risk assessment findings

Species	Risk estimation on nursery stock of *Citrus* spp.	Likelihood of: Entry	Exposure	Establishment	Spread	Consequences of establishment: Economic	Environmental	Socio-cultural	Human health
Liberibacters:									
'*Candidatus* Liberibacter americanus'	Non-negligible	Low	High	Moderate-high	Moderate-high	Low (no vector) Moderate (vector)	Negligible (no vector) Low (vector)	Low	Negligible
Phytoplasmas:									
'*Candidatus* Phytoplasma asteris'	Non-negligible	Low	High	Moderate-high	Moderate-high	Low-high (uncertain)	Negligible-high (uncertain)	Low	Negligible
'*Candidatus* Phytoplasma' 16SrIX subgroup A	Non-negligible	Low	High	Moderate-high	Moderate-high	Low-moderate (uncertain)	Negligible-high (uncertain)	Low	Negligible
Viroids:									
Citrus viroid V	Non-negligible	Low	High	Moderate-high	Moderate-high	Low	Negligible	Negligible-low	Negligible
Citrus viroid VI	Non-negligible	Low	High	Moderate-high	Moderate-high	Low	Negligible	Negligible-low	Negligible
Viruses:									
Citrus sudden death-associated virus	Non-negligible	Low	High	Moderate-high	Moderate-high	Low	Negligible	Low	Negligible
Indian citrus ringspot virus	Non-negligible	Low	High	Moderate	Moderate (limited area)	Low (limited area)	Negligible	Low (limited area)	Negligible
Olive latent virus 1	Non-negligible	Low	High	Moderate-high	Moderate-high	Low-moderate (uncertain)	Negligible-low (uncertain)	Negligible-low (uncertain)	Negligible

1 Risk assessment background

1.1 Purpose

A review is being carried out of the Import Health Standard 155.02.06: Importation of Nursery Stock, specific *Citrus* schedule, which is out of date. Pest risk assessments are required for several organisms that have been identified as pests of *Citrus* but do not currently have a requirement for specific tests.

1.2 Scope

The scope of this risk assessment includes the following:

- the commodity is nursery stock (dormant budwood and tissue culture plantlets) of *Citrus*, *Fortunella* (kumquat), *Poncirus* (trifoliate orange), and *Microcitrus australasica*[1] (Australian finger-lime);
- all countries are being considered;
- it is assumed that the commodity is of unknown phytosanitary status; no assumptions are made about pre-export pest and disease monitoring or control;
- it is assumed that imported cuttings will be treated for insects and mite either prior to export or on arrival in New Zealand, prior to entering a post entry quarantine facility (PEQ);
- it is assumed that the commodity will enter a post entry quarantine facility where it will be inspected for visually obvious signs of pests and diseases;
- the following points are addressed in this risk assessment:
    - Hazard identification: name and taxonomy of the organism, New Zealand status, indicative geographic distribution, commodity association, potential for establishment or spread;
    - Risk assessment: biology, assessment of entry, exposure, establishment and spread, consequence assessment (economic, environmental, human health and social/cultural);
- Risk management: Specific details for risk management options are provided in the *Citrus* testing manual (MAF 2010) for most of the pathogens. Therefore this information will not be repeated in the risk assessments which will provide only information to allow evaluation of the different options in relation to the level of risk. The exception is Citrus sudden death where options will be briefly presented and discussed in a risk management section at the end of the pest risk assessment for that disease.

The risk assessment in this document is consistent with the process and methodology undertaken for import risk analyses. For a more detailed description of that process and methodology please refer to the Biosecurity New Zealand Risk Analysis Procedures (Version 1, 12 April 2006) which is available on the Ministry for Primary Industries web site (MAF 2006).

1.3 Description of current requirements and assumptions for the importation of *Citrus* nursery stock

The following gives an overview of the requirements for importing citrus nursery stock and of procedures in post-entry quarantine (PEQ) upon arrival in New Zealand. In addition, assumptions made for the purposes of this risk assessment have been documented along with

1 *Microcitrus australasica* (Australian finger-lime) is a new organism under the Hazardous Substances and New Organisms Act (1996), and will not be eligible for import until approved by the Environmental Protection Agency.

figures outlining the overall process of importation of citrus nursery stock from an overseas facility to New Zealand.

Import requirements for *Citrus* nursery stock

The import requirements for nursery stock (dormant cuttings and plants in tissue culture only) of *Citrus*, *Fortunella* and *Poncirus* are set out in MPI's import health standard (IHS) 155.02.06: Importation of Nursery Stock (the version issued on 21 January 2015 is referred to in this analysis).

The imported nursery stock must meet:

> (i) basic conditions that apply to all nursery stock (outlined in sections 2.2.1 and 2.2.2 of the IHS 155.02.06)
>
> AND
>
> (ii) additional specific requirements as detailed in the "*Citrus*", "*Fortunella*" or "*Poncirus*" schedule as appropriate.

In summary, an import permit is required and phytosanitary certificate must accompany all consignments certifying that the nursery stock has been inspected and found to be free of any visually detectable regulated pests and has been treated for regulated insects and mites (cuttings only). Additional declarations are required for material sourced from MPI approved offshore facilities, and to certify material of the same variety has been sourced from a single mother plant.

Upon arrival in New Zealand, all consignments are inspected to verify the documentation is compliant and the consignment is free from visually detectable pests and disease.

All material from non-accredited facilities must be actively grown for a minimum of 16 months in a Level 3 post-entry quarantine facility where it will be inspected, treated and/or tested for regulated pests and/or diseases. Material from accredited facilities must be grown for a minimum period of either 6 months (for plants in tissue culture or dormant cuttings sourced from mother plants grown in insect-proof houses) or 16 months (for dormant cuttings sourced from mother plants in open ground) in a Level 2 post-entry quarantine facility where it will be inspected, treated and /or tested for regulated pests and/or diseases. Specifically, material sourced from the approved offshore facility Elizabeth Macarthur Agricultural Institute, Australia, must undergo a minimum 12 month active growth period in post-entry quarantine, as the time to complete pre-determined testing has meant the minimum quarantine period has been extended.

Import requirements for nursery stock from non-accredited facilities

In this risk assessment it is assumed that the plant material is of unknown phytosanitary status; therefore, no assumptions are made about pest and disease monitoring or control before importation (i.e., it is assumed that material has been sourced from a non-approved offshore facility, and will be entering level 3 post-entry quarantine).

The entry conditions set out in the schedule for *Citrus*[2], section 3.2, apply to *Citrus* cuttings from non-accredited facilities in any country, and the entry conditions in section 3.4 apply to *Citrus* plants in tissue culture from non-accredited facilities in any country. These conditions are summarised below (see the schedule for exact conditions).

Citrus cuttings from non-accredited facilities in any country

1. Import permit required.

2 The requirements for *Fortunella* and *Poncirus* are identical to those for *Citrus*.

2. Phytosanitary certificate certifying that the nursery stock has been inspected and found to be free of any visually detectable regulated pests. (It will also include any additional declarations required to conform to the current phytosanitary requirements of MPI).

3. Cuttings must be sprayed/dipped in MPI-approved miticides and insecticides as described in section 2.2.1.6 of the basic conditions. If this treatment occurs prior to export, the treatment details must be endorsed on the phytosanitary certificate. Alternatively the treatment may occur on arrival in New Zealand, prior to entry to the post entry quarantine facility.

4. On arrival in New Zealand the consignment is inspected to verify the documentation is compliant and the consignment is free from visually detectable pests and disease.

5. The cuttings are imported into a Level 3 post-entry quarantine (PEQ) facility, where they are grafted onto New Zealand origin rootstocks. The quarantine period is the time required to complete inspections and/or indexing to detect regulated pests. 16 months is an indicative minimum quarantine period. The quarantine period may be extended if material is slow growing, pests are detected, or treatment/testing are required. Note that the quarantine period starts from when plants are actively growing, not from when the plant material first enters the PEQ facility.

6. Testing for specified regulated pests, in accordance with the import health standard and permit to import, must be undertaken on samples collected from actively growing plants during the quarantine period. Testing must occur at an MPI approved diagnostic facility.

Citrus plants in tissue culture from non-accredited facilities in any country

1. Import permit is required

2. Cultures imported in growing media must have been grown in the vessel in which they have been imported; the container must be pest-proof and the tissue culture media must not contain charcoal.

2. Phytosanitary certificate certifying that the nursery stock has been inspected and found to be free of any visually detectable regulated pests. (It will also include any additional declarations required to conform to the current phytosanitary requirements of MPI).

3. On arrival in New Zealand the consignment is inspected to verify the documentation is compliant and the consignment is free from visually detectable pests and disease.

4. The cuttings are imported into a Level 3 post-entry quarantine (PEQ) tissue culture laboratory, and must be deflasked into a Level 3 PEQ. The quarantine period begins when the plants are deflasked into the greenhouse, and is the time required to complete inspections and/or indexing to detect regulated pests. 16 months is an indicative minimum quarantine period. The quarantine period may be extended if material is slow growing, pests are detected, or treatment/testing are required.

5. Testing for specified regulated pests, in accordance with the import health standard and permit to import, must be undertaken on samples collected from actively growing plants during the quarantine period. Testing must occur at an MPI approved diagnostic facility.

Level 3 post-entry quarantine procedures

The following information has been taken largely from the *Citrus* (Citrus), *Fortunella* (Kumquat) & *Poncirus* (Trifoliate orange) Post-Entry Quarantine Testing Manual (MAF 2010) to provide an overview of the procedures undergone while material is in the post-entry quarantine (PEQ) facility. Refer to the manual for more detailed information. The manual is not a legal document and provides guidance only. Any information is superseded by specific requirements for the IHS, *Citrus* schedule or on the permit to import.

Note that as it is assumed that the plant material is from a non-accredited facility, it will be treated for insects and mites prior to entry into the level 3 PEQ facility and then inspected for visually obvious signs of pests and diseases during the quarantine period. As a result, it is expected that the plant material will be vector-free while in the facility.

Propagation, care, maintenance:

Plants must be maintained in a healthy, vigorous and free-growing state for adequate expression of any disease symptoms, without any nutrient or water stress. All citrus material must be held at temperatures suitable for liberibacters. Although the current IHS 155.02.06 *Citrus* schedule does not stipulate temperatures for plant material from non-accredited facilities (held in Level 3 PEQ), the schedule does require that *Citrus* cuttings from off-shore MPI-accredited facilities (held in Level 2 PEQ) be held at 18–25ºC throughout the quarantine period[3]. When working with the plants, it is important that pruning and cutting tools are disinfected between each plant, or that disposable razor blades used.

Dormant cuttings:

New elite genetic and promising cultivars are most efficiently imported as budwood, although tissue-cultured material can be imported. It is advisable to import budwood which arrives as bud-sticks containing 8–12 buds. When in PEQ, it is most convenient to propagate new plants by budding, which is quick and reliable. Budding is performed by slicing off a bud from the imported bud stick, complete with bark and a slice of wood, and inserting this into the "T" cut in the stem of the rootstock/indicator plant. The bud is prepared 21 days before the stem of the rootstock is cut off above the bud. The new scion bud should start to grow in 14 days if given sufficient heat and light, allowing disease status monitoring to start.

Plants in tissue culture:

Plantlets are established using conventional tissue culture techniques. On arrival, plantlets which are growing in media contained in flasks are carefully removed in the quarantine facility. The plantlets are washed to remove all traces of the old growing media from the developing roots and planted into 50 mm diameter pots containing a sterile 1:1 (v:v) peat:pumice or similar mixture with fertiliser pellets. The plants are initially grown for 3 weeks in incubator or glasshouse under specific conditions of temperature, light and humidity. Once established, the plants can be maintained in the quarantine facility with increasing light intensity and repotted as required.

Inspection:

Inspection is required for all plants during the quarantine period growing season. The quarantine period begins when all plants are actively growing. The operator of the facility inspects the plants regularly, generally weekly. In addition, the MPI biosecurity inspector undertakes scheduled inspections, with a minimum of 10 inspections during a 16 month quarantine period (active growth).

Inspection requirements for the operator of the facility are set out in the MPI Facility Standard, Post Entry Quarantine for Plants (MPI.STD.PEQ).

Testing:

Each of the specific pre-determined tests required by the IHS must be performed irrespective of whether plants exhibit symptoms. This testing is required to detect latent infections. Tests options given in the IHS, and in the *Citrus* testing manual (MAF 2010), have included graft inoculation (biological indexing), ELISA, PCR, shoot-tip grafting, detached leaf bioassay, and

3 All citrus nursery stock imports since 2010 have come from an MPI-approved facility overseas and therefore have entered Level 2 PEQ. The IHS requires that plants in Level 2 PEQ must be held at 18–25ºC throughout the quarantine period (incorporated in IHS amendment 27 November 2014). However the CTO has required operational measures in PEQ to effectively manage temperature to these levels since April 2012.

return PAGE, depending on the organism being tested. However, the only tests being currently being used by the MPI Level 3 PEQ facility are biological indexing and PCR[4]. Each plant in the greenhouse must be tested, although it is possible to have composite samples tested in some cases, provided the specific conditions on the permit to import (including pre-export certification, and traceability in PEQ) are maintained.

In addition to required pre-determined tests, if symptoms of pests or diseases are observed then samples are collected for diagnostic tests/identification.

Specific tests for nursery stock:

Citrus budwood is the primary source of tissue used for testing. It is recommended budwood is not collected during excessively hot weather because some graft-transmissible pathogens can be temporarily inactive or suppressed by heat in the peripheral branches of field trees. Seasons with cooler temperatures are preferable because pathogens actively replicate and titre increases, however citrus viroids are best sampled for in late summer when temperatures are warmer.

Graft indexing must be done in early spring using young, vigorous indicator plants (*Citrus* spp. as appropriate for each disease). Lab tests for viruses (ELISA and RT-PCR) must be carried out in the spring using the new flush of spring growth. Lab tests for phytoplasmas, viroids and bacteria (RT-PCR and PCR) must be carried out at the end of summer.

Graft indexing:

Each plant must be tested by bud-grafting onto 3–5 replicate indicator species. Plants must be grown under moderate to warm temperatures and with supplemented lighting to ensure a 16 hr photoperiod. Growing temperatures and conditions and the times from inoculation to the appearance of the first symptoms are detailed in the *Citrus* testing manual (MAF 2010). Most symptoms will be apparent within 2 to 3 months; however, symptoms on indicators grafted with material infected with '*Ca*. Liberibacter spp.' or 'Ca. *Phytoplasma* aurantifolia' may take between 5 months to 2 years, or longer, to show symptoms. It is important the grafted plants are kept properly watered and not exposed to cold temperatures during the test period.

As some field sources may contain several viroids, the described symptoms may vary to those described. The synergistic and inhibitory interactions of multiple infections may delay or enhance symptoms causing pronounced dwarfing and epinasty or variable leaf symptoms.

Assumptions for these risk assessments

For the purposes of these assessments the following assumptions have been made:

- As it is assumed that the plant material is from a non-accredited facility, then upon arrival the plant material will enter a Level 3 PEQ facility. It will be treated for insects and mites prior to entry to the facility, and inspected for visually obvious signs of pests and diseases during the quarantine period. As a result, the plant material is expected to be vector-free.
- The imported material will be either dormant cuttings (budwood) or plants in tissue culture.
- It is assumed that a small amount of budwood material will be imported because facilities are small and testing is expensive. It is likely that there would be around 5 to 12 buds per stick of budwood. In the past not much tissue culture has been imported relative to budwood.

4 Accordimg to the *Citrus* schedule in the IHS 155.02.06, MPI will accept other internationally recognised testing methods as equivalent to those given in the schedule, with prior notification.

- Buds will be taken from imported dormant cuttings and grafted onto rootstock.
- Imported tissue culture plantlets will be removed from the media flask and replanted and acclimatised in greenhouse.
- Growing plants will spend a minimum of 16 months in Level 3 PEQ where they will be inspected, treated and/or tested for regulated pests. This period allows time for biological indexing.
- Tests will include biological indicators and PCR tests.
- PCR tests will take place in spring (September/October) for viruses, and in summer (March/April) for phytoplasmas/bacteria/viroids.
- Plants will be inspected during the growing season. The minimum number of inspections by an MPI biosecurity inspector is 10 over a 16 month growing season. Plants will be checked weekly by the operator of the facility
- Organisms that are the subject of pest risk assessment will not be specifically tested for by PCR before release from PEQ[5]. However, it is possible that they could be detected through visually obvious symptoms on test plants.

The following figures outline the overall process of importation of citrus nursery stock from an overseas facility to New Zealand (figure 1: dormant cuttings (budwood); figure 2: tissue culture).

5 PCR tests are specified for some organisms on current permits to import

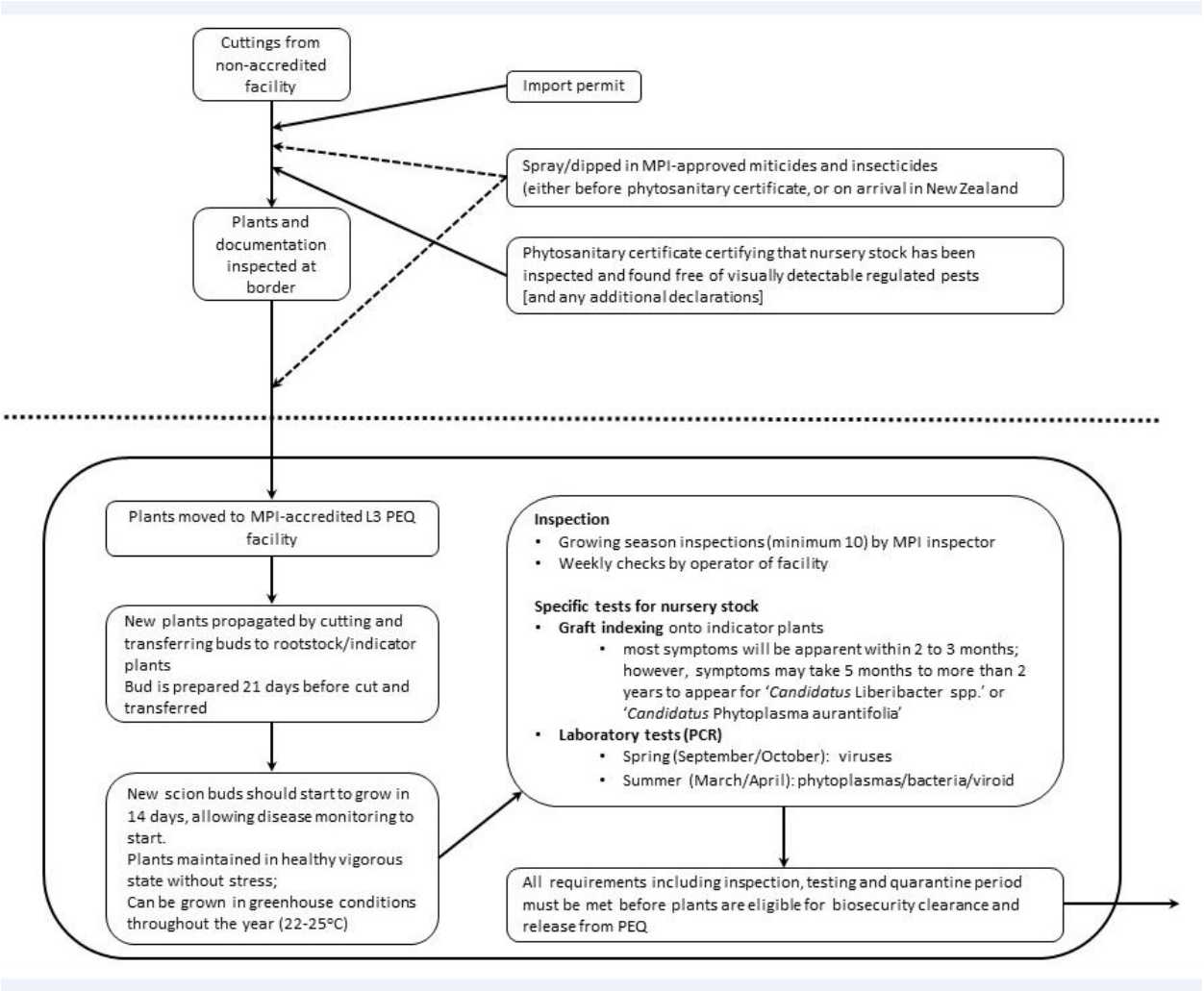

Figure 1. The process of importation of citrus nursery stock (dormant cuttings (budwood)) from an overseas facility to New Zealand.

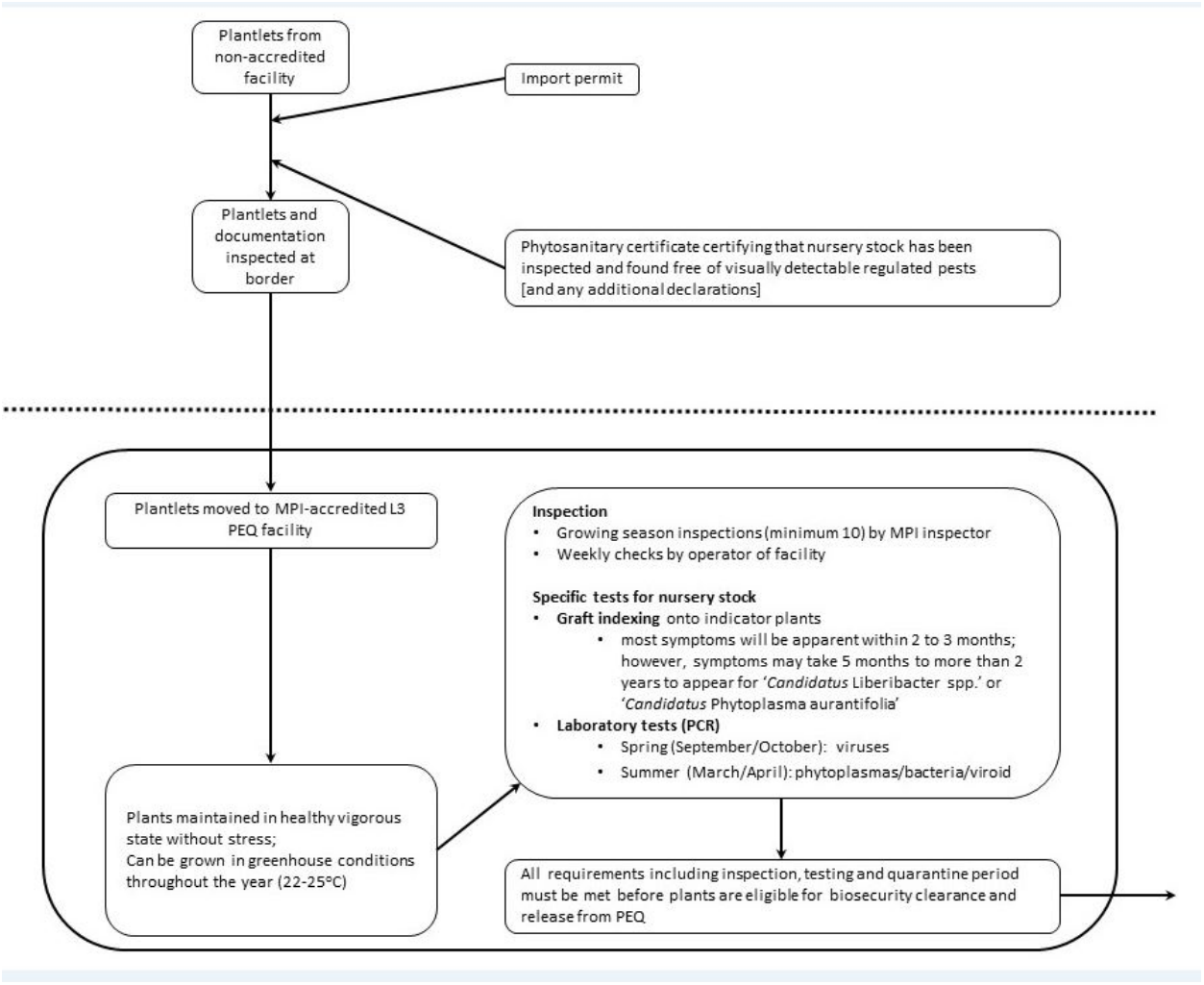

Figure 2. The process of importation of citrus nursery stock (tissue culture) from an overseas facility to New Zealand.

1.4 References

MAF (2006) Biosecurity New Zealand risk analysis procedures. Ministry of Agriculture and Forestry, New Zealand, 201 pp. Available online at https://www.mpi.govt.nz/document-vault/2031

MAF (2010) *Citrus* (Citrus), *Fortunella* (Kumquat) & *Poncirus* (Trifoliate orange) Post-Entry Quarantine Testing Manual (July 2010) https://mpi.govt.nz/document-vault/13630

Import health standard 155.02.06 Importation of Nursery Stock (the version issued on 21 January 2015). http://www.biosecurity.govt.nz/files/ihs/155-02-06.pdf

MPI Facility Standard: Post Entry Quarantine for Plants (MPI.STD.PEQ) (issued 1 March 2016) https://mpi.govt.nz/document-vault/11368

2 Risk assessments of potential hazard organisms

2.1 '*Candidatus* Liberibacter americanus' – huanglongbing

Scientific name: '*Candidatus* Liberibacter americanus' (Proteobacter: Rhizobiales: Rhizobiaceae)
Other relevant scientific names: *Candidatus* Liberobacter americanus
Common names: huanglongbing (HLB), citrus greening
Abbreviations: CLam, Lam

2.1.1 Hazard identification

2.1.1.1 Description

'*Candidatus* Liberibacter americanus' is an unculturable bacterium found in the phloem of citrus and is transmitted by psyllid vectors. It is thought to be one of the causal organisms of huanglongbing, or citrus greening disease, which is regarded as one of the most devastating diseases of citrus in the world

2.1.1.2 Taxonomic issues

'*Candidatus* Liberibacter americanus', '*Candidatus* Liberibacter africanus' and '*Candidatus* Liberibacter asiaticus' are thought to be the causal organisms of the disease huanglongbing (also known as citrus greening). These species are fastidious phloem-limited bacteria which means that they have not been cultured on artificial media and can only survive in phloem sieve tubes in the host plant, and in the insect vector.

Candidatus is the name used for bacterial species that have not yet been successfully cultured. New names will be assigned to the bacteria once they have been cultured and taxonomically described (Halbert and Manjunath 2004).

'*Ca*. L africanus' and '*Ca*. L. asiaticus' are already listed in the IHS for citrus nursery stock and the risk posed by these two species will not be addressed in this risk assessment. However, much of the literature refers to these species, particularly '*Ca*. L. asiaticus', and will be used to inform this risk assessment.

2.1.1.3 New Zealand status

'*Ca*. L. americanus' is not known to be present in New Zealand. Not recorded in: Veerakone et al. (2015), NZFungi (2015) or PPIN (2015).

2.1.1.4 General geographic distribution

'*Ca*. L. americanus' occurs in Brazil (Gottwald 2010), and has recently been reported in Hunan, China, although it is not clear if the latter has been confirmed (Bové et al. 2008).

2.1.1.5 Commodity association

'*Ca*. L. americanus' bacteria are found in the phloem sieve tubes which transport sap containing sugars and other nutrients around the plant. Therefore it is assumed that they can be found in any parts of the plant that contain phloem tissue, including stems, leaves, roots, flowers and fruit.

2.1.1.6 Plant associations

'*Ca*. L. americanus' has been recorded from *Citrus reticulata*, *Citrus sunki*, *Citrus reticulata x C. sinensis*, *Citrus reshni*, *Poncirus trifoliata x C. paradisi*, *Citrus sinensis*, *Citrus limonia*, *Citrus latifolia*, (Lopes and Frare 2008); *Murraya exotica* (Lopes et al. 2010); *Murraya paniculata* (Gasparoto et al. 2008, Lopes et al. 2009b); *Nicotiana tabacum* [experimental host] (Beattie and Barkley 2009, cited in Plant Biosecurity 2011); *Catharanthus roseus* [experimental host], *Cuscuta campestris* [experimental host] (Teixeira et al. 2005).

In addition, the host range for the three '*Ca*. Liberibacter'spp. is presented below as it is not clear how thoroughly the host range for '*Ca*. L. americanus' has been assessed.

The host range under natural conditions appears to be restricted to Rutaceae, although dodder (*Cuscuta*), periwinkle (*Catharanthus roseus*) and tobacco (*Nicotiana*) have been successfully infected under experimental conditions (Floyd and Krass 2006). The bacteria can infect most citrus cultivars, species and hybrids as well as some citrus relatives (Halbert and Manjunath 2004). Some species and hybrids appear to be more severely affected than others. Most sweet oranges (*Citrus sinensis*), mandarins (*C. reticulata*) and mandarin hybrids are severely affected. Grapefruit (*C.* × *paradisi*), Rangpur lime (*C.* × *limonia*), lemons (*C. limon*), calamondin (*C. microcarpa*) and some pummelos (*C. maxima*) are less severely affected. Mexican lime (*C. aurantifolia*), some pummelos, trifoliate orange (*Poncirus trifoliata*) and trifoliate orange hybrids are the most tolerant and may show only slight symptoms on the leaves (Garnier and Bové 2000, Polek 2007). However, calmondins, pummelos, grapefruit and limes have also developed severe symptoms in Florida (Polek 2007). It is possible that strains of the pathogen can adapt to citrus species and cultivars over time (Gottwald et al. 2007). Kumquat (*Fortunella* spp.) is also a host (Floyd and Krass 2006).

Other rutaceous plants that have been observed as hosts, either experimentally or naturally, include: *Severinia buxifolia*, *Balsamocitrus dawei*, *C. grandis*, *C. hystrix*, *C. jambhiri*, *Citrus* × *nobilis*, *Clausena indica*, *Cl. lansium*, *Microcitrus australasica*, *Triphasia trifolia*, *Atalantia missionis*, *Limonia acidissima* (= *Feronia limonia*), *Swinglea glutinosa*, *Murraya paniculata*, *Calodendrum capense* (host for a distinct subspecies of '*Ca*. L. africanus') and *Vepris lanceolata* (= *Toddalia lanceolata*, *V. undulata*) (a host for '*Ca*. L. africanus') (Halbert and Manjunath 2004; Floyd and Krass 2006).

2.1.1.7 Potential for establishment and impact

'*Ca*. L. americanus' is found in countries that have areas with climates similar to parts of New Zealand and can potentially establish in New Zealand. '*Ca*. L. americanus' is believed to be a causal agent of HLB, a major disease of *Citrus* spp. which is an important horticultural crop in New Zealand.

2.1.1.8 Hazard identification conclusion

Given that '*Candidatus* Liberibacter americanus':

- is associated with nursery stock of *Citrus* spp. and citrus-related species;
- is recorded from two countries that grow *Citrus* spp.;
- is not recorded from New Zealand;
- can potentially establish in New Zealand;
- can potentially cause unwanted impacts in New Zealand;

'*Candidatus* Liberibacter americanus' is considered a hazard on *Citrus* nursery stock in this risk analysis.

2.1.2 Biology

'*Ca*. L. americanus' is one of the three '*Candidatus* Liberibacter' species associated with the devastating citrus disease huanglongbing (HLB), also known as citrus greening. It was first detected in São Paulo State, Brazil, in sweet orange (*Citrus sinensis*) that showed symptoms of HLB. Early studies found '*Ca*. L. americanus' in 216 of 218 symptomatic leaf samples from 47 farms in 35 municipalities, while '*Ca*. L. asiaticus' was detected in only four of the 218 samples, indicating that '*Ca*. L. americanus' was the major cause of HLB in São Paulo State (Teixeira et al. 2005). However, '*Ca*. L. asiasticus' is now more prevalent in São Paulo State (Lopes et al. 2009b).

The following information on biology is largely based on the most common form of HLB and on '*Ca*. L. asiaticus', which is believed to be a causative agent of the disease in Asia and elsewhere, and is the most geographically widespread of the liberibacter species associated with HLB (Wang et al. 2015). It is assumed that the information will largely apply to the American form, '*Ca*. L. americanus'. Where these are known to differ, it will be noted in the text. At the end of this section, more detailed information will be provided on temperature effects on the liberibacters.

Huanglongbing disease is regarded as one of the most devastating diseases of citrus in the world. Premature fruit drop on infected trees results in decreased production. Infected fruit that remains on the tree can be small, hard, discoloured and misshapen with a very bitter unpleasant taste that makes it useless. Trees become stunted and have a much shortened life-span. Nearly all commercial citrus species and cultivars are sensitive regardless of rootstock (Bové 2006). There is no cure for the disease and in parts of Asia it has substantially reduced the amount of citrus that can be grown (Grafton-Cardwell et al. 2006).

Disease symptoms are variable and can resemble other diseases and conditions (Gottwald 2010). Infected trees have leaves that are blotchy and mottled and develop into yellow shoots which are an early and characteristic symptom of the disease (Bové 2006). In some trees, the yellow shoots remain confined to one part of the tree giving a sectored appearance. The leaves can appear to have zinc deficiency symptoms. Chronically infected trees can be stunted with extensive twig and limb die-back, tend to drop fruit prematurely and have sparse foliage with small leaves that point upwards (Polek 2007). Trees may bloom off-season (Halbert and Manjunath 2004). Symptomatic fruit are small, underdeveloped and lopsided. As they mature they tend to remain green, at least in part, and colouring starts at the stem end (peduncle) rather than from the stylar end as seen in healthy fruit (Gottwald et al. 2007, Polek 2007). The fruit frequently contains small, dark aborted seeds, and the vascular bundles in the fruit axis can be discoloured (Gottwald et al. 2007). Fruit can have a mottled appearance and if the peel is pressed with a finger the depressed area can turn silvery in appearance (Gottwald et al. 2007). The juice is low in soluble solids, high in acid, and abnormally bitter, leaving the fruit inedible (Polek 2007).

HLB is associated with three phloem-limited, unculturable bacteria species, '*Candidatus* Liberibacter africanus', '*Candidatus* Liberibacter americanus' and '*Candidatus* Liberibacter asiaticus'. The bacteria are found in the phloem sieve tubes which transport sap containing sugars and other nutrients around the plant. They are transmitted naturally by two psylloid[6] vectors: the Asian citrus psyllid *Diaphorina citri* which occurs in Asia and America, and *Trioza erytreae* which occurs in Africa (Bové 2006). The psyllids acquire the bacteria when they pierce the phloem cells with their mouthparts to feed on the contents. The bacteria multiply in the salivary glands and haemolymph of the psyllids. Both vectors are capable of transmitting all three of the bacteria, experimentally at least (Gottwald 2010, Bové 2006). Although other psyllid species have been recorded on citrus, none have been shown to be vectors (Halbert and

6 Psylloids are members of the superfamily Psylloidea. The superfamily contains several families including Psyllidae and Triozidae. Members of the Psyllidae (e.g., *Diaphorina citri*) are known as psyllids and members of the Triozidae (e.g., *Trioza erytreae*) are known as triozids.

Manjunath 2004, Gottwald 2007). '*Ca*. L. asiaticus' has been recently detected in the psyllid *Diaphorina communis* which occurs in India, Nepal and Bhutan (Donovan et al. 2012). However, it is unproven whether *D. communis* is capable of transmitting the bacterium to host plants.

'*Ca*. L. asiaticus', associated with Asian HLB disease is the most prevalent bacteria, and Asian HLB is the most prevalent disease worldwide. Its major insect vector is *D. citri*. '*Ca*. L. americanus' occurs in Brazil (Gottwald 2010) and has been recently reported in Hunan, China although it is not clear if this report has been confirmed (Bové et al. 2008). It is also transmitted by *D. citri*. '*Ca*. L. africanus', associated with African HLB disease, is found in Africa, especially South Africa, and in Saudi Arabia and a few islands in the Indian Ocean. It is transmitted by the African citrus psyllid, *T. erytreae* (Gottwald 2010, Bové 2006).

The HLB pathogens show some temperature sensitivity. Experiments and field observations show that HLB in Africa is heat-sensitive, occurring only in cool areas where temperatures stay below 30–32ºC. Its vector, *T. erytreae*, only does well in cool environments and is sensitive to high temperature combined with low humidity. In contrast, HLB in Asia and its vector *D. citri* are both heat-tolerant, standing temperatures well above 30ºC (Bové 2006). The American form, also vectored by *D. citri*, shows heat-sensitivity (Bové 2006). Further information on the effects of temperature is given at the end of this Biology section under the heading "Temperature sensitivity".

In addition to temperature sensitivity, DNA hybridisations, genomic properties and serology have been used to distinguish the liberibacters indicating that they are different species (Bové 2006). Electron microscopy has been used to detect the bacteria in plant tissues but is not able to distinguish between species. Molecular methods, such as DNA hybridisation and polymerase chain reaction (PCR), have been used to detect and identify liberibacter species in both plant tissue and psyllid vectors (Bové 2006). PCR is the main method used now for detection and identification (Dr LW Liefting, personal communication, 07/06/2013) and there is a specific Taqman assay for each of the three liberibacter species (Dr LW Liefting, personal communication, 07/06/2013, Li et al. 2006, Teixeira et al. 2008).

Psyllids are typically associated with new growth on the host plant. *D. citri* eggs are laid on the tips of growing shoots or in the crevices on unfolded "feather flush" leaves. The nymphs feed exclusively on new growth. Waxy secretions, honeydew and associated sooty mould growth can be signs of their presence. Developing shoots on the host plant can become malformed, twisted, curled, or laterally notched, and sometimes die (Halbert and Manjunath 2004). Adults usually feed on the undersides of leaves. Adult *D. citri* have been found on fruit during transport (Halbert et al. 2010) but they are not thought to feed on fruit and in this situation can be regarded as hitchhikers. *T. erytreae* nymphs live in individual depressions on the undersides of citrus leaves and remain there until adult (Halbert and Manjunath 2004). The upper sides of these leaves can appear lumpy. It is assumed that adults could be associated with citrus fruit as hitchhikers in the same manner as for *D. citri*.

Once the psyllid vector has acquired the bacteria, as a nymph or adult, it is maintained in the psyllid for its lifetime. For *T. erytreae*, there is evidence that transovarial transmission occurs (Gottwald 2007).

Transmission by psyllid vectors is considered to be the primary mode of HLB spread in the field and where psyllids are present the disease often appears soon after (Bové 2006). Adult psyllids are very mobile and can move from tree to tree in the field, and further afield. There is some evidence that high winds and storms can move psyllids considerable distances (Gottwald 2010). The other major method of transmission is by grafting infected budwood. There are some indications that transmission occurs infrequently through infected seed but more research is required in this area (Tirtawidjaja 1981, Benyon, et al. 2008, Benyon et al. 2009, Federal

Register 2010, National Research Council 2010, Hartung et al. 2010, Hilf 2011, Hilf et al. 2013). In experiments, the parasitic plant, dodder, has been shown to transmit the bacteria from citrus to periwinkle, tobacco and tomato (Floyd and Krass 2006).

If HLB is absent from a region, then it is most likely to be introduced by the transport of citrus for propagation, either whole plants or budwood, and citrus relatives. These plants may be infected by the bacteria and may carry psyllid eggs and nymphs. Introduction of alternative psyllid host plants such as *Murraya paniculata* and *M. koenigii* may also introduce the vector to a new region (Bové 2006; Halbert and Manjunath 2004). The introduction of both HLB and *D. citri* to both Brazil and Florida are believed to be the direct result of movement of plant material (Gottwald 2010). Psyllids may also arrive actively or passively by other means such as wind currents, and in commercial and military aircraft (Beattie et al. 2010). They may either introduce the infection or enable the transmission and spread of bacteria that are introduced by other means.

HLB can have a long incubation period in the host plant before symptoms are expressed. The length of the incubation period can be variable as it is influenced by tree age and health. As a result there may be visually asymptomatic infections established at the same time as symptomatic infections. Although bacteria can be detected in asymptomatic trees using polymerase chain reaction (PCR), the concentration of bacteria is not always above the threshold of detection for PCR. At the same time, the asymptomatic trees still appear to be infective, with bacteria capable of being picked up and transmitted by the psyllid vectors. Once infected, trees can produce inoculum for a number of years until they die or are removed. These factors contribute to the disease being difficult to control commercially (Gottwald 2010).

HLB disease is controlled by preventing trees from becoming infected. This is largely based on: production of citrus propagation materials in insect-proof facilities; elimination of inoculum by removal of infected trees; the use of systemic and contact insecticides, mineral oils and other methods to strongly reduce vector populations; and quarantine measures to counter spread of both pathogens and vectors (Bové 2006, National Research Council 2010). When an orchard is severely affected, it may be best to remove the whole stand and replant with healthy trees (Bové 2006). Although very few countries have been able to control Asian HLB, São Paulo State in Brazil seems to be successfully managing the disease through tree removal and insecticide treatments (Bové 2006). It has been proposed that the most powerful long-term management tool for Asian HLB is likely to be the development of citrus cultivars resistant to '*Ca.* L. asiaticus' and perhaps its vector (National Research Council 2010).

Temperature sensitivity:

Symptom expression of HLB is affected by temperature (Bové 2006, Gasparoto et al. 2008, Lopes et al. 2009a, Lopes et al. 2009b) and light (Folimonova et al. 2009), and varies with season (Lopes et al. 2009b). '*Ca*. L. americanus' has similar environmental tolerances to '*Ca*. L. africanus' (Lopes et al. 2009a) which survives in cool regions at high elevations (Bové 2006). In unfavourable environments symptoms may not be expressed (Bové 2006).

Studies indicate that '*Ca*. L. americanus' and '*Ca*. L. africanus' are heat sensitive (Bové 2006, Lopes et al. 2009a) and do not produce symptoms above 27°C. Temperatures in the range of 22–24°C are optimum for the development of '*Ca*. L. africanus' (Bové 2006) and '*Ca*. L. americanus' (Gasparoto et al. 2008, Lopes et al. 2009a). '*Ca*. L. asiaticus' is heat tolerant and produces symptoms above 27°C (Bové 2006, Lopes 2009b). Temperatures in the range of 17–32°C are optimum for the development of '*Ca*. L. asiaticus'.

Studies by Gasparoto et al. (2012) on the influence of temperature on the infection and establishment of '*Ca*. L. americanus' and '*Ca*. L. asiaticus' in citrus plants, found that '*Ca*. L. americanus' did not infect the plants maintained at 27/32°C (8/16 h dark/light photoperiod).

Infection by '*Ca*. L. asiaticus' occurred at all tested temperatures (17/22, 22/27 and 27/32ºC, at 8/16 h D/L), however the highest bacterial titre occurred under cooler (17/22ºC) temperatures in one experiment, and over time in mature leaves from infected plants maintained at 22/27ºC (compared with leaves at 27/32ºC) in another experiment. '*Ca*. L. asiaticus' colonisation of citrus plants was negatively affected by daily temperatures of 27/32ºC.

2.1.3 Risk assessment

2.1.3.1 Entry assessment

'*Ca*. L. americanus' has been recorded from *Citrus* spp. in São Paulo State, Brazil. Early studies found '*Ca*. L. americanus' in 216 of 218 symptomatic leaf samples from 47 farms in 35 municipalities, while '*Ca*. L. asiasticus' was detected in only four of the 218 samples, indicating that '*Ca*. L. americanus' was the major cause of HLB in São Paulo State (Teixeira et al. 2005). However, '*Ca*. L. asiasticus' is now more prevalent in São Paulo State (Lopes et al. 2009b). It has also been reported from China although it is not clear if this has been confirmed (Bové et al. 2008).

Citrus nursery stock may enter New Zealand as either budwood (dormant cuttings) or tissue culture plantlets, although budwood is more usual. It is likely that a small amount of budwood material will be imported in a consignment, with probably 5–12 buds per stick of wood. Tissue culture plants will arrive as plantlets in flasks of media. Because '*Ca*. L. americanus' infects citrus systemically it can be present in the imported nursery stock.

For this assessment it is assumed that the plant material is imported from a non-accredited facility, and therefore is of an unknown phytosanitary status. In this case the material enters a Level 3 post-entry quarantine facility (PEQ) for a minimum of 16 months to allow time for biological indexing (import health standard (IHS) 155.02.06, *Citrus* schedule).

Overview of Level 3 PEQ for citrus nursery stock:

The overall process for importation of citrus nursery stock from a non-accredited facility to New Zealand is outlined in section 1.3, including figures 1 and 2. In brief, upon arrival in Level 3 PEQ, the plant material is inspected for visually obvious signs of regulated pests and diseases. For dormant cuttings, buds are grafted on to rootstock, to allow tests to take place. For tissue culture material, plants are removed from media flasks and replanted into pots. Plants are grown in greenhouse conditions throughout the year, and inspected frequently by the facility operator for signs of regulated pests and diseases. In addition, pre-determined specific tests are carried out for some regulated organisms before plants can be cleared for release from quarantine.

'*Ca*. L. americanus' on citrus nursery stock in Level 3 PEQ:

The ability of '*Ca*. L. americanus' to infect plants asymptomatically (Lopes et al. 2009b) means that it can enter New Zealand undetected on citrus nursery stock such as budwood (dormant cuttings) or plants in tissue culture. Upon arrival the symptomless citrus budwood or plants in tissue culture material without any visually detectable regulatory pathogens will be taken to the PEQ facility for propagation. Buds from the asymptomatic budwood will be grafted onto New Zealand origin rootstock to develop sufficient material to allow buds to be grafted to New Zealand origin indicator plants. The asymptomatic tissue culture plantlets will be deflasked in a quarantine greenhouse and planted in pots.

Plants are maintained in a healthy vigorous state without stress and can be grown in greenhouse conditions throughout the year. They are held at temperatures considered suitable for

liberibacters. However, the appropriate temperatures are not stipulated in the current IHS 155.02.06 for plant material from non-accredited facilities[7].

Pre-determined tests are currently required by the IHS for '*Ca*. L. africanus' and '*Ca*. L. asiaticus'[8]. Graft indexing for '*Ca*. Liberibacter spp.' is done in the early spring using young, vigorous indicator plants. Laboratory tests for '*Ca*. Liberibacter spp.' (PCR) are then carried out at the end of summer. Although specific tests are available for '*Ca*. L. americanus' (MAF 2010), pre-determined tests for this species are not required by the IHS[9].

For graft indexing, sweet orange 'Pineapple' (*C. sinensis*) is used as the indicator plant (incubation temperature 18–25ºC) for all three liberibacter species[10]. Specific symptoms such as chlorotic mottling on the leaves can appear within 5 months, but may take more than 2 years to appear. Therefore it is possible that symptoms of '*Ca*. L. americanus' infection or co-infection could be produced and detected, and any indicator plant that shows symptoms is tested to diagnose the cause. However the bacterial titre may be too low for symptoms to become apparent during the testing period. Studies show that high temperatures can suppress symptom expression or eliminate '*Ca*. L. americanus' from host plants and therefore the conditions under which the plants are held is critical for effective detection of the bacteria.

The original imported plant material is also tested for the presence of '*Ca*. Liberibacter spp.' by means of real-time TaqMan PCR. This is not a generic test for liberibacter as a separate set of specific primer pairs is used for each species tested. As noted earlier, pre-determined tests for '*Ca*. L. americanus' are not required by the IHS. As with any laboratory-based test there are limitations in the ability to detect asymptomatic infection, such as uneven distribution within the plants (i.e., the liberibacter is not present in the leaves submitted for analysis) or the titre of the liberibacter is not high enough to be detected. Every attempt is made to ensure the likelihood of testing and detecting liberibacter is increased, including the type of material selected for testing, the time of year samples are collected, the temperatures under which the plants are held and using the most sensitive detection method.

In post-entry quarantine (PEQ), the nursery stock will be kept and monitored for a minimum of 16 months from the start of plant growth before being released. During this period plants are checked weekly by the facility operator and any plants showing symptoms of '*Ca*. L. americanus' would be tested to diagnose the cause.

Given that:

- although '*Ca*. L. americanus' has been recorded in citrus in Brazil, and possibly China, the liberibacter has not yet been recorded in citrus elsewhere;
- '*Ca*. L. americanus' is systemic in its citrus host and therefore can occur in nursery stock;
- infected plants may show disease symptoms during the growing period of at least 16 months in Level 3 PEQ, however, infected citrus plants can be asymptomatic;
- high temperatures can suppress symptom expression or eliminate '*Ca*. L. americanus' from host plants;
- no specific pre-determined tests for '*Ca*. L. americanus' are required by the IHS to be carried out in Level 3 PEQ[11];

The likelihood of entry is considered to be low but non-negligible.

7 All citrus nursery stock imports since 2010 have come from an MPI-approved facility overseas and therefore have entered Level 2 PEQ. The IHS requires that plants in Level 2 PEQ must be held at 18–25ºC throughout the quarantine period (incorporated in IHS amendment 27 November 2014). However the CTO has required operational measures in PEQ to effectively manage temperature to these levels since April 2012.

8 The *Citrus* schedule in the IHS 155.02.06 states that 'Country freedom is accepted as equivalence to a treatment'

9 Current *Citrus* import permits do require PCR tests for '*Ca*. L. americanus'

10 Stipulated for '*Ca*. L. africanus' and '*Ca*. L. asiaticus' in the *Citrus* schedule in IHS 155.02.06; considered suitable for '*Candidatus* Liberibacter spp.' in the *Citrus* Testing Manual (MAF 2010) which considers all three species

11 Pre-determined tests for '*Ca*. L. americanus' have been stipulated on the import permit in the past

2.1.3.2 Exposure assessment

'*Ca*. L. americanus' is borne internally and will survive for as long as the host plant remains alive. Infected plants that are symptomless are likely to be released from PEQ for future propagation. As the liberibacter is a systemic pathogen that is graft-transmissible, it is likely that infected plant material used for grafting will expose '*Ca*. L. americanus' to other susceptible plants in the New Zealand environment.

Given that:

- '*Ca*. L. americanus' can survive in its host for as long as the plant remains alive;
- symptomless plants systemically infected with '*Ca*. L. americanus' that are released from the PEQ facility are likely to be used for further propagation;

The likelihood of exposure is considered to be high.

2.1.3.3 Assessment of establishment and spread

'*Ca*. L. americanus' can remain systemically in the host plant for the lifetime of that plant unless held under unfavourable conditions of high temperature. If infected plants are used for propagation of new infected plants that are planted, then '*Ca*. L. americanus' can be considered to have established.

'*Ca*. L. americanus' was the main cause of HLB in citrus orchards in São Paulo State, Brazil, before '*Ca*. L. asiaticus' became more prevalent. The region has some climatic similarities to parts of New Zealand. There is no evidence that climate would limit the ability of '*Ca*. L. americanus' to establish in at least some regions of New Zealand. Citrus species are grown both commercially and domestically, with the main citrus growing areas occurring in warmer northern regions such as Northland, Auckland, Bay of Plenty and Gisborne. Host plant availability is unlikely to limit the establishment of '*Ca*. L. americanus' in New Zealand.

Liberibacter are most commonly transmitted by propagation or grafting of infected material, and by psyllid vectors. Plants that show HLB symptoms are unlikely to be used for propagation, especially in a commercial situation. However, infected plants may be asymptomatic, or at least initially, and imported nursery stock is likely to be used to propagate a large number of plants. Infected but symptomless plant material could be distributed without disease being evident or before symptoms become apparent thereby spreading the bacteria to different locations.

Diaphorina citri, the only recorded vector of *Ca*. L. americanus', is not present in New Zealand. It is unlikely that other species could vector the bacteria from citrus to citrus as so far no psylloids have been recorded from citrus species in New Zealand (Dr P J Dale, personal communication, 16/05/2014). If *D. citri* established then it is likely that the disease would spread to all citrus trees within its distribution as transmission by psyllid vectors is considered to be the primary mode of HLB spread in the field and where psyllids appear the disease often appears soon after (Bové 2006).

Given that:

- '*Ca*. L. americanus' can remain systemically in the plant for as long as the plant remains alive;
- imported citrus nursery stock is used for the propagation of new plants for planting;
- infected host plants may be asymptomatic and used for propagation;
- '*Ca*. L. americanus' is spread by propagation or grafting;
- the New Zealand climate is unlikely to prevent the establishment of '*Ca*. L. americanus';
- the availability of host plants is unlikely to limit the establishment of '*Ca*. L. americanus';

- known psylloid vectors of '*Ca*. L. americanus' are not present in New Zealand and no other psylloids have been recorded from citrus in New Zealand;

*The likelihood of establishment and spread of '*Ca*. L. americanus' is considered to be moderate to high.*

2.1.3.4 Consequence assessment

The degree of impact from '*Ca*. L. americanus' depends on presence of a suitable vector. The known psylloid vectors, *D. citri*, *T. erytreae*, and potentially *D. communis*, are not present in New Zealand. Elsewhere, other species of psylloid have been recorded on citrus, but none have been shown to be vectors (Halbert and Manjunath 2004, Gottwald et al. 2007). So far, no psylloids have been recorded from citrus species in New Zealand (Dr P J Dale, personal communication, 16/05/2014). In the following sections, consequences are considered in both the presence and absence of a suitable vector in New Zealand.

Economic consequences

The New Zealand citrus industry comprises around 1,700 hectares divided between approximately 500 orchards, most of which are in the Bay of Plenty, Gisborne and Northland regions (NZCGI 2013). In 2013/14, the domestic sales of fresh citrus fruit were $49.1m and in 2014 export sales of fresh fruit were $6.9m (Fresh Facts 2014). The discovery of the presence of '*Ca*. L. americanus' could have an immediate impact on exports of citrus to some countries. It is currently considered a quarantine pest on fruit in trade for Japan, Thailand and Taiwan. If the HLB disease is expressed, then the loss of production from unmarketable symptomatic fruit would affect both export and domestic sales. The disease, however, may affect only some of the citrus production regions if temperature requirements limit the appearance of symptoms. If there is no suitable vector present, then control could be achieved by tracing diseased plants, removing and replacing them. This could be a major cost to some producers if they have invested in diseased stock. If a vector is present, management activities would be much greater through having to control vectors and replace diseased plants. A greater and longer term effect on the citrus industry would be expected through lowered production, increased management activities and to some extent limited access to or increased requirements for overseas markets where the disease is absent. If there is no vector, '*Ca*. L. americanus' could have a high impact on individual citrus growers. If a vector is present, '*Ca*. L. americanus' could have high impact on the citrus industry. However, within the context of the New Zealand economy as a whole, the consequences are considered to be low (without a vector) to moderate (if vector present).

The potential economic consequences within New Zealand are considered to be low (without a vector) to moderate (with a vector).

Environmental consequences

'*Ca*. L. americanus' has been recorded from *Citrus* spp. and *Murraya* spp. which are members of the Rutaceae. There are two genera within Rutaceae endemic to New Zealand: *Leionema* and *Melicope*. Neither the *Leionema* nor *Melicope* species are considered threatened (NZPCN 2015). However, if these species were susceptible, they would be unlikely to be affected in the natural environment in the absence of an effective insect vector. If an effective vector was present, there is a possibility that these species would be affected.

The potential environmental consequences within New Zealand are considered to be negligible (without a vector) to low (with a vector).

Socio-cultural consequences

'*Ca*. L. americanus' has been recorded from *Citrus* spp. and *Murraya* spp. which are members of the Rutaceae. Both genera are grown domestically in New Zealand, particularly in warmer regions. Symptomatic infected citrus trees would have blotchy mottled and yellowing leaves, small lopsided and bitter tasting fruit with aborted seeds, with branches and eventually whole plants dying after a few years. In the absence of a vector, this would occur through the purchase of infected plants or by grafting infected plant material onto existing plants. Infected plants could be replaced by healthy plants. If an effective vector was present, any plant could potentially be infected and control would be difficult.

The potential socio-cultural consequences within New Zealand are considered to be low.

Human health consequences

There are no known human health consequences.

2.1.3.5 Risk estimation

The likelihood of entry of '*Candidatus* Liberibacter americanus' is considered to be low, and the likelihood of exposure is considered to be high. The likelihood of establishment and spread is considered to be moderate to high. The potential economic consequences within New Zealand are considered to be low unless a vector is present in which case they are considered to be moderate. The potential environmental consequences within New Zealand are considered to be negligible without a vector and low if a vector is present. The potential socio-cultural consequences within New Zealand are considered to be low. The potential health consequences within New Zealand are considered to be negligible.

As a result the overall risk estimate for 'Candidatus *Liberibacter americanus' on nursery stock of* Citrus *and related species is non-negligible and it is considered to be a risk on the commodity. Therefore the risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

Risk estimation table

'Candidatus Liberibacter americanus' on imported nursery stock of *Citrus* and related species				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment				
Spread				
Consequences of establishment				
Economic		no vector	vector	
Environmental	no vector	vector		
Socio-cultural				
Human Health				

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2.2 '*Candidatus* Phytoplasma asteris'

Scientific name: '*Candidatus* Phytoplasma asteris' (Class: Mollicutes)
Other relevant scientific names: 16SrI, '*Candidatus* Phytoplasma asteris' subgroup B, 16SrI subgroup B, 16SrI-B, '*Candidatus* Phytoplasma asteri'
Common names: aster yellows, aster yellows subgroup B

2.2.1 Hazard identification

2.2.1.1 Description

Phytoplasmas are phloem-limited bacteria that lack cell walls and have not been cultured (Lee et al. 2000, Bertaccini 2007). They are associated with a wide range of symptoms affecting all plant parts; typical symptoms include 'little leaf', abnormal root growth, overall stunting, shoot proliferation, leaf yellowing, flower phyllody and virescence, as well as a general decline that is sometimes fatal (Agrios 2005).

2.2.1.2 Taxonomic issues

Phytoplasmas form a distinct clade in the Class *Mollicutes*. The phytoplasma clade has been proposed to represent at least a genus, with each subclade (16S rRNA group) proposed to represent at least one species (Gundersen et al. 1994). The '*Candidatus* Phytoplasma asteris' group (16SrI) is the largest of these groups, but it has also been divided into subgroups (A, B, C, D, E and others: Lee et al. 2004, Zhao et al. 2010).

The species concept is defined within the phytoplasmas (IRPCM 2004), but the appropriate level at which to conduct a risk assessment is often unclear. Since the subgroups of the '*Ca*. P. asteris' 16SrI group phytoplasmas appear to represent distinct phylogenetic lineages, differing in distribution and host range, Lee et al. (2004) suggest that for quarantine purposes it would be appropriate to consider them separately. This assessment therefore primarily considers subgroup B of '*Ca*. P. asteris' (16SrI-B) because it is associated with disease in citrus[12]. However, some of the information in this risk assessment refers to '*Ca*. P. asteris' and is not specified as relating to a particular subgroup.

Subgroup B is widespread and diverse. There are dozens of isolates within this subgroup, described from different hosts in different regions (Lee et al. 2004). It is unclear whether any of these also differ in host range, distribution or other properties that may affect a risk analysis. It is assumed here that they do not differ to any significant degree, but further information characterising variation within '*Ca*. P. asteris' subgroup B may affect the results of this risk analysis.

2.2.1.3 New Zealand status

None of the subgroups of '*Ca.* P. asteris' is known to be present in New Zealand. Not recorded in: Veerakone et al. 2015, Liefting et al. 2007, NZFungi 2015, PPIN 2015.

2.2.1.4 General geographic distribution

The '*Ca*. P. asteris' subgroup B is the most widespread subgroup of '*Ca*. P. asteris', and is found in Europe, Asia, North America, South America and Africa (Lee et al. 2004, Engelbrecht et al. 2010).

12 Since this risk assessment was prepared, another subgroup of '*Ca*. P. asteris', subgroup S (16SrI-S), has been reported along with subgroup B (16SrI-B) on citrus in association with HLB-like symptoms, in Mexico (Arratia-Castro et al. 2014).

A phytoplasma in the '*Ca*. P. asteris' subgroup B has been detected in citrus in Guangdong Province, China. This is the first report of '*Ca*. P. asteris' in citrus (Chen et al. 2009)[13].

2.2.1.5 Commodity association

'*Ca*. P. asteris' has been detected in citrus leaves (midribs) by PCR (Chen et al. 2009). As phytoplasmas are systemic within infected plants, although limited to phloem, '*Ca*. P. asteris' can potentially be present in citrus nursery stock (budwood or tissue culture).

2.2.1.6 Plant associations

The '*Ca*. P. asteris' group of phytoplasmas (16SrI) has the most diverse and widespread host range of any group of phytoplasmas, reported to be associated with more than 100 plant species and many economically important diseases worldwide (Lee et al. 2004, Lee et al. 2006). '*Ca*. P. asteris' subgroup B infects a wide range of hosts including food crops, trees, ornamentals and weeds (Lee et al. 2004). The wide host range of subgroup B appears to be a result of the large number and polyphagous nature of its insect vectors, rather than the specificity of the phytoplasma itself.

Recorded hosts for subgroup B (from Lee et al. 2004, unless indicated) include, amongst others, *Allium cepa* (onion), *Apium graveolens* (celery), *Brassica* spp., *Daucus carota* (carrot), *Hydrangea macrophylla* (hydrangea), *Solanum lycopersicum* (tomato), *Malus domestica* (apple) (Jomantiene and Davis 2005), *Medicago sativa* (alfalfa), *Nasturtium microphyllum* (watercress), *Olea europaea* (olive), *Plantago major* (broad-leaved plantain), *Populus nigra* 'Italica' (Lombardy poplar), *Pyrus communis* (pear), *Rosa* spp. (rose) (Kamińska et al. 2006), *Salix* spp. (willows), *Solanum tuberosum* (potato), *Trifolium* spp. (clover), *Vitis* spp. (grape) (Alma et al. 1996), *Zea mays* (maize).

'*Ca*. P. asteris' subgroup B has been detected in the following citrus species: *Citrus reticulata* Blanco (mandarin), *C. sinensis* (L.) Osbeck (sweet orange) and *C. maxima* ((Burn.) Merrill (pummelo) (Chen et al. 2009)[14]. The isolates studied by Chen et al. (2009), infected the following experimental hosts: *Catharanthus roseus* (L.) G. Don. (periwinkle) and *Cuscuta campestris* Yunck (dodder).

2.2.1.7 Potential for establishment and impact

'*Ca*. P. asteris' subgroup B occurs in countries with climates similar to parts of New Zealand. Further, as there are potentially vectors in New Zealand that can transmit '*Ca*. P. asteris' subgroup B (see Biology section), and many recorded hosts, including citrus species, occur in New Zealand, it can potentially establish in New Zealand. '*Ca*. P. asteris' subgroup B causes damage to its host plants, so can potentially cause unwanted impacts on economically important species in New Zealand.

2.2.1.8 Hazard identification conclusion

Given that '*Ca*. P. asteris':

- is reported from *Citrus* spp.;
- is present in many regions of the world including countries from which *Citrus* nursery stock may be imported;

13 Since this risk assessment was prepared, '*Ca*. P. asteris' subgroup 16SrI-B has been reported in citrus in Mexico (Arratia-Castro et al. 2014, Poghosyan et al. 2015). At the same time, subgroup 16SrI-S has also been reported in citrus in Mexico (Arratia-Castro et al. 2014)
14 Since this risk assessment was prepared, '*Ca*. P. asteris' subgroup 16SrI-B has been reported in *Citrus aurantiifolia* (Christm.) Swingle (Mexican lime) (Arratia-Castro et al. 2014) and *C. japonica* (kumquat) (Poghosyan et al. 2015), and subgroup 16SrI-S has been reported in *C. aurantiifolia* and *C. sinensis* (Valencia sweet orange) (Arratia-Castro et al. 2014).

- is not known to occur in New Zealand;
- can potentially establish in New Zealand;
- can potentially cause unwanted impacts in New Zealand;

'*Candidatus* Phytoplasma asteris' is considered a hazard on *Citrus* nursery stock in this risk analysis.

2.2.2 Biology

Huanglongbing (HLB) is one of the most destructive diseases worldwide to affect citrus production and it has generally been accepted that '*Candidatus* Liberibacter spp.' are the most probable aetiological agents of HLB. A study by Chen et al. (2009) indicates that '*Ca.* P. asteris' subgroup B is also associated with citrus HLB along with '*Ca*. L asiaticus' in Guangdong Province, China. However, the true aetiological role(s) of the phytoplasma and the liberibacter in HLB will remain uncertain until Koch's postulates are fulfilled (Chen et al. 2009). Adding weight to the association of phytoplasmas with citrus HLB is the report of another citrus HLB-associated phytoplasma in São Paulo State in Brazil (Teixeira et al. 2008)[15]. This phytoplasma is closely related to the pigeon pea witches'-broom phytoplasma (16SrIX), and more distantly related to '*Ca.* Phytoplasma asteris' subgroup B (Chen et al. 2009). It is associated with citrus HLB symptoms and has been detected both separately and in mixed infection with '*Ca*. L. spp.' in symptomatic sweet orange trees (Teixeira et al. 2008).

'*Ca*. P. asteris' has been detected in field-collected samples of mandarin (*Citrus reticulata* Blanco), sweet orange (*C. sinensis* (L.) Osbeck) and pummelo (*C. maxima* (Burn.) Merrill), as well as in experimentally infected periwinkle (Chen et al. 2009). Nested PCR using phytoplasma-specific primer sets (P1/P7 followed by fU5/rU3), has been used to detect the phytoplasma within infected plant material. A 1785 bp amplicon of the 16Sr RNA gene region was obtained using the primers P1/P7. DNA sequence analysis of amplicons (using primer set fU5/rU3) from 18 symptomatic leaf samples from mandarin, sweet orange, pummelo and periwinkle, were identical (Chen et al. 2009). The 16S rDNA and 16S-23S intergenic spacer gene region sequence showed 100% identity to three strains of '*Ca*. P. asteris': onion yellows phytoplasma OY-M from Japan, aster yellows phytoplasma 'watercress' from Hawaii, and valeriana yellows phytoplasma clone VlY2 from Lithuania (Chen et al. 2009). The phytoplasma from citrus is therefore considered to be '*Ca*. P. asteris', and belongs in the 16SrI-B subgroup (Chen et al. 2009).

Of 141 samples from citrus plants showing typical HLB symptoms, 110 (78%) samples were positive for '*Ca*. P. asteris', 89 (63%) were positive for '*Ca*. L asiaticus', and 69 (49%) were positive for both bacteria, indicating mixed infection. Transmission electron microscope (TEM) analysis of infected periwinkle showing virescence and phyllody symptoms revealed many sieve tubes were filled by cells that were indicative of phytoplasmas. These cells were also observed in HLB citrus samples (Chen et al. 2009).

Disease symptoms:

Chen et al. (2009) were not able to clearly identify symptoms specifically associated with each bacterium alone or together. HLB symptoms described in Chen et al. (2009) included leaf mottling and yellowing ("leaves with characteristic symptoms of blotchy mottle"), and 'symptomatic' fruit. The authors describe the trees infected by the phytoplasma as showing leaf and fruit symptoms indistinguishable from those produced by the liberibacters. Although not explicitly stated, HLB disease symptoms typically includes lopsided and abnormally coloured

15 There have been further reports of phytoplasmas found in association with HLB-like symptoms in citrus: '*Ca*. P. asteris' subgroup B (Arratia-Castro et al. 2014; Poghosyan et al. 2015); '*Ca*. P. asteris' subgroup S (Arratia-Castro et al. 2014); '*Ca*. P.' 16SrII-A* (Lou et al. 2014).

fruits carrying aborted seed (Teixiera et al. 2008), branch dieback and even whole plant death, and it is assumed these may also occur in the affected plants in Guangdong.

Graft-inoculated periwinkle showed leaf yellowing/mottling symptoms 1 month after grafting. Virescence and phyllody symptoms appeared as the infected plants began to flower. None of these symptoms were apparent in non-inoculated periwinkle control plants (Chen et al. 2009).

Disease latency:

'*Ca.* P. asteris' has been detected by PCR in asymptomatic mandarin leaves from non-HLB-affected trees in Guangdong (Chen et al. 2009). It is assumed that these plants were not showing any symptoms of other non-HLB phytoplasma diseases. Evidence that '*Ca.* P. asteris' infection can occur asymptomatically in other species are the reports of '*Ca.* P. asteris' being present in asymptomatic ashleaf maple trees in Poland (Kamińska and Śliwa 2005), and of '*Ca.* P. asteris' being present in asymptomatic grapevines in Canada (Olivier et al. 2009).

Disease transmission:

There are two main mechanisms of transmission known for phytoplasmas: propagation or grafting of infected material, and transmission by phloem-feeding insect vectors. Experimentally, phytoplasmas can be transmitted between plants via the vascular connection formed by dodder (*Cuscuta* spp.). Phytoplasmas are not reported to be spread by mechanical transmission (Lee et al. 2000).

Phloem-feeding leafhoppers (family Cicadellidae) belonging to the genera *Macrosteles, Euscelis, Scaphytopius* and *Aphrodes* are the main vectors of '*Ca*. P. asteris' (Lee et al. 2004); [there is a *Macrosteles* species in NZ (Gordon, 2010)]. The subgroups vary in the specificity of their relationships with insect vectors; subgroup B is reported to have a low vector specificity (Lee et al. 1998).

Insect vectors have been identified for relatively few phytoplasma diseases and typically when new phytoplasma diseases are discovered little is known about the disease epidemiology (Weintraub and Beanland 2006). It is still unknown which insect vectors may be involved in the transmission of HLB-associated '*Ca*. P. asteris' (Chen et al. 2009, Arratia-Castro et al. 2014). As with other phytoplasmas, dodder has been used to transmit the phytoplasma from HLB-affected mandarin to periwinkle in experiments (Chen et al. 2009).

2.2.3 Risk assessment

2.2.3.1 Entry assessment

'*Ca*. P. asteris' has been recorded from *Citrus* spp. only in China where it has been detected during surveys of citrus species in Guangdong Province (Chen et al. 2009). It is not known how widespread it is through citrus in China, nor if it occurs in *Citrus* spp. in other countries[16].

Citrus nursery stock may enter New Zealand as either budwood (dormant cuttings) or tissue culture plantlets, although budwood is more usual. It is likely that a small amount of budwood material will be imported in a consignment, with probably 5–12 buds per stick of wood. Tissue culture plants will arrive as plantlets in flasks of media. Because '*Ca*. P. asteris' infects citrus systemically it can be present in the imported nursery stock.

For this assessment it is assumed that the plant material is imported from a non-accredited facility, and therefore is of an unknown phytosanitary status. In this case the material enters a

16 Since this risk assessment was prepared, '*Ca*. P. asteris' subgroup 16SrI-B has been reported in citrus in Mexico (Arratia-Castro et al. 2014, Poghosyan et al. 2015). At the same time, subgroup 16SrI-S has also been reported in citrus in Mexico (Arratia-Castro et al. 2014)

Level 3 post-entry quarantine facility (PEQ) for a minimum of 16 months to allow time for biological indexing (import health standard (IHS) 155.02.06, *Citrus* schedule).

Overview of Level 3 PEQ for citrus nursery stock:

The overall process for importation of citrus nursery stock from a non-accredited facility to New Zealand is outlined in section 1.3, including figures 1 and 2. In brief, upon arrival in Level 3 PEQ, the plant material is inspected for visually obvious signs of regulated pests and diseases. For dormant cuttings, buds are grafted on to rootstock, to allow tests to take place. For tissue culture material, plants are removed from media flasks and replanted into pots. Plants are grown in greenhouse conditions throughout the year, and inspected frequently by the facility operator for signs of regulated pests and diseases. In addition, pre-determined specific tests are carried out for some regulated organisms before plants can be cleared for release from quarantine.

'*Ca.* P. asteris' on citrus nursery stock in Level 3 PEQ:

The ability of phytoplasmas to infect plants asymptomatically means that '*Ca*. P. asteris' can enter New Zealand undetected on citrus nursery stock such as budwood (dormant cuttings) or plants in tissue culture. Upon arrival the symptomless citrus budwood or plants in tissue culture material without any visually detectable regulatory pathogens will be taken to the PEQ facility for propagation. Buds from the asymptomatic budwood will be grafted onto New Zealand origin rootstock to develop sufficient material to allow buds to be grafted to New Zealand origin indicator plants. The asymptomatic tissue culture plantlets will be deflasked in a quarantine greenhouse and planted in pots.

Plants are maintained in a healthy vigorous state without stress and can be grown in greenhouse conditions throughout the year. They are held at temperatures considered suitable for liberibacters. However, the appropriate temperatures are not stipulated in the current IHS 155.02.06 for plant material from non-accredited facilities[17].

Graft indexing for phytoplasmas is done in the early spring using young, vigorous indicator plants. Laboratory tests for phytoplasmas (PCR) are then carried out at the end of summer. No pre-determined specific tests for '*Ca*. P. asteris' are stipulated in the current IHS. However, specific tests are currently required for '*Candidatus* Phytoplasma aurantifolia' and Australian citrus dieback (phytoplasma-associated). The IHS gives graft inoculation onto lime (*C. aurantiifolia*) (and indicator plants grown at cool temperatures 18 to 25ºC) as an acceptable method for '*Ca.* P. aurantifolia', and states that a suitable test is required for Australian citrus dieback[18]. PCR tests are not currently specified on the IHS for phytoplasmas although they are given in the *Citrus* testing manual (MAF 2010)[19].

The citrus host range of the '*Ca*. P. asteris' isolates found in association with HLB is not known; however, the phytoplasma has been recorded from mandarin (*Citrus reticulata* Blanco), sweet orange (*C. sinensis* [L.] Osbeck), and pummelo (*C. maxima* [Burn.] Merrill) displaying HLB-like symptoms (Chen et al. 2009)[20]. As varieties of sweet orange and several other citrus species/varieties are used as indicator plants, then it is possible that symptoms could be produced and detected. Any indicator plant that shows symptoms is tested to diagnose the cause.

17 All citrus nursery stock imports since 2010 have come from an MPI-approved facility overseas and therefore have entered Level 2 PEQ. The IHS requires that plants in Level 2 PEQ must be held at 18–25ºC throughout the quarantine period (incorporated in IHS amendment 27 November 2014). However the CTO has required operational measures in PEQ to effectively manage temperature to these levels since April 2012.

18 The *Citrus* schedule in the IHS 155.02.06 states that 'Country freedom is accepted as equivalence to a treatment' for both species.

19 PCR tests are included as a mandatory requirement on current import permits: nested-PCR using universal phytoplasma primers is listed for all three phytoplasmas – '*Ca*. P. asteris', '*Ca*. P. aurantifolia' and Australian citrus dieback. The nested-PCR and real-time PCR using universal phytoplasma primers would be considered equivalent measures and so either could be used.

20 '*Ca*. P. asteris' subgroup B has since been detected in Mexican lime (*C. aurantiifolia*) and kumquat (*C. japonica*) displaying HLB-like symptoms in Mexico (Arratia-Castro et al. 2014, Poghosyan et al. 2015), and '*Ca*. P. asteris' subgroup S has been detected in Valencia sweet orange (*C. sinensis*) and Mexican lime displaying HLB-like symptoms in Mexico (Arratia-Castro et al. 2014).

In post-entry quarantine (PEQ), the nursery stock will be kept and monitored for a minimum of 16 months from the start of plant growth before being released. During this period plants are checked weekly by the facility operator and any plants showing symptoms of '*Ca*. P. asteris' would be tested to diagnose the cause.

Although it is not certain that '*Ca*. P asteris' would produce detectable symptoms on the current suite of indicator plants, it is highly likely that the phytoplasma would be detected with universal phytoplasma primers during routine PCR testing of the original imported plant material. These tests are not required by the IHS, however. As with any laboratory based test there are limitations in the ability to detect asymptomatic infection, such as uneven distribution within the plants (i.e. the phytoplasma is not present within the leaves submitted for analysis) or the titre of the phytoplasma is not high enough to be detected. Every attempt is made to ensure the likelihood of testing and detecting phytoplasma is increased, including the type of material selected for testing, the time of year samples are collected, the temperatures at which plants are held and using the most sensitive detection method.

Given that:

- although '*Ca*. P. asteris' has been recorded in citrus in China, the phytoplasma has not yet been recorded in citrus elsewhere[21];
- '*Ca*. P. asteris' is systemic in its citrus host and therefore can occur in nursery stock;
- infected plants may show disease symptoms during the growing period of at least 16 months in Level 3 PEQ, however, infected citrus plants can be asymptomatic;
- no specific predetermined tests for '*Ca*. P. asteris' are required by the IHS to be carried out in Level 3 PEQ[22, 23];

The likelihood of entry is considered to be low but non-negligible.

2.2.3.2 Exposure assessment

'*Ca*. P. asteris' is borne internally and will survive for as long as the host plant remains alive. Infected plants that are symptomless are likely to be released from PEQ for future propagation. As the phytoplasma is a systemic pathogen that is graft-transmissible, it is likely that infected plant material used for grafting will expose the phytoplasma to other susceptible plants in the New Zealand environment.

Given that:

- '*Ca*. P. asteris' can survive in its host for as long as the plant remains alive;
- symptomless plants systemically infected with '*Ca*. P. asteris' that are released from the PEQ facility are likely to be used for further propagation;

The likelihood of exposure is considered to be high.

2.2.3.3 Assessment of establishment and spread

'*Ca*. P. asteris' can remain systemically in the plant for as long as the plant remains alive. If infected plants are used for propagation of new infected plants that are planted, then the phytoplasma can be considered to have established.

21 Since this risk assessment was prepared, '*Ca*. P. asteris' (subgroups 16SrI-B and 16SrI-S) has been reported in association with HLB in citrus in Mexico (Arratia-Castro et al. 2014).
22 However, tests may be stipulated on the import permit.
23 It is highly likely that the phytoplasma would be detected with universal phytoplasma primers during routine PCR testing of the original imported plant material: however, this is not required by the IHS and is excluded from consideration for the risk estimate.

'*Ca.* P. asteris' occurs in tropical to cool temperate regions. There is no evidence that its distribution is limited by climate and so in New Zealand it is unlikely that climate would limit the ability of '*Ca*. P. asteris' subgroup B to establish. Citrus species are grown both commercially and domestically in New Zealand ensuring that host plants would be available. Many other reported host plants for the '*Ca*. P. asteris' subgroup B phytoplasmas are common in New Zealand as either agricultural, horticultural or amenity species (e.g. maize, barley, wheat, tomato, onion, carrots, roses, pear, poplar and willow apple); weeds (e.g. plantain) or garden plants (e.g. hydrangea, roses, marigolds). Host plant availability is very unlikely to limit the establishment of '*Ca*. P. asteris' subgroup B in New Zealand.

Phytoplasmas are most commonly transmitted by propagation or grafting of infected material, and by insect vectors. These are the two potential routes for 'Ca. P. asteris' subgroup B to get from infected citrus nursery stock into other host plants in New Zealand.

Plants that show symptoms of a phytoplasma infection are unlikely to be propagated from. However, hosts may be asymptomatic, at least initially, and plant material could be distributed without disease being evident or before symptoms become apparent thereby inadvertently spreading the phytoplasma. Distribution of infected plants throughout the country also increases the spread and exposure to different suites of potential vectors.

The other potential route for '*Ca*. P. asteris' subgroup B to reach a suitable host plant and new environments is via a vector. There is a species present in New Zealand from the vector genus *Macrosteles* (*M. fieberi*) (Larivière, 2005, and updates), and given the reported low specificity of subgroup B and the incomplete knowledge of vectors, it is possible that other leafhoppers in New Zealand are capable of transmitting the phytoplasma. If suitable vectors are present then vector transmission is likely to be an important means of spread in New Zealand.

Given that:

- '*Ca*. P. asteris' can remain systemically in the plant for as long as the plant remains alive;
- imported citrus nursery stock is used for propagation of new plants for planting;
- the New Zealand climate is unlikely to limit the establishment of '*Ca*. P. asteris' subgroup B;
- the availability of host plants is unlikely to limit the establishment of '*Ca*. P. asteris' subgroup B;
- phytoplasmas are spread by propagation or grafting, and by insect vectors;
- some hosts may be asymptomatic and be used for propagation;
- known vectors of '*Ca*. P. asteris' subgroup B are not present in New Zealand but other potential insect vectors are present;

*The likelihood of establishment and spread of '*Ca. *Phytoplasma asteris' is considered to be moderate to high.*

2.2.3.4 Consequence assessment

Phytoplasmas are associated with a wide range of symptoms affecting all plant parts; typical symptoms include 'little leaf', abnormal root growth, overall stunting, shoot proliferation, leaf yellowing, flower phyllody and virescence, as well as a general decline that is sometimes fatal (Agrios 2005). Symptoms can be misleading, with similar symptoms in the same host caused by different phytoplasmas, different strains causing different symptoms in the same host (Davis and Sinclair 1998), and different symptoms in different hosts caused by very similar phytoplasmas (Lee et al. 2004).

Lee et al. (2004) describe the typical symptoms of aster yellows (caused by members of subgroups 16SrI-A and 16SrI-B) as including virescence (greening of flower petals) and

phyllody (development of floral parts into leaf-like structures), flower streaking and malformation, yellowing and upright posture of leaves, elongation of and etiolation of internodes, excessive branching of axillary shoots, witches'-broom and general stunting of plants. However some infected plants may exhibit only some of these symptoms and plants infected with mild strains may show no obvious symptoms.

The consequences of '*Ca*. P. asteris' subgroup B in New Zealand will depend on which vectors transmit it. If there are few or no vectors, or if the vectors are confined to a limited range of plants, the impacts will be minimal. If '*Ca*. P. asteris' subgroup B is transmitted by one of the widespread and common vector species with a wide host range, the impacts will be large. There is currently not enough known about the potential vectors of '*Ca*. P. asteris' subgroup B in New Zealand to determine how significant an impact it would have in New Zealand, and there is a high degree of uncertainty about the impacts.

Economic consequences

In 2013/14, the New Zealand domestic sales of fresh citrus fruit were $49.1m and in 2014 export sales of fresh fruit were $6.9m (Fresh Facts 2014). Although '*Ca*. P. asteris' subgroup B is associated with HLB in China, it has not been proven using Koch's postulates to be the causal agent, and it is not certain how the phytoplasma alone would affect the productivity of citrus trees. As a result, it is not clear what the impact the establishment of '*Ca*. P. asteris' subgroup B would have on the New Zealand citrus industry. If it caused HLB symptoms, infected plants are likely to become decreasingly productive with poor quality fruit and later decline and partial dieback or plant death. In this scenario the impact on the citrus industry could be either low or high depending on whether or not effective vectors are present. If there were no vector, affected plants could be replaced with phytoplasma-free plants which could pose a high cost for some producers until diseased stock is eliminated. The cost to the citrus industry would be much greater and longer term if effective vectors were present.

Many of the other reported hosts of '*Ca*. P. asteris' subgroup B are important commercial crops for New Zealand. The largest horticultural export earner for 2014, was grapes (as wine), worth $1321.4 million. Fresh apples were worth $536.4 million during the same period. Export onions earned $97.1 million during 2014, frozen potatoes earned $105.4 million and frozen/dried sweet corn $38.3 million. Fresh potatoes and tomatoes jointly earned $25.8 million during 2014. On the domestic market potatoes, corn, tomatoes, carrots, brassicas and onions combined earned $655.3 million during 2014 (Fresh Facts 2014). Because it depends very much on which leafhoppers are involved in transmission, the host plants in New Zealand may be different from those reported overseas.

The impacts of phytoplasmas are uncertain because symptoms are varied, and there is a tendency for infections to become latent and for plants to recover. However, some of the typical symptoms of '*Ca*. P. asteris' subgroup B infection affect flowers, such as: virescence (greening of flowers), phyllody (conversion of petals and sepals to more leaf-like structures) and sterility of flowers. These types of symptoms would have severe impacts for seed or ornamental crops. In 2014, the export value was $26.6m for cut flowers and foliage, $36.0m for flower bulbs, and $66.2m for vegetable seeds (Fresh Facts 2014). Other symptoms are likely to reduce the yield and quality of fruit.

The potential economic consequences within New Zealand are highly uncertain and depend on the phytoplasma/vector relationship. The economic consequences could range from very low (if there are few or no vectors, or if vectors transmit the phytoplasma ineffectively) to high (if it were to be transmitted by a widespread, polyphagous vector affecting high value perennial crops such as grape).

Environmental consequences

Phytoplasmas are one of the few pathogen groups in New Zealand to have been associated with a serious epidemic in native plant populations (Liefting et al. 2007, Phillips et al. in prep.). If infected leafhoppers feed on native plants then infection is likely. Because the host range of '*Ca*. P. asteris' appears to be largely determined by the specificity of the insect vectors, the native plants infected and the level of damage that occurs will depend on which leafhoppers in New Zealand act as a vector. This is difficult to predict given the limited information on the ability of insects in New Zealand to transmit '*Ca*. P. asteris' subgroup B. However, given that a phytoplasma interacting with a native polyphagous vector produced a serious epidemic in the endemic cabbage tree (*Cordyline australis*) (Beever et al. 2004), there is cause for concern about the impacts of other phytoplasmas in native ecosystems.

The potential environmental consequences within New Zealand are highly uncertain and depend on the phytoplasma/vector relationship. The environmental consequences could range from negligible (if there are few or no vectors or if vectors transmit the phytoplasma ineffectively) to high (if it were to be transmitted by a widespread, polyphagous vector affecting native plants).

Socio-cultural consequences

'*Ca*. P. asteris' subgroup B is reported to infect amenity plants such as willow and poplar (section 2.2.1.6). Other reported hosts include roses, hydrangeas, delphiniums, marigolds, tomatoes, carrots and potatoes which are commonly grown by home gardeners. If '*Ca*. P. asteris' subgroup B affects similar species in New Zealand then it is expected that there will be impacts upon amenity and domestic plantings. However, these impacts are difficult to quantify.

The potential socio-cultural consequences within New Zealand are considered to be low.

Human health consequences

There are no known human health consequences associated with any '*Ca*. P. asteris' subgroups.

2.2.3.5 Risk estimation

The likelihood of entry of '*Ca*. P. asteris' is considered to be low. The likelihood of exposure is considered to be high and the likelihood of establishment and spread moderate-high. The potential economic and environmental consequences within New Zealand are uncertain and could be low to high for economic consequences and negligible to high for environmental consequences depending on factors detailed in section 2.2.3.4. The potential sociocultural consequences within New Zealand are considered to be low, and potential human health consequences are considered to be negligible.

As a result the overall risk estimate for 'Ca*. P. asteris' on nursery stock of* Citrus *and related species is non-negligible and it is considered to be a risk on the commodity. Therefore the risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

Risk estimation table

'*Candidatus* Phytoplasma asteris' on imported nursery stock of *Citrus* and related species				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment				
Spread				
Consequences of establishment				
Economic			uncertain	
Environmental			uncertain	
Socio-cultural				
Human Health				

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2.3 '*Candidatus* Phytoplasma' 16SrIX subgroup A

Scientific name: '*Candidatus* Phytoplasma' 16SrIX subgroup A (Class: Mollicutes)
Other relevant scientific names: pigeon pea witches'-broom phytoplasma 16SrIX-A
Common names: HLB-associated 16SrIX phytoplasma; HLB-associated phytoplasma; HLB-phytoplasma

2.3.1 Hazard identification

2.3.1.1 Description

Phytoplasmas are phloem-limited bacteria that lack cell walls and have not been cultured (Lee et al. 2000, Bertaccini 2007). They are associated with a wide range of symptoms affecting all plant parts; typical symptoms include 'little leaf', abnormal root growth, overall stunting, shoot proliferation, leaf yellowing, flower phyllody and virescence, as well as a general decline that is sometimes fatal (Agrios 2005).

2.3.1.2 Taxonomic issues

Phytoplasmas form a distinct clade in the Class *Mollicutes*. The phytoplasma clade has been proposed to represent at least a genus, with each subclade (16S rRNA group) proposed to represent at least one species (Gundersen et al. 1994). The pigeon pea witches' broom group (16SrIX) is one of the smaller groups, and is further divided into several subgroups (A, B, C, D; E and F) (Lee et al. 2012). The 16SrIX group includes the recognised species '*Candidatus* Phytoplasma phoenicium'. Strains of '*Ca*. P. phoenicium' in subgroup B (16SrIX-B) are associated with the devastating almond witches'-broom disease in the Middle East (Lee et al. 2012, Abou-Jawdah et al. 2009).

The species concept is defined within the phytoplasmas (IRPCM 2004), but the appropriate level at which to conduct a risk assessment is often unclear. Lee et al. (2012) have carried out multilocus sequence analyses that include the moderately conserved ribosomal protein (rp) and *secY* genes, as well as the more conserved 16Sr RNA gene. These indicate that there are at least six distinct lineages in the 16SrIX group, corresponding with the A, B, C and E 16SrIX subgroups along with two new subgroups, D and F (tentative). The rp and *secY* gene-based trees have overall topologies similar to that of the 16Sr RNA gene-based trees, with the '*Ca*. P. phoenicium' strain cluster (16SrIX-B) separated from the remaining members of the 16SrIX group. The distinct lineages cannot be readily differentiated on the basis of 16Sr RNA gene sequences alone. The authors suggest the use of multiple genetic markers to develop a better classification system that allows unambiguous identification of lineages and strains for quarantine purposes.

A phytoplasma belonging to the pigeon pea witches' broom subgroup A (16SrIX-A) has been found in citrus showing huanglongbing (HLB) symptoms in the state of São Paulo, Brazil (Teixeira et al. 2008, Lee et al. 2012). This assessment therefore considers subgroup A (referred to as '*Candidatus* Phytoplasma' 16SrIX subgroup A in this risk assessment) along with the citrus HLB-associated isolates within the subgroup.

There are several isolates within '*Ca*. P.' 16SrIX subgroup A, described from different hosts in different locations (Lee et al. 2012). It is unclear to what degree these differ in host range, distribution or other properties that may affect a risk analysis. It is assumed here that they do not differ to any significant degree, but further information characterising variation within '*Ca*. P.' 16SrIX subgroup A may affect the results of this risk analysis.

2.3.1.3 New Zealand status

'*Ca.* P.' 16SrIX subgroup A is not known to be present in New Zealand. Not recorded in: PPIN (2015), NZFungi (2015), Veerakone et al. 2015, Liefting et al. (2007).

2.3.1.4 General geographic distribution

'*Ca.* P.' 16SrIX subgroup A has been recorded in USA, Jamaica, Puerto Rico, Colombia, Brazil (Lee et al. 2012).

'*Ca.* P.' 16SrIX subgroup A has been detected in citrus in the State of São Paulo, Brazil (Teixeira et al. 2008).

2.3.1.5 Commodity association

'*Ca.* P.' 16SrIX subgroup A has been detected in citrus leaves (midribs) by PCR (Teixeira et al. 2008). As phytoplasmas are systemic within infected plants, occurring in the phloem sieve elements (Lee et al. 2000), '*Ca.* P.' 16SrIX subgroup A can potentially be present in citrus nursery stock (budwood or tissue culture).

2.3.1.6 Plant associations

'*Ca.* P.' 16SrIX subgroup A has been recorded from *Citrus sinensis* (Teixeira et al. 2008).

Other reported hosts of '*Ca.* P.' 16SrIX subgroup A include *Catharanthus roseus* (periwinkle) (Family Apocyancaeae) (Barbosa et al. 2012); *Cajanus cajan* (pigeon pea), *Crotolaria juncea*, *Rhynchosia* (Family Leguminosae [Fabaceae]) (Wulff et al. 2009, cited in Montano et al. 2011; Lee et al. 2012).

A naturally occurring strain in periwinkle (*Catharanthus roseus*) in Brazil could infect *Citrus limonium* experimentally (Barbosa et al. 2012).

2.3.1.7 Potential for establishment and impact

'*Ca.* P.' 16SrIX subgroup A has been recorded in a region of Brazil (State of Sao Paulo) that has some climatic similarities to parts of New Zealand and is therefore potentially able to establish in New Zealand. The phytoplasma has been found in association with citrus plants that have huanglongbing (HLB) or HLB-like symptoms; however, it is not yet understood exactly what role the phytoplasma has in relation to the disease, or what the potential is for the phytoplasma to have an impact on citrus species. HLB and HLB-like diseases would be damaging to New Zealand's citrus industry.

2.3.1.8 Hazard identification conclusion

Given that '*Candidatus* Phytoplasma' 16SrIX subgroup A:

- is recorded on *Citrus sinensis*;
- is associated with citrus nursery stock;
- is present in at least one citrus-producing country;
- is not known to occur in New Zealand;
- can potentially establish in New Zealand;
- is associated with huanglongbing, a damaging disease of citrus which is of economic importance to New Zealand;

'*Candidatus* Phytoplasma' 16SrIX subgroup A is considered a hazard on *Citrus* nursery stock in this risk analysis.

2.3.2 Biology

Huanglongbing (HLB) is one of the most destructive diseases worldwide to affect citrus production and it has generally been accepted that '*Candidatus* Liberibacter spp.' are the most probable aetiological agents of HLB. A study by Teixiera et al. (2008) has found that a phytoplasma "closely related to the pigeon pea witches'-broom phytoplasma (16SrIX)" ['*Ca*. P. 16SrIX subgroup A] is associated with citrus HLB symptoms in the State of São Paulo, Brazil. The phytoplasma has been detected both separately and in mixed infection with '*Ca*. L. spp.' in symptomatic sweet orange trees (Teixeira et al. 2008). The association of phytoplasmas with citrus HLB is supported by the discovery of a citrus strain of '*Candidatus* Phytoplasma asteris' associated with citrus HLB in Guangdong Province, China (Chen et al. 2009)[24]. However, the latter phytoplasma has not been found separately from '*Ca*. L. asiaticus' and the true aetiological role(s) of the phytoplasma and the liberibacter in HLB will remain uncertain until Koch's postulates are fulfilled (Chen et al. 2009).

Association with HLB

In 2007, samples from sweet orange (*C. sinensis*) trees with characteristic symptoms of HLB in a region of the state of São Paulo previously free of HLB were found to test negative for the three liberibacter species associated with HLB. However, a phytoplasma [subsequently assigned to 16SrIX subgroup A] was detected (Teixeira et al. 2008). Two primers (D7f2/D7r2), based on the 16SrDNA sequence, were used to screen over 100 samples from 16 municipalities in the state of São Paulo by polymerase chain reaction (PCR). Samples positive for phytoplasmas were negative for liberibacters, except for four that were positive for both the phytoplasma and '*Ca*. Liberibacter asiaticus'. The phytoplasma was detected in sieve tubes of midribs from symptomatic plants using electron microscopy. These results indicate that the phytoplasma is associated with citrus HLB symptoms in northern, central and southern regions of the state of São Paulo (Teixeira et al. 2008).

Disease symptoms

Sweet orange trees infected with '*Ca.* P.' 16SrIX subgroup A show symptoms indistinguishable from those produced when infected with liberibacter species, and occur in plants that are negative for the liberibacter species. The leaves show a blotchy mottle which is a characteristic symptom of HLB. Symptomatic fruits are lopsided, with abnormal coloration, brownish aborted seeds, and the vascular bundles at the peduncular end of the fruit axis (columella) are stained brown (Teixeira et al. 2008).

Leaves and normal fruits collected from symptomless trees adjacent to symptomatic trees were screened by PCR and none tested positive for phytoplasmas (or liberibacters) in the survey by Teixiera et al. (2008). However, there are no reports in the literature of tests from symptomless leaves from symptomatic trees. Nor are there descriptions of experimental inoculations of test plants to describe symptoms, length of time until symptom expression, or titre detection using molecular techniques.

The periwinkle '*Ca*. P.' 16SrIX subgroup A was isolated from naturally infected *Catharanthus roseus* plants exhibiting typical symptoms of phytoplasma infection, virescence, phyllody and variegation, in public gardens in the states of Minas Gerais and São Paulo, Brazil (Barbosa et al. 2012). The PwK-AR1 strain was transmitted by graft from an infected periwinkle to healthy periwinkle plants. Grafted periwinkle plants showed leaf yellowing symptoms. This strain was also transmitted by dodder (*Cuscuta campestris*) from the grafted periwinkle plant to a *Citrus*

24 Since this risk assessment was prepared, there have been further reports of phytoplasmas found in association with HLB-like symptoms in citrus: '*Ca*. P. asteris' subgroup B (Arratia-Castro et al. 2014; Poghosyan et al. 2015); '*Ca*. P. asteris' subgroup S (Arratia-Castro et al. 2014); '*Ca*. P.' 16SrII-A* (Lou et al. 2014).

limonia plant, which also exhibited leaf yellowing symptoms, described as in part similar to those in citrus plants infected by '*Ca*. P.' 16SrIX subgroup A (Barbosa et al. 2012).

Transmission

There are two main mechanisms of transmission of phytoplasmas: propagation or grafting of infected material and transmission by phloem-feeding insect vectors. Experimentally, phytoplasmas can be transmitted between plants via the vascular connection formed by dodder (*Cuscuta* spp.). Phytoplasmas are not reported to be spread by mechanical transmission (Lee et al. 2000).

Phytoplasmas are vectored primarily by phloem-feeding leafhoppers, planthoppers and psyllids (Weintraub and Beanland 2006). Insect vectors have been identified for relatively few phytoplasma diseases and typically when new phytoplasma diseases are discovered little is known about the disease epidemiology (Weintraub and Beanland 2006). No vectors have yet been confirmed for '*Ca*. P.' 16SrIX subgroup A in citrus in Brazil. However, a leafhopper, *Scaphytopius* (*Convelinus*) *marginelineatus* (Cicadellidae: Deltocephalinae), was one of several leafhopper species sampled in a survey of potential vectors and their associated host plants in sweet orange groves ['Valencia' grafted on to Rangpur lime rootstock (*Citrus limonia* Osbeck), and 'Pera' plants grafted on to rangpur lime roots stock] affected by '*Ca*. P.' 16SrIX subgroup A (Marques et al. 2012). DNA samples of field-collected *S. marginelineatus* were positive by PCR and sequencing tests for the presence of '*Ca*. P.' 16SrIX subgroup A, indicating that this species is a potential vector. There may also be other leafhoppers (or other hemiptera) that could act as vectors for the phytoplasma.

According to Teixeira et al. (2008), '*Ca*. P.' 16SrIX subgroup A is very likely to have been transmitted to citrus from an external source of inoculum. Analysis of the spatial distribution of the phytoplasma within sweet orange orchards in northern São Paulo State indicated that no secondary infections from citrus to citrus seem to have occurred (Teixiera et al 2008). Therefore, *D. citri*, which is the psyllid vector for the liberibacter, is so far unlikely to have been involved in transmitting the phytoplasma. In addition, the disease incidence was low in the surveyed blocks, ranging from 0.01 to 1.76% affected trees per block with an average of 0.14%. Phytoplasma-infected trees have now been found in northern, central and southern São Paulo State, suggesting that the external source on which the putative vector becomes infected, such as a weed or a crop, is fairly well-distributed through the state. Teixeira et al. (2008) suggest that legumes such as *Cajanus* sp. or *Crotalaria* sp., which are used as cover crop plants in the sugar cane industry and have a wide distribution in the state, could be the candidate host plants of '*Ca*. P.' 16SrIX subgroup A associated with citrus HLB in Brazil.

No information relating to experimental transmission by graft or dodder was found in the literature for the citrus HLB-associated '*Ca*. P.' 16SrIX subgroup A. However, '*Ca*. P.' 16SrIX subgroup A from periwinkle (PwK-AR1 strain) has been experimentally transmitted by graft from an infected periwinkle plant to healthy periwinkle plants, and by dodder (*Cuscuta campestris*) from infected periwinkle to a *Citrus limonia* plant (Barbosa et al. 2012).

Control

In the state of São Paulo, control of liberibacter-associated HLB has been based on two strategies (Teixeira et al. 2008): firstly, to remove the sources of inoculum (liberibacter-infected citrus trees and the ornamental *Murraya paniculata*, a citrus relative), and secondly to use insecticides to control *Diaphorina citri*, the psyllid vector of the liberibacter. Because the psyllid has a limited host range, insecticide sprays can be restricted to these plants (Teixeira et al. 2008). However, the vector(s) of the HLB-associated phytoplasma, '*Ca*. P.' 16SrIX subgroup A, is unknown. Teixeira et al (2008) suggest that if it is more polyphagous than *D. citri*, it may be much more difficult to control by the use of targeted insecticide sprays, unless the major source

of inoculum is a crop plant that could eventually be replaced by one resistant to the phytoplasma and not used by the vector.

2.3.3 Risk assessment

2.3.3.1 Entry assessment

'*Ca.* P.' 16SrIX subgroup A has been recorded from sweet orange (*Citrus sinensis*) in 16 municipalities in the State of São Paulo, Brazil (Teixiera et al. 2008). It is not known how widespread it is through citrus in Brazil, nor if it occurs in *Citrus* spp. in other countries.

Citrus nursery stock may enter New Zealand as either budwood (dormant cuttings) or tissue culture plantlets, although budwood is more usual. It is likely that a small amount of budwood material will be imported in a consignment, with probably 5–12 buds per stick of wood. Tissue culture plants will arrive as plantlets in flasks of media. Because '*Ca.* P.' 16SrIX subgroup A infects citrus systemically it can be present in the imported nursery stock.

For this assessment it is assumed that the plant material is imported from a non-accredited facility, and therefore is of an unknown phytosanitary status. In this case the material enters a Level 3 post-entry quarantine facility (PEQ) for a minimum of 16 months to allow time for biological indexing (import health standard (IHS) 155.02.06, *Citrus* schedule).

Overview of Level 3 PEQ for citrus nursery stock:

The overall process for importation of citrus nursery stock from a non-accredited facility to New Zealand is outlined in section 1.3, including figures 1 and 2. In brief, upon arrival in Level 3 PEQ, the plant material is inspected for visually obvious signs of regulated pests and diseases. For dormant cuttings, buds are grafted on to rootstock, to allow tests to take place. For tissue culture material, plants are removed from media flasks and replanted into pots. Plants are grown in greenhouse conditions throughout the year, and inspected frequently by the facility operator for signs of regulated pests and diseases. In addition, pre-determined specific tests are carried out for some regulated organisms before plants can be cleared for release from quarantine.

'*Ca.* P.' 16SrIX subgroup A on citrus nursery stock in Level 3 PEQ:

The ability of phytoplasmas to infect plants asymptomatically means that '*Ca.* P.' 16SrIX subgroup A can enter New Zealand undetected on citrus nursery stock such as budwood (dormant cuttings) or plants in tissue culture. Upon arrival the symptomless citrus budwood or plants in tissue culture material without any visually detectable regulatory pathogens will be taken to the PEQ facility for propagation. Buds from the asymptomatic budwood will be grafted onto New Zealand origin rootstock to develop sufficient material to allow buds to be grafted to New Zealand origin indicator plants. The asymptomatic tissue culture plantlets will be deflasked in a quarantine glasshouse and planted in pots.

Plants are maintained in a healthy vigorous state without stress and can be grown in greenhouse conditions throughout the year. They are held at temperatures considered suitable for liberibacters. However, the appropriate temperatures are not stipulated in the current IHS 155.02.06 for plant material from non-accredited facilities[25].

Graft indexing for phytoplasmas is done in the early spring using young, vigorous indicator plants. Laboratory tests for phytoplasmas (PCR) are then carried out at the end of summer. No specific pre-determined tests for '*Ca.* P.' 16SrIX subgroup A are stipulated in the current IHS.

25 All citrus nursery stock imports since 2010 have come from an MPI-approved facility overseas and therefore have entered Level 2 PEQ. The IHS requires that plants in Level 2 PEQ must be held at 18–25ºC throughout the quarantine period (incorporated in IHS amendment 27 November 2014). However the CTO has required operational measures in PEQ to effectively manage temperature to these levels since April 2012.

However, specific tests are currently required for '*Candidatus* Phytoplasma aurantifolia' and Australian citrus dieback (phytoplasma-associated). The IHS gives graft inoculation onto lime (*C. aurantiifolia*) (and indicator plants grown at cool temperatures 18 to 25°C) as an acceptable method for '*Ca.* P. aurantifolia', and states that a suitable test is required for Australian citrus dieback[26]. PCR tests are not currently specified on the IHS for phytoplasmas although they are given in the *Citrus* testing manual (MAF 2010)[27].

The host range of '*Ca.* P.' 16SrIX subgroup A is not known; however, the phytoplasma is recorded from sweet orange, *C. sinensis* (including 'Valência' and 'Pêra' grafted onto rangpur lime (*C. limonia* Osbeck) rootstock) and is associated with HLB symptoms in the absence of liberibacter species (Teixeira et al. 2008, Marques et al. 2012). As varieties of sweet orange along with other citrus species/varieties are used as indicator plants, then it is possible that symptoms could be produced and detected in PEQ. Any indicator plant that shows symptoms is tested to diagnose its cause.

In post-entry quarantine (PEQ), the nursery stock will be kept and monitored for a minimum of 16 months from the start of plant growth before being released. During this period plants are checked weekly by the facility operator and any plants showing symptoms of infection by '*Ca.* P.' 16SrIX subgroup A would be tested to diagnose the cause.

Although it is not certain that '*Ca.* P.' 16SrIX subgroup A would produce detectable symptoms on the current suite of indicator plants, it is highly likely that the phytoplasma would be detected with universal phytoplasma primers during routine PCR testing of the original imported plant material. These tests are not required by the IHS, however. As with any laboratory based test, there are limitations in the ability to detect asymptomatic infection, such as uneven distribution within the plants (i.e. the phytoplasma is not present within the leaves submitted for analysis) or the titre of the phytoplasma is not high enough to be detected. Every attempt is made to ensure the likelihood of testing and detecting phytoplasma is increased, including the type of material selected for testing, the time of year samples are collected, the temperatures at which plants are held and using the most sensitive detection method.

Given that:

- '*Ca.* P.' 16SrIX subgroup A has been recorded in sweet orange in São Paulo State, Brazil, but the phytoplasma has not yet been recorded in citrus elsewhere;
- '*Ca.* P.' 16SrIX subgroup A is systemic in its citrus host and therefore can occur in nursery stock;
- infected plants may show disease symptoms during the growing period of at least 16 months in Level 3 PEQ, however, infected citrus plants can be asymptomatic;
- no specific tests for '*Ca.* P.' 16SrIX subgroup A are carried out in Level 3 PEQ[28];

The likelihood of entry is considered to be low but non-negligible.

2.3.3.2 Exposure assessment

'*Ca.* P.' 16SrIX subgroup A is borne internally and will survive for as long as the host plant remains alive. Infected plants that are symptomless are likely to be released from PEQ for future propagation. As the phytoplasma is a systemic pathogen that is graft-transmissible, it is likely

26 The *Citrus* schedule in the IHS 155.02.06 states that 'Country freedom is accepted as equivalence to a treatment'
27 PCR tests are included as a mandatory requirement on current import permits: nested-PCR using universal phytoplasma primers is listed for three phytoplasmas – '*Ca.* P. asteris', '*Ca.* P. aurantifolia' and Australian citrus dieback. The nested-PCR and real-time PCR using universal phytoplasma primers would be considered equivalent measures and so either could be used.
28 It is highly likely that the phytoplasma would be detected with universal phytoplasma primers during routine PCR testing of the original imported plant material; however, this is not required by the IHS and is excluded from consideration for the risk estimate.

that infected plant material used for grafting will expose the phytoplasma to other susceptible plants in the New Zealand environment.

Given that:

- '*Ca.* P.' 16SrIX subgroup A can survive in its host for as long as the plant remains alive;
- symptomless plants systemically infected with '*Ca.* P.' 16SrIX subgroup A that are released from the PEQ facility are likely to be used for further propagation;

The likelihood of exposure is considered to be high.

2.3.3.3 Assessment of establishment and spread

'*Ca.* P.' 16SrIX subgroup A can remain systemically in the plant for as long as the plant remains alive. If infected plants are used for propagation of new infected plants that are planted, then the phytoplasma can be considered to have established.

'*Ca.* P.' 16SrIX subgroup A has been reported in association with citrus HLB from the state of São Paulo, Brazil. All reports of '*Ca.* P.' asteris 16SrIX subgroup A in other plant hosts have been from locations in the Americas with subtropical to tropical climates. It is assumed in this assessment that the phytoplasma could exist wherever its host plants occur and that climate is likely to affect host plant distribution, vector distribution and disease expression. As citrus is grown in New Zealand, it is unlikely that climate would limit the ability of citrus isolates to establish in New Zealand but it could affect disease expression and insect vector transmission if suitable vectors are present. At least some regions of New Zealand may be suitable for establishment and some micro-climates may allow disease expression.

Citrus species are grown both commercially and domestically in New Zealand, especially in the warmer northern regions, ensuring that host plants would be available. Other reported host plants for '*Ca.* P.' 16SrIX subgroup A include members of the Leguminosae (pigeon pea, *Crotalaria juncea, Rhynchosia*) and Apocyancaeae (periwinkle, *Catharanthus roseus*). Representatives of these families occur in New Zealand as agricultural, horticultural or amenity species, and as weeds or garden plants and are potentially hosts for '*Ca.* P.' 16SrIX subgroup A.

Phytoplasmas are most commonly transmitted by propagation or grafting of infected material, and by insect vectors. These are the two potential routes for '*Ca.* P.' 16SrIX subgroup A to get from infected citrus nursery stock into other host plants in New Zealand.

Plants that show symptoms of a phytoplasma infection are unlikely to be propagated from. However, hosts may be asymptomatic, or at least initially, and plant material could be distributed without disease being evident or before symptoms become apparent thereby inadvertently spreading the phytoplasma. The distribution of infected but symptomless plants throughout the country will increase the spread and exposure to different suites of potential vectors.

The other potential route for '*Ca.* P.' 16SrIX subgroup A to reach suitable host plants and new environments is via an insect vector. The vector for the phytoplasma in Brazil has not been confirmed but studies indicate that the leaf hopper *S. marginelineatus* (Deltocephalini) is a likely vector. This does not exclude other leafhoppers (or other hemiptera) from also being vectors, although *Diaphorina citri*, the vector of the citrus liberibacters, is not believed to vector the phytoplasma. Observations in Brazil suggest that there is no spread by vectors from citrus to citrus, and that it is likely that citrus is infected by vectors transmitting the phytoplasma from other plants, either weeds or crops, possibly Leguminosae, grown in proximity to citrus groves. *S. marginelineatus* is not recorded in New Zealand. However, other leafhoppers in New Zealand may be capable of transmitting the phytoplasma. If suitable vectors are present then the extent of

vector transmission is likely to be dependent on both host range of the vector and on suitable reservoir plants being nearby.

Given that:

- '*Ca.* P.' 16SrIX subgroup A can remain systemically in the plant for as long as the plant remains alive;
- imported citrus nursery stock is used for propagation of new plants for planting;
- the New Zealand climate is unlikely to prevent the establishment of '*Ca.* P.' 16SrIX subgroup A;
- the availability of host plants is unlikely to limit the establishment of '*Ca.* P.' 16SrIX subgroup A;
- phytoplasmas are spread by propagation of infected nursery plants or through grafting, and by insect vectors;
- some hosts may be asymptomatic and be used for propagation;
- known vectors of '*Ca.* P.' 16SrIX subgroup A are not present in New Zealand but other potential insect vectors are present;

The likelihood of establishment and spread is considered to be moderate to high.

2.3.3.4 Consequence assessment

Phytoplasmas are associated with a wide range of symptoms affecting all plant parts; typical symptoms include 'little leaf', abnormal root growth, overall stunting, shoot proliferation, leaf yellowing, flower phyllody and virescence, as well as a general decline that is sometimes fatal (Agrios 2005). Symptoms can be misleading, with similar symptoms in the same host caused by different phytoplasmas, different strains causing different symptoms in the same host (Davis and Sinclair 1998), and different symptoms in different hosts caused by very similar phytoplasmas (Lee et al. 2004).

The consequences of '*Ca.* P.' 16SrIX subgroup A in New Zealand will depend on which vectors transmit it, along with the susceptibility of plants to the phytoplasma. If there are few or no vectors, or if the vectors are confined to a limited range of plants, the impacts will be minimal. If the phytoplasma is transmitted by one of the widespread and common vector species with a wide host range, the impacts will be larger. There is currently not enough known about the potential vectors of the phytoplasma in New Zealand that will help determine how significant an impact it would have, and there is a high degree of uncertainty about the impacts.

Economic consequences

In 2013/14, the New Zealand domestic sales of fresh citrus fruit were $49.1m and in 2014 export sales of fresh fruit were $6.9m (Fresh Facts 2014). Although the phytoplasma is associated with HLB in sweet orange in Brazil, it is has not been proven using Koch's postulates to be the causal agent, and it is not certain how the phytoplasma would affect the productivity of citrus trees of the same or other species. In addition, any disease expression may be limited by temperature and therefore may not occur throughout the potential distribution of the phytoplasma. As a result, it is not clear what impact the establishment of the phytoplasma would have on the New Zealand citrus industry. If it caused HLB symptoms, infected plants are likely to become decreasingly productive with poor quality fruit and later decline and partial dieback or plant death. In this scenario the impact on the citrus industry could be either low or high depending on whether or not effective vectors are present. If there were no effective vector, affected plants could be replaced with phytoplasma-free plants which could pose a high cost for some producers until

diseased stock is eliminated. The cost to the citrus industry would be much greater and longer term if effective vectors were present.

Other reported hosts within '*Ca.* P.' 16SrIX subgroup A include members of the Leguminosae (pigeon pea, *Crotalaria juncea*, *Rhynchosia*) and Apocyancaeae (periwinkle, *Catharanthus roseus*). Representatives of the Leguminosae occur in New Zealand as commercial crops. For example, exported processed peas were worth $79.8 million in 2014 and domestic sales of peas were $50 million (Fresh Facts 2014). Exports of processed beans were worth $44.6 million in 2014 (Fresh Facts 2014). In addition, Leguminosae and Apocyanceae, either weeds or crop species, could act as reservoirs for citrus infections if suitable vectors are present. Because it depends very much on which leafhoppers, or other hemiptera, are involved in transmission, the host plants in New Zealand may be different from those reported overseas.

It is assumed in this analysis that host plants of strains in other subgroups of the 16SrIX group are much less likely to be hosts; the subgroups are regarded as distinct lineages by Lee et al. (2012) with the implication being that they have distinct biological and ecological properties from strains in subgroup A. These include isolates found on blueberry, brassicas, lettuce (*Lactuca* spp.) and juniper, and the well known strains of almond witches' broom which can cause disease in almonds, nectarines and peaches.

The potential economic consequences within New Zealand are highly uncertain and depend on the phytoplasma/vector relationship and disease expression. The economic consequences could range from low (if there are few or no vectors, or if vectors transmit the phytoplasma ineffectively) to moderate (if it were to be transmitted by a widespread, polyphagous vector(s) affecting several crops).

Environmental consequences

Although the host range of '*Ca.* P.' 16SrIX subgroup A associated with citrus HLB has not been described, it is known to infect sweet orange, *Citrus sinensis*; other isolates in the subgroup are reported to affect plant species in the Apocyanaceae and Leguminosae. There are no native species of citrus in New Zealand. However, there are two genera in the Rutaceae that have endemic species: *Melicope* and *Leionema*. Neither species is considered threatened (NZPCN 2015). The Apocyanceae includes three endemic species of *Parsonsia*, *P. heterophylla* and *P. capsularis*, and *P. praeruptis*. The latter species is considered to be threatened and nationally endangered, while the others are considered to be not threatened (NZPCN 2015). In the Leguminosae, there are many endemic species of *Carmichaelia*, of which 3 species are considered nationally endangered, 3 species nationally critical and 4 species or subspecies nationally vulnerable (NZPCN 2015). Both endemic species of *Clianthus* (kakabeak) have a nationally critical status (NZPCN 2015).

Phytoplasmas are one of the few pathogen groups in New Zealand to have been associated with a serious epidemic in native plant populations (Liefting et al. 2007, Phillips et al. in prep.). If infected leafhoppers, or other vectors, feed on native plants then exposure to infection can occur. Because the host range of phytoplasmas is determined to some degree by the specificity of insect vectors, then the native plants infected and the level of damage that occurs will depend on what potential vectors are present in New Zealand. This is difficult to predict given the limited information on vectors that transmit '*Ca.* P.' 16SrIX subgroup A. However, given that a phytoplasma interacting with a native polyphagous vector produced a serious epidemic in the endemic cabbage tree (*Cordyline australis*) (Beever et al. 2004), there is cause for concern about the impacts of other phytoplasmas in native ecosytems.

The potential environmental consequences within New Zealand are highly uncertain and depend on the phytoplasma/vector relationship. The environmental consequences could range from

negligible (if there are few or no vectors or if vectors transmit the phytoplasma ineffectively) to high (if it were to be transmitted by a widespread, polyphagous vector affecting native plants).

Socio-cultural consequences

Although the host range of '*Ca*. P.' 16SrIX subgroup A associated with citrus HLB has not been described, it is known to affect sweet orange, *C. sinensis*, and it is likely that it could affect other *Citrus* species as well. Citrus is grown domestically in New Zealand, particularly in the warmer regions of the country. However, the potential host range and impact of the phytoplasma are uncertain and are to some degree dependant on the availability of suitable vectors. Other isolates in the subgroup are reported to affect plant species in the Apocyanaceae and Leguminosae. A number of genera from the Apocyanaceae are cultivated in New Zealand for ornamental purposes (Webb et al. 1988), for example, oleander, *Nerium oleander*, and periwinkles, *Vinca* spp. Numerous species from the Leguminosae are grown domestically either for ornamental purposes (e.g., ornamental brooms, kowhai – *Sophora* spp., kakabeak – *Clianthus puniceus*, wisteria, lupins and sweet peas) or for consumption (e.g., peas and beans). If '*Ca*. P.' 16SrIX subgroup A from citrus can infect species from these families in New Zealand and there are suitable vectors available then it expected that there will be impacts upon domestic plantings. However, these impacts are difficult to quantify.

The potential socio-cultural consequences within New Zealand are considered to be low.

Human health consequences

There are no known human health consequences associated with '*Ca*. P.' 16SrIX subgroup A phytoplasmas.

2.3.3.5 Risk estimation

The likelihood of entry of 'Candidatus *Phytoplasma' 16SrIX subgroup A* is considered to be low. The likelihood of exposure is considered to be high. The likelihood of establishment and spread is considered to be moderate to high. The potential economic consequences within New Zealand are considered to be uncertain but could be low to moderate depending on factors detailed in section 2.3.3.4). The potential environmental consequences within New Zealand are considered to be uncertain but could be negligible to high depending on factors detailed in section 2.3.3.4). The potential socio-cultural consequences are considered to be low, and the potential human health consequences are considered to be negligible.

As a result the overall risk estimate for 'Candidatus *Phytoplasma' 16SrIX subgroup A on nursery stock of* Citrus *and related species is non-negligible and it is considered to be a risk on the commodity. Therefore the risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

Risk estimation table

'*Candidatus* Phytoplasma' 16SrIX subgroup A on imported nursery stock of *Citrus* and related species				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment				
Spread				
Consequences of establishment				
Economic			uncertain	
Environmental			uncertain	
Socio-cultural				
Human Health				

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2.4 *Citrus viroid V* (CVd-V)

Scientific name: *Citrus viroid V*
(Family: *Pospiviroidae*; Genus: *Apscaviroid*)

Acronym: CVd-V

2.4.1 Hazard identification

2.4.1.1 Description

The viroid *Citrus viroid V* is a plant pathogen that infects species of citrus and citrus relatives.

2.4.1.2 Taxonomic issues

CVd-V has recently been accepted as a new species of the genus *Apscaviroid*. All known citrus viroid species belong to the family *Pospiviroidae* (Eiras et al. 2009, Owens et al. 2012).

2.4.1.3 New Zealand status

Citrus viroid V is not known to be present in New Zealand. Not recorded in: Veerakone et al. 2015, PPIN 2015.

2.4.1.4 General geographic distribution

CVd-V has been reported from:

Asia: China (Cao et al. 2010), Japan (Ito and Ohta 2010), Iran (Bani Hashemian et al. 2010b), Nepal (Serra et al. 2008b), Oman (Serra et al. 2008b), Pakistan (Cao et al. 2013), Turkey (Önelge and Yurtmen 2012);
North America: USA (Serra et al. 2008b);
Europe: Spain (Serra et al. 2008b).

2.4.1.5 Commodity association

Citrus viroid V is reported from bark tissue, budwood and rootstock (Bani Hashemian et al. 2010a). As viroids are systemic within infected plants (Ding 2009), CVd-V can potentially be present in citrus nursery stock (budwood or tissue culture).

2.4.1.6 Plant associations

The known host range of CVd-V is restricted to citrus and citrus-related species (Serra et al. 2008a and b).

In a study by Serra et al. (2008b), CVd-V was able to infect all citrus species and cultivars tested, including: 'Sanguinelli', 'Salustiana' and 'Ricart navelina' sweet oranges (*C. sinensis* (L.) Osb.); 'Oroval' and 'Hernandina' clementines (*C. clementina* Hort. Ex Tan.); 'Fino' and 'Verna' lemons (*C. limon* (L.) Burn.f.); 'Sevillano' and 'Cajel' sour oranges (*C. aurantium* L.); 'Clausellina' satsuma (*C. unshiu* (Mak.) Marc.); Temple mandarin (*C. temple* Hort. Ex Tan.); Tahiti lime (*C. latifolia* Tan); Palestine sweet lime (*C. limettioides* Tan); calamondin (*C. madurensis* Lour); 'Calabria' bergamot (*C. bergamia* Risso and Pot.); 'Orlando' tangerine (*C. paradisi* x *C. tangerina*); 'Nova' tangerine (*C. clementina* x [*C. paradisi* x *C. tangerina*]); 'Page' mandarin ((*C. paradisi* x *C. tangerina*) x *C. clementina*); 'Nagami' kumquat (*Fortunella margarita* (Lour.) Swing.).

CVd-V has also been detected in: *C. tamuranua* (cvs. Nishirokaori, Haruka) (Cao et al. 2010), *C. unshiu* x *C. sinensis* (Kiyomi) (Cao et al. 2010), Seminole tangelo (*C. paradisi* x C. *tangerina* (Serra et al. 2008b).

Etrog citron (*C. medica* L.), the classical indicator plant for citrus viroids, is susceptible to CVd-V. Both Etrog citron and the susceptible rootstock rough lemon (*C. jambhiri*) have been used in studies of viroid infection mechanisms (Bani Hashemian et al. 2010a).

Eremocitrus glauca and *Microcitrus australis*, two non-*Citrus* species in Rutaceae, are poor hosts of CVd-V, but they are capable of transporting viroids through intergeneric grafted scions and stocks (Bani Hashemian et al.2010a).

CVd-V is capable of replicating in *Atalantia citroides*, a citrus relative (Serra et al 2008a).

Citrus and citrus relatives are usually graft-propagated and the resulting plant is composed of two species, which may have different responses to infection (Bani Hashemian et al. 2010a).

2.4.1.7 Potential for establishment and impact

Citrus viroid V is found in countries some of which have climates similar to parts of New Zealand and can potentially establish in New Zealand. It is pathogenic to species of *Citrus* which is an important horticultural crop in New Zealand.

2.4.1.8 Hazard identification conclusion

Given that *Citrus viroid V*:

- is reported from *Citrus* spp. and citrus-related species;
- is recorded from several countries that grow *Citrus* spp.;
- is not recorded from New Zealand;
- can potentially establish in New Zealand;
- can potentially cause unwanted impacts in New Zealand;

Citrus viroid V is considered a hazard on *Citrus* nursery stock in this risk analysis.

2.4.2 Biology

Viroids are the smallest known plant pathogens. They consist of a non-encapsidated, circular, single-stranded non-coding RNA that replicates autonomously in their host plants. Viroids can move from cell to cell through plasmodesmata and travel long-distance through phloem to establish systemic infection (Ding 2009).

All citrus viroids belong to the family *Pospiviroidae* which replicate in the nucleus of the infected cell. Citrus viroids are graft-transmitted and they are spread mainly by propagation of contaminated material. Seed transmission and vector transmission of citrus viroids have not been demonstrated in citrus (Duran-Vila and Semancik 2003). Citrus viroids are usually present as complex mixtures co-infecting the same plant (Duran-Vila and Semancik 2003).

Citrus viroid V (CVd-V) is a member of the genus *Apscaviroid* which includes *Citrus bent leaf viroid* (CBLVd), *Citrus dwarfing viroid* (CDVd) (formerly known as *Citrus viroid III*, CVd-III), and *Citrus viroid VI* (CVd-VI) (formerly known as Citrus viroid Original Source, CVd-OS; Ito et al. 2001).

CVd-V has been recently discovered and has been recorded from *Citrus* spp. in several countries. However, it may be more widespread than first anticipated (Serra et al. 2008b). In

several places it appears to have been present but overlooked until surveys have been undertaken or collections analysed for its presence (Cao et al. 2013, Bani Hashemian et al. 2010b, Ito and Ohta 2010; Serra et al. 2008b). A survey in Punjab Province, Pakistan by Cao et al. (2013) found a high incidence with 93% of samples infected. The authors suggest the high infection rate was probably due to more than 90% of the rootstock used in Punjab being rough lemon which could have been infected with CVd-V for a long time.

In a host range study, CVd-V was able to infect all the 18 genotypes of citrus species and cultivars tested (Serra et al. 2008b) (see section 2.4.1.6). For citrus viroids in general, the host range includes *Citrus* species and close *Citrus* relatives (Duran-Vila and Semancik 2003). There is little information on the occurrence of citrus viroids in wild citrus. However, experiments involving *Eremocitrus glauca* and *Microcitrus australis*, two species of true citrus fruits native to Australia, found that these two species were poor citrus viroid hosts in which viroid replication/accumulation does not occur or is extremely inefficient. Of the six citrus viroids tested, CVd-V had the highest affinity for the two non-citrus hosts (Bani Hashemian et al. 2010). In an earlier study, *Atalantia citroides*, a non-citrus host, was a poor host for all citrus viroids with the exception of CVd-V (Barbosa et al. 2005b, Serra et al. 2008a).

CVd-V induced mild but typical symptoms on Etrog citron, (*Citrus medica* L.), the sensitive indicator plant for detection of citrus viroids, in a study by Serra et al. (2008a). No symptoms were seen 6 months after inoculation. However, 10 months after inoculation the stems of the plants showed very small necrotic, gum-filled lesions. All citrus viroids induce a few characteristic symptoms such as epinasty, leaf bending, stunting and mid-vein and petiole necrosis in 'Etrog' citron. However, symptoms can range from mild to severe, depending on viroid species or variant (Ito et al. 2001). In another experiment in the same study, CVd-V infection resulted in mild stunting of plant growth, and mild stem symptoms (necrotic lesions, cracking and gum exudates), but no leaf symptoms (Serra et al. 2008a).

Most citrus viroid/host combinations are symptomless and disease is only perceived when a specific viroid infects a sensitive species (Duran-Vila and Semancik 2003). Two well-known diseases of citrus, exocortis and cachexia, are caused by viroids in other viroid genera (*Citrus exocortis viroid*, CEVd: *Pospiviroid*; and *Hop stunt viroid*, HSVd: *Hostuviroid*, respectively). Members of the genus *Aspscaviroid* do not induce specific diseases of citrus but can result in moderate effects on tree size and crop yield (Vernière et al. 2004). The effect of a single citrus viroid on its host plant is influenced by a number of factors including temperature, light intensity and quality, presence of other citrus viroid species, host plant, and rootstock cultivar. However, there are few data on the effects induced by individual viroids in citrus grown under field conditions (Eiras et al. 2010), and CVd-V has not been evaluated under natural conditions in Pakistan due to mixture with other viroid species in commercial citrus (Cao et al. 2013).

In the field, viroids in citrus are often found in combination as has been the case with CVd-V in Pakistan (Cao et al. 2013). Impact on the plant is affected by many factors including which viroid variants and species predominate in terms of accumulation and symptom expression. Multiple infections of citrus viroids can result in synergistic or antagonistic interactions, depending on the combination (Vernière et al. 2006). Experimental co-inoculation of Etrog citron with pairs of citrus viroids in the genus *Apscaviroid* (CVd-V with either CBLVd or CDVd), resulted in synergistic interactions in which leaf symptoms were enhanced and dwarfing was very pronounced (Serra et al. 2008a). This suggests that interactions of CVd-V with other citrus apscaviroids may result in reduced tree size and yield in the field. Both antagonistic and synergistic effects on symptom expression and field performance have been reported for clementine trees grafted on trifoliate orange (*Poncirus trifoliata*) co-infected with different combination of viroids (CEVd, CBLVd, HSVd, CDVd (named as CVd-III), CBCVd (named as CVd-IV)) (Vernière et al. 2006). CVd-V could potentially affect commercial rootstock-scion combinations in the same way (Serra et al. 2008b). In Japan, some combinations of citrus viroids

other than CEVd have been found to induce exocortis-like symptoms as severe as those caused by CEVd, affecting plants with trifoliate orange (*Poncirus trifoliata*) rootstocks (Ito et al. 2002b; Ito et al. 2003).

CVd-V can be transmitted by both graft inoculation and by mechanical transmission (Serra et al. 2008a). Dissemination of citrus viroids occurs mainly by propagation of symptomless, viroid-infected bud-wood (Duran-Vila and Semancik 2003). Mechanical transmission to uninfected plants occurs through using contaminated tools for pruning and harvesting (Barbosa et al. 2005a). Some viroids (*Potato spindle tuber viroid* PSTVd, *Avocado sunblotch viroid* ASBVd, and *Coleus blumei viroid 1* CbVd1) are vertically transmitted through pollen and/or true seed, however, it is not clear the significance of this in the natural spread of disease (Flores and Owens 2008). Seed transmission and vector transmission of citrus viroids have not been demonstrated in citrus (Duran-Vila and Semancik 2003). There is no indication in the literature surveyed that CVd-V is transmitted by seed, pollen or insect vectors.

CVd-V has been reported in several countries so far, and it has been discovered to have a high incidence rate in Punjab Province, Pakistan (Cao et al. 2013). Given the range of countries it has been reported from, it seems likely that the viroid can survive anywhere that its host plants can survive, although temperature will affect titre levels and any consequent symptom expression. Viroid diseases are more common in semi-tropical than in temperate climates (Semancik 2003) and viroid replication and symptom development is enhanced as temperature increases above 20ºC to at least 35ºC (Singh et al. 2003). Although diseases in the field are probably more prevalent in warmer climates, some viroids perform better at lower temperatures (Singh et al. 2003).

Control of citrus viroids is based on preventive measures such as the use of viroid-free budwood as propagation material and adequate indexing procedures (Eiras et al. 2009), and through the disinfection of pruning and harvesting tools (Barbosa et al. 2005a).

2.4.3 Risk assessment

2.4.3.1 Entry assessment

CVd-V has only been recently discovered and has been recorded from *Citrus* spp. in several countries including Japan, China, Nepal, Iran, Sultanate of Oman, Spain, USA, Turkey and Pakistan (see section 2.4.1.4). It is not known if it occurs on *Citrus* elsewhere, but it may be more widespread than first anticipated (Serra et al. 2008b). A survey in Punjab Province, Pakistan (Cao et al. 2013) found a high incidence with 93% of samples infected.

Citrus nursery stock may enter New Zealand as either budwood (dormant cuttings) or tissue culture plantlets, although budwood is more usual. It is likely that a small amount of budwood material will be imported in a consignment, with probably 5–12 buds per stick of wood. Tissue culture plants will arrive as plantlets in flasks of media. Because CVd-V infects citrus systemically it can be present in the imported nursery stock.

For this assessment it is assumed that the plant material is imported from a non-accredited facility, and therefore is of an unknown phytosanitary status. In this case the material enters a Level 3 post-entry quarantine facility (PEQ) for a minimum of 16 months to allow time for biological indexing (import health standard (IHS) 155.02.06, *Citrus* schedule).

Overview of Level 3 PEQ for citrus nursery stock:

The overall process for importation of citrus nursery stock from a non-accredited facility to New Zealand is outlined in section 1.3, including figures 1 and 2. In brief, upon arrival in Level 3 PEQ, the plant material is inspected for visually obvious signs of regulated pests and diseases.

For dormant cuttings, buds are grafted on to rootstock, to allow tests to take place. For tissue culture material, plants are removed from media flasks and replanted into pots. Plants are grown in greenhouse conditions throughout the year, and inspected frequently by the facility operator for signs of regulated pests and diseases. In addition, pre-determined specific tests are carried out for some regulated organisms before plants can be cleared for release from quarantine.

CVd-V on citrus nursery stock in Level 3 PEQ:

The ability of citrus viroids to infect plants asymptomatically means that CVd-V can enter New Zealand undetected on citrus nursery stock such as budwood (dormant cuttings) or plants in tissue culture. Upon arrival the symptomless citrus budwood or plants in tissue culture material without any visually detectable regulatory pathogens will be taken to the PEQ facility for propagation. Buds from the asymptomatic budwood will be grafted onto New Zealand origin rootstock to develop sufficient material to allow buds to be grafted onto New Zealand origin indicator plants. The asymptomatic tissue culture plantlets will be deflasked in a quarantine greenhouse and planted in pots.

Plants are maintained in a healthy vigorous state without stress and can be grown in greenhouse conditions throughout the year. They are held at temperatures considered suitable for liberibacters. However, the appropriate temperatures are not stipulated in the current IHS 155.02.06 for plant material from non-accredited facilities[29].

Pre-determined tests are currently required by the IHS for the following citrus viroids: CVd-I, CVd-III, CVd-IV, and HSVd (formerly named CVd-II). The IHS gives SPAGE and PCR on grafted inoculated citron extract (with citron grown at hot temperature, 27 to 32ºC) as an acceptable method[30]. Graft indexing for viroids is done in the early spring using young, vigorous indicator plants. Laboratory tests for viroids (polymerase chain reaction, PCR) are then carried out at the end of summer as viroid activity and, therefore, titre in the sampled tissues is higher when temperatures are warmer. However, no pre-determined tests for CVd-V are required by the IHS.

For graft indexing, citron 'Etrog Arizona 861' (*Citrus medica*) is used as the indicator plant and is grown at high temperatures to enhance titre build-up and symptom expression (the *Citrus* testing manual (MAF 2010) states incubation temperatures of 32–40ºC max. day and 25–30ºC min. night, and the IHS states 27 to 32ºC). This citrus species is the classical indicator for citrus viroids, including CVd-V, with each viroid inducing specific symptoms which are apparent within 6 to 12 months or longer. However, citrus viroids can occur as multiple infections; the synergistic and inhibitory interactions can delay or enhance the symptoms produced and these may vary from the described symptoms (Semancik and Duran-Vila 1991). CVd-V induces very small necrotic lesions and cracks in the stem which are sometimes filled with gum. These symptoms are mild compared with other citrus viroids, but there can be synergistic interactions such as enhanced leaf symptoms and pronounced dwarfing if there is co-infection with more than one citrus viroid of the *Apscaviroid* genus (Serra et al. 2008a). It is possible that symptoms of CVd-V infection or co-infection could be produced and detected. However, given that CVd-V appears to have gone unnoticed until recently, this may not always be the case. In a study by Cao et al. (2013), budwoods from seven random selected trees of different cultivars were grafted onto Arizona 861-S1 'Etrog' citron on rough lemon rootstock. After more than 12 months, five of the seven samples revealed typical viroid symptoms on the indicator plants. However, reverse transcription (RT)-PCR which revealed the presence of several citrus viroids, showed that six of the samples were infected with CVd-V.

29 All citrus nursery stock imports since 2010 have come from an MPI-approved facility overseas and therefore have entered Level 2 PEQ. The IHS requires that plants in Level 2 PEQ must be held at 18–25ºC throughout the quarantine period (incorporated in IHS amendment 27 November 2014). However the CTO has required operational measures in PEQ to effectively manage temperature to these levels since April 2012..

30 The *Citrus* schedule in the IHS 155.02.06 states that 'Country freedom is accepted as equivalence to a treatment'

Indicator plants are also tested for the presence of citrus viroids by means of conventional reverse transcription (RT)-PCR, with primer pairs specific for each citrus viroid tested (Serra et al. 2008b, Ito et al. 2002a, Hadidi et al. 1992). Specific tests are available for CVd-V (MAF 2010). However they are not required by the current IHS and if they are not used then CVd-V is unlikely to be detected by PCR. As with any laboratory-based test there are limitations in the ability to detect asymptomatic infection, such as uneven distribution within the plants (i.e. the viroid is not present in the leaves submitted for analysis) or the titre of the viroid is not high enough to be detected. Every attempt is made to ensure that the likelihood of testing and detecting viroids is increased, including the type of material selected for testing, the number of replicates, and the time of year samples are collected. For citrus viroids, temperature is perhaps the most influential factor to reach a high titre in the sample.

In post-entry quarantine (PEQ), the nursery stock will be kept and monitored for a minimum of 16 months from the start of plant growth before being released. During this period plants are checked weekly by the facility operator and any plants showing symptoms of CVd-V would be tested to diagnose the cause.

Given that:

- although CVd-V has been recorded in citrus in several countries, it may be more widespread than current records suggest;
- CVd-V is systemic in its citrus host and therefore can occur in nursery stock;
- no pre-determined tests for CVd-V are required by the IHS to be carried out in Level 3 PEQ, however, the indicator plant Etrog citron used for other citrus viroids is suitable for CVd-V;
- infected plants may show disease symptoms during the growing period of at least 16 months in Level 3 PEQ, however, infected citrus plants can also be asymptomatic;

The likelihood of entry is considered to be low but non-negligible.

2.4.3.2 Exposure assessment

CVd-V is borne internally and will survive for as long as the host plant remains alive. Infected plants that are symptomless are likely to be released from PEQ for future propagation. As the viroid is a systemic pathogen that is graft-transmissible, it is likely that infected plant material used for grafting will expose the viroid to other susceptible plants in the New Zealand environment.

Given that:

- CVd-V can survive in its host for as long as the plant remains alive;
- symptomless plants systemically infected with CVd-V that are released from the PEQ facility are likely to be used for further propagation;

The likelihood of exposure is considered to be high.

2.4.3.3 Assessment of establishment and spread

CVd-V can remain systemically in the plant for as long as the plant remains alive. If infected plants are used for propagation of new infected plants that are planted, then the viroid can be considered to have established.

CVd-V has been recorded from several countries with a variety of climatic conditions. There is no evidence from its current known distribution that climate would limit the ability of the viroid to establish in at least some regions in New Zealand. The known hosts of CVd-V are *Citrus*

species and their close relatives, and in host range studies CVd-V has infected all the *Citrus* species and cultivars tested (Serra et al. 2008b). It is likely that the movement of infected plant material, both whole nursery plants and cuttings or budwood, would be the most effective means of the viroid spreading among regions of New Zealand where citrus is grown, both commercially and domestically. The majority of commercial citrus orchards are in the Bay of Plenty, Gisborne, and Northland.

Viroids are also spread through mechanical transmission and therefore improper sanitation such as the indiscriminate use of non-sterile pruning shears would allow CVd-V to spread from infected to clean plants. Some viroids (PSTVd, ASBVd, and CbVd1) are vertically transmitted through pollen and/or true seed, however, it is not clear the significance of this in the natural spread of disease (Flores and Owens 2008). Seed transmission of citrus viroids has not been demonstrated in citrus (Duran-Vila and Semancik 2003). There is no indication in the literature surveyed that CVd-V is transmitted by seed, pollen or insect vectors.

Infected plants that show disease symptoms are unlikely to be propagated from. However, hosts may be asymptomatic, or at least initially, and plant material could be distributed without disease being evident or before symptoms become apparent thereby inadvertently spreading the viroid both locally and longer distances. As CVd-V has been overlooked until recently and induces mild symptoms, it is likely that the viroid could be widespread before it is detected.

Given that:

- CVd-V can remain systemically in the plant for as long as the plant remains alive;
- imported citrus nursery stock is used for propagation of new plants for planting;
- the New Zealand climate is unlikely to limit the establishment of CVd-V;
- the availability of host plants is unlikely to limit the establishment of CVd-V;
- citrus viroids are spread by propagation or grafting;
- some hosts may be asymptomatic and be used for propagation;

The likelihood of establishment and spread of CVd-V is considered to be moderate to high.

2.4.3.4 Consequence assessment

Economic consequences

CVd-V is able to infect a wide range of citrus species. The New Zealand citrus industry comprises around 1,700 hectares divided between approximately 500 orchards, most of which are in the Bay of Plenty, Gisborne and Northland regions (NZCGI 2013). In 2013/14, the New Zealand domestic sales of fresh citrus fruit were $49.1m and in 2014 export sales of fresh fruit were $6.9m (Fresh Facts 2014).

Transmission occurs by graft propagation and by mechanical transmission with contaminated tools; there is no evidence that CVd-V is transmitted by vectors, seed or pollen. If this assumption is found to be incorrect, then the conclusions in this analysis would need to be reassessed.

Without vectors, any impact is unlikely to affect existing plantings except through mechanical transmission by contaminated tools. For those growers who acquire infected plants, the effects of CVd-V alone are likely to be subtle, perhaps affecting moderately the tree size and yield. Any symptom expression is more likely in warmer regions of New Zealand as viroid activity and therefore titre is higher when temperatures are warmer.

There is, however, the potential for synergy in the presence of other citrus viroids resulting in a greater impact on production. Two viroids have been previously reported in citrus in New

Zealand: *Citrus exocortis viroid*, CEVd (Pearson et al. 2006) and *Citrus dwarfing viroid*, CDVd (Syn. *Citrus viroid III*, CVd-III) (Quemin et al. 2011). In addition, citrus viroids are frequently present in mixed infections, and if CVd-V established in New Zealand, it could be in combination with another viroid(s). If infected plants were replaced with viroid-free plants then this could be a major cost to some producers. The presence of viroids could also affect future options of suitable rootstocks.

The potential economic consequences within New Zealand are considered to be low but non-negligible.

Environmental consequences

Although CVd-V has a restricted host range it is able to infect a wide range of citrus and closely related species. *Citrus* species are members of the Rutaceae. There are two genera within Rutaceae endemic to New Zealand: *Leionema* and *Melicope*. Neither the *Leionema* nor *Melicope* species are considered threatened (NZPCN 2015). However, if these species were susceptible, they would be unlikely to be affected in the natural environment as the natural transmission of CVd-V is assumed to occur only by graft propagation and by mechanical transmission with contaminated tools.

The potential environmental consequences within New Zealand are considered to be negligible.

Socio-cultural consequences

CVd-V is known to affect only *Citrus* spp. and closely related species. Citrus is grown domestically in New Zealand, particularly in warmer regions of the country. CVd-V alone may be asymptomatic or produce very mild symptoms affecting trees size and yield. However, as transmission is known only to occur by grafting and mechanical means and as there is no known vector, many plants may be unaffected. Infected plants can be replaced by healthy plants.

The potential socio-cultural consequences within New Zealand are considered to be negligible to low.

Human health consequences

There are no known human health consequences associated with CVd-V.

2.4.3.5 Risk estimation

The likelihood of entry of CVd-V is considered to be low, the likelihood of exposure is considered to be high, and the likelihoods of establishment and spread are considered to be moderate to high. The potential economic consequences within New Zealand are considered to be low, the potential environmental consequences are considered to be negligible, the potential socio-cultural consequences are considered to be negligible to low, and the human health consequences are considered to be negligible.

As a result the overall risk estimate for CVd-V on nursery stock of Citrus *and related species is non-negligible and it is considered to be a risk on the commodity. Therefore the risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

Risk estimation table

CVd-V on imported nursery stock of *Citrus* and related species				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment				
Spread				
Consequences of establishment				
Economic				
Environmental				
Socio-cultural				
Human Health				

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2.5 *Citrus viroid VI* (CVd-VI)

Scientific name: *Citrus viroid VI*
(Family: *Pospoviroidae*; Genus: *Apscaviroid*)
Other relevant scientific names: Citrus viroid original source (CVd-OS)
Acronym: CVd-VI

2.5.1 Hazard identification

2.5.1.1 Description

Citrus viroid VI is a plant pathogen that infects species of citrus and citrus relatives.

2.5.1.2 Taxonomy

CVd-VI (formerly known as *Citrus viroid original source*, CVd-OS) (Ito et al. 2001) has been accepted as a new species of the genus *Apscaviroid*. All known citrus viroid species belong to the family *Pospiviroidae* (Eiras et al. 2009, Owens et al. 2012).

2.5.1.3 New Zealand status

Citrus viroid VI is not known to be present in New Zealand. Not recorded in: Veerakone et al. 2015, PPIN 2015.

2.5.1.4 General geographic distribution

CVd-VI has been recorded in Japan (Ito et al. 2001).

2.5.1.5 Commodity association

Viroids are systemic within infected plants (Ding 2009) and citrus viroids are readily graft-transmissible (Duran-Vila and Semancik 2000), including CVd-VI (Ito et al. 2001, Ito et al. 2002b). Therefore, CVd-VI can potentially be present in citrus nursery stock (budwood and tissue culture plantlets).

2.5.1.6 Plant associations

CVd-VI has been isolated from *Citrus reticulata* Blanco x *C. sinensis* (L.) Osb. x *C. reticulata* 'Shiranui' (Ito et al. 2001, 2002b), *C. reticulata* x *C. sinensis* 'Kiyomi', *C. reticulata* 'Ueno Wase' and *C. sinensis* 'Tsutsumi navel'(Ito et al. 2002b).

CVd-VI also infects Etrog citron (*C. medica* L.) (Ito et al. 2001) which is the generic indicator plant for citrus viroids (Duran-Vila et al. 2000).

It is not clear how wide the host range is for CVd-VI. For citrus viroids in general, the host range includes *Citrus* species and close *Citrus* relatives (Duran-Vila and Semancik 2003).

CVd-VI has been detected in Japanese persimmon (*Diospyros kaki* Thunb.) (Nakaune and Nakano 2008).

2.5.1.7 Potential for establishment and impact

Citrus viroid VI is found in Japan, which has some climatic similarities with parts of New Zealand, and can potentially establish in New Zealand. It is pathogenic to species of *Citrus* some of which are important horticultural crops in New Zealand.

2.5.1.8 Hazard identification conclusion

Given that *Citrus viroid VI*:

- is reported from *Citrus* spp.;
- is recorded from Japan;
- is not recorded from New Zealand;
- can potentially establish in New Zealand;
- can potentially cause unwanted impacts in New Zealand;

Citrus viroid VI is considered a hazard on *Citrus* nursery stock in this risk analysis.

2.5.2 Biology

Viroids are the smallest known plant pathogens. They consist of a non-encapsidated, circular, single-stranded non-coding RNA that replicates autonomously in their host plants. Viroids can move from cell to cell through plasmodesmata and travel long-distance through phloem to establish systemic infection (Ding 2009).

All citrus viroids belong to the family *Pospiviroidae* which replicate in the nucleus of the infected cell. Citrus viroids are graft-transmitted and they are spread mainly by propagation of contaminated material. Seed transmission and vector transmission of citrus viroids have not been demonstrated in citrus (Duran-Vila and Semancik 2003). Citrus viroids are usually present as complex mixtures co-infecting the same plant (Duran-Vila and Semancik 2003).

Citrus viroid VI (CVd-VI) was first collected from a tree of 'Shiranui' [*C. reticulata* Blanco x *C. sinensis* (L.) Osb.) x *C. reticulata*] in Japan (Ito et al. 2001). It was found as a component of a mixture of viroids within a single sample. In a later study of the presence of viroids in citrus trees in Japan, Ito et al. (2002b) found several instances of CVd-VI occurring in combination with other citrus viroids in the same host plant. Ito et al (2002a) developed a multiplex reverse transcription polymerase chain reaction (RT-PCR) to detect CVd-VI and other citrus viroids in citrus plants.

CVd-VI has been detected in hybrids of *C. reticulata* and *C. sinensis*. However, it is not clear how wide the host range is for this viroid. Ito et al. (2002b) surveyed several citrus species in Japan for the presence of citrus viroids, including CVd-VI, but it was not detected in all the citrus species surveyed. However, this study was not a test of the ability of CVd-VI to infect these species and no studies to determine the host range of CVd-VI have been found in the literature. For citrus viroids in general, the host range includes *Citrus* species and close *Citrus* relatives (Duran-Vila and Semancik 2003). There is little information on the occurrence of citrus viroids in wild citrus. However, experiments involving *Eremocitrus glauca* and *Microcitrus australis*, two species of true citrus fruits native to Australia, found that these two species were poor citrus viroid hosts in which viroid replication/accumulation does not occur or is extremely inefficient (Bani Hashemian et al. 2010).

Viroids with 92.1–94.3% nucleotide sequence identity with CVd-VI [named as CVd-OS] have been detected in Japanese persimmon (*Diospyros kaki* Thunb.) which can be considered a natural host (Nakaune and Nakano 2008). According to the criteria suggested by the International Committee for Taxonomy of Viruses (ICTV), these viroids can be considered variants of the

species CVd-VI as they exceed the arbitrary level of 90% nucleotide sequence identity that has been accepted as separating species from variants (Owens et al. 2012). Nakaune and Nakono (2008) note that persimmons are widely distributed throughout Japan, overlapping with the cultivation areas for citrus, but that there is no information about viroid transmission from persimmon to other crops or from others to persimmon. Results from their study could not be used to determine a relationship between viroid infection and symptom expression, with further study required to assess the impact of viroid infection on persimmon and other crops.

CVd-VI, by itself, induced only moderate symptoms such as mild petiole necrosis, characteristically very mild leaf bending (epinasty), and stunting in Arizona 861-S1 Etrog citron, (*Citrus medica* L.), the sensitive indicator for detection of citrus viroids. The plants were held over one year under high temperature conditions (28ºC for 6 months, followed by 32ºC day and 28ºC night for 8 months) (Ito et al. 2001). According to Ito et al. (2001), the degree of leaf bending differed from that induced by other citrus viroids: clearer leaf bending has been previously reported for CEVd, CBLVd, CVD-III, CVd-IV, and CVd-I-LSS, and an absence of leaf bending for HSVd. All citrus viroids induce a few characteristic symptoms such as epinasty, leaf bending, stunting and mid-vein and petiole necrosis in 'Etrog' citron. However, symptoms can range from mild to severe, depending on viroid species or variant (Ito et al. 2001).

There is no description of the impact of CVd-VI by itself on other *Citrus* spp., either experimentally or in the field. Other citrus viroids infect species of *Citrus* as well as citrus relatives. Most of these viroid/host combinations are symptomless and disease is only perceived when a specific viroid infects a sensitive species (Duran-Vila and Semancik 2003). Members of the genus *Aspscaviroid* do not induce specific diseases of citrus but can result in moderate effects on tree size and crop yield (Vernière et al. 2004). Two well-known diseases of citrus, exocortis and cachexia, are caused by viroids in other genera (*Citrus exocortis viroid*, CEVd: *Pospiviroid*; and *Hop stunt viroid*, HSVd: *Hostuviroid*, respectively). The effect of a single citrus viroid on its host plant is influenced by a number of factors including temperature, light intensity and quality, presence of other citrus viroid species, host plant, rootstock. However, there are few data on the effects induced by individual viroids in citrus grown under field conditions (Eiras et al. 2010).

In the field, viroids in citrus are often found in combination as has been the case with CVd-VI (Ito et al. 2002b). Impact on the plant is affected by many factors including which viroid variants and species predominate in terms of accumulation and symptom expression. Multiple infections of citrus viroids can result in synergistic or antagonistic interactions, depending on the combination (Vernière et al. 2006). For example, some combinations of citrus viroids other than CEVd can induce exocortis-like symptoms as severe as those caused by CEVd, affecting plants with trifoliate orange (*Poncirus trifoliata*) rootstocks (Ito et al. 2002b; Ito et al. 2003). The presence of CVd-VI in combination with other citrus viroids could affect options of suitable rootstocks, although the role of CVd-VI, if any, in exocortis-like diseases is not yet clear (Ito et al. 2002b).

CVd-VI can be transmitted by both graft inoculation (Ito et al. 2002b) and by mechanical transmission (Ito et al. 2001). Dissemination of citrus viroids occurs mainly by propagation of symptomless, viroid-infected bud-wood (Duran-Vila and Semancik 2003). Mechanical transmission to uninfected plants occurs through using contaminated tools for pruning and harvesting (Barbosa et al. 2005). Some viroids (*Potato spindle tuber viroid* PSTVd, *Avocado sunblotch viroid* ASBVd, and *Coleus blumei viroid 1* CbVd1) are vertically transmitted through pollen and/or true seed, however, it is not clear the significance of this in the natural spread of diseases (Flores and Owens 2008). Seed transmission and vector transmission of citrus viroids have not been demonstrated in citrus (Duran-Vila and Semancik 2003). There is no indication in the literature surveyed that CVd-VI is transmitted by seed, pollen or insect vectors.

CVd-VI has been described as prevalent on citrus in Japan where it was first detected and it may be present but not yet detected in other countries (Ito et al. 2002b). It is assumed that it can survive anywhere that its host plants can survive. Viroid diseases are more common in semi-tropical than in temperate climates (Semancik 2003) and viroid replication and symptom development is enhanced as temperature increases above 20ºC to at least 35ºC (Singh et al. 2003). Although diseases in the field are probably more prevalent in warmer climates, some viroids perform better at lower temperatures (Singh et al. 2003).

Control of citrus viroids is based on preventive measures such as the use of viroid-free budwood as propagation material and adequate indexing procedures (Eiras et al. 2009), and through the disinfection of pruning and harvesting tools (Barbosa et al. 2005).

2.5.3 Risk assessment

2.5.3.1 Entry assessment

CVd-VI has been recorded only from Japan. However, Ito et al. (2002b) point out that CVd-OS [CVd-VI] is prevalent in Japan and could well be present in other countries; variants of some of the other citrus viroids in Japan have similar or identical nucleotide sequences to those in other countries suggesting that the variants had been imported from or exported to those countries via citrus budwood.

Citrus nursery stock may enter New Zealand as either budwood (dormant cuttings) or tissue culture plantlets, although budwood is more usual. It is likely that a small amount of budwood material will be imported in a consignment, with probably 5–12 buds per stick of wood. Tissue culture plants will arrive as plantlets in flasks of media. Because CVd-VI can infect citrus systemically it can be present in the imported nursery stock.

For this assessment it is assumed that the plant material is imported from a non-accredited facility, and therefore is of an unknown phytosanitary status. In this case the material enters a Level 3 post-entry quarantine facility (PEQ) for a minimum of 16 months to allow time for biological indexing (import health standard (IHS) 155.02.06, *Citrus* schedule).

Overview of Level 3 PEQ for citrus nursery stock:

The overall process for importation of citrus nursery stock from a non-accredited facility to New Zealand is outlined in section 1.3, including figures 1 and 2. In brief, upon arrival in Level 3 PEQ, the plant material is inspected for visually obvious signs of regulated pests and diseases. For dormant cuttings, buds are grafted on to rootstock, to allow tests to take place. For tissue culture material, plants are removed from media flasks and replanted into pots. Plants are grown in greenhouse conditions throughout the year, and inspected frequently by the facility operator for signs of regulated pests and diseases. In addition, pre-determined specific tests are carried out for some regulated organisms before plants can be cleared for release from quarantine.

CVd-VI on citrus nursery stock in Level 3 PEQ:

The ability of citrus viroids to infect citrus without any external symptoms (asymptomatically) means that CVd-VI can enter New Zealand undetected on citrus nursery stock such as budwood (dormant cuttings) or plants in tissue culture. Upon arrival the symptomless citrus budwood or plants in tissue culture material without any visually detectable regulatory pathogens will be taken to the PEQ facility for propagation. Buds from the asymptomatic budwood will be grafted onto New Zealand origin rootstock to develop sufficient material to allow buds to be grafted on to New Zealand origin indicator plants. The asymptomatic tissue culture plantlets will be deflasked in a quarantine glasshouse and planted in pots.

Plants are maintained in a healthy vigorous state without stress and can be grown in greenhouse conditions throughout the year. They are held at temperatures considered suitable for liberibacters. However, the appropriate temperatures are not stipulated in the current IHS 155.02.06 for plant material from non-accredited facilities[31].

Pre-determined tests are currently required by the IHS for the following citrus viroids: CVd-I, CVd-III, CVd-IV, and Hop stunt viroid (formerly named CVd-II). The IHS gives SPAGE and PCR on grafted inoculated citron extract (with citron grown at hot temperature, 27 to 32ºC) as an acceptable method[32]. Graft indexing for viroids is done in the early spring using young, vigorous indicator plants. Laboratory tests for viroids (polymerase chain reaction, PCR) are then carried out at the end of summer as viroid activity and, therefore, titre in the sampled tissues is higher when temperatures are warmer. However, no pre-determined tests for CVd-VI are required in the IHS.

For graft indexing, citron 'Etrog Arizona 861' (*Citrus medica*) is used as the indicator plant and is grown at high temperatures to enhance titre build-up and symptom expression (the *Citrus* testing manual (MAF 2010) states incubation temperatures of 32–40ºC max. day and 25–30ºC min. night, and the IHS states 27 to 32ºC). This citrus species is the classical indicator for citrus viroids, including CVd-VI, with each viroid inducing specific symptoms which are apparent within 6 to 12 months or longer. However, citrus viroids can occur as multiple infections; the synergistic and inhibitory interactions can delay or enhance the symptoms produced and these may vary from the described symptoms (Semancik and Duran-Vila 1991). Ito et al. (2001) found CVd-VI on its own induced only mild petiole necrosis and characteristically very mild leaf bending in the 'Etrog' citron indicator plants over one year under high temperature conditions, with the degree of leaf bending differing from the clear leaf bending previously reported for many other citrus viroids, and the absence of leafbending reported for HSVd. CVd-VI may therefore produce detectable symptoms in the citrus viroid indicator plants, particularly in combination with other citrus viroids, but would be overlooked in infected plants that are asymptomatic.

Indicator plants are also tested for the presence of citrus viroids by means of conventional reverse transcription (RT)-PCR, with primer pairs specific for each citrus viroid tested (Serra et al. 2008, Ito et al. 2002a, Hadidi et al. 1992). Specific tests are available for CVd-VI (MAF 2010). However they are not required by the current IHS and if they are not used then CVd-VI is unlikely to be detected by PCR. As with any laboratory-based test there are limitations in the ability to detect asymptomatic infection, such as uneven distribution within the plants (i.e. the viroid is not present in the leaves submitted for analysis) or the titre of the viroid is not high enough to be detected. Every attempt is made to ensure the likelihood of testing and detecting viroid is increased, including the type of material selected for testing, the number of replicates, and the time of year samples are collected. For citrus viroids, temperature is perhaps the most influential factor to reach a high titre in the sample.

In post-entry quarantine (PEQ), the nursery stock will be kept and monitored for a minimum of16 months from the start of plant growth before being released. During this period plants are checked weekly by the facility operator and any plants showing symptoms of CVd-VI would be tested to diagnose the cause.

Given that:

- CVd-VI is reported as prevalent in citrus in Japan, however, it is not known if it occurs in citrus elsewhere;

31 All citrus nursery stock imports since 2010 have come from an MPI-approved facility overseas and therefore have entered Level 2 PEQ. The IHS requires that plants in Level 2 PEQ must be held at 18–25ºC throughout the quarantine period (incorporated in IHS amendment 27 November 2014). However the CTO has required operational measures in PEQ to effectively manage temperature to these levels since April 2012.

32 The *Citrus* schedule in the IHS 155.02.06 states that 'Country freedom is accepted as equivalence to a treatment'

- CVd-VI can remain systemic in its citrus host and therefore occur in nursery stock;
- no pre-determined tests for CVd-VI are required by the IHS to be carried out in Level 3 PEQ, however, the indicator plant Etrog citron used for other citrus viroids is suitable for CVd-VI;
- infected plants may show disease symptoms during the growing period of at least 16 months in Level 3 PEQ, however, infected citrus plants can be asymptomatic;

The likelihood of entry is considered to be low but non-negligible.

2.5.3.2 Exposure assessment

CVd-VI is borne internally and will survive for as long as the host plant remains alive. Infected plants that are symptomless are likely to be released from PEQ for future propagation. As the viroid is a systemic pathogen that is graft-transmissible, it is likely that infected plant material used for grafting will expose the viroid to other susceptible plants in the New Zealand environment.

Given that:

- CVd-VI is systemic and can remain in the host as long as the host remains alive;
- host plant material will be kept alive as nursery stock and used for propagation;

The likelihood of exposure is considered to be high.

2.5.3.3 Assessment of establishment and spread

CVd-VI can remain systemically in the plant for as long as the plant remains alive. If infected plants are used for propagation of new infected plants that are planted, then the viroid can be considered to have established.

CVd-VI has been recorded from Japan where it is considered to be prevalent (Ito et al 2002b). There is no evidence that climate would limit the ability of the viroid to establish in at least some regions in New Zealand. Any symptom expression is more likely in the warmer regions of New Zealand as viroid activity and, therefore, titre is generally higher when temperatures are warmer. *Citrus* is mostly grown in the warmer regions. The reported hosts of CVd-VI include sweet orange and mandarin hybrid/varieties. Other *Citrus* spp. may be hosts as the full host range does not appear to have been determined. It is likely that the movement of infected plant material, both whole plants and cuttings or budwood, would be the most effective means of the viroid spreading among regions of New Zealand where citrus is grown, both commercially and domestically. The majority of commercial citrus orchards are in the Bay of Plenty, Gisborne, and Northland. In Japan, a variant of CVd-VI has also been detected in persimmons, which are grown in New Zealand.

Viroids are also spread through mechanical transmission and therefore improper sanitation such as the indiscriminate use of non-sterile pruning shears would allow the CVd-VI to spread from infected to clean plants. Some viroids (PSTVd, ASBVd, and CbVd1) are vertically transmitted through pollen and/or true seed, however, it is not clear the significance of this in the natural spread of disease (Flores and Owens 2008). Seed transmission of citrus viroids has not been demonstrated in citrus (Duran-Vila and Semancik 2003). There is no indication in the literature surveyed that CVd-VI is transmitted by seed, pollen or insect vectors.

Infected plants that show disease symptoms are unlikely to be used for propagation. However, hosts may be asymptomatic, or at least initially, and plant material could be distributed without disease being evident or before symptoms become apparent thereby inadvertently spreading the

viroid both locally and longer distances. As CVd-VI has not been recognised until recently and induces mild symptoms, it is likely that the viroid could be widespread before it is detected.

Given that:

- CVd-VI can remain systemically in the plant for as long as the plant remains alive;
- imported citrus nursery stock is used for propagation of new plants for planting;
- the New Zealand climate is unlikely to limit the establishment of CVd-VI;
- the availability of host plants is unlikely to limit the establishment of CVd-VI;
- citrus viroids are spread by propagation or grafting;
- some hosts may be asymptomatic and be used for propagation;

The likelihood of establishment and spread of CVd-VI is considered to be moderate to high.

2.5.3.4 Consequence assessment

Economic consequences

CVd-VI is known to infect sweet orange and mandarin hybrid/varieties and may infect other *Citrus* spp. A variant of CVd-VI has also been detected in persimmon although there is no information on any impact on persimmon or other crops. The New Zealand citrus industry comprises around 1,700 hectares divided between approximately 500 orchards, most of which are in the Bay of Plenty, Gisborne and Northland regions (NZCGI 2013). In 2013/14, the New Zealand domestic sales of fresh citrus fruit were $49.1m and in 2014 export sales of fresh fruit were $6.9m (Fresh Facts 2014). Natural transmission of citrus viroids occurs by graft propagation and by mechanical transmission with contaminated tools, and there is no evidence that CVd-VI is transmitted by vectors, seed or pollen. Without vectors, any impact is unlikely to affect existing plantings except through mechanical transmission by contaminated tools. For those growers who acquire infected plants, the effects of CVd-VI alone are likely to be subtle, perhaps affecting moderately the tree size and yield. Any symptom expression is more likely in warmer regions of New Zealand as viroid activity and therefore titre is higher when temperatures are warmer. There is, however, the potential for synergy in the presence of other citrus viroids resulting in a greater impact on production. For example, some combinations of citrus viroids other than CEVd can induce exocortis-like symptoms as severe as those caused by CEVd, affecting plants with trifoliate orange (*Poncirus trifoliata*) rootstocks (Ito et al. 2002b, Ito et al. 2003). Viroids currently reported from citrus in New Zealand are: *Citrus dwarfing viroid*, formerly known as *Citrus viroid III* (Quemin et al. 2011), and *Citrus exocortis viroid* (Pearson et al. 2006). Citrus viroids are frequently present in mixed infections, and if CVd-VI established in New Zealand, it could be in combination with another viroid(s). If infected plants were replaced with viroid-free plants then this could be a major cost to some producers. The presence of CVd-VI in combination with other citrus viroids could also affect options of suitable rootstocks, although the role of CVd-VI, if any, in exocortis-like diseases is not yet clear (Ito et al. 2002b).

The potential economic consequences within New Zealand are considered to be low but non-negligible.

Environmental consequences

CVd-VI is known to infect *Citrus* spp. (Rutaceae) with a variant known to infect persimmon (*Diospyros kaki*, Ebenacaeae), and given that these known hosts occur in different families there could be other hosts. There are two genera within Rutaceae endemic to New Zealand: *Leionema* and *Melicope*. Neither the *Leionema* nor *Melicope* species are considered threatened (NZPCN 2015). However, if these species were susceptible, they would be unlikely to be affected in the

natural environment as the natural transmission of CVd-VI is assumed to occur only by graft propagation and by mechanical transmission with contaminated tools.

The potential environmental consequences within New Zealand are considered to be negligible.

Socio-cultural consequences

CVd-VI is only known to infect *Citrus* spp. with a variant known to infect persimmons, although other hosts could be affected given these hosts are from two different families. Citrus is grown domestically in New Zealand, particularly in warmer regions of the country, and persimmon is grown less frequently. CVd-VI alone may be asymptomatic or produce very mild symptoms affecting tree size and yield. However, as transmission is known only to occur by grafting and mechanical means and as there is no known vector, many plants may be unaffected. Should plants show visible symptoms they can be replaced by healthy plants.

The potential socio-cultural consequences within New Zealand are considered to be negligible to low.

Human health consequences

There are no known human health consequences.

2.5.3.5 Risk estimation

The likelihood of entry of CVd-VI is considered to be low and the likelihood of exposure is considered to be high. The likelihoods of establishment and spread are considered to be moderate to high. The potential economic consequences within New Zealand are considered to be low, the potential environmental consequences in New Zealand are considered to be negligible, the potential socio-cultural consequences are considered to be negligible to low, and the potential human health consequences are considered to be negligible.

As a result the overall risk estimate for CVd-VI on nursery stock of Citrus *and related species is non-negligible and it is considered to be a risk on the commodity. Therefore the risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

Risk estimation table

CVd-VI on imported nursery stock of *Citrus* and related species				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment				
Spread				
Consequences of establishment				
Economic				
Environmental				
Socio-cultural				
Human Health				

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2.6 *Citrus sudden death-associated virus* / citrus sudden death

Scientific name (virus): *Citrus sudden death-associated virus* (Order *Tymovirales*: Family: *Tymoviridae*: Genus: *Marafivirus*)
Disease name: Citrus sudden death
Acronyms: CSDaV, CSD

2.6.1 Hazard identification

2.6.1.1 Description

Citrus sudden death-associated virus (CSDaV) is found in plants affected with the disease citrus sudden death (CSD) and has been considered the putative causal agent of the disease. However, this has not been proven by Koch's postulates and its exact role in the disease is not understood.

2.6.1.2 New Zealand status

CSDaV has not been reported from New Zealand, nor has the disease CSD (Veerakone et al. 2015, PPIN 2015).

2.6.1.3 General geographic distribution

CSDaV has been reported from São Paulo State and Minas Gerais State in Brazil (Loeza-Kuk et al. 2008). The associated disease CSD also occurs in the same states of São Paulo and Minas Gerais in Brazil (Yamamoto et al. 2011).

2.6.1.4 Commodity association

CSDaV has been detected by reverse transcriptase PCR (RT-PCR) in roots, young flush, bark (scion and rootstock), fruit peduncles, and leaves of trees infected with CSD (Maccheroni et al. 2005). Therefore it can potentially be present in citrus nursery stock (budwood and tissue culture).

2.6.1.5 Plant associations

CSDaV:

CSDaV has no known hosts other than citrus.

Species in which CSDaV has been detected include:

- sweet orange trees (Hamlin and Pera varieties) grafted onto Rangpur lime (*C. limonia*) rootstock (Maccheroni et al. 2005, Yamamoto et al. 2011);
- sweet orange (Natal variety) on Rangpur rootstock (Yamamoto et al. 2011);
- CSDaV has also been detected in asymptomatic sweet orange trees grafted onto tolerant rootstocks such as Cleopatra mandarin and *P. trifoliata* hybrids (Maccheroni et al. 2005, Yamamoto et al. 2011).

CSD:

CSD has been detected in sweet orange (*Citrus sinensis*) cultivars (Hamlin, Natal, Pera, Rubi, Westin, Pineapple, Valencia) on Rangpur lime (*C. limonia*) rootstock (Bassanezi et al. 2003, Roman et al. 2004).

It also affects other species, varieties and hybrids grafted on Rangpur rootstock, including: Ponkan and Cravo mandarin (*C. reticulata*), Murcott tangor (*C. sinensis* x *C. reticulata*), sweet lime (*C. limettioides*) and Tahiti lime (*C. latifolia*) (Yamamoto et al. 2011).

Trees grafted on Volkamer lemon (*C. volkameriana*) and rough lemon (*C. jambhiri*) rootstocks are also affected by CSD (Yamamoto et al. 2011).

In the CSD-affected region, sweet orange trees known to be infected with the CSD agent are symptomless on Cleopatra mandarin (*C. reticulata*) rootstock indicating that they are tolerant to CSD (Yamamoto et al. 2011). Sweet orange trees grafted onto Sunki mandarin (*C. sunki*), Swingle citrumelo (*P. trifoliata* × *C. paradisi*), and *Poncirus trifoliata* rootstocks are also considered to be tolerant to CSD (Yamamoto et al. 2011, Bové and Ayres 2007).

2.6.1.6 Potential for establishment and impact

CSDaV and the associated disease CSD have been reported from Brazil. There may be areas within the distribution of CSDaV that have climates similar to parts of New Zealand where citrus is grown and the virus may potentially establish in New Zealand. The disease is a serious threat to the citrus industry in Brazil and therefore could have an impact on the citrus industry in New Zealand.

The aphid (*Toxoptera citricida*), which is known to vector CSDaV (Loeza-Kuk et al. 2008), is present in New Zealand (PPIN 2015, Gordon 2010) and therefore will aid the establishment and spread of CSDaV.

2.6.1.7 Hazard identification conclusion

Given that *Citrus sudden death-associated virus*:

- is reported from *Citrus* spp. and citrus-related species;
- is recorded from Brazil;
- is not recorded from New Zealand;
- can potentially establish in New Zealand;
- can potentially cause unwanted impacts in New Zealand;

Citrus sudden death-associated virus is considered a hazard on *Citrus* nursery stock in this risk analysis.

2.6.2 Biology

Citrus sudden death (CSD) is a disease that causes decline in sweet orange (*Citrus sinensis*) and some mandarins (*C. reticulata*) grafted on either Rangpur lime (*C. limonia*) or Volkamerian lemon (*C. volkameriana*) (Bassanezi et al. 2007). The disease was first confirmed as a problem in Brazil in 1999, killing millions of orange trees (Futch et al. 2011). Although the aetiology of the disease is unknown, it has been suggested that the disease is caused by *Citrus sudden death-associated virus* (CSDaV) which is graft transmissible (Maccheroni et al. 2005).

CSD is considered to be a bud-union disease (Yamamoto et al. 2011). The disease is characterized by pale green coloration of leaves throughout the canopy, increased defoliation, reduction in new shoots, absence of internal shoots in the canopy, rot and death of a large portion of the root system, and the characteristic development of a yellow stain in the phloem of the

rootstock (Bassanezi et al. 2007). Such symptoms can be observed within a minimum of two years incubation period (Román et al. 2004).

The sudden appearance of CSD symptoms and the rapid disease progression have similarities to that of the quick-decline form of citrus tristeza caused by *Citrus tristeza virus* (CTV) (Rocha-Peña et al. 1995). Yamamoto et al. (2011) reported that all CSD infected trees and leaves tested positive to the new virus CSDaV and CTV at the same time. It may therefore be possible that a particular strain of CTV could be involved in this lethal disease. Although all plants infected by CSDaV are co-infected by CTV, to date, no reports of a single inoculation of CSDaV causing CSD have been made (Cantú et al. 2008). This implies that CSDaV may not be solely responsible in CSD aetiology and it has been suggested that CTV may help in the transmission of the CSD agent from citrus to citrus by aphids (Yamamoto et al. 2011).

The CSD disease is currently known to be associated with the *Marafivirus* CSDaV. Bové and Ayres (2007) reported that there was a 99.7% correlation between the disease symptoms (CSD) and presence of the virus (CDSaV). Since inoculated indicator plants with bark symptoms are always positive for the virus while indicator plants positive for the virus are not always positive for bark symptoms, CSDaV is probably an earlier indicator for the disease than bark symptoms (Yamamoto et al. 2011).

A graft-inoculation transmission study as well as natural transmission study was carried out by Yamamoto et al. (2011) using young sweet orange trees on Rangpur rootstock as indicator plants. The plants were examined regularly for one or two characteristic markers of CSD: (i) presence of a yellow-stained layer of thickened bark on the Rangpur rootstock, and (ii) infection with the CSD-associated *Marafivirus*. Based on these two markers, transmission of CSD was obtained, not only when budwood for graft-inoculation was taken from symptomatic sweet orange trees on Rangpur, but also when the budwood sources were asymptomatic sweet orange trees on Cleopatra mandarin, indicating that the latter trees are symptomless carriers of the CSD agent (Yamamoto et al. 2011).

Experiments using insect-proof cages strongly suggest that under natural conditions the disease is transmitted by an aerial vector such as an insect (Yamamoto et al. 2011). Results showed that 29 out of 40 uncaged indicator plants became infected as compared to only two out of 40 indicator plants that were put into insect-proof cages. Román et al. (2004) also reported that CSD is transmitted by grafting and that fungi, viroids, and endogenous bacteria were not the causal agents.

Maccheroni et al. (2005) reported that CSDaV was detected in aphid vectors such as *Toxoptera citricida*, *Aphis gossypii*, and *Aphis spiraecola* that were found feeding on CSD-affected trees; these species are also known to vector CTV. In 2007, Loeza-Kuk et al. (2008) confirmed the presence of CSDaV in aphids (*T. citricida* and *A. gossypii*) colonizing trees with or without CSD symptoms. However, detection of CSDaV was more frequent under the CSD condition and five times higher in *T. citricida*.

The virus has also been detected in leafhoppers (N. A. Wulff, unpublished as cited in Yamamoto et al. 2011). However, no particular species were named as vectors of CSDaV. Although the virus has been detected in both aphids and leafhoppers, direct transmission from insect to the citrus plant and vice versa has not been demonstrated to confirm Koch's postulates (Bassanezi et al. 2007).

Citrus sudden death once established cannot be treated with pesticides. It appears that the disease mainly affects trees on Rangpur lime (*C. limonia*) rootstocks. In Brazil where the disease has already established, control consists of replacing Rangpur lime with compatible rootstocks, such as Swingle citrumelo, Cleopatra mandarin, Sunki mandarin and trifoliate orange that appear to

tolerate the disease. In addition, approach grafting[33] (inarching) with compatible rootstock seedlings to the scions of trees on Rangpur lime is used.

2.6.3 Risk assessment

2.6.3.1 Entry assessment

Both CSDaV and CSD are known only from Brazil, having been reported from São Paulo State and Minas Gerais State. CSDaV is regarded as an excellent marker of CSD (Yamamoto et al. 2011). Citrus sudden death is known to be associated with many different citrus varieties grafted onto either Rangpur lime (*C. limonia*) or Volkamerian lemon (*C. volkameriana*) rootstock. However, some citrus varieties that are carriers of CSDaV do not show symptoms of the virus or disease.

Citrus nursery stock may enter New Zealand as either budwood (dormant cuttings) or tissue culture plantlets, although budwood is more usual. It is likely that a small amount of budwood material will be imported in a consignment, with probably 5–12 buds per stick of wood. Tissue culture plants will arrive as plantlets in flasks of media. Because CSDaV can infect citrus systemically it can be present in the imported nursery stock.

For this assessment it is assumed that the plant material is imported from a non-accredited facility, and therefore is of an unknown phytosanitary status. In this case the material enters a Level 3 post-entry quarantine facility (PEQ) for a minimum of 16 months to allow time for biological indexing (import health standard (IHS) 155.02.06, *Citrus* schedule).

Overview of Level 3 PEQ for citrus nursery stock:

The overall process for importation of citrus nursery stock from a non-accredited facility to New Zealand is outlined in section 1.3, including figures 1 and 2. In brief, upon arrival in Level 3 PEQ, the plant material is inspected for visually obvious signs of regulated pests and diseases. For dormant cuttings, buds are grafted on to rootstock, to allow tests to take place. For tissue culture material, plants are removed from media flasks and replanted into pots. Plants are grown in greenhouse conditions throughout the year, and inspected frequently by the facility operator for signs of regulated pests and diseases. In addition, pre-determined specific tests are carried out for some regulated organisms before plants can be cleared for release from quarantine.

CSDaV on citrus nursery stock in Level 3 PEQ:

The ability of CSDaV to infect citrus without any external symptoms means that it can enter New Zealand undetected on citrus nursery stock such as budwood (dormant cuttings) or plants in tissue culture. Although plants with CSD have external symptoms, these are unlikely to be visible on budwood which will be free of leaves. Upon arrival the symptomless citrus budwood or plants in tissue culture material without any visually detectable regulatory pathogens will be taken to the PEQ facility for propagation. Buds from the asymptomatic budwood will be grafted onto New Zealand origin rootstock to develop sufficient material to allow buds to be grafted to New Zealand origin indicator plants. The asymptomatic tissue culture plantlets will be deflasked in a quarantine greenhouse and planted in pots.

Plants are maintained in a healthy vigorous state without stress and can be grown in greenhouse conditions throughout the year. They are held at temperatures considered suitable for

33 Approach grafting or inarching is when plants are grown close together, and then joined so that each plant has roots below and growth above the point of union.

liberibacters. However, the appropriate temperatures are not stipulated in the current IHS 155.02.06 for plant material from non-accredited facilities[34].

No specific pre-determined tests for CSDaV or CSD are required by the current IHS. However, testing is currently required for strains of *Citrus tristeza virus* (CTV) not in New Zealand. CTV is routinely found in plants that carry CSDaV and may have a role in CSD, possibly helping in the transmission of the CSD agent from citrus to citrus by aphids (Yamamoto et al. 2011). For CTV, the IHS gives ELISA, graft inoculated Mexican lime (*Citrus aurantifolia*), sour orange (*Citrus aurantium*) and *Citrus excelsa* (with indicator plants grown at cool temperatures, 18 to 25ºC) as acceptable methods[35] (the *Citrus* testing manual (MAF 2010) recommends Mexican lime). Graft indexing is done in the early spring using young, vigorous indicator plants. Laboratory tests for viruses (PCR[36]) are then carried out in the spring using the new flush of spring growth. Any indicator plant that shows disease symptoms is tested to diagnose the cause.

In post-entry quarantine (PEQ), the nursery stock will be kept and monitored for a minimum of 16 months from the start of plant growth before being released. During this period plants are checked weekly by the facility operator and any plants showing symptoms of CSDaV or CSD would be tested to diagnose the cause. Symptoms are characterised by pale green colouration of leaves, reduction in new shoots and absence of shoots internal in the canopy are likely to appear. A symptom specific to CSD, a conspicuous yellow-stained layer either on the cambium side of the bark or within the bark, shows in the rootstock immediately below the bud-union of all CSD-sensitive scion/rootstock combinations (Gimenes-Fernandes and Bassanezi 2001, cited in Yamamoto et al. 2011). However, if either Rangpur lime (*C. limonia*) or Volkamerian lemon (*C. volkameriana*) are not used as rootstock for indicator plant in PEQ, then no symptoms relating to a bud-union disease would be expressed.

It is also likely that infected plants would not show symptoms within the 16 month growing period in PEQ as infected plants can take some time to show symptoms. Such infected but symptomless plant material is likely to be released for future propagation.

Given that:

- CSDaV and CSD are known only from Brazil;
- CSDaV is a systemic pathogen and can therefore occur in citrus nursery stock;
- CSDaV can occur asymptomatically both in citrus varieties in which the disease (CSD) symptoms do not occur and in sensitive scion/rootstock combinations;
- Citrus sudden death disease is a bud-union disease and can show characteristic symptoms in rootstock bark immediately below bud-union of sensitive scion-rootstock combinations;
- infected plants may not show disease symptoms during the growing period of at least 16 months in Level 3 PEQ;
- the indicator plants may not be sensitive to the disease;
- no specific tests for CSDaV are carried out in Level 3 PEQ;
- infected plants may also be co-infected with *Citrus tristeza virus* which is tested for in Level 3 PEQ;

The likelihood of entry is considered to be low but non-negligible.

34 All citrus nursery stock imports since 2010 have come from an MPI-approved facility overseas and therefore have entered Level 2 PEQ. The IHS requires that plants in Level 2 PEQ must be held at 18–25ºC throughout the quarantine period (incorporated in IHS amendment 27 November 2014). However the CTO has required operational measures in PEQ to effectively manage temperature to these levels since April 2012.

35 The *Citrus* schedule in the IHS 155.02.06 states that 'Country freedom is accepted as equivalence to a treatment'

36 PCR is accepted as an equivalent testing method

2.6.3.2 Exposure assessment

CSDaV is borne internally and can survive for as long as the host plant remains alive. Infected plants that are symptomless are likely to be released from PEQ for future propagation. As CSDaV is a systemic pathogen that is graft-transmissible, it is likely that infected plant material used for grafting will expose the virus to other susceptible plants in the New Zealand environment.

Given that:

- CSDaV can survive in its host for as long as the plant remains alive;
- symptomless plants systemically infected with CSDaV that are released from PEQ are likely to be used for further propagation and distribution;

The likelihood of exposure of CSDaV is considered to be high.

2.6.3.3 Assessment of establishment and spread

CSDaV is borne internally in the citrus plant and therefore may survive for as long as the host remains alive. If infected plants are used for propagation of new infected plants that are planted, then the virus can be considered to have established.

CSDaV and CSD have not been reported outside two states in Brazil (São Paulo State and Minas Gerais State) but it is not clear how much climatic conditions contribute to the current distribution of the virus and disease. This region is also the largest region for production of sweet orange (*C. sinensis*) in the world which, along with the disease being relatively new, could be more influential than climate. However, it is likely that some areas within their distribution have climates similar to parts of New Zealand where citrus is grown.

Some of the major *Citrus* species that are commercially grown in New Zealand include *C. sinensis* (sweet orange – both navel and Valencia), *C. limon* (lemon), *C. latifolia* and *C. aurantiifolia* (limes), *C. paradisi* (grapefruit), *C. reticulata* (Clementine mandarin), and *C. unshiu* (Satsuma mandarin). *Citrus sinensis, C. reticulata,* and *C. latifolia* have a recorded association with either CSDaV or CSD, or both. Furthermore, *Toxoptera citricida* a putative aphid vector for CSDaV, and CTV (also vectored by *T. citricida*) are already well-established where citrus is grown in New Zealand. It has been proposed that CTV may have a role in the disease, possibly helping in the transmission of the CSD agent from citrus to citrus by aphids (Yamamoto et al. 2011).

CSD is considered a bud union disease that seems to appear only on certain susceptible scion/rootstock combinations such as sweet orange or some mandarins grafted on either Rangpur lime or Volkamerian lemon rootstocks. Bové and Ayres (2007) noted that transmission of the CSD agent by graft-inoculation has been obtained with budwood inoculum taken not only from CSD-affected trees (grafted on Rangpur lime), but also from symptomless trees (grafted on Cleopatra mandarin) from the same citrus block. The propagation of budwood taken from symptomless but tolerant trees could therefore serve as inoculum donor trees.

Bassanezi et al. (2007) reported that after the appearance of the first CSD-symptomatic trees in the grove, disease symptoms could show up in 60–100% of the trees in the next 2 to 3 years.

The disease CSD is not likely to be expressed unless susceptible scion/rootstock combinations are used. The rootstocks commonly used in New Zealand such as *Poncirus trifoliata* and citrange (a hybrid of the sweet orange and the trifoliate orange) (Currie and Harty 2001) are tolerant to CSD. However, different rootstocks provide different characteristics and if new rootstock varieties that are susceptible to CSD are taken up by the industry for other reasons, then the disease is likely to be expressed.

Given that:

- CSDaV can remain systemically in the plant for as long as the plant remains alive;
- imported citrus nursery stock is used for propagation of new plants for planting;
- the climate is unlikely to be a barrier to establishment in at least some parts of New Zealand where citrus is grown;
- many different varieties of citrus including sweet oranges and mandarin are grown in New Zealand;
- the aphid *Toxoptera citricida*, which is thought to vector CSDaV, possibly in combination with CTV, is widespread in New Zealand;
- CSDaV and CSD are spread by propagation or grafting;
- hosts are likely to be asymptomatic (unless a susceptible scion/rootstock combination is used) and used for propagation;
- however, the New Zealand citrus industry has largely used *Poncirus trifoliata* and citrange which are rootstocks tolerant to CSD;

The likelihood of establishment and spread of CSDaV in New Zealand is considered to be moderate to high and the likelihood of expression of the disease is considered to be low due to the low prevalence of susceptible scion/rootstock combinations.

2.6.3.4 Consequence assessment

Economic consequences

CSDaV is known only from *Citrus* species and the disease CSD causes decline in sweet orange (*Citrus sinensis*) and some mandarins (*C. reticulata*) grafted on either Rangpur lime (*C. limonia*) or Volkamerian lemon (*C. volkameriana*) rootstocks. Economic losses would come from the expression of the disease and possibly impacts on trade with countries that do not have the virus or the disease. The New Zealand citrus industry comprises around 1,700 hectares divided between approximately 500 orchards, most of which are in the Bay of Plenty, Gisborne and Northland regions (NZCGI 2013). In 2013/14, the New Zealand domestic sales of fresh citrus fruit were $49.1m and in 2014 export sales of fresh fruit were $6.9m (Fresh Facts 2014). In Brazil, CSD has been responsible directly or indirectly for decline and eradication of almost 4 million trees (Bassanezi et al 2007). The symptoms decline and death can't be controlled except by changing to a tolerant rootstock (or by inarching). In New Zealand, rootstock varieties that are tolerant to CSD are widely used, and in these cases the disease is unlikely to be expressed and have an impact. However, different rootstocks are suitable in different situations such as tolerance to disease or drought resistance. For example, the economically important rootstock species *Poncirus trifoliata* is resistant to most isolates of CTV. However, it is not resistant to members of a CTV resistance-breaking (RB) strain presently found in New Zealand (Harper et al. 2010). The presence of the disease CSD may limit future rootstock options.

The potential economic consequences within New Zealand are considered to be low but non-negligible.

Environmental consequences

CSDaV is only known to affect *Citrus* spp. As there are no *Citrus* spp. native to New Zealand it is assumed that it will not infect New Zealand native plants.

The potential environmental consequences within New Zealand are considered to be negligible.

Socio-cultural consequences

CSDaV may affect home gardeners growing citrus, especially if the virus is vectored by *Toxoptera citricida*, which is present in New Zealand. CSD, however, is only expressed when susceptible scion/rootstock combinations are grown. In such cases, plants could decline and die. However, they could be replaced by plants on resistant rootstock.

The potential socio-cultural consequences within New Zealand are considered to be low.

Human health consequences

There are no known human health consequences.

2.6.3.5 Risk estimation

The likelihood of entry of *Citrus sudden death-associated virus* is considered to be low and exposure is considered to be high. The likelihood of establishment and spread is considered to be moderate to high. The potential economic consequences within New Zealand are considered to be low. The potential environmental consequences are considered to be negligible and the socio-cultural consequences within New Zealand are considered to be low. The potential health consequences within New Zealand are considered to be negligible.

As a result the overall risk estimate for Citrus sudden death-associated virus *on nursery stock of* Citrus *and related species is non-negligible and it is considered to be a risk on the commodity. Therefore the risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

Risk estimation table

CSDaV on imported nursery stock of *Citrus* and related species				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment				
Spread				
Consequences of establishment				
Economic				
Environmental				
Socio-cultural				
Human Health				

2.6.4 Risk management options

Options for risk management of CSDaV and CSD were requested, and are presented below.

Nursery stock can be sourced from areas free from the virus CSDaV and the disease citrus sudden death. The current known distribution of both the virus and the disease is the states of São Paulo State and Minas Gerais State in Brazil. Both the disease and the virus are relatively newly known and they appear to be of limited distribution. However, the virus CSDaV can be

systemic without showing symptoms in certain rootstock/scion combinations which means that it could be more widespread in Brazil, and possibly elsewhere, than currently recognised.

Specific predetermined tests for CSDaV can be carried out on imported citrus nursery stock in Level 3 PEQ. CSDaV can be detected in plant tissues by RT-PCR using the primers in Maccheroni et al. (2005) which were designed on the basis of the nucleotide sequence of the CSDaV RNA-dependent RNA polymerase (RdRp) domain. CSDaV is regarded as an excellent marker of CSD (Yamamoto et al. 2011). However, it should be noted that CSDaV is not confirmed as the causal agent of CSD and this could be a drawback to adopting the option of detecting the virus to indicate presence of CSD (or CSD causal agent). If it is not the causal agent, then there is potential for false positives or false negatives.

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2.7 *Indian citrus ringspot virus*

Scientific name: *Indian citrus ringspot virus* (Order *Tymovirales*; Family: *Alphaflexiviridae*; Genus: *Mandarivirus*)

Acronym: ICRSV

2.7.1 Hazard identification

2.7.1.1 Description

Indian citrus ringspot virus (ICRSV) is a viral pathogen that infects some species and/or cultivars of *Citrus* causing Indian citrus ring spot disease. Leaves of infected plants exhibit typical chlorotic rings of variable diameter and drop prematurely. Severely affected trees suffer reduced fruit yield and decline with plant dieback symptoms leading to death after a few years.

2.7.1.2 Taxonomy and nomenclature

ICRSV was initially known as Citrus ringspot virus in India, the only country from which it has been reported. Its name was changed to avoid confusion with a strain of *Citrus psorosis virus* (CPsV) called Citrus ringspot virus (Rustici et al. 2000). CPsV is of widespread occurrence and is the type species of the *Ophiovirus* genus.

Until recently, ICRSV has been the only recognised species in the genus *Mandarivirus* (ICTV 2013). A new member of *Mandarivirus*, *Citrus yellow vein clearing virus* (CYVCV), has been reported in Pakistan, India, China and Turkey. ICRSV and CYVCV are similar with regard to genome organization, viral particles and herbaceous host range (Loconsole et al. 2012). However, the two viruses are serologically distinct, have different natural host plants, cause two distinct diseases, and have different symptoms on citrus indicator plants (Loconsole et al. 2012). At least two cases of CYVCV have been incorrectly identified as ICRSV in India (Loconsole et al. 2012).

2.7.1.3 New Zealand status

ICRSV has not been reported in New Zealand (not listed in: Veerakone et al. 2015 PPIN 2015).

2.7.1.4 General geographic distribution

ICRSV has been reported only from India and occurs predominantly in northern India (Prabha and Baranwal 2011).

2.7.1.5 Commodity association

The commodity for this risk analysis is *Citrus* nursery stock which could either be budwood or tissue culture material. ICRSV has been isolated from budwood (Singh et al. 2008) and from mature leaves (Rustici et al. 2000) It has also been found localised in the testa (seed coat) of kinnow mandarin seeds (Prabha and Baranwal 2011)

2.7.1.6 Plant associations

ICRSV has been found in citrus species and varieties, especially kinnow mandarin (*Citrus nobilis* Lour x *C. deliciosa* Tenora), a hybrid of ‘King’ and ‘Willow’ mandarins, and Mosambi sweet orange (*C. sinensis*) (Milne et al. 2007).

Other citrus species that have shown susceptibility to ICRSV include: *C. aurantiifolia*, *C. reticulata* (Khasi mandarin), *C. reshni* (Cleopatra mandarin), *C. medica* (citron), and *C. jambhiri* (rough lemon) (Milne et al. 2007; Pant and Ahlawat 1998, cited in Singh et al. 2008; Thind et al. 1999, cited in CPC 2015).

The virus has produced symptoms in the following herbaceous plants after experimental mechanical inoculation: *Phaseolus vulgaris* cv Saxa, *Chenopodium quinoa*, *C. amaranticolor*, *Glycine max* (soybean cv Hodgson) and *Vigna unguiculata* (cowpea cv Black) (Rustici et al. 2000).

2.7.1.7 Potential for establishment and impact

ICRSV has been reported only from India, including states such as Punjab, Haryana, Delhi, Uttar Pradesh, Orissa, Maharashtra, Karnataka and Andhra Pradesh, (references in Prabha and Baranwal 2011). The disease occurs predominantly in the northern states with disease incidence of up to 100% recorded in kinnow mandarin orchards (Prabha and Baranwal 2011).

It is likely that the virus is capable of establishing in New Zealand wherever susceptible citrus species are grown. However, there may be some regions with micro-climates similar to some of the affected states in India that particularly favour disease expression to cause unwanted impacts.

2.7.1.8 Hazard identification conclusion

Given that *Indian citrus ringspot virus*:

- is associated with nursery stock of *Citrus* spp.;
- is recorded from India;
- is not recorded from New Zealand;
- can potentially establish in New Zealand;
- can potentially cause unwanted impacts in New Zealand;

Indian citrus ringspot virus is considered a hazard on *Citrus* nursery stock in this risk analysis.

2.7.2 Biology

Indian citrus ringspot virus has flexuous filamentous particles 650 nm in length with a single stranded RNA genome of 7560 nucleotides and a capsid protein of 34 kDa. It has six open reading frames (ORFs) (Rustici et al. 2002). ICRSV and the recently accepted species CYVCV are the only two recognised species in the genus *Mandarivirus* (ICTV 2013).

Sharma et al. (2007) reported that one of the main causes of the spread of the disease is the use of infected budwood. ICRSV can easily be transmitted by grafting and, among susceptible herbaceous hosts, by mechanical inoculation if greenhouse temperatures are not too high. The virus is also transmissible between citrus plants by dodder.

So far, ICRSV has not been found to have any natural vectors for its transmission (Pant and Ahlawat 1998, Thind et al. 1999: both cited in Singh et al 2008). A vector is suspected to occur because infected seedling trees have been found in nature (Ahlawat, personal observation, cited in Milne et al. 2007). However, in a study by Byadgi and Ahlawat (1995, cited in Milne et al. 2007), no transmission was obtained with four aphid species tested: *Aphis gossypii*, *A. citricola*, *A. craccivora* and *Myzus persicae*.

The virus does not appear to be seed-transmitted. Prabha and Baranwal (2011) detected the virus in the seed coats, but not the embryo or endosperm of seed collected from fruit of ICRSV-infected kinnow mandarin. Seedlings grown under favourable conditions from infected seed did not show any symptoms over a 2-year observation period and the virus could not be detected in

the seedlings by DAC-ELISA and RT-PCR. They concluded that it is likely that ICRSV is localised in the seed testa of kinnow mandarin. However, although no transmission of the virus to progeny was observed in their study of 160 seedlings, they note that an examination of a much greater number of seedlings is needed to confirm the absolute absence of seed transmission. They also note that seed transmission is uncommon in the Alphaflexiviridae.

ICRSV has been shown to be associated with the pollen of naturally infected flowers of kinnow mandarins (RP Pant, 1995 thesis, cited in Prabha and Baranwal, 2011). However, this does not appear to result in seed transmission (Prabha and Baranwal, 2011).

ICRSV induces the development of conspicuous chlorotic rings in the leaves of naturally infected mandarin (*Citrus* spp.) and sweet orange (*C. sinensis*) trees. Symptoms observed on young leaves of Mosambi sweet orange are vein-clearing, vein-banding and flecking which then persist in mature leaves (Milne et al. 2007). Infected kinnow mandarin develops conspicuous yellow ringspots with green centers in mature leaves. Necrotic spots develop in leaves of graft-inoculated King mandarin (Hoa and Ahlawat, 2004, cited in Milne et al. 2007). Trees affected by Indian citrus ringspot disease develop a thin canopy, and dieback symptoms, thus becoming less productive. Fruit is reduced in quantity and quality, but without specific symptoms (Milne et al. 2007). Dieback and decline symptoms lead to death after a few years (Milne et al., 2007).

Methods used to detect ICRSV include diagnostic field symptoms, biological indexing, serological and molecular methods (Prabha and Baranwal 2011, CPC 2015, Sharma et al. 2009, Singh et al. 2008, Milne et al. 2007, Roy et al. 2005, Rustici et al. 2000). The clearest diagnostic symptoms, especially in the winter months, are the yellow rings with green centres that develop in mature leaves of Kinnow mandarin and also in Mosambi and Malta sweet orange; however, ringspots disappear in summer (Milne et al 2007). In northern India ringspots are seen on Kinnow from December to February when the temperature is low; for the rest of the year no symptoms are visible on field trees. (Milne et al. 2007). Symptoms, in early stages, may be confused with those of citrus psorosis (Milne et al. 2007). When ICRSV is present along with other viruses in *Citrus* spp., it is not possible to rely on symptoms to identify the virus with certainty (CPC 2015).

In experimental infections of herbaceous plants, leaves of *Chenopodium quinoa* mechanically inoculated with a preparation of infected citrus leaves developed necrotic local lesions about 10 days after inoculation. When local lesion material from *C. quinoa* was mechanically inoculated to a range of test plants, *C. amaranticolor, Glycine max* (soybean cv Hodgson) and *Vigna unguiculata* (cowpea cv Black) developed only local lesions; however, inoculations onto *Phaseolus vulgaris* cv Saxa gave local lesions, then systemic infection with symptoms of vein clearing and mosaic. Other test plants appeared not to be infected (Rustici et al. 2000).

There is no cure for ICRSV infection and so far, the only effective measure to overcome the disease is to eradicate virus-affected plants (Singh et al. 2008). The production of healthy virus-free propagation material and the use of appropriate sanitation and cultural practices are therefore important.

2.7.3 Risk assessment

2.7.3.1 Entry assessment

ICRSV has been recorded only from India where it is reported as widespread in a number of states.

Citrus nursery stock may enter New Zealand as either budwood (dormant cuttings) or tissue culture plantlets, although budwood is more usual. It is likely that a small amount of budwood

material will be imported in a consignment, with probably 5–12 buds per stick of wood. Tissue culture plants will arrive as plantlets in flasks of media. Because ICRSV can infect citrus systemically it can be present in the imported nursery stock.

For this assessment it is assumed that the plant material is imported from a non-accredited facility, and therefore is of an unknown phytosanitary status. In this case the material enters a Level 3 post-entry quarantine facility (PEQ) for a minimum of 16 months to allow time for biological indexing (import health standard (IHS) 155.02.06, *Citrus* schedule).

Overview of Level 3 PEQ for citrus nursery stock:

The overall process for importation of citrus nursery stock from a non-accredited facility to New Zealand is outlined in section 1.3; figures 1 and 2. In brief, upon arrival in Level 3 PEQ, the plant material is inspected for visually obvious signs of regulated pests and diseases. For dormant cuttings, buds are grafted on to rootstock, to allow tests to take place. For tissue culture material, plants are removed from media flasks and replanted into pots. Plants are grown in greenhouse conditions throughout the year, and inspected frequently by the facility operator for signs of regulated pests and diseases. In addition, pre-determined specific tests are carried out for some regulated organisms before plants can be cleared for release from quarantine.

ICRSV on citrus nursery stock in Level 3 PEQ:

The ability of ICRSV to infect citrus without any external symptoms means that it can enter New Zealand undetected on citrus nursery stock such as budwood (dormant cuttings, without leaves) or plants in tissue culture. Upon arrival the symptomless citrus budwood or plants in tissue culture material without any visually detectable regulatory pathogens will be taken to the PEQ facility for propagation. Buds from the asymptomatic budwood will be grafted onto New Zealand origin rootstock plants to develop sufficient material to enable grafting of buds onto New Zealand origin indicator plants. The asymptomatic tissue culture plantlets will be deflasked in a quarantine greenhouse and planted in pots.

Plants are maintained in a healthy vigorous state without stress and can be grown in greenhouse conditions throughout the year. They are held at temperatures considered suitable for liberibacters. However, the appropriate temperatures are not stipulated in the current IHS 155.02.06 for plant material from non-accredited facilities[37]. The literature indicates that temperature affects the expression of ICRSV symptoms. In Northern India where the disease is most common, ringspots are seen on Kinnow in December to February when temperatures are low; for the rest of the year no symptoms are visible on field trees (Milne et al 2007). Some mechanical inoculation attempts have been unsuccessful because greenhouse temperatures were too high (Milne et al 2007). Kinnow trees are symptomless in warm weather and can appear to offer good material for propagation (Milne et al 2007). There is little information in the literature to indicate the optimum temperatures for symptom expression. However, Prabha and Baranwhal (2011) carried out an experiment in which they investigated the possibility of seed transmission of ICRSV in Kinnow mandarin. They state that they used favourable conditions for the grow-out test: the seedlings were grown in a glasshouse for 2 years at 25–28ºC day and 15–18ºC night temperatures with 70–80% RH.

Graft indexing for viruses is done in the early spring using young, vigorous indicator plants. Laboratory tests for viruses (PCR) are then carried out in the spring using the new flush of spring growth. However, no specific tests for ICRSV are required by the IHS and it will escape detection and identification in plants infected with ICRSV that are symptomless.

37 All citrus nursery stock imports since 2010 have come from an MPI-approved facility overseas and therefore have entered Level 2 PEQ. The IHS requires that plants in Level 2 PEQ must be held at 18–25ºC throughout the quarantine period (incorporated in IHS amendment 27 November 2014). However the CTO has required operational measures in PEQ to effectively manage temperature to these levels since April 2012.

In post-entry quarantine (PEQ), the nursery stock will be kept and monitored for a minimum of 16 months from the start of plant growth before being released. During this period plants are checked weekly by the facility operator and any plants showing symptoms of ICRSV would be tested to diagnose the cause.

Given that:

- although ICRSV appears to be widespread in states in India, the virus has not yet been recorded elsewhere;
- ICRSV is systemic in its citrus host and therefore can occur in nursery stock;
- infected budwood without leaves will not show symptoms;
- infected plants may show disease symptoms during the growing period of at least 16 months in Level 3 PEQ; but
- infected citrus plants can be asymptomatic; and
- and no specific tests for ICRSV are carried out in Level 3 PEQ;

The likelihood of entry is considered to be low but non-negligible.

2.7.3.2 Exposure assessment

ICRSV is borne internally and will survive for as long as the host plant remains alive. Infected plants that are symptomless are likely to be released from PEQ for future propagation. As the virus is a systemic pathogen that can be transmitted mechanically, it is likely that infected plant material used for grafting will expose the virus to other susceptible plants in the New Zealand environment.

Given that:

- ICRSV can survive in its host for as long as the plant remains alive;
- symptomless plants systemically infected with ICRSV are likely to be released from the PEQ facility and used for further propagation;

The likelihood of exposure is considered to be high.

2.7.3.3 Assessment of establishment and spread

ICRSV can remain systemically in the plant as long as the plant remains alive. If infected plants are used for propagation and distribution of new infected plants then ICRVS is highly likely to establish. As hosts may be asymptomatic, or at least initially, plant material could be distributed without disease being evident or before symptoms become apparent. Symptoms can also be similar to other diseases such as citrus psorosis in the early stages and may not be initially recognised as Indian citrus ringspot disease.

The known natural hosts of ICRSV are confined to species of citrus. It is likely that the movement of infected plant material, both whole plants and cuttings or budwood, would be the most effective means of the virus spreading among regions in New Zealand where citrus is grown, both commercially and domestically. The New Zealand citrus industry comprises some 1,700 hectares divided between approximately 500 orchards, most of which are in the Bay of Plenty, Gisborne, and Northland regions (NZCGI 2013).

Although mechanisms for natural spread are not known, this may occur since citrus seedlings have been found to be infected in India indicating the possibility of an insect vector (Ahlawat, personal communication, cited in Milne et al. 2007); however, no vector has yet been identified. The virus is transmissible between citrus plants by dodder experimentally. There are several

species of dodder which have been introduced into New Zealand and one native species, *Cuscuta densiflora* which is closely related to, if not identical with, those introduced (Aiken 1957). The presence of these species can provide an avenue for the exposure of the virus to other susceptible host plants. However, this seems to be an unlikely mechanism in the field, particularly in situations such as commercial orchards.

Climate may not be a barrier to establishment of ICRSV and it is likely that the virus is capable of establishing in New Zealand wherever susceptible citrus species are grown. However, there may be some regions with micro-climates similar to some of the affected states in India that particularly favour disease expression to cause unwanted impacts.

Given that:

- ICRSV can remain systemically in the plant for as long as the plant remains alive;
- IRSCV can spread to other plants through grafting;
- longer distance spread can be achieved through the movement of infected plant material;
- natural infection may be possible although no vector has yet been identified;
- climate is unlikely to be a barrier to establishment;
- suitable hosts are grown both commercially and domestically in regions of New Zealand where microclimates suitable for disease expression may occur;

The likelihood of establishment and spread within New Zealand is considered to be moderate, and disease expression would possibly occur within a limited area.

2.7.3.4 Consequence assessment

Economic consequences

The New Zealand citrus industry comprises around 1,700 hectares divided between approximately 500 orchards, most of which are in the Bay of Plenty, Gisborne, and Northland regions (NZCGI 2013). In 2013/14, the New Zealand domestic sales of fresh citrus fruit were $49.1m and in 2014 export sales of fresh fruit were $6.9m (Fresh Facts 2014). If ICRSV became established in New Zealand there is likely to be some negative impact on the industry. Assuming there is no insect vector any impact is likely to not affect existing plantings. For those who are affected, yield is likely to decrease with infected plants becoming decreasingly productive with poor quality fruit and later decline with dieback symptoms. Affected plants would have to be replaced with virus-free plants and this could be a major cost to some producers. It is not clear how well the climate requirements for ICRSV would be met in New Zealand but it is very likely that at least some regions of the country would have microclimates suitable for the virus and disease expression on susceptible citrus species/varieties. Although there could be high impact on some citrus producers, in the context of the overall New Zealand economy, the economic consequences are likely to be low.

The potential economic consequences within New Zealand are considered to be low but non-negligible, probably in a limited area.

Environmental consequences

ICRSV has been recorded only from *Citrus* spp. which are members of the Rutaceae. There are two genera within Rutaceae endemic to New Zealand: *Leionema* and *Melicope*. Neither the *Leionema* nor *Melicope* species are considered threatened (NZPCN 2015). However, if these species were susceptible, they would be unlikely to be affected in the natural environment as the natural transmission of ICRSV is assumed to occur only by graft propagation.

The potential environmental consequences within New Zealand are considered to be negligible.

Socio-cultural consequences

ICRSV is known to affect only *Citrus* spp. Citrus is grown domestically in New Zealand, particularly in the warmer regions of the country. Severely affected trees can suffer a significant loss of fruit yield and eventually decline with dieback symptoms. However, as transmission is only known to occur by grafting and there is no known vector, many plants may be unaffected. Infected plants can be replaced by healthy plants. If climate requirements are not widely met then ICRSV, or its disease impacts, could have a limited distribution.

The potential socio-cultural consequences within New Zealand are considered to be low, possibly in a limited area depending on climatic requirements.

Human health consequences

There are no known human health consequences.

2.7.3.5 Risk estimation

The likelihood of entry of *Indian citrus ringspot virus* is considered to be low and exposure is considered to be high. The likelihood of establishment and spread is considered to be moderate, with disease expression possibly occurring within a limited area. The potential economic consequences within New Zealand are considered to be low, possibly within a limited area, and the potential socio-cultural consequences within New Zealand are considered to be low, possibly within a limited area. The potential environmental and health consequences within New Zealand are considered to be negligible.

As a result the overall risk estimate for Indian citrus ringspot virus *on nursery stock of* Citrus *and related species is non-negligible and it is considered to be a risk on the commodity. Therefore the risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

Risk estimation table:

ICRSV on imported nursery stock of *Citrus* and related species				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment				
Spread			Limited area	
Consequences of establishment				
Economic		Limited area		
Environmental				
Socio-cultural		Limited area		
Human Health				

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2.8 *Olive latent virus 1*

Scientific name: *Olive latent virus 1* (Order: unassigned; Family: *Tombusviridae*; Genus: *Alphanecrovirus*)
Other relevant scientific names: Olive latent sobemovirus
Acronym: OLV-1

2.8.1 Hazard identification

2.8.1.1 Description

Olive latent virus 1 (OLV-1) is a viral pathogen known to infect several citrus species. Infection can occur either with no symptoms or in association with symptoms characteristic of citrus chlorotic dwarf disease (CCD) such as leaf deformation and interveinal chlorotic flecking (Martelli et al. 1996, Félix et al. 2012).

2.8.1.2 Taxonomic changes

The former genus *Necrovirus* has recently been divided into two genera, *Alphanecrovirus* and *Betanecrovirus* (ICTV 2013). Six of the seven species in *Necrovirus* have been reassigned to either one of these two new genera:

Alphanecrovirus:	*Tobacco necrosis virus A* (TNV-A, the type member)
	Olive mild mosaic virus (OMMV)
	Olive latent virus 1 **(OLV-1)**
Betanecrovirus:	*Tobacco necrosis virus D* (TNV-D, the type member)
	Beet black scorch virus (BBSV)
	Leek white stripe virus (LWSV).

The seventh species, *Chenopodium necrosis virus* (ChNV) is currently unassigned within the family *Tombusviridae*.

The division into new genera was supported by the high level of sequence diversity in the polymerase MPI and MP2 sequences, forming two phylogenetically distinct groups (ICTV 2013). All necroviruses are soil-transmitted. Some (e.g., TNV-A, TNV-D, BBSV) are naturally transmitted by the chytrid fungus genus *Olpidium*; others (e.g., OLV-1) are transmitted through soil without an apparent vector (King et al. 2012).

2.8.1.3 New Zealand status

Olive latent virus 1 is not known to be present in New Zealand. Not recorded in Veerakone et al. 2015 or PPIN (2015).

2.8.1.4 General geographic distribution

Olive latent virus 1 has been recorded in:

- Europe: Italy (Gallitelli and Savino 1985, cited in Félix et al. 2007; Martelli et al. 1996), Portugal (Félix and Clara 2002, cited in Félix et al. 2007), Poland (Borodynko et al. 2010)
- Middle East: Turkey (Martelli et al. 1996); Jordan (Martelli et al. 1995, cited in Félix et al. 2007); Lebanon (Fadel et al. 2005); Syria (Al Abdullah et al. 2005)

- Africa: Tunisia (El Air et al. 2011); Egypt (Youssef et al. 2010)
- Asia: Japan (Kanematsu et al. 2001).

The citrus isolates have been reported from Turkey (Martelli et al. 1996).

2.8.1.5 Commodity association

Olive latent virus 1 is a systemic viral pathogen that has been detected in asymptomatic citrus plants (Martelli et al. 1996) and can therefore be associated with nursery stock of citrus.

2.8.1.6 Plant associations

The natural host range of OLV-1, though apparently limited, includes taxonomically diverse species. It has been recorded from olive (*Olea europaea* L., Oleaceae) (Martelli et al. 1996), citrus (*Citrus* spp., Rutaceae) (Martelli et al. 1996), tulips (Liliaceae) (Kanematsu et al. 2001), and tomato (*Solanum lycopersicum* L., Solanaceae) (Borodynko et al. 2010).

Additionally, OLV-1 can infect a variety of experimental hosts usually causing local necrosis: *Celosia cristata*, *Chenopodium amaranticolor*, *Ch. quinoa*, *Cucumis sativus*, *Cucurbita pepo*, *Datura stramonium*, *Gomphrena globosa*, *Momordica balsamina*, *Nicotiana cavicola*, *N. clevelandii*, *N. glutinosa*, *N. megalosiphon*, *N. occidentalis*, *N. rotundifolia*, *N. rustica*, *N. tabacum*, *Ocymum basilicum*, *Petunia hybrida*, *Phaseolus aureus*, *Ph. vulgaris* and *Vigna unguiculata* (Félix et al. 2007, Félix et al. 2012). Systemic symptoms are exhibited by *Nicotiana benthamiana* and *N. occidentalis*, following mechanical inoculation with almost all OLV-1 isolates with the exception of the Portuguese isolate GM6 that does not systemically invade these indicator plants (Félix et al. 2007, Félix et al. 2012).

2.8.1.7 Potential for establishment and impact

Parts of New Zealand where citrus, olive, tulip or tomato are grown may have climatic conditions similar to countries where OLV-1 has been found. Therefore OLV-1 can potentially establish in New Zealand. Although its impact is not yet known, its known hosts are grown commercially in New Zealand.

2.8.1.8 Hazard identification conclusion

Given that *Olive latent virus 1*:

- can be associated with nursery stock of *Citrus* spp.;
- is recorded from several countries that grow *Citrus* spp.;
- is not recorded from New Zealand;
- can potentially establish in New Zealand;
- can potentially cause unwanted impacts in New Zealand;

Olive latent virus 1 is considered a hazard on *Citrus* nursery stock in this risk analysis.

2.8.2 Biology

Olive latent virus 1 (OLV-1) is a viral plant pathogen which, although it has an apparently restricted natural host range, has been detected in species from several plant families. It was first detected in olive (*Olea europaea*) trees growing in Southern Italy. However in Turkey OLV-1 was detected in several citrus species in plants that appeared symptomless as well as many plants that had been affected by the chlorotic dwarf disease (CCD) (Félix et al. 2007, Martelli et al. 1996).

The citrus isolate of OLV-1 possesses biological, morphological, physico-chemical, and ultrastructural properties similar, if not identical, to those of the olive isolate-OLV-1 type strain and is also serologically indistinguishable from it (Martelli et al. 1996).

Symptoms

Symptoms caused by OLV-1 vary among hosts and infected plants can remain symptomless. For example in Portugal several isolates were obtained from leaves of different olive cultivars showing low vigour, leaf chlorosis and occasionally no symptoms (Félix et al. 2007). The virus is often associated with olive decline (Félix et al. 2007). In Japan, OLV-1 was recovered from tulips exhibiting mottling and yellow streak leaf symptoms (Kanematsu et al. 2001). In Turkey, OLV-1 was detected in leaves of several citrus species where it appeared symptomless (Martelli et al. 1996), as well as from plants that showed citrus dwarfism. In western Poland, OLV-1 has been recovered from greenhouse-grown tomato plants with necrotic leaf spots (Borodynko et al. 2010).

In a citrus transmission study, inoculation of citrus seedlings by subcortical injections resulted in systemically infected plants with later recovery of the virus from upper leaves that did not show any symptoms. Mexican lime and sour orange seedlings inoculated mechanically with purified virus preparations all reacted with small necrotic local lesions (Martelli et al. 1996).

OLV-1 infects a wide variety of experimental hosts usually causing local necrosis (see section 2.8.1.6), although systemic symptoms have been shown by *Nicotiana benthamiana* and *N. occidentalis* (Félix et al. 2007, Félix et al. 2012).

Transmission

OLV-1 is easily mechanically transmissible to indicator plants (see section 2.8.1.6) in which it causes necrotic lesions (Félix et al. 2007). So far there are no indications that biological vectors such as fungi, nematodes or arthropods are involved in the transmission of the virus (Félix et al. 2007). However, it can be transmitted to plant roots through the soil in the absence of vectors (Félix et al. 2007). The family *Tombusviridae* has a number of members that are waterborne (e.g., Mukherjee et al. 2012, Horvath et al. 1999, Nienhaus and Castello 1989), therefore it is possible that OLV-1 is transmitted by water also.

Martelli et al. (1996) observed that roots of *Vigna unguiculata* seedlings grown in pots containing autoclaved river sand, and with no soil-borne fungal structures, showed necrotic streaks on some rootlets after seedlings had been inoculated. The inoculation was carried out by pouring infected plant extracts or purified virus preparations into small holes made in the vicinity of the seedlings and immediately watering the pots with distilled sterilised water. Control plants did not show any necrotic streaks on rootlets. The OLV-1 pathogen was later recovered from the rootlets showing necrosis, but not from stems or leaves.

Laboratory experiments using *N. benthamiana* as the plant host showed that leaf inoculation with OLV-1 resulted in local lesions and downwards symptomless invasion of the plant roots followed by virus release into the surrounding soil. The virus was then able to invade healthy roots of new *N. benthamiana* plants grown in that soil in the absence of any biological vector (Félix et al. 2007). It is likely that this mode of dissemination can also occur in citrus fields and especially in nurseries where seedlings are closely packed in a rooting substrate before transplanting to individual containers.

OLV-1 has been detected by RT-PCR analysis in whole olive flowers (Lobão et al. 2002, cited in Félix et al. 2007), pollen (Saponari et al. 2002, cited in Félix et al. 2007), fruit pulp (Felix et al. 2007) and a high percentage on seedlings grown from seed from an infected olive tree (Saponari et al. 2002, cited in Félix et al. 2007). This is an indication that the virus is also seed-borne and seed transmitted in olive. Félix et al. (2007) also state that data indicates that one of the means by

which OLV-1 is spreading among field olive plants may be by ovule fertilisation with infected pollen.

Interaction with other viruses

OLV-1 is the second necrovirus known to infect citrus (Martelli et al. 1996), the first being a strain of TNV-D (which is known to be water-borne) (Yot-Dauthy et al. 1969, cited in Martelli et al. 1996). Félix et al. (2007) noted that an interesting feature of OLV-1 genome is its potential ability to act as a 'parental' molecule together with that of *Tobacco necrosis virus* D (TNV-D), to give rise to a new recombinant virus, as in the case of *Olive mild mosaic virus* (OMMV); all three viruses have been recorded in olive (Félix et al. 2012).

Economic importance

Although OLV-1 is present in olive and citrus crops in the Mediterranean region and the Middle East, and has often been associated with olive decline and citrus dwarfism, its economic importance is unknown as it is not clear what the impact of the virus is on yield and quality of these economic crops (Félix et al. 2007). However, in the case of olives, international demand for olive plants has resulted in legislation enacted by the European Union as the Directive 93/48 dated 23-06-93 as cited from Félix et al. (2007) requiring that all propagative material of olive produced in nurseries must be free of all viruses.

2.8.3 Risk assessment

2.8.3.1 Entry assessment

OLV-1 appears to be widespread in countries through the Mediterranean and Middle East regions, as well as reported in Poland and Japan. Although the citrus isolates have been recorded only from Turkey, olive isolates have been demonstrated to infect citrus (Martelli et al. 1996). As OLV-1 can occur asymptomatically it may be present more widely than recognised and its prevalence is unknown.

Citrus nursery stock may enter New Zealand as either budwood (dormant cuttings) or tissue culture plantlets, although budwood is more usual. It is likely that a small amount of budwood material will be imported in a consignment, with probably 5–12 buds per stick of wood. Tissue culture plants will arrive as plantlets in flasks of media. Because OLV-1 can infect citrus systemically it can be present in the imported nursery stock.

For this assessment it is assumed that the plant material is imported from a non-accredited facility, and therefore is of an unknown phytosanitary status. In this case the material enters a Level 3 post-entry quarantine (PEQ) facility for a minimum of 16 months to allow time for biological indexing (import health standard (IHS) 155.02.06, *Citrus* schedule).

Overview of Level 3 PEQ for citrus nursery stock:

The overall process for importation of citrus nursery stock from a non-accredited facility to New Zealand is outlined in section 1.3, including figures 1 and 2. In brief, upon arrival in Level 3 PEQ, the plant material is inspected for visually obvious signs of regulated pests and diseases. For dormant cuttings, buds are grafted on to rootstock, to allow tests to take place. For tissue culture material, plants are removed from media flasks and replanted into pots. Plants are grown in greenhouse conditions throughout the year, and inspected frequently by the facility operator for signs of regulated pests and diseases. In addition, pre-determined specific tests are carried out for some regulated organisms before plants can be cleared for release from quarantine.

OLV-1 on citrus nursery stock in Level 3 PEQ:

The ability of OLV-1 to infect citrus without symptoms means that it can enter New Zealand undetected on citrus nursery stock such as budwood (dormant cuttings) or plants in tissue culture. Upon arrival the citrus material without any visually detectable regulatory pathogens will be taken to the PEQ facility for propagation. Buds from the asymptomatic budwood will be grafted onto New Zealand origin rootstock to develop sufficient material to allow buds to be grafted into New Zealand origin indicator plants. The asymptomatic tissue culture plantlets will be deflasked in a quarantine greenhouse and planted in pots.

Plants are maintained in a healthy vigorous state without stress and can be grown in greenhouse conditions throughout the year. They are held at temperatures considered suitable for liberibacters. However, the appropriate temperatures are not stipulated in the current IHS 155.02.06 for plant material from non-accredited facilities[38].

Graft indexing for viruses is done in the early spring using young, vigorous indicator plants. Laboratory tests for viruses (PCR) are then carried out in the spring using the new flush of spring growth. However, no specific predetermined tests for OLV-1 are required by the IHS and it can escape detection and identification in any plants infected with OLV-1 that are symptomless. Only plants showing symptoms of OLV-1 would be tested to diagnose the cause.

In PEQ, the nursery stock will be kept and monitored for a minimum of 16 months from the start of plant growth before being released. During this period plants are checked weekly by the facility operator.

Given that:

- although OLV-1 has been recorded from citrus in Turkey, its prevalence on this host is not known;
- OLV-1 can remain systemic in its citrus host and therefore can occur in nursery stock;
- infected plants may show disease symptoms during the growing period of at least 16 months in Level 3 PEQ, however, infected citrus plants can be asymptomatic;
- and no specific predetermined tests for OLV-1 are carried out in Level 3 PEQ;

The likelihood of entry is considered to be low but non-negligible.

2.8.3.2 Exposure assessment

OLV-1 is systemic and will survive for as long as the host plant remains alive. Infected plants that are symptomless are likely to be released from PEQ for future propagation. As the virus is graft-transmissible, it is likely that infected plant material used for grafting will expose the virus to other susceptible plants in the New Zealand environment.

Given that:

- OLV-1 can survive in its host for as long as the plant remains alive;
- symptomless but systemically OLV-1 infected plants that are released from the PEQ facility are likely to be used for further propagation;

The likelihood of exposure is considered to be high.

38 All citrus nursery stock imports since 2010 have come from an MPI-approved facility overseas and therefore have entered Level 2 PEQ. The IHS requires that plants in Level 2 PEQ must be held at 18–25ºC throughout the quarantine period (incorporated in IHS amendment 27 November 2014). However the CTO has required operational measures in PEQ to effectively manage temperature to these levels since April 2012.

2.8.3.3 Assessment of establishment and spread

OLV-1 can remain systemically in the plant for as long as the plant remains alive. If infected plants are used for propagation of new infected plants that are planted, then the virus can be considered to have established.

OLV-1 is known to naturally infect citrus, olives, tulips, and tomato, with systemic infection recorded in citrus and assumed for olive at least. The known natural hosts occur in New Zealand and are grown both as commercial crops and in domestic gardens. It is likely that in a commercial situation an infected plant would be in close proximity to other plants of the same species, especially in nurseries. In a domestic environment, it is possible for an infected plant to be near any of the known host species. The finding of a virus infecting tulips and showing extensive genomic identity with OMMV (thought to be a recombinant of OLV-1 and TNV-D) indicates that this virus is more widespread than anticipated and that additional hosts may emerge (Félix et al. 2007).

OLV-1 can be transmitted through propagation. So far there are no indications that biological vectors such as fungi, nematodes or arthropods are involved in the transmission of OLV-1 (Félix et al. 2007). However, OLV-1 can transmit gradually from infected plants through the soil to infect other susceptible host roots. Any establishment of citrus orchards infected with OLV-1 is likely to spread the virus via the soil to other nearby host crops. Excessive soil irrigation is likely to aid spread of the viral particle to roots of other susceptible hosts. The family *Tombusviridae* has a number of members that are waterborne, including the closely related TNV, and OLV-1 may prove to be waterborne as well.

OLV-1 has been detected in seedlings originating from seeds of infected olive tree and in pollen (Lobão et al. 2002, Saponari et al. 2002, both cited in Félix et al. 2007). This is an indication that the virus is also seed-borne and seed transmitted in olive. It is assumed here that it is possible it can behave in a similar manner in citrus plants[39]. Félix et al. (2007) also state that data indicates that one of the means by which OLV-1 is spreading among field olive plants may be by ovule fertilisation with infected pollen. It is also likely that pollinating insects can transfer infected pollen to new hosts. Therefore, it is assumed here that this could be a possibility for spread among citrus.

Given the current known distribution of OLV-1 in Mediterranean countries, Middle East and in Japan, it is likely climate will not be a barrier to establishment in at least some of the regions of New Zealand where citrus and other hosts are frequently grown.

Given that OLV-1:

- can remain systemically in the host plant as long as the plant remains alive;
- imported citrus nursery stock is used for the propagation of new plants for planting;
- can spread through propagation, through roots and infected soil and is probably waterborne;
- can combine with other viruses (e.g. TNV) to give rise to new recombinant viruses;
- suitable hosts are grown both commercially and domestically in regions of New Zealand where climate is unlikely to be a barrier to establishment;

The likelihood of establishment and spread within New Zealand is considered to be moderate to high.

39 As there is no evidence that OLV-1 is seed or pollen transmitted in *Citrus*, the possibility has not been considered in the estimate for likelihood of establishment and spread withn New Zealand.

2.8.3.4 Consequence assessment

Economic consequences

All the known hosts of OLV-1 are grown in New Zealand commercially.

The New Zealand citrus industry comprises around 1,700 hectares divided between approximately 500 orchards, most of which are in the Bay of Plenty, Gisborne, and Northland regions (NZCGI 2013). In 2013/14, the New Zealand domestic sales of fresh citrus fruit were $49.1m and in 2014 export sales of fresh fruit were $6.9m (Fresh Facts 2014).

New Zealand grows tomato for domestic consumption and exports with about 160 growers. Greenhouse tomatoes had a domestic sales value of $5m in 2014; outdoor tomatoes had a domestic sales value of $95m in 2014 and an export value of $8.9m (fresh) and $3.0m (processed) in 2014 (Fresh Facts 2014).

Olives are grown in many regions of New Zealand. Domestic sales of olive oil were worth $2.3m in 2013/2014 and export sales were $0.4m in 2014. Exports of tulip bulbs were worth $11.6m in 2014 (Fresh Facts 2014).

If OLV-1 became established in New Zealand there is likely to be some negative impact on these industries however, it is unclear what the magnitude would be. Although OLV-1 is present in olive and citrus crops in the Mediterranean region and the Middle East, and has often been associated with olive decline and citrus dwarfism, its economic importance is unknown as it is not clear what the impact of the virus is on yield and quality of these economic crops (Félix et al. 2007).

The potential economic consequences within New Zealand are uncertain but considered to be low to moderate.

Environmental consequences

Although the natural host range of OLV-1 is apparently limited, it includes taxonomically diverse species. Citrus species are members of the Rutaceae. There are two genera within Rutaceae endemic to New Zealand these are *Leionema* and *Melicope.* Neither the *Leionema* nor *Melicope* species are considered threatened (NZPCN 2015). In the Solanaceae the indigenous species *Solanum avicularae* (poroporo) (not endemic) is listed as declining in the New Zealand Conservation database of endangered plants (13 July 2012). The database does not list any members of the Rutaceae, Liliaceae or Oleaceae. However, potential host range and impact of OLV-1 are uncertain. If there is no discernible effect on these plants then the consequences are likely to be negligible. If there is a noticeable effect on appearance, growth, or fruit production the consequences from a national perspective are likely to be low.

The potential environmental consequences within New Zealand are considered to be uncertain but likely to be negligible to low.

Socio-cultural consequences

Known hosts (citrus, olives, tulips and tomatoes) are all grown in home gardens and may also be used as amenity plants (for example, tulips and olives). However, potential host range and impact are uncertain. If there is no discernible effect on these plants then the consequences are likely to be negligible. If there is a noticeable effect on appearance, growth, or fruit production the consequences from a national perspective are likely to be low. Should recombination with other viruses to produce new species take place (such as has been postulated with OMMV) there could potentially be some impact, however this scenario and its impacts are uncertain.

The potential socio-cultural consequences within New Zealand are uncertain but considered likely to be negligible to low.

Human health consequences

There are no known human health consequences.

2.8.3.5 Risk estimation

The likelihood of entry of OLV-1 is considered to be low, the likelihood of exposure is considered to be high, and the likelihood of establishment and spread is considered to be moderate to high. The potential economic consequences within New Zealand are uncertain but considered to be low to moderate, and the potential environmental consequences within New Zealand are uncertain but considered to be negligible to low. The potential socio-cultural consequences are uncertain but considered to be negligible to low and potential human health consequences within New Zealand are considered to be negligible.

As a result the overall risk estimate for OLV-1 on nursery stock of Citrus *and related species is non-negligible and it is considered to be a risk on the commodity. Therefore the risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

Risk estimation table

OLV-1 on imported nursery stock of *Citrus* and related species				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment				
Spread				
Consequences of establishment				
Economic		uncertain		
Environmental		uncertain		
Socio-cultural		uncertain		
Human Health				

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