Ministry for Primary Industries Manatū Ahu Matua



Risk Management Proposal: Import health standard (IHS) 155.02.06: Importation of Nursery Stock, schedule of special conditions for Citrus (including *Citrus, Fortunella*, and *Poncirus*) from all countries

FOR PUBLIC CONSULTATION

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New Zealand Government

Growing and Protecting New Zealand

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Submissions

The Ministry for Primary Industries (MPI) invites comment from interested parties on the proposed revision of the import health standard (IHS) 155.02.06: Importation of Nursery Stock, schedule of special conditions for *Citrus*, *Fortunella*, and *Poncirus* which is supported by this Risk Management Proposal document.

MPI has developed this proposal based on the best available scientific evidence and assessment of this evidence. If you disagree with the measures proposed to manage the risks, please provide either data or published references to support your comments. This will enable MPI to consider additional evidence which may change how risks are proposed to be managed.

The following points may be of assistance in preparing comments:

- Comments should be specific to a particular change in IHS measures or a question asked in this document (referencing section numbers or commodity names as applicable);
- Justifications, data and supporting published references to support comments are requested;
- The use of examples to illustrate particular points is encouraged.

MPI encourages respondents to forward comments electronically. Please include the following in your submission:

- The title of the consultation document in the subject line of your email;
- Your name and title (if applicable);
- Your organisation's name (if applicable); and
- Your address.

Send submissions to: plantimports@mpi.govt.nz.

However, should you wish to forward submissions in writing, please send them to the following address to arrive by close of business on **21 October 2016**.

Plant Germplasm (Imports) Plants, Food & Environment Ministry for Primary Industries PO Box 2526 Wellington 6140 New Zealand

Official Information Act 1982

Please note that your submission is public information and it is MPI policy to publish submissions and the review of submissions on the MPI website. Submissions may also be the subject of requests for information under the Official Information Act 1982 (OIA).

The OIA specifies that information is to be made available to requesters unless there are sufficient grounds for withholding it, as set out in the OIA. Submitters may wish to indicate grounds for withholding specific information contained in their submission, such as the information is commercially sensitive or they wish personal information to be withheld. Any decision to withhold information requested under the OIA is reviewable by the Ombudsman.

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Purpose

- 1. The purpose of this document is to provide information about the proposed revision of the *Citrus, Fortunella* and *Poncirus* schedules of the Import Health Standard (IHS) 155.02.06: Importation of Nursery Stock.
- 2. The IHS is the subject of consultation but MPI will accept comments and suggestions on this risk management proposal in order to improve future consultations.

Scope

- 3. This document provides the rationale for the proposed amendments to the *Citrus, Fortunella* and *Poncirus* schedules associated with imported *Citrus, Fortunella* and *Poncirus* nursery stock from all countries. It includes:
 - a) an update on the taxonomy and regulatory status of the pest list;
 - b) an assessment of the biosecurity risks posed to New Zealand;
 - c) proposed risk management measures for pests associated with imported *Citrus* nursery stock.
- 4. This document also provides an assessment of the risks associated with Australian fingerlime (*Citrus australasica*, syn. *Microcitrus australasica*), and proposes phytosanitary measures which would allow imports into New Zealand.

Background

- 5. The phytosanitary measures for all nursery stock species approved for importation into New Zealand are specified in the IHS 155.02.06: Importation of Nursery Stock. The IHS specifies the import requirements approved in accordance with New Zealand MPI's obligations under the Biosecurity Act 1993 and the International Plant Protection Convention (IPPC).
- 6. The IHS identifies the requirements for the import of *Citrus*, *Fortunella*, and *Poncirus* species from any country under separate schedules. All nursery stock (cuttings and tissue culture) species of *Citrus*, *Fortunella*, and *Poncirus* approved for entry in New Zealand are listed on the MPI Plants Biosecurity Index (PBI, <u>https://www1.maf.govt.nz/cgi-bin/bioindex/bioindex.pl</u>)
- 7. The regulated pest list for *Citrus* is out of date with current scientific knowledge, including the phytosanitary and testing measures for regulated bacteria, fungi, phytoplasmas, viroids, viruses and diseases of unknown aetiology.
- 8. Industry's request for access to new germplasm of Australian finger-lime (*Citrus australasica*, syn. *Microcitrus australasica*) requires the phytosanitary risks to be assessed and incorporated in the *Citrus* schedule.

CURRENT MEASURES

- 9. A summary of the current measures for importing *Citrus*, *Fortunella* and *Poncirus* nursery stock (cuttings and tissue culture) is provided below. The complete requirements can be found in the IHS at the following link: <u>http://www.mpi.govt.nz/document-vault/1152</u>.
- Citrus, Fortunella and Poncirus nursery stock eligible for import into New Zealand are listed in the MPI Plants Biosecurity Index (PBI): <u>https://www1.maf.govt.nz/cgibin/bioindex/bioindex.pl</u>.

^{1 •} Risk Management Proposal: Citrus schedule of the IHS nursery stock

- 11. *Citrus, Fortunella* and *Poncirus* nursery stock must meet the general requirements (Part 1) of the IHS 155.02.06 and the specific requirements listed in Part 2 under the '*Citrus*', '*Fortunella*' and '*Poncirus*' schedules, respectively.
- 12. *Citrus, Fortunella* and *Poncirus* nursery stock can be imported from any country. Plants from a MPI accredited offshore facility must enter Level 2 post entry quarantine (PEQ) for a minimum growing period of six months. Plants from non-accredited facilities must enter Level 3 PEQ for a minimum growing period of 16 months.
- 13. A permit to import issued by MPI is required for all *Citrus*, *Fortunella* and *Poncirus* nursery stock imported into New Zealand.
- 14. A phytosanitary certificate with the appropriate additional declarations must be issued by the National Plant Protection Organisation (NPPO) of the exporting country.
- 15. *Citrus, Fortunella* and *Poncirus* cuttings must be treated for insects and mites either prior to export or on arrival in New Zealand, before entering a post entry quarantine facility.
- 16. The nursery stock must be inspected, tested and treated during the post entry quarantine period.

COMMODITY

17. The genus *Citrus*, and the closely related genera *Poncirus* and *Fortunella*, belong to the subfamily Aurantioideae, family Rutaceae of the order Sapindales. The following species which are listed on the Plant Biosecurity Index, are permitted entry into New Zealand, with the exception of *C. australasica* for which a phytosanitary assessment is required (part of this review):

Citrus aurantifolia	Citrus meyerii
Citrus aurantium	Citrus myrtifolia
Citrus australasica	Citrus nobilis
Citrus bergamia	Citrus reshni
Citrus deliciosa	Citrus reticulata
Citrus excelsa	Citrus sinensis
Citrus grandis	Citrus unshiu
Citrus hystrix	Citrus volkameriana
Citrus jambhiri	Citrus × paradisi (syn. Citrus paradisi)
Citrus junos	Citrus imes reticulata
Citrus latifolia	Citrus × sinensis
Citrus limon	Citrus imes tangelo
Citrus limonia	Fortunella crassifolia
Citrus macrophylla	Fortunella japonica
Citrus madurensis	Fortunella margarita
Citrus medica	Poncirus trifoliata (syn. Citrus trifoliata)

18. Citrus nursery stock imported into New Zealand has been sourced from Australia, USA and South Africa.

TRADE VALUE

19. Citrus is an important crop in New Zealand and is the third largest fresh fruit crop after kiwifruit and apples. The New Zealand citrus industry comprises around 1,000 hectares divided between approximately 500 orchards, most of which are located in Bay of Plenty, Gisborne and Northland regions. In 2014, the domestic and export sales of fresh citrus fruit were \$49.1 million and \$6.9 million, respectively (Fresh Facts, 2014).

SOURCE INFORMATION

- 20. In the development of the risk management proposal the following information was used to identify the risk organisms and the appropriate measures to mitigate their entry and establishment into New Zealand:
 - a) *Citrus, Fortunella*, and *Poncirus* nursery stock schedules in the IHS 155.02.06, <u>http://www.mpi.govt.nz/document-vault/1152</u>.
 - MPI Plant Health and Environment Laboratory (PHEL) *Citrus* (citrus), *Fortunella* (kumquat) & *Poncirus* (trifoliate orange) Post-Entry Quarantine Testing Manual, July 2010, <u>http://www.biosecurity.govt.nz/files/regs/imports/plants/high-value-crops/citrus-testing-manual.pdf</u>.
 - c) MPI Pest Risk Assessments for '*Candidatus* Liberibacter americanus', '*Candidatus* Phytoplasma' 16SrIX subgroup A, *Citrus sudden death-associated virus, Indian citrus ringspot virus, Olive latent virus 1, Citrus viroid V* and *Citrus viroid VI*.
 - d) Risk management proposal for temperature sensitivity of high impact bacteria and bacteria-like organisms.
 - e) MAF Biosecurity New Zealand (MPI) Audit report Importation of nursery stock from the Elizabeth Macarthur Agricultural Institute (EMAI), New South Wales, Australia, Audit date: 26 February 2009.
 - f) Recommended amendments to the *Citrus* schedule identified during the audit of EMAI.
 - g) MPI databases: BORIC, PBI
 - h) Relevant literature and database searches.
 - i) Additional technical advice from scientific experts.
 - j) Stakeholder discussions prior to, and during the development of this Risk Management Proposal.

INTERNATIONAL SETTING

- 21. Where possible, phytosanitary measures are aligned with international standards, guidelines, and recommendations as per New Zealand's obligations under Article 3.1 of the World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), WTO 1995 and section 23(4)(c) of the Biosecurity Act 1993.
- 22. The SPS Agreement states that phytosanitary measures must not discriminate unfairly between countries or between imported or domestically produced goods, and where there is a choice of phytosanitary measures to reduce risk to an acceptable level, WTO members must select the least trade restrictive measures.

Objective

23. To ensure the phytosanitary measures are used to effectively manage the biosecurity risk associated with *Citrus* nursery stock, while enabling trade and are consistent with New Zealand's domestic legislation and international obligations.

Summary

- 24. Schedule: MPI proposes:
 - a) to combine the schedules of *Citrus* (citrus), *Fortunella* (kumquat) and *Poncirus* (trifoliate orange) into one schedule, referred to as *Citrus* (Paragraphs 31-32);
 - b) to include *Citrus australasica* (syn. *Microcitrus australasica*, Australian fingerlime) under the *Citrus* schedule (Paragraphs 33-35).
- 25. Pest list: MPI proposes:
 - a) to remove 22 citrus pests which are present in New Zealand and have a non-regulated status; 1 bacterium, 9 fungi, 4 insects, 1 viroid, 3 viruses and 4 diseases of unknown aetiology (Paragraph 37);
 - b) to include 11 organisms to the *Citrus* pest list with specific phytosanitary measures;
 1 liberibacter, 2 phytoplasmas, 3 viroids and 5 viruses (Paragraphs 81-83, 90-91, 94-95, 100-105, 118-121, 125-128, 132-137);
 - c) to include 5 organisms to the *Citrus* pest list without specific phytosanitary measures but with active monitoring by the International Biosecurity Intelligence System; 1 fungus, 1 liberibacter and 3 viruses (Paragraphs 71-73, 84-85, 122).

26. Phytosanitary measures: MPI proposes:

- a) to replace specific phytosanitary measures with active monitoring by the International Biosecurity Intelligence System for 2 viruses and 9 diseases of unknown aetiology (Paragraphs 113-114, 141).
- b) to replace specific with generic (i.e. visual inspection) phytosanitary measures for 2 bacteria (Paragraphs 56-57).
- c) to replace generic with specific phytosanitary measures for 3 fungi (Paragraphs 69-70, 74-77).
- d) to reword the current phytosanitary measure for Citrus blight (Paragraphs 138).
- e) to include a molecular test (PCR) as an alternative or compulsory test depending on the biosecurity risk associated with an organism.
- f) to reduce the list of woody indicator species from 15 to 4 species (Paragraphs 47-50).
- g) to include country freedom and pest free areas as alternative measures to the specific phytosanitary measures for fungi, viruses, viroids, phytoplasmas and disease of unknown aetiology listed in the Testing requirements table of the IHS *Citrus* schedule (Paragraphs 41-43).
- 27. **Post entry quarantine:** MPI proposes:

- a) to include a sodium hypochlorite treatment for cuttings on arrival at the quarantine facility in New Zealand (Paragraphs 51-52).
- b) changing the Level of PEQ (based on the new standard) to range from Level 2 with additional temperature control, to Level 3A or Level 3B depending on the pest biology, type and origin of nursery stock (Paragraphs 143-147).
- c) to adjust the period of time in quarantine to better manage the biosecurity of citrus nursery stock, ranging from a minimum of 8 months to 18 months depending on the type and origin of nursery stock (Paragraphs 148-151).

Risk management of pests associated with imported Citrus nursery stock

- 28. The proposed phytosanitary measures with significant changes are detailed below and summarized in Table 1. Minor changes (e.g. taxonomy) are only provided in Table 1.
- 29. Appropriate phytosanitary measures are required to minimize the risks posed by regulated pests associated with citrus. Asymptomatic plants present a serious problem in spreading important diseases such as Huanglongbing (Roistacher, 1991).
- 30. A range of diagnostic methods for detecting citrus pests is available. Some background information on each method with their advantages and disadvantages is provided in Appendix 2. In addition, some general information on the types of organism infecting citrus can be found in Appendix 3.

PHYTOSANITARY REQUIREMENTS FOR CITRUS, FORTUNELLA AND PONCIRUS

- 31. MPI propose to combine the *Citrus*, *Fortunella* and *Poncirus* schedules into a single shcedule, referred to as *Citrus*. These genera share the same pest list and phytosanitary requirements.
- 32. The Plant Biosecurity Index will be updated so that the import specifications for nursery stock of *Fortunella* and *Poncirus* species is listed as "see 155.02.06 under *Citrus*".

PHYTOSANITARY REQUIREMENTS FOR CITRUS AUSTRALASICA

- 33. There is little information about the pests infecting *Citrus australasica* (Australian fingerlime). Hardy *et al.*, (2010) provided a brief overview of the main pests in Australia. However, it is assumed that the types of pests and diseases which are able to infect other citrus are also able to infect finger-lime, as it belongs to the same genus. Therefore MPI proposes that *Citrus australasica* (syn. *Microcitrus australasica*, Australian finger lime) is eligible for import under the IHS *Citrus* schedule. The MPI Plants Biosecurity Index will be updated for nursery stock of *C. australasica* as "see 155.02.06 under *Citrus*".
- 34. C. australasica has also been reported to be a host to Citrus viroid V (Bani Hashemian et al., 2010) which is proposed as a new addition to the Citrus pest list (Paragraphs 100-103). The main disease affecting C. australasica, is "melanose" (Diaporthe citri), a fungal disease that is already present in New Zealand and its regulatory status is non-regulated (Hardy et al., 2010; BORIC; Ngā Harore o Aotearoa New Zealand Fungi Databases, 2014).
- 35. MPI is confident that the management measures proposed for other Citrus are applicable to *C. australasica*. Insects commonly found causing damage on *C. australasica* such as scale insects, spined citrus bug (Hardy *et al.*, 2010) are currently effectively managed under the basic conditions of the IHS (i.e. pesticide treatments) and under the *Citrus* schedule (i.e. the requirement for phytosanitary inspection and certification as being free of any visually detectable regulated pests, and the growing season inspection during post entry quarantine).

TAXONOMY AND REGULATORY STATUS UPDATES

- 36. The taxonomy of the regulated pests of citrus and updates to regulatory status are presented in Appendix 1. Pests with an undetermined regulatory status are proposed to be regulated.
- 37. Pests which have been reported to be present in New Zealand or have a non-regulated status since the last *Citrus*, *Fortunella* and *Poncirus* schedules update, have been removed from the pest list; these are:
 - a) Bacterium (1): *Burkholderia cepacia*;
 - b) Fungus (9): Aureobasidium pullulans, Colletotrichum coccodes (syn Gloeosporium foliicolum), Debaryomyces hansenii, Elsinoë fawcettii (stat. anam. Sphaceloma fawcettii var. scabiosa), Galactomyces citri-aurantii (anamorph Geotrichum citriaurantii), Rhytidhysteron rufulum, Sporobolomyces roseus, Syncephalastrum racemosum, Ulocladium obovoideum;
 - c) Insect (4): *Lepidosaphes beckii*, *Signiphora flavella*, *Siphoninus phillyreae*, *Stethorus histrio*;
 - d) Viroid (1): *Citrus dwarfing viroid* (syn. Citrus viroid III);
 - e) Virus (3): *Apple stem grooving virus* (syn. Citrus tatter leaf virus), *Citrus psorosis virus* (syn. Citrus ringspot virus), *Citrus tristeza virus*.
- 38. Any synonyms and obsolete names have been removed and organism names have been updated according to the latest taxonomy. Any updates on aetiology of organisms have also been included and details are provided in the section 'Diseases of unknown aetiology' (Paragraphs 139-142). Furthermore six fungi and three insect pests which were previously only identified to the genus level have now been identified to the species level (Appendix 1).
- 39. MPI have not reviewed the phytosanitary measures for insects, mites, spiders or molluscs as these are currently effectively managed under the basic conditions (i.e. pesticide treatments) and under the *Citrus* schedule (i.e. pre-export phytosanitary inspection) set out in the IHS.
- 40. MPI propose to remove the list of regulated insect, mite, mollusc and spider in the *Citrus* schedule and replace with the following wording: 'Check the MPI database, Biosecurity organisms register for imported commodities (BORIC) for regulatory status, <u>http://archive.mpi.govt.nz/applications/boric</u>' (refer to Section 'Proposed Citrus schedule in the Import Health Standard 155.02.06: Nursery stock').

PHYTOSANITARY MEASURES

Country freedom and pest free areas declarations

- 41. MPI propose to include country freedom and pest free areas declarations as alternative measures to the specific phytosanitary measures proposed for fungi, viruses, viroids, phytoplasmas and disease of unknown aetiology listed in the Testing requirements table of the IHS *Citrus* schedule. These declarations provide good evidence that the pest is not present in the country or the area.
- 42. The declarations would not be applicable to bacteria and liberibacters. MPI take a more precautionary approach for these organisms because of the high biosecurity risks posed by these groups of organisms (refer to paragraphs 54-64 for bacteria and 78-85 for liberibacters).
- 43. The exporting NPPO must endorse additional declarations on the phytosanitary certificate, to be considered equivalent to testing in post entry quarantine. The exporting NPPO must

^{6 •} Risk Management Proposal: Citrus schedule of the IHS nursery stock

meet the specifications set out in ISPM No. 4 – Requirements for the establishment of pest free areas, <u>https://www.ippc.int/en/publications/614/</u>.

Molecular test (PCR)

- 44. MPI propose to add PCR (Polymerase chain reaction) as an alternative test, wherever appropriate, to allow greater flexibility on the choice of method of diagnostic (Table 1). PCR is a widely used and internationally recognised method (Appendix 2). The organisms for which PCR is considered an appropriate alternative to other phytosanitary measures are *Citrus leaf rugose virus*, *Citrus variegation virus* (syn. Citrus infectious variegation virus), *Elsinoë australis* and *Satsuma dwarf virus*.
- 45. MPI propose to have PCR as a compulsory test for high impact pest organisms, Liberibacter spp. and *Xylella fastidiosa* because of it is more sensitive and reliable when compared to other diagnostic tests (Appendix 2). PCR is also compulsory when MPI consider it to be the best method of detection (Table 1); organisms to which this apply are *Citrus sudden deathassociated virus*, *Citrus chlorotic dwarf-associated virus*, *Citrus leprosis virus C*, *Citrus leprosis virus* nuclear type, *Citrus leprosis virus* cytoplasmic type 2, *Citrus yellow mosaic virus*, *Citrus yellow vein clearing virus*, *Indian citrus ringspot virus*, *Olive latent virus 1*, *Phyllosticta citricarpa* (syn. *Guignardia citricarpa*), *Plenodomus tracheiphilus* (syn. Phoma tracheiphila), *Spiroplasma citri*, *Xanthomonas citri* subsp. *citri* (syns. *X. campestris* pv. *citri*, *X. axonopodis* pv. *citri*), phytoplasmas and viroids.
- 46. MPI propose to refer to the *Citrus* post-entry quarantine testing manual (<u>http://www.biosecurity.govt.nz/files/regs/imports/plants/high-value-crops/citrus-testing-manual.pdf</u>) developed by the MPI Plant Health and Environement Laboratory for the recommended PCR protocols and primers (refer to section 'Guidance' below for details).

Woody indexing

- 47. Woody indicator species have been selected based on their suitability for detecting the targeted organisms and the range of diseases which can be transmitted to the indicator, reducing the number of species listed on the *Citrus* schedule to four (instead of 15). The woody indicator species are: *Citrus limon* 'Eureka' (lemon), *C. medica* 'Arizona 861' (citron), *C. reticulata* 'Parson's special' (mandarin) and *C. sinensis* 'Pineapple' (sweet orange). Alternative indicator species may be accepted by MPI with prior notification.
- 48. MPI propose that a minimum of two woody indicator species are used for each of the organisms which require this test. This would enhance the likelyhood of detecting the pathogens, if present. Further information on woody indexing can be found in Appendix 2.
- 49. MPI propose to name the woody indicators by their scientific name as defined by the International Association for Plant Taxonomy (<u>http://iapt-taxon.org/index_layer.php.</u> accessed 08 June 2015) rather than the common name.
- 50. MPI propose to refer to the *Citrus* post-entry quarantine testing manual (<u>http://www.biosecurity.govt.nz/files/regs/imports/plants/high-value-crops/citrus-testing-manual.pdf</u>) developed by the MPI Plant Health and Environement Laboratory for the recommended protocol for woody indexing (refer to paragraph 53 on 'Guidance' for details).

Treatment

51. MPI propose to include a sodium hypochlorite treatment for cuttings on arrival in New Zealand (Table 1). It is a generic treatment used to remove potential pathogenic bacteria and fungi that may be found on the surfaces of cuttings that are not visible to the naked eye. The sodium hypochlorite treatment will add an additional level of assurance for cuttings sourced

from an open field which are subject to wind and rain, the main mode of transmission of bacteria and fungi.

52. This treatment is already in place in the IHS nursery stock for other high value crops including *Fragaria*, *Malus*, *Prunus*, *Rubus* and *Vitis* with cuttings being dipped in a 1% sodium hypochlorite for 2 minutes upon arrival at the post entry quarantine facility. This treatment is also used by Elizabeth Macarthur Agricultural Institute (EMAI), New South Wales, Australia before sending citrus cuttings within Australia (Nerida Donovan, phone comm. dated 06 April 2016).

Guidance

- 53. MPI propose to re-name the 'Notes' section as Guidance under the Testing requirement table in the *Citrus* schedule of the IHS and update them as follows:
 - a) **Country freedom and pest free areas declarations**: These endorsments are only for regulated fungi, viruses, viroids, phytoplasmas and disease of unknown aetiology listed in the Testing requirements table of the IHS *Citrus* schedule and must be assessed by MPI prior to permit issue. The exporting NPPO must endorse the additional declarations on the phytosanitary certificate, to be considered equivalent to testing in post entry quarantine.
 - b) **Unit**: The unit for testing is defined in section 2.3.2.1.
 - c) Testing methods: The recommended woody indexing and PCR protocols (including primers) can be found at the following link: <u>http://www.biosecurity.govt.nz/files/regs/imports/plants/high-value-crops/citrus-testing-manual.pdf</u>. The term PCR includes conventional (simplex or duplex) as well as real-time PCR unless it is specified (e.g. phytoplasmas). Recommended sample collection and time of testing are also provided in the manual. Note the specific temperature range for liberibacters and viroids in the Table.
 - d) Other internationally recognised testing methods including woody indicator species may be accepted by MPI with prior notification.

BACTERIA

Spiroplasma citri (Stubborn disease)

- 54. MPI propose PCR as phytosanitary measure for *Spiroplasma citri* as it is very sensitive, fast and more reliable than woody indexing and culturing, which are the current phytosanitary measures (Table 1; Yokomi *et al.*, 2008). Detection of *S. citri* by woody indexing or the traditional culture method is not always reliable because of the uneven distribution of the organism in the plant and seasonal fluctuations in titre. Also, there is a risk of contamination by other organisms when culturing, producing false results (Rangel *et al.*, 2005).
- 55. *S. citri* infects most citrus species causing Stubborn disease which primarily affects young citrus trees in hot and arid climate, including California, North Africa, the Mediterranean basin and the Middle East (CABI datasheet; Timmer *et al.*, 2000).

Xanthomonas alfalfae subsp. citrumelonis (Bacterial spot) and X. fuscans subsp. aurantifolii (Canker)

56. MPI propose to replace the specific phytosanitary measures with visual inspections of actively growing plants during post entry quarantine for *Xanthomonas alfalfae* subsp. *citrumelonis* (syn. *X. campestris* pv. *citrumelo*) and *X. fuscans* subsp. *aurantifolii* (syn. *X. campestris* pv. *aurantifolii*) (Table 1). The bacteria cause typical symptoms on leaves: flat with water-soaked margins and necrotic centers for *X. alfalfae* subsp. *citrumelonis*

(responsible for citrus bacterial spot in USA) and raised lesions surrounded by a yellow halo for *X. fuscans* subsp. *aurantifolii* (responsible for cankers in South America) (CABI datasheet; Schaad *et al.*, 2006; Timmer *et al.*, 2000).

57. These two bacteria are not major citrus pathogens. They have a limited host range, rarely produce lesions on fruit and cause less aggressive symptoms than *X. citri* subsp. *citri* (Schaad *et al.*, 2006). In Florida, *X. alfalfae* subsp. *citrumelonis* was deregulated based on the low disease incidence in citrus and its limited host range (Timmer *et al.*, 2000). The disease caused by *X. fuscans* subsp. *aurantifolii* may even no longer exist in nature because of the pathogen's non-competitiveness with *X. citri* subsp. *citri* when both diseases occur in the same area (CABI datasheet).

Xanthomonas citri subsp. citri (Canker)

- 58. MPI propose to retain PCR as the only phytosanitary measure for *Xanthomonas citri* subsp. *citri* (syns. *X. axonopodis* pv. *citri*, *X. campestris* pv. *citri*) (Table 1). PCR is the most sensitive, reliable and rapid detection method for this bacteria (Mavrodieva *et al.*, 2004). The detached leaf bioassay, woody indexing and shoot tip grafting bioassay which are other phytosanitary measures currently listed in the IHS, are not recommended because of the lack of sensitivity of these techniques.
- 59. The detached leaf bioassay is used only from symptomatic material due to its low sensitivity (ISPM 27/DP6, 2014). There is no mention of woody indexing in the International Standards for Phytosanitary Measures (ISPM) protocol as it is not a reliable diagnostic technique (Rob Taylor, PHEL, pers. coms., June 2015). The shoot tip grafting bioassay is a tissue culture technique for pathogen elimination and not a diagnostic assay.
- 60. *X. citri* subsp. *citri* causes bacterial canker of citrus which infects many citrus species from Asia, South America, Pacific Islands and the USA, resulting in defoliation, premature fruit drops and blemished fruit. Young trees are very susceptible while the disease appears sporadically on mature trees. The bacterium can survive as a latent infection on woody branches for many years. Mexican lime, grapefruit and trifoliate orange are highly susceptible while sour orange, lemon and sweet orange are moderately susceptible (CABI datasheet; ISPM 27/DP6, 2014; Schaad *et al.*, 2006; Timmer *et al.*, 2000).

Xylella fastidiosa (Variegated chlorosis)

- 61. MPI propose to keep PCR as the only phytosanitary measure for *X. fastidiosa*. PCR is the only diagnostic method to reliably detect the bacterium (Table 1; Harper *et al.*, 2010; Li *et al.*, 2013). ELISA, detached leaf bioassay and woody indexing, currently listed in the IHS, are not recommended because of the lack of sensitivity of these techniques which could result in false negative results (Li *et al.*, 2013; Timmer *et al.*, 2000; Rob Taylor, pers. comms., June 2015). Woody indexing and detached leaf bioassay are not even mentioned in the European standard diagnostic protocol for *X. fastidiosa* (EPPO standards PM7/24, 2004). The shoot tip grafting bioassay is a tissue culture technique for pathogen elimination and not a diagnostic assay.
- 62. MPI propose to remove *Xylella fastidiosa* pv. *citri* from the *Citrus* pest list and retain the name *Xylella fastidiosa* only. *X. fastidiosa* pv. *citri* is not a valid name. The sub-specie infecting citrus is called *X. fastidiosa* subsp. *pauca* causing Citrus variegated chlorosis, one of the most damaging disease of citrus. The distribution of the disease is limited to South America. The bacterium affects many, if not all, citrus species. In 2005, 43% (approximately 80 million) citrus trees in the state of São Paulo, Brazil were infected with the disease, resulting in loss of trees and production plus annual control costs estimated at US\$138 million (Harper et al., 2010; Li et al., 2013; Timmer et al., 2000).

- 63. *X. fastidiosa* has a wide host range including wild plants and has been found in latent state in many symptomless hosts. It causes serious damage on grapevine, peach and olive. It inhabits the xylem and is transmitted by several sharpshooter species which are not known to be present in New Zealand. It can also be transmitted by spittle bugs and New Zealand may have native and naturalised vectors. There are currently four recognised sub-species of *X. fastidiosa* which can be detected with the proposed PCR test (CABI datasheet; Janse and Obradovic, 2010; Loconsole *et al.*, 2014; Timmer *et al.*, 2000).
- 64. MPI will be introducing urgent measures in the next few months to apply the measures for *X. fastidiosa* to all host plants *in vitro*, including Citrus hosts. The urgent measures will likely be in place before this *Citrus* schedule is re-issued, so the requirement has been indicated in red font in the draft IHS on page 44 of this RMP.

FUNGI

Genera to species

- 65. MPI propose that four genera of fungi currently on the *Citrus* pest list be identified to species level which are associated with citrus (Appendix 1). Another two genera are proposed to be removed as there are no records of disease association with citrus (Farr and Rossman, 2006; Ngā Harore o Aotearoa New Zealand Fungi Databases, 2014). The fungi genera are:
 - a) *Aureobasidium* sp.: It has been removed from the pest list. *A. pullulans*, the only *Aureobasidium* species associated with citrus, is present in New Zealand and has a non-regulated status.
 - b) *Didymosphaeria* sp.: It has been removed from the pest list. No *Didymosphaeria* species have been found to be associated with citrus.
 - c) *Isaria* sp.: It has been removed from the pest list. No *Isaria* species have been found to be associated with citrus.
 - d) *Phomopsis* sp.: It has been replaced with *P. cytosporella*, which is the only regulated *Phomopsis* species associated with citrus.
 - e) *Septoria* sp.: It has been replaced with *S. limonum*, which is the only regulated *Septoria* species associated with citrus.
 - f) *Stenella* sp.: It has been replaced with *S. citri-grisea* (anamorph is *Mycosphaerella citri* that is already on the pest list), which is the only regulated *Stenella* species associated with citrus.

Ceratocystis fimbriata sensu lato complex (Ceratocystis blight)

- 66. MPI propose to add the testing method for *Ceratocystis fimbriata* in order to meet the requirements of the special conditions of section 2.2.8. The proposed testing method is: plating on arrival from the cuttings and on actively growing plants while in PEQ for *Ceratocystis fimbriata* with any suspect *C. fimbriata sensu lato* complex confirmed by PCR (Table 1). This testing only apply to cuttings of the genus *Citrus*. The combined testing of the original budwood and actively growing plant material in PEQ would increase the likelihood of detecting *C. fimbriata sensu lato* complex if it was present in the material.
- 67. *C. fimbriata* causes serious dieback on *citrus* in Colombia (CABI datasheet). The pathogen has a wide host range (at least 43 genera) including agricultural crops (e.g. *Actinidia* sp. [kiwifruit], *Colocasia* [taro], *Ficus carica* [fig], *Mangifera* [mango] and *Theobroma* [cacao]), ornamentals (e.g. *Metrosideros polymorpha* [same genus as Pōhutukawa],

Platanus [London plane]) and forestry species (e.g. *Acacia* and *Eucalyptus*). *C. fimbriata* has been recorded from many countries (at least 61) (CABI datasheet; Harrington, 2015).

68. *C. fimbriata* infection typically occurs through fresh wounds by tools and equipment, insects, wind or rain. Root infections are also common. For some host species, transmission by Ambrosia beetle frass is an important means of transmission. The fungus causes dark reddish-brown to purple to deep-brown or black staining in the xylem (CABI datasheet).

Elsinoë australis (Sweet orange scab)

- 69. MPI propose to replace the current phytosanitary requirement for growing season inspection with plating on semi-selective medium or PCR for *Elsinoë australis* (Table 1). The fungus only induces symptoms on fruit and the post entry quarantine period is too short for plants to produce fruits. Furthermore the fungus cannot be reliably distinguished by morphological or cultural characteristics from *E. fawcettii* which is present in New Zealand and is non-regulated (Chung, 2011; Hyun *et al.*, 2009).
- 70. *E. australis* causes Sweet orange scab which affects the fruit but *not* the leaves of most sweet orange and some mandarin cultivars. The fungus is common in humid citrus-growing areas of South America but it has not been reported elsewhere (Chung, 2011). The introduction and establishment of *E. australis* in a new area could have quarantine implications for the marketing of fresh fruit.

Eremothecium coryli (Dry rot)

- 71. MPI propose to add *Eremothecium coryli* (syn. *Nematospora coryli*) to the *Citrus* pest list. The fungus is associated with citrus (Fawcett, 1929; Shivas *et al.*, 2005) and it is regulated in New Zealand (BORIC, Table 1). *E. coryli* was isolated from seeds of native lime (*Citrus australis*), mandarin and lemon in Australia with evidence of being present for decades (Shivas *et al.*, 2005). The fungus was previously reported on grapefruit, lemon, orange and tangerines in California, USA (Fawcett, 1929).
- 72. As management measure, MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this fungus and will re-assess if the situation changes (Table 1). At this stage, MPI consider that there is insufficient justification for requiring specific phytosanitary measures. MPI believe that the importation of citrus cuttings and tissue culture is an unlikely pathway because symptoms have only been reported on fruit and nuts suggesting that the infection is localised (Shivas *et al.*, 2005).
- 73. *E. coryli* is a serious pathogen of seeds of many species of tropical and sub-tropical plants including bean, cashew, coffee, cotton, cowpea, hazelnut, macadamia, pigeon pea, soybean, tomato. The fungus is mainly transmitted by sap-sucking pentatomid (Hemiptera) insects feeding on fruits and nuts. It is present in Africa, Asia, Europe, North and South America (CABI datasheet; Miles *et al.*, 2009; Shivas *et al.*, 2005).

Phyllosticta citricarpa (Black spot)

- 74. MPI propose to replace the current phytosanitary requirement for growing season inspection with PCR for *Phyllosticta citricapra* (syn. *Guignardia citricarpa*, Table 1). Latent infections are common on leaves and symptoms are mainly observed on fruits (EPPO datasheet; CABI datasheet). The post entry quarantine period is too short for plants to produce fruits. Plating is not an option as this is a generic method and additional testing (e.g. morphologically, PCR) would be required for identification at species level.
- 75. *P. citricarpa* causes Black spot disease which produces external blemishes of fruit making them unsuitable for the fresh market. Sometimes the internal quality of fruit may also be

affected. Susceptible citrus species include grapefruit, lemon, lime, mandarin and sweet orange. The disease is present in Australia, South Africa and some part of Asia. Once black spot disease is well established, fruit losses may periodically be severe (EPPO datasheet; CABI datasheet).

Plenodomus tracheiphilus (Mal socco)

- 76. MPI propose to replace the current phytosanitary requirement for the growing season inspection with PCR for *Plenodomus tracheiphilus* (syn. *Phoma tracheiphila*) (Table 1). Although *P. tracheiphilus* can induce symptoms on plants, symptoms may not have time to develop during the post entry quarantine period. Furthermore by the time the symptoms are visible, the disease is well established. The fungus has also been detected from asymptomatic citrus plants. PCR is the only technique that is sensitive, reliable and rapid for the early detection of the fungus (Cacciola and di San Lio, 2007; Donovan *et al.*, 2014).
- 77. *P. tracheiphilus* causes a destructive vascular disease of citrus named Mal secco which is present in the Mediterranean countries and the Black Sea region. The principal host is lemon, but the fungus has also been reported on many other citrus species. The disease reduces the quantity and quality of lemon production. Symptoms range from leaf and shoot chlorosis, wilting and dieback to complete canopy collapse (Cacciola and di San Lio, 2007; Donovan *et al.*, 2014).

LIBERIBACTERS

'Candidatus Liberibacter africanus' and 'Ca. Liberibacter asiaticus' (Huanglongbing)

- 78. MPI propose to include PCR in addition to woody indexing for the detection of '*Ca*. Liberibacter africanus' and '*Ca*. Liberibacter asiaticus' (Table 1). Woody indexing alone is not appropriate for the detection of '*Candidatus* Liberibacter spp.' because the liberibacter titre can be very low in the early stages of infection and may be irregularly distributed in the host plant, which may produce false negative results (Timmer *et al.*, 2000). However, woody indexing is still a valuable test for the detection of unknown organisms. PCR can sometimes be too specific resulting in false negative results as in the case of the recently discovered '*Ca*. Liberibacter americanus' (Paragraphs 81-83).
- 79. The candidate plants for biosecurity clearance must be tested by PCR after they have been grown at 18-25°C for a minimum of five months (Folimonova *et al.*, 2009; Razi *et al.*, 2012). This is because PCR can detect liberibacters from two to five months after grafting depending on the liberibacter species and the citrus species while symptoms may take longer to develop (Teixeira *et al.*, 2005; Tsai *et al.*, 2008). The temperature at which the plants are grown is also very important for the optimal development of symptoms and increase in titre as liberibacters are temperature sensitive (Bové, 2006).
- 80. '*Ca.* Liberibacter africanus' and '*Ca.* Liberibacter asiaticus' are the causal agents of Huanglongbing, also known as citrus greening, one of the most damaging diseases of citrus. Many, if not all, citrus species are susceptible to this disease. Liberibacters are phloem-limited bacteria causing blotchy mottle on leaves and are efficiently transmitted by several psyllid species (not known to be present in New Zealand). '*Ca.* Liberibacter africanus' is only found in Africa and '*Ca.* Liberibacter asiaticus' is found in Asia, Brazil and in Florida, USA. In the absence of vectors, the disease is spread mainly through asymptomatic plants. (CABI datasheet; GISD, 2014; Timmer *et al.*, 2000).

'Candidatus Liberibacter americanus' (Huanglongbing) (new)

81. MPI propose to include '*Ca*. Liberibacter americanus', a new species of liberibacters on the *Citrus* pest list. This liberibacter has been associated with Huanglongbing disease, one of the

most damaging diseases of citrus (Lopes and Frare, 2008). MPI's pest risk assessment of '*Ca*. Liberibacter americanus' reported that the presence of '*Ca*. Liberibacter americanus' in New Zealand would have an immediate impact on export and domestic sales (Table 2).

- 82. MPI propose PCR and woody indexing as phytosanitary measures for '*Ca*. Liberibacter americanus' (Table 1). The candidate plants for biosecurity clearance must be tested by PCR after they have been grown at 18-25°C for a minimum of five months (Folimonova *et al.*, 2009; Razi *et al.*, 2012). The time and temperature are critical for the optimal development of symptoms and increase in titre of liberibacters (Bové, 2006).
- 83. '*Ca.* Liberibacter americanus' was detected by PCR on several *Citrus* species from Brazil in 2005 (Lopes and Frare, 2008). Huanglongbing disease has recently been identified in two areas of La Guajira region, Columbia, threatening the citus industry of 83,000 hectares (ProMed alert, February 2016, <u>http://www.promedmail.org/</u>). The liberibacter was also detected in the psyllid vector for '*Ca.* Liberibacter asiaticus', *Diaphorina citri*, which is established in South, Central and North America and the Caribbean (Teixeira et al., 2005). *D. citri* is not known to be present in New Zealand and it has a regulated status (BORIC).

'Candidatus Liberibacter caribbeanus' (Huanglongbing-like) (new)

- 84. MPI propose to include '*Ca*. Liberibacter caribbeanus' on the *Citrus* pest list. The new liberibacter species has been recently reported during the American Phytopathological Society Annual Meeting held in California, USA in August 2015 (abstract publication). It was found on sweet orange with typical symptom of Huanglongbing disease from South America. The liberibacter was also detected in *Diaphorina citri*, the Asian psyllid vector that is not known to be present in New Zealand (BORIC; Keremane *et al.*, 2015).
- 85. As management measure, MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this liberibacter and will re-assess if the situation changes (Table 1). This is because '*Ca*. Liberibacter caribbeanus' can be detected by real-time PCR using the same primers and probes routinely used for the detection of '*Ca*. Liberibacter asiaticus' which is already on the pest list (Table 1; Keremane *et al.*, 2015).

PHYTOPLASMAS

Generic PCR for phytoplasmas

- 86. MPI propose to replace the current phytosanitary requirement for woody indexing with PCR for phytoplasmas. The proposed PCR is a generic nested-PCR or real-time PCR which would detect any phytoplasmas, including Australian citrus dieback and the newly reported phytoplasmas in citrus (Chen *et al.*, 2009; Liefting *et al.*, 2011; Teixeira *et al.*, 2008). Furthermore, PCR is much more sensitive than woody indexing for the detection of phytoplasmas (Appendix 2; Weintraub and Jones, 2010).
- 87. Woody indexing is not suitable because the period of time for symptoms to develop is very variable, ranging from five months to more than two years (Ochoa-Corona and Ward, 2010). Furthermore, phytoplasmas are often in low titre and unevenly distributed in the plant which may result in false negative results when using woody indexing (EPPO standards PM7/61, 2006; Weintraub and Jones, 2010).

Australian citrus dieback

88. MPI propose to move Australian citrus dieback from the category of diseases of unknown aetiology to the phytoplasma group with a generic phytoplasma PCR as phytosanitary

measure (refer to paragraphs 86-87 for details; Table 1). A phytoplasma was identified using molecular technique (Davis *et al.*, 1997).

89. Australian citrus dieback produces symptoms similar to Huanglongbing disease. Grapefruit is the most susceptible species but the disease can affect most citrus species. The phytoplasma is graft transmissible with difficulty (Broadbent *et al.* 1976; Timmer *et al.* 2000).

'Candidatus Phytoplasma asteris' (Huanglongbing-like) (new)

- 90. MPI propose to include '*Candidatus* Phytoplasma asteris' on the *Citrus* pest list with a generic phytoplasma PCR (refer to paragraphs 86-87 for details) as a specific phytosanitary measure (Table 1). The phytoplasma has been detected by sequence analysis and electron microscopy on mandarin, sweet orange and pummelo showing Huanglongbing-like symptoms (i.e. leaf yellowing) and also in asymptomatic mandarin leaves from China (Chen *et al.*, 2009). More recently, it was also detected on kumquat and Mexican lime with Huanglongbing-like symptoms from Mexico (Arratia-Castro *et al.* 2014; Poghosyan *et al.* 2015). Huanglongbing and Huanglongbing-like diseases are devastating diseases of citrus that would be damaging to the New Zealand's citrus industry.
- 91. MPI's pest risk assessment of the 16SrI-B phytoplasma group also called aster yellows subgroup B (not known to be present in New Zealand) to which '*Ca*. Phytoplasma asteris' belongs to, reported that given the wide host range of this group of phytoplasmas and the low vector specificity (phloem-feeding leafhoppers; one *Macrosteles* species, *M. fieberi* is present in New Zealand [Gordon, 2010]), it could potentially have an important economic impact on other crops in New Zealand including apples, carrots, grapes, onions, potatoes, sweet corn and tomatoes (Table 2). Some symptoms can affect flowers and sterility of flowers which would have severe impacts on seed production or ornamental crops.

'Candidatus Phytoplasma aurantifolia' (Witches'-broom)

- 92. MPI propose to replace the current phytosanitary requirements for woody indexing with PCR for *'Candidatus* Phytoplasma aurantifolia' (Table 1). PCR is much more sensitive than woody indexing (refer to paragraphs 86-87 for details).
- 93. '*Ca.* Phytoplasma aurantifolia' was the first phytoplasma detected in citrus more than 30 years ago. It causes witches'-broom disease, resulting in important losses in Mexican lime trees from Oman. It also naturally infects citron and is experimentally graft-transmissible to a number of citrus species (EPPO standards PM7/61, 2006; Timmer *et al.*, 2000). It is transmitted by a leafhopper, *Hishimonus phycitis* which is not known to occur in New Zealand and has a regulated status (BORIC). The phytoplasma was then reported from India, Iran and United Arab Emirates (Timmer *et al.*, 2000).

'Huanglongbing-associated 16SrIX-A phytoplasma (new)

- 94. MPI propose to add the HLB (Huanglongbing)-associated 16SrIX-A phytoplasma on the *Citrus* pest list with a generic phytoplasma PCR (refer to paragraphs 86-87 for details) as a specific phytosanitary measure (Table 1). The phytoplasma was identified by sequence analysis and electron microscopy on Huanglongbing symptomatic sweet orange separately and in mixed infection with '*Candidatus* Liberibacter asiaticus' from Brazil (Teixeira *et al.*, 2008). Huanglongbing disease is a devastating disease of citrus that would be damaging to the New Zealand's citrus industry. Disease expression may be limited by temperature but symptomless plants may be distributed throughout the country.
- 95. MPI's pest risk assessment of the 16SrIX-A phytoplasma group also called pigeon pea witches' broom subgroup A (not known to be present in New Zealand) reported that given the limited host range of this group of phytoplasmas and the possibility of transmission by

leafhoppers, it could potentially have an economic impact on other crops of the Leguminosae family including peas and beans in New Zealand (Table 2). Phytoplasmas of the 16SrIX-A phytoplasma group are present in Brazil, Colombia, Jamaica, Puerto Rico and USA (Teixeira *et al.*, 2008; Dickinson and Hodgetts, 2013).

Variant (A*) of subgroup 16Srll-A phytoplasma (new)

96. A phytoplasma named as variant (A*) of subgroup 16SrII-A is a new phytoplasma that is not proposed to be added to the *Citrus* pest list. There is insufficient evidence to justify regulating; only molecular data are available (Lou *et al.*, 2014). At least two methods of detection (e.g. morphology and molecular test) should be used to confirm the identification of a new organism. Furthermore, it would still be detected by the same PCR assay used for the other regulated phytoplasmas. The phytoplasma was detected by PCR on citrus with Huanglongbing-like symptoms from China that also tested positive for '*Ca*. liberibacter asiaticus'.

VIROIDS

Citrus bark cracking viroid, Citrus bent leaf viroid and Hop stunt viroid [citrus strain]

- 97. MPI propose the replace the current phytosanitary measures of sPAGE and PCR from graft inoculated citron extract with PCR after the candidate plants for biosecurity clearance have been grown at 28-32°C temperature for a minimum of 3 months and woody indexing (Table 1) for *Citrus bark cracking viroid* (syn. Citrus viroid IV), *Citrus bent leaf viroid* (syn. Citrus viroid I, Citrus variable viroid), and *Hop stunt viroid* [citrus strain] (syns. Citrus viroid II, Citrus cachexia viroid).
- 98. Woody indexing is a valuable test for the detection of known and unknown viroids. This has been demonstrated by the recent identification of a new viroid, *Citrus viroid-VII* by Chambers *et al.* (2016; refer to paragraphs 104-105 for details). PCR is more sensitive, less time consuming and reliable than sPAGE. PCR is also the only method which can differentiate strains of *Hop stunt viroid*, some of which are present in New Zealand and have a non-regulated status (BORIC; Hadidi *et al.*, 2003; Veerakone *et al.*, 2015).
- 99. The PCR must be carried out after the candidate plants for biosecurity clearance have been grown at 28-32°C for a minimum of three months which is equivalent to a full summer (Table 1). Temperature at which the plants are grown is very important for the increase in titre as viroids are temperature sensitive (Barbosa *et al.*, 2002; Hadidi *et al.*, 2003).

Citrus viroid V and Citrus viroid VI (new)

- 100. MPI propose to add *Citrus viroid V* (CVd-V) and *Citrus viroid VI* (CVd-VI; syn. Citrus viroid-OS [original sample]) to the *Citrus* pest list with the following phytosanitary measures: PCR after the candidate plants for biosecurity clearance have been grown at 28-32°C temperature for a minimum of 3 months and woody indexing (Table 1; refer to paragraphs 98-99 for details).
- 101. MPI's pest risk assessment of CVd-V and CVd-VI reported that the viroids could be widespread before they are detected because the affects of CVd-V and CVd-VI alone are likely to be subtle and even symptomless (Hadidi *et al.*, 2003; Serra *et al.*, 2008b). Furthermore there is the potential for synergy in the presence of other citrus viroids resulting in a greater impact on citrus production (Table 2). CVd-VI has been found in mixed infection with another four viroids causing exocortis-like symptoms; the viroids were *Citrus bent leaf viroid* (regulated), *Citrus bark cracking viroid* (regulated), *Citrus dwarfing viroid* (non-regulated status) and *Hop stunt viroid* [citrus strain] (regulated) (Ito *et al.*, 2002).

Synergetic interactions has also been demonstrated for CVd-V with *Citrus bent leaf viroid* (regulated) and *Citrus dwarfing viroid* (non-regulated) (Serra *et al.*, 2008a).

- 102. CVd-V was found on citron, desert-lime, finger-lime, lemon, lime, mandarin, sweet orange, tangelo, and tangor. The viroid was reported from Japan, China, Nepal, Iran, Sultanate of Oman, Spain, USA and Pakistan (Table 2, Serra *et al.*, 2008a & 2008b). CVd-V was also detected on a citrus relative, *Atalantia citroides* (Bani Hashemian *et al.*, 2010).
- 103. CVd-VI was detected on hybrids of *C. reticulata* and *C. sinensis* from Japan (Table 2, Ito et al., 2001). CVd-VI is graft transmissible to *C. medica* cv. Eureka (Ito et al., 2001) which is the generic indicator plant for citrus viroids (Duran-Vila et al., 2000). The viroid was also detected from an asymptomatic and symptomatic Japanese persimmon (*Diospyros kaki*) (Nakaune and Nakano, 2008). Persimmon sales were worth NZ\$4 and 8.2 millions in domestic and export markets, respectively (Fresh Facts, 2014).

Citrus viroid VII (new)

- 104. MPI propose to add *Citrus viroid VII* (CVd-VII) to the *Citrus* pest list with the following phytosanitary measure: PCR after the candidate plants for biosecurity clearance have been grown at 28-32°C temperature for a minimum of 3 months and woody indexing (Table 1; refer to paragraphs 98-99 for details).
- 105. CVd-VII was recently reported during the 20th International Organization of Citrus Virologists, held in China in May 2016 (abstract publication). The viroid was found on symptomless Lisbon lemon by woody indexing on citron 'Etrog' in Australia. The current PCR failed to detect using the specific primers developed for other citrus viroids. A specific PCR test was developed and it successfully detected CVd-VII from Lisbon lemon in the field (Chambers *et al.*, 2016).

VIRUSES

Apple stem grooving virus (tatter leaf)

- 106. MPI propose to remove Apple stem grooving virus (ASGV, syn. Citrus tatter leaf virus) from the Citrus pest list (Table 1) and to change its regulatory status to non-regulated. Citrus tatter leaf virus is considered to be the same specie as ASGV by the International Committee on Taxonomy of Viruses (ICTV, 2014) based on morphology, serology and molecular data (Yoshikawa et al., 1993). ASGV is present in New Zealand, it was isolated from apple (Malus × domestica) and nandina (Nandina domestica) (Veerakone et al., 2015).
- 107. A recent sequence analysis of ASGV and CTLV sequences available from the GenBank database showed that they are closely related with no evidence of strains (Dr Bénédicte Lebas, PHEL, pers. coms, March 2016). CTLV from citrus and lily is indistinguishable from ASGV from *Rosaceae* fruit trees biologically, serologically, in genome organization and in nucleotide sequence (Yoshikawa *et al.*, 1993). Furthermore an ASGV isolate of Japanese pear induced symptoms similar to those by CTLV from citrus on Rusk citrange (Yoshikawa *et al.*, 1993; Yoshikawa, 2000).
- 108. ASGV can affect many citrus species without any symptoms. Sensitive tree becomes stunted or often dies. CTLV has been reported from Australia, China, Japan, South Africa and USA (Frison and Taher, 1991; Roistacher, 1991; Timmer *et al.*, 2000). ASGV has also been reported from apple, apricot, kiwifruit, pear and lily plants. ASGV is probably present in all apple growing regions (Clover *et al.*, 2003; Yoshikawa, 2000).

Citrus chlorotic dwarf-associated virus (Citrus chlorotic dwarf)

- 109. MPI propose to update the name from Citrus chlorotic dwarf to *Citrus chlorotic dwarf-associated virus* (CCDaV) and to replace the current specific phytosanitary measures for woody indexing with PCR which is more sensitive and reliable than woody indexing (Table 1; Appendix 2). CCDaV was detected by next generation sequencing from sample with citrus chlorotic dwarf disease. The virus was consistently detected from samples with citrus chlorotic dwarf disease symptoms by PCR. Furthermore typical symptoms of citrus chlorotic dwarf disease were observed when inoculated with CCDaV. The virus is a new member of the *Geminiviridae* family (Loconsole *et al.*, 2012).
- 110. The disease incidence is 60-70% in Mersin, one of the main citrus growing region of Turkey. Production losses of up to 50% have been reported on grapefruit due to the reduction in the number and size of the fruits. The disease is present in Turkey and it can affect nearly all cultivars of citrus with lemons, grapefruit and some mandarin and tangelo being the most susceptible hosts (Kersting *et al.*, 1996; Korkmaz *et al.*, 1995; Loconsole *et al.*, 2012).

Citrus leaf rugose virus, Citrus variegation virus, Satstuma dwarf virus

- 111. MPI propose to include PCR as an alternative test to woody indexing for the specific phytosanitary measures of *Citrus leaf rugose virus*, *Citrus variegation virus* (syn. Citrus infectious variegation virus) and *Satsuma dwarf virus* (Table 1). This will allow greater flexibility on the choice of method of detection.
- 112. Some background information of these viruses is provided below:
 - a) *Citrus leaf rugose virus* (CiLV): CiLRV can affect a wide range of citrus cultivars but there is little evidence for rapid natural spread in citrus. It was reported from USA, the Mediterranean basin and Australia (Roistacher, 1991; Timmer *et al.*, 2000).
 - b) *Citrus variegation virus* (CVV, syn. Citrus infectious variegation virus): CVV can affect a wide range of citrus cultivars but there is little evidence for rapid natural spread in citrus. It was reported from Australia, USA and the Mediterranean basin (Frison and Taher, 1991; Roistacher, 1991; Timmer *et al.*, 2000).
 - c) *Satsuma dwarf virus* (SDV; syn. Citrus mosaic virus, Navel infectious mottle virus, Natsudaidai dwarf virus): Satsuma mandarin is very susceptible to SDV, resulting in stunted trees and reduction of fruit yields. SDV may induce mild symptoms or no symptoms on other cultivars. SDV is present in Japan and has recently been reported in China on lemon (Roistacher, 1991; Timmer *et al.*, 2000; Guo *et al.*, 2015).

Citrus leathery leaf virus and Citrus yellow mottle virus

- 113. MPI propose to remove the specific phytosanitary measures for Citrus leathery leaf virus and Citrus yellow mottle virus but retain the name on the *Citrus* pest list (Table 1). These viruses have never been found since their initial reports. There is not enough evidence to justify retaining the specific phytosanitary measures. MPI also propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to these viruses and will re-assess if the situation changes.
- 114. Some background information of these viruses is provided below:
 - a) **Citrus leathery leaf virus** was reported in one publication from India in 1979. It affects mandarin, sweet orange, Rangpu lime and lemons causing thickening of the

new leaves, death of apical buds and dieback (Frison and Taher, 1991). Furthermore woody indicators which would detect this disease, are required for the detection of other *Citrus* pests (e.g. *Citrus reticulata* for liberibacters).

b) Citrus yellow mottle virus was reported on a few, very old citrus trees in one publication from Japan in 1984 (Bové *et al.*, 2010). Furthermore woody indicators which would detect this disease, are required for the detection of other *Citrus* pests (e.g. *Citrus sinensis* for liberibacters).

Citrus leprosis virus C

- 115. MPI propose to replace the specific phytosanitary measure for woody indexing with PCR as the phytosanitary measure for *Citrus leprosis virus C* (CiLV-C, Table 1). The virus is not easily graft transmissible from symptomatic citrus material. However the virus can be detected by PCR which is a more sensitive method than woody indexing (Appendix 2; Brlansky and Hartung, 2013; Locali-Fabris, 2006; Lovisolo, 2001).
- 116. CiLV-C causes a serious disease in Brazil and has now spread in several South and Central American countries. It was initially described in Florida, USA but has not been found since the 1960s (Roistacher 2003; Timmer *et al.*, 2000; Bastianel *et al.*, 2010). Trees infected with CiLV-C have severe defoliation with premature fruit frop leading to trees death within 3-5 years (Lovisolo, 2001; Roy *et al.*, 2015). Sweet orange is the most susceptible host of CiLV-C which has also been identified on mandarin and sour orange (Frison and Taher, 1991).
- 117. The most important method for spread and transmission of CiLV-C is through the mite vector, *Brevipalpus* spp., including *B. californicus*, *B. obovatus* and *B. phoenicis* which are present in New Zealand but vector regulated (BORIC). Furthermore a considerable number of plant species have been shown under experimental conditions to be susceptible to CiLV-C by vector transmission: 59 species (from 24 families) including ornamental plants, vegetable and fruit crops and herbaceous, bushy and woody wild species (Bastianel *et al.*, 2010; Nunes *et al.*, 2012; Garita *et al.*, 2014).

Citrus leprosis virus cytoplasmic type 2 and Citrus leprosis virus nuclear types (new)

- 118. MPI propose to add *Citrus leprosis virus* cytoplasmic type 2 (CiLV-C2) and *Citrus leprosis virus* nuclear type (CiLV-N) to the *Citrus* pest list with PCR as a specific phytosanitary measure (Table 1). PCR is the only method suitable for detecting these viruses. Symptoms produced by CiLV-C2 and CiLV-N are not distinguishable by woody indexing and woody indexing is not as sensitive as PCR (Appendix 2; Hartung *et al.*, 2013; Roy *et al.*, 2014).
- 119. CiLV-C, CiLV-N and *Citrus leprosis virus C* (CiLV-C, already on the pest list) cause Citrus leprosis disease, one of the most economically important viral diseases of citrus in South and Central America. Brazilian citrus growers manage the disease by applying acaricides costing ≈ US\$80 million every year to control the mite vector, *Brevipalpus* spp. (Roy *et al.*, 2013). Some of the *Brevipalpus* spp. are present in New Zealand but vector regulated, such as *B. californicus* which is vector of CiLV-C, CiLV-C2 and CiLV-N (BORIC; Hartung *et al.*, 2013; Roy *et al.*, 2013, 2014 & 2015).
- 120. CiLV-C2 and CiLV-N are rare viruses detected on sweet orange from Columbia and Brazil, respectively. CiLV-N was also detected on grapefruit, lemon, lime, mandarin, sour orange and sweet lime while CiLV-C2 was also found on *Hibiscus rosa-sinensis*. CiLV-N was found in mixed infection with CiLV-C2 on sweet orange and on two species growing around orchards, *Dieffenbachia* sp. and *Swinglea glutinosa*. Symptoms induced by both viruses are not distinguishable from *Citrus leprosis virus C* (Hartung *et al.*, 2013; Roy *et al.*, 2014 & 2015; Timmer *et al.*, 2000).

121. Another virus associated to citrus leprosis nuclear type disease was recently reported at the 20th International Organization of Citrus Virologists, held in China in April 2016 (Chabi-Jesus *et al.*, 2016). The virus was identified by morphology and next generation sequencing on sweet orange from Brazil. At this stage, MPI do not propose to include this virus on the *Citrus* pest list. MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this virus and will re-assess if the situation changes.

Citrus necrotic spot virus, Hibiscus green spot virus 2 and Iranian citrus ringspot-associated virus (new)

- 122. MPI propose to add *Citrus necrotic spot virus*, *Hibiscus green spot virus* and *Iranian citrus ringspot-associated virus* on the *Citrus* pest list because they have been found to be associated with citrus (Table 1). At this stage, MPI consider that there is insufficient justification for imposing specific phytosanitary measures based on the reasons described below. MPI propose to set up active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to these viruses and will re-assess if the situation changes.
 - a) *Citrus necrotic spot virus* (CiNSV; tentative name) is not a recognised species by the International Committee on Taxonomy of Viruses (ICTV, 2014). There is only one published paper by Cruz-Jaramillo *et al.* (2014) from Mexico, from which citrus nursery stock has never been sourced from in the last 15 years (QuanCargo). CiNSV was detected on grapefruit, sour orange, sweet orange and tangerine trees with leprosis-like symptoms from Mexico. The virus was identified by morphology and by sequencing the full genome using next generation sequencing (NGS). The authors concluded that there is a strong correlation between the presence of CiNSV and leprosis symptoms, considering that no other extant virus was detected using PCR and ELISA assays, and given that no additional RNA sequences were identified by NGS (Cruz-Jaramillo *et al.*, 2014).
 - b) *Hibiscus green spot virus 2* (HGSV-2) was initially identified by sequencing and morphology on a single 20+ year old *Citrus volkameriana* (lemon) tree with leprosis-like symptoms and on *Hibiscus arnottianus* leaves with symptoms of hibiscus green spot from Hawaii. *H. arnottianus* was found to be infested with *Brevipalpus* spp., the leprosis-virus vector, some of which are present in New Zealand but vector regulated (BORIC; Melzer *et al.*, 2012). More recently, HGSV-2 was found on mandarin, navel orange and *H. tiliaceus* from Hawaii (Roy *et al.*, 2015). Citrus nursery stock has never been sourced from Hawaii in the last 15 years (QuanCargo). Furthermore, *C. volkameriana* is not known to be grown in New Zealand (Nikki Johnson, pers. comm., 24 June 2015).
 - c) *Iranian citrus ringspot-associated virus* (IrCRSaV; tentative name) is not a recognised species by the International Committee on Taxonomy of Viruses (ICTV, 2014). There is only one published paper by Sadeghi *et al.* (2016) from Iran, from which citrus nursery stock has never been sourced from in the last 15 years (QuanCargo). IrCRSaV is a newly discovered virus causing chlorotic ringspot and necrotic spots on leaves and twigs on sweet orange in Iran. The effected plants are less vigorous although no specific symptoms were found on fruit. The virus was identified by electron microscopy (morphology), molecular and biological assays (Sadeghi *et al.*, 2016).

Citrus psorosis virus

123. MPI propose to remove *Citrus psorosis virus* (CPsV; syn. Citrus ringspot virus) from the *Citrus* pest list (Table 1). CPsV has been found on two sweet orange trees and one tangerine

tree from a collection of 273 citrus trees in New Zealand (Quemin *et al.*, 2011). MPI propose to update the BORIC database with *Citrus psorosis virus* being non-regulated and list Citrus psorosis virus A and B as synonyms.

124. CPsV causes a complex of diseases. Citrus psorosis A is the most common form of psorosis, characterized by the presence of bark-scaling in the trunk and limbs of infected trees. Citrus psorosis B is less common but more aggressive than psorosis A. Psorosis A and B may occur in the same tree at the same time but in different tissues (Achachi *et al.*, 20104; Velázquez *et al.* 2012). They are considered isolates of CPsV by the International Committee on Taxonomy of Viruses (ICTV, 2014).

Citrus sudden death-asssociated virus (new)

- 125. MPI propose to add *Citrus sudden death-associated virus* (CSDaV) on the *Citrus* pest list with PCR as a specific phytosanitary measure (Table 1). CSDaV can be detected by PCR from symptomatic and asymptomatic material. Woody indexing is not an option because it may take more than two years for symptoms to develop (Maccheroni *et al.*, 2005; Yamamoto *et al.*, 2001).
- 126. CSDaV is associated with Citrus sudden death which has been responsible for the decline and elimination of almost four million trees, representing 10% of the 40 million trees in the affected region or 2% of the 200 million sweet orange trees in Brazil, posing a serious threat to the citrus industry (Bassanezi *et al.*, 2007; Yamamoto *et al.*, 2001).
- 127. MPI's pest risk assessment of CSDaV concluded that the economic losses would come from the expression of the disease and possible impacts on trade with countries (Table 2). Citrus sudden death disease 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 the disease. However, the presence of the disease may limit future rootstock options.
- 128. The disease effects sweet orange and some mandarins grafted on either Rangpur lime or Volkamerian lemon causing symptoms such as defoliation, reduction in new shoots and death of the root system. CSDaV was detected in aphid vectors such as *Aphis gossypii*, *A. spiraecola* and *Toxoptera citricida*; these species which are vectors regulated in New Zealand (BORIC). The virus has also been detected from leafhoppers but the species names were not provided (Maccheroni *et al.*, 2005; Yamamoto *et al.*, 2011).

Citrus tristeza virus

- 129. MPI propose to remove *Citrus tristeza virus* (CTV) from the *Citrus* pest list (Table 1). A recent publication by Harper and Pearson (2015) showed that all the major CTV strains are present in New Zealand and that they exist as complex mixture of strains. The economically important rootstock species *Poncirus trifoliata* is resistant to most isolates of CTV, but not to members of the CTV resistance-breaking strain presently found in New Zealand (Harper *et al.*, 2010).
- 130. CTV isolates vary in pathogenicity and are often present in mixed-infection (Timmer *et al.* 2000). Symptom expression in citrus is highly variable and affected by environmental conditions, host species and the population of CTV strains (EPPO standards PM7/31, 2004). Most citrus species are susceptible to CTV including *Citrus australasica* (finger-lime) and it is present in all citrus-growing countries (EPPO standards PM7/31, 2004). CTV is transmitted by grafting and by its aphid vectors, such as *Aphis gossypii* which is present in New Zealand but vector regulated (BORIC).

Citrus yellow mosaic virus, Citrus yellow vein clearing virus

- 131. MPI propose to replace woody indexing with PCR as specific phytosanitary measure of *Citrus yellow mosaic virus* (CYMV) and *Citrus yellow vein clearing virus* (CYCV; syn. Yellow vein clearing of lemon) (Table 1). PCR is more reliable and sensitive than woody indexing for the detection of these viruses (Barman, 2013; Chen *et al.*, 2014), which can cause significant damages:
 - a) *Citrus yellow mosaic virus*: CYMV can affect most citrus species. It has been reported from India with a disease incidence of 10-70%. Yield reduction of up to 77% has occurred in 10 year old trees (Ahlawat *et al.*, 1996; Timmer *et al.*, 2000).
 - b) Citrus yellow vein clearing virus: CYCV (syn. Yellow vein clearing of lemon) causes almost 20% yield reduction in lemon plants with CYCV symptoms from China. The virus was also reported in lemon from Pakistan. CYCV was transmitted by grafting to dweet tangor, mash grapefruit, sour orange, sweet orange with the development of variable symptoms and no symptoms were observed on Etrog citron, Mexican lime and Ponkan (Chen *et al.*, 2014; Timmer *et al.*, 2000).

Indian citrus ringspot virus (new)

- 132. MPI propose to add *Indian citrus ringspot virus* (ICRSV) on the *Citrus* pest list with PCR as phytosanitary measure (Table 1). PCR is the most reliable and sensitive method of detection for this virus (CABI, 2015; Milne *et al.*, 2007; Rustici *et al.*, 2000).
- 133. Indian citrus ring spot disease is limited to India where incidence of up to 100% in Kinnow mandarin (*Citrus nobilis* Lour x *C. deliciosa* Tenora) orchards in Northern India were reported. Mosambi sweet orange (*C. sinensis*) is another highly susceptible host while citron and rough lemon are also natural hosts. Severely affected trees suffer reduced fruit yield and decline with plant dieback symptoms leading to death after a few years. ICRSV appears to be seed-borne but not seed transmissible and natural vectors for its transmission have not been found so far (Milne *et al.*, 2007; Prabha and Baranwal, 2011; Rustici *et al.*, 2000).
- 134. MPI's pest risk assessment of ICRSV reported that if the virus became established in New Zealand there is likely to be some negative impact on the citrus industry (Table 2). As hosts may be asymptomatic, at least in the initial stages of infection, plant material could be distributed 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. Another mode of spread is through mechanical transmission and therefore improper sanitation such as the indiscriminate use of non-sterile pruning shears could lead to infection of other host plants.

Olive latent virus 1 (new)

- 135. MPI propose to add *Olive latent virus 1* (OLV-1) to the *Citrus* pest list with PCR as phytosanitary measure (Table 1). PCR is more sensitive and reliable than other diagnostic methods for the detection of this virus (Martelli, 2013). OLV-1 was detected by PCR from asymptomatic and symptomatic trees of citrus and olive (Martelli *et al.*, 1996). Woody indexing is not an option because indicators may not develop symptoms (Félix *et al.*, 2007) while herbaceous indexing is challenging due to the presence of inhibitors in citrus and the low titre of viruses in woody perennial hosts (Roistacher, 1991).
- 136. MPI's pest risk assessment of OLV-1 reported that if OLV-1 became established in New Zealand there is likely to be some negative impact on several horticultural industries. The known natural hosts of OLV-1 to date include species from several plant families: citrus (*Citrus* sp., Rosaceae), olive (*Olea europaea*, Oleaceae), tomato (*Solanum lycopersicum*,

Solanaceae) and tulip (*Tulipa* sp., Liliaceae) which are all grown both as commercial crops and in domestic gardens in New Zealand. OLV-1 is present in Turkey,

137. OLV-1 has no known vector however it has been shown to be seed and pollen transmissible in olive. It has also been demonstrated that the virus is released in soil from the roots of infected *Nicotiana benthamiana* plants and infected healthy roots of new *N. benthamiana* plants grown in that soil. The virus is also transmitted by grafting and mechanically (Félix *et al.*, 2007). To date, OLV-1 is present in Europe, Middle East, USA and Japan (Martelli *et al.*, 1996; Kanematsu *et al.*, 2001; Borodynko *et al.*, 2010).

DISEASES OF UNKNOWN AETIOLOGY

- 138. MPI propose to retain specific phytosanitary measures for only one disease of unknown aetiology (Table 1), namely **Citrus blight**, for the following reasons:
 - a) Citrus blight is an economically important disease; it is responsible for the loss of about thousands of trees annually in Florida with estimated losses at more than US\$60 million per year and perhaps as many as 10 million trees in Brazil every year. The disease only appears on bearing trees with first symptoms observed from 4 to 10 years old trees and affects all citrus cultivars (some are more susceptible than others). There is no known vectors. It is present in Australia, Central and South America and South Africa (Roistacher, 1991; Timmer and Brlansky, 2006).
 - b) MPI propose to re-word the phytosanitary measure as follows: 'Growing season inspection of mother plants prior to production of cuttings/ tissue cultures for export' (Table 1). The current phytosanitary measure of inspecting source tree after two years before releasing from quarantine is not suitable; it is more appropriate to inspect plants before material is sent to New Zealand. The proposed measure is not ideal as symptoms are only observed on bearing trees. However, nursery stock budwood and tissue culture are unlikley to be infected because the causal agent seems to be restricted to the roots (Timmer and Brlansky, 2006).
 - c) Two viruses were detected from citrus blight diseased tissue using molecular technique by two separate studies, a virus related to Idaeovirus and a virus related to *Petunia vein clearing virus* (Brlansky and Wang, 2014; Derrick, 2006). However there is no transmission studies to confirm their association with the disease. Therefore, MPI do not propose adding them to the pest list. Instead, MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to these viruses and will re-assess if the situation changes.
- 139. MPI propose to remove **Lemon sieve tube necrosis** from the *Citrus* pest list (Table 1). It is not a transmissible bud union problem of citrus but rather a physiological disorder. It is a typical bud union incompatibility of lemon reported from California in 1951 (Roistacher, 2012).
- 140. MPI propose to remove from the *Citrus* pest list three diseases of unknown aetiology which have been associated with viroids that are already included on the *Citrus* pest list with specific phytosanitary measures (Table 1); these are:
 - a) **Gummy bark**: *Citrus bark cracking viroid* (syn. Citrus viroid IV) is the only candidate for the putative aetiology of gummy bark. The disease was first reported in 1954 from Egypt on sweet orange scions with sour orange rootstocks, then in several countries from North African and near Eastern countries (Bernad *et al.*, 2005; Mohamed *et al.*, 2009; Roistacher, 1991).

- b) Kassala: Different viroids have been isolated from kassala diseased samples including *Citrus bark cracking viroid* (syn. Citrus viroid IV), *Citrus bent leaf viroid* (syn. Citrus viroid I), *Citrus dwarfing viroid* (syn. Citrus viroid III), *Citrus exocortis viroid* and *Hop stunt viroid* (syn. Citrus viroid II). The disease was found on grapefruit from Sudan in 1995 (Mohamed *et al.*, 2009).
- c) **Shell bark**: A number of citrus viroids including *Citrus exocortis viroid* have been detected on old-line lemon trees with shell bark disease. The disease has never been found since published in two papers between 1959 and 1979 (Timmer *et al.*, 2000).
- 141. MPI propose to remove specific phytosanitary measures of eight diseases of unknown aetiology but retain the name on the *Citrus* pest list (Table 1). Instead, MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: http://biointel.org/) to gather any new information related to these diseases and will re-assess if the situation changes. These diseases have never been found since their initial reports. There is not enough evidence to justify retaining the specific phytosanitary measures. The diseases are:
 - a) **Bud union**: It has never been found since published in eight papers between 1937 and 1993. It was reported on sweet orange and/or rough lemon in Brazil, Egypt, Florida, Palestine, Spain and South Africa. The disease was discovered on several old Spanish cultivars by chance. Symptoms include swelling at the bud union, cracks in the bark, deep crease on trunk and tree stunting, leaf chlorosis and dieback (Navarro *et al.*, 1993; Roistacher, 1991).
 - b) **Citrus fatal yellows**: It has never been found since published in four papers between 1984 and 1988. It was reported on lemon trees with *Citrus macrophylla* rootstocks in California. Symptoms include vein clearing, vein corking, leaf epinasty, and stunting (Schneider, 1984 & 1988).
 - c) **Citrus impietratura**: It is a relatively rare disease that has never been found since published in ten papers early 1990s. The infection results in off-season fruit drop and affected fruit is not marketable. It mainly affects grapefruit and sweet orange, but also induces symptoms on clementines, sour orange, tangelo and *C. volkameriana*. It is present in all countries of the Mediterranean basin and also in India, Iran, South Africa and Venezuela (Caruso *et al.*, 1993; Roistacher, 1991).
 - d) **Citrus sunken vein**: It has never been found since its initial report in 1988 from California. It affects lemon trees with *Citrus macrophylla* rootstocks (Schneider, 1988).
 - e) Concave gum blind pocket disease: Concave gum and blind pocket have separate entries on the current *Citrus* pest list, however the two names are now combined into one name because they are caused by a similar, if not identical agent. The disease has not been found since the early 1990s. It affects mandarin, sweet orange and tangelos from most citrus growing countries. As the disease is not transmitted by insects or mechanically, it has disappeared from orchards started with citrus material certified free of graft-transmissible agents (Roistacher, 1991; Roistacher and Bové, 2009).
 - f) Cristacortis: It has never been found since published in six papers between 1964 and 1980. It is a non-destructive disease, in spite of severe stem pitting symptoms reported in the Mediterranean basin and Brazil. Susceptible varieties are grapefruit, mandarin, rough lemon, siamelo, sour orange, sweet lime, sweet orange, tangelo, tangor and occasionally lemon (Bové, 2008; Roistacher, 1991).

- g) Gum pocket: It has never been found since published in four papers between 1969 and 2005. It was reported on Trifoliate orange rootstock from South Africa. It may be caused by one or more viroids (e.g. *Hop stunt viroid* [syn. CVd-II] or *Citrus dwarfing virus* [syn. CVd-III]) for which specific phytosanitary measures are already in place (or present in New Zealand). It has also been suggested that it could be physiological or stress origin symptoms which may be enhanced by a viroid infection (Duran-Vila *et al.*, 2002; van Vuuren *et al.* 2005).
- h) Zonate chlorosis: It has never been found since mentioned in three papers between 1991 and 2003. It is a rare disease found in the coastal area of Brazil and somewhat similar to, but distinct from leprosis. It produces chlorotic areas on leaves and chlorotic rings on fruits but it is apparently not harmful. It affects grapefruit, lime, sweet orange and tangerine. The disease is transmitted by *Brevipalpus phoenicis*, the vector of leprosis disease (Frison and Taher, 1991; Roistacher, 2003; Timmer *et al.*, 2000).
- 142. MPI propose to move **Citrus rubbery wood** (syn. Rubbery wood) from the phytoplasma category to the diseases of unknown aetiology category with the removal of specific phytosanitary measures but retaining the name on the *Citrus* pest list (Table 1), for the following reasons:
 - a) There is not enough evidence to justify retaining the specific phytosanitary measures. The disease which effect lemon and lime by becoming unproductive has never been found since published in four papers between 1969 and 1985 from India. (Frison and Taher, 1991; Timmer *et al.*, 2000). MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: http://biointel.org/) to gather any new information related to this disease and will reassess if the situation changes.
 - b) Phytoplasmas have sometimes been found in citrus with Rubbery wood symptoms, however it has never been confirmed (Dr Lia Liefting, PHEL, pers. com., February 2015). If it is a phytoplasma, it would be detected using the generic PCR used for the detection of the other phytopalsmas infecting citrus (refer to paragraphs 86-87).

LEVEL OF POST ENTRY QUARANTINE

- 143. At present, citrus cuttings and tissue cultures from offshore MPI-accredited facilities are eligible for quarantine in a Level 2 PEQ facility with additional temperature control. Under the revised IHS sechedule for *Citrus*, material from an approved facility would require quarantine in a Level 3A PEQ facility or Level 2 facility with additional temperature control at the direction of the CTO (Table 3). Nursery stock imported from the offshore MPI-accredited facility, Elizabeth Macarthur Agricultural Institute (EMAI, Australia) will remain the same (i.e. Level 2 quarantine facility with additional temperature control) because liberibacters are not present in Australia (Table 3).
- 144. Cuttings and tissue cultures from offshore non-accredited facilities currently imported into a Level 3 PEQ facility would now be imported into Level 3B or Level 3A facility at the direction of the CTO (Table 3). This is because there is no testing history of the mother-plants. The smallest vectors of citrus regulated organisms are *Brevipalpus* mites, the vectors of citrus leprosis viruses. Some species of mites are present in New Zealand and they can only be excluded by HEPA filtration.
- 145. A lower level of quarantine may be appropriate when some testing is conducted overseas and/or from which country the nursery stock is imported from. This would be assessed on a case by case basis. It would require discussion with the exporting NPPO on the additional declarations for absence of pathogens and an assessment for the CTO at the time of the

import permit application process. Table 3 identifies criteria that may be used to determine the appropriate level of quarantine.

- 146. Tissue culture plants from accredited and non-accredited facilities that are multipled and/or maintained as tissue culture during the quarantine period must be held in a Level 3 tissue culture laboratory as set out in the revised PEQ standard for plants.
- 147. The level of quarantine for citrus nursery stock was determined based on:
 - a) The type of nursery stock (i.e. cuttings or tissue culture);
 - b) The type of offshore facilities (i.e. MPI-accredited versus non-accredited);
 - c) The testing conducted offshore;
 - d) The country of origin;
 - e) The potential impact of pathogens (e.g. *Xylella fastidiosa*);
 - f) The mode of transmission of regulated citrus pathogens (Appendix 5);
 - g) The conditions for a regulated citrus pathogen to develop and cause a disease whilst in post-entry quarantine; and
 - h) The physical requirements of the recently reviewed standard for post entry quarantine (PEQ) of plants (*Post Entry Quarantine for Plants Facilities Standard*, Appendix 4).

PERIOD IN POST ENTRY QUARANTINE

- 148. MPI propose some modifications of the PEQ period which are summarised in Table 4.
- 149. MPI propose to change the time in PEQ from a minimum of six months (which may be extended to allow for testing to be completed) to a minimum of eight months for material sourced from mother plants that have been kept in insect-proof plant houses and tissue culture plants at offshore MPI-accredited facilities. This is because liberibacters are proposed to be tested by PCR directly from the candidate plants after five months at 18-25°C followed by viroid testing by PCR after three months at 28-32°C (Table 1). Furthermore, the proposed PEQ period should be long enough for the completion of woody indexing which require symptoms observation of up to seven months for liberibacters.
- 150. MPI propose to reduce the PEQ period to a minimum of 12 months (instead of 16 months) for tissue culture plants imported from non-accredited facilities. This is because citrus tissue culture plants are grown in sterile conditions, eliminating the risk of insects and mites infections as well as surface contaminants such as bacteria and fungi. Furthermore certain pathogens (i.e. fungi, viruses, viroids, phytoplasmas) may also be eliminated.
- 151. MPI propose to change the PEQ period from a minimum of 16 months to a minimum of 18 months for cutting sourced from mother plants that have been kept in insect-proof plant houses and from open ground mother plants at non-accredited facilities. The risks associated is considered to be higher than material sourced from offshore MPI-accredited facilities because of the absence of testing history of the mother-plants. This will provide time for completing two complete spring and summer periods, for potential disease development in quarantine.

Feasibility of Measures

152. The cost of the proposed phytosanitary measures for citrus nursery stock is anticipated to be similar to the current measures when material is obtained from the MPI offshore accredited facility, Elizabeth Macarthur Agricultural Institute (EMAI) in Australia. The proposed

addition of 10 new organisms to the *Citrus* pest list is not likely to have any effect on the cost of material from EMAI as these organisms are not known to occur in Australia providing that the NPPO is able to endorse the additional declaration of absence of these organisms in Australia.

- 153. Citrus nursery stock imported from other countries such as Brazil, India, USA from which a greater number of citrus pathogens have been reported and are regulated, would likely result in increased costs. However, to date, citrus materials have never been sourced from Brazil and India. As above, all phytosanitary measures are consistent with the risk and necessary in order to prevent them entering New Zealand.
- 154. It is not yet known whether country freedom or pest free areas declarations could be supplied by all trading partners. Declarations for pest free area for *Xylella fastidiosa* have successfully been sourced from a number of exporting countries for a number of years. However, in countries where area freedom declarations for pests are not possible, testing options have been provided.
- 155. The number of indicator species for woody indexing has been reduced from 15 to 4 species, to reduce the cost without compromising the quality of the test.
- 156. All proposed PCR tests are established and they can be integrated into the *Citrus* schedule immediately. Samples of the same species can be bulked up to five for PCR testing as specified in Section 2.3.2.1 of the IHS. This will keep the cost of testing down.
- 157. Another example in saving time and cost is the testing measures for viroids which have been replaced with PCR directly from the nursery stock rather than conducting woody indexing followed by r-PAGE or PCR on indicator species.

Table 1. Proposed inspection, testing and treatment measures for *Citrus*.

Note: Country freedom and pest free area declarations: These endorsments are only for regulated fungi, viruses, viroids, phytoplasmas and disease of unknown aetiology listed in the Testing requirements table of the IHS *Citrus* schedule and must be assessed by MPI prior to permit issue. The exporting NPPO must endorse the additional declarations on the phytosanitary certificate, to be considered equivalent to testing in post entry quarantine.

Organism currently on the IHS and proposed new addition (in bracket)	Current phytosanitary measures	Proposed changes to IHS	Note	References
Insects	Visual inspection AND approved insecticide treatments (Refer to section 2.2.1.6 of the basic conditions).	Visual inspection (Refer to section 5(ii) of the <i>Citrus</i> schedule) AND approved insecticide treatments (Refer to section 2.2.1.6 of the basic conditions). Replace list of regulated insects with the following wording: 'Check the MPI database, Biosecurity organisms register for imported commodities (BORIC) for regulatory status, http://archive.mpi.govt.nz/applications/boric'	Insects are currently effectively managed under the basic and under the <i>Citrus</i> schedule set out in the IHS. Replace the list of regulated insects in the <i>Citrus</i> schedule with the wording 'Check the MPI database, Biosecurity organisms register for imported commodities (BORIC) for regulatory status, <u>http://archive.mpi.govt.nz/applications/boric</u> '. Refer to paragraph 40 for further details.	IHS 155.02.06
Mites	Visual inspection AND approved miticide treatments (Refer to section 2.2.1.6 of the basic conditions).	Visual inspection (Refer to section 5(ii) of the <i>Citrus</i> schedule) AND approved miticide treatments (Refer to section 2.2.1.6 of the basic conditions). Replace list of regulated mites with the following wording: 'Check the MPI database, Biosecurity organisms register for imported commodities (BORIC) for regulatory status, http://archive.mpi.govt.nz/applications/boric	Mites are currently effectively managed under the basic conditions and under the <i>Citrus</i> schedule set out in the IHS. Replace the list of regulated mites in the <i>Citrus</i> schedule with the wording 'Check the MPI database, Biosecurity organisms register for imported commodities (BORIC) for regulatory status, <u>http://archive.mpi.govt.nz/applications/boric</u> '. Refer to paragraph 40 for further details.	IHS 155.02.06
Bacteria				
Bacteria		All cuttings must be dipped in 1% sodium hypochlorite for 2 minutes upon arrival in the post entry quarantine facility.	Generic treatment used to remove potential pathogenic bacteria and fungi that may be found on the surfaces of cuttings that are not visible to the naked eye (refer to paragraphs 51-52 for further details).	IHS 155.02.06
Burkholderia cepacia	Growing season inspection for symptom expression.	Remove from pest list.	This bacteria is present in New Zealand and is non-regulated.	BORIC MPI database
Spiroplasma citri	Country freedom/shoot tip grafting. Graft inoculated sweet orange, 27 to 32°C. Bioassay = culture petiole new flush tissue. Collect tissue after several days at hot temperature (> 30°C) and incubate cultures at 32°C.	Replace the current phytosanitary measures with PCR.	PCR is very sensitive, fast and more reliable than woody indexing and culturing, the current phytosanitary measures for <i>Spiroplasma citri</i> (Stubborn disease). Refer to paragraphs 54-55 for further details.	CABI datasheet Rangel <i>et al.</i> 2005 Timmer <i>et al.</i> 2000 Yokomi <i>et al.</i> 2008

Organism currently on the IHS and proposed new addition (in bracket)	Current phytosanitary measures	Proposed changes to IHS	Note	References
Xanthomonas alfalfae subsp. citrumelonis	Country freedom/shoot tip grafting bioassay/detached leaf bioassay/ PCR OR suitable citrus indicator.	Update name from <i>Xanthomonas campestris</i> pv. <i>citrumelo</i> to <i>X. alfalfae</i> subsp. <i>citrumelonis.</i> Replace the current phytosanitary measures with growing season inspection for symptom expression.	Xanthomonas campestris pv. citrumelo is synonym of X. alfalfae subsp. citrumelonis (Bacterial spot). Visual inspection is the most appropriate phytosanitary measure for this bacterium. It causes typical symptoms on leaves: flat with water-soaked margins and necrotic centers. Refer to paragraphs 56-57 for further details.	Schaad <i>et al.</i> 2006 Timmer <i>et al.</i> 2000
Xanthomonas fuscans subsp. aurantifolii	Country freedom/shoot tip grafting bioassay/detached leaf bioassay/ PCR OR suitable citrus indicator.	Update name from <i>Xanthomonas campestris</i> pv. <i>aurantifolii</i> to <i>X. fuscans</i> subsp. a <i>urantifolii</i> . Replace the current phytosanitary measures with growing season inspection for symptom expression.	Xanthomonas campestris pv. aurantifolii is synonym of X. fuscans subsp. aurantifolii (Canker). Visual inspection is the most appropriate phytosanitary measure for this bacterium. It causes typical symptoms on leaves: raised lesions surrounded by a yellow halo. Refer to paragraphs 56-57 for further details.	Schaad <i>et al.</i> 2006 Timmer <i>et al.</i> 2000
Xanthomonas citri subsp. citri	Country freedom/shoot tip grafting bioassay/detached leaf bioassay/ PCR OR suitable citrus indicator.	Update name from <i>Xanthomonas axonopodis</i> pv. <i>citri</i> to <i>X. citri</i> subsp. <i>citri</i> . Keep PCR as the only phytosanitary measure.	<i>Xanthomonas axonopodis</i> pv. <i>citri</i> is synonym of <i>X. citri</i> subsp. <i>citri</i> (Canker). PCR is the most sensitive, reliable and rapid detection method for this bacterium. Refer to paragraphs 58-60 for further details.	ISPM27/DP6 2014 Mavrodieva <i>et al.</i> 2004 Schaad <i>et al.</i> 2006 Timmer <i>et al.</i> 2000
Xylella fastidiosa	Special conditions (section 2.2.1.12) with the following phytosanitary requirements for Citrus: Country freedom/shoot tip grafting bioassay/ PCR/ELISA OR suitable citrus indicator.	Refer to <i>X. fastidiosa</i> (not <i>X. fastidiosa</i> pv. <i>citri</i>) in the pest list. Keep PCR as the only phytosanitary measure. Add after PCR: 'two sets, samples to be collected at least four weeks apart while in PEQ'.	 Xylella fastidiosa pv. citri is synonym of Xylella fastidiosa PCR is the only diagnostic method to reliably detect Xylella fastidiosa (Variegated chlorosis). Plants must be tested two times while in quarantine. Refer to paragraphs 61-64 for further details. Note that measures are intended to be brought in under emergency in the next few months to include requirements for <i>X. fastidiosa</i> in plants <i>in vitro</i> of all hosts, including <i>Citrus</i> hosts. 	Bull <i>et al.</i> 2012 EPPO standards PM7/24 2004 Harper <i>et al.</i> 2010 Li <i>et al.</i> 2013
Fungi	Country freedom OR growing season inspection for symptom expression.	All cuttings must be dipped in 1% sodium hypochlorite for 2 minutes upon arrival in the post entry quarantine facility.	Generic treatment used to remove potential pathogenic bacteria and fungi that may be found on the surfaces of cuttings that are not visible to the naked eye (refer to paragraphs 51-52 for further details). Six genera identified to species level (refer to paragraph 65 for further details). Addition of specific phytosanitary measures for three fungi (refer to separate entries below).	IHS 155.02.06
Ceratocystis fimbriata sensu lato complex	Special conditions (section 2.2.1.8)	Add the testing required for the special conditions of section 2.2.1.8: Plating from original budstick and on actively growing plants while in PEQ (any suspect <i>C. fimbriata</i> will be confirmed by PCR) [only apply to cuttings of the genus <i>Citrus</i>].	Plating on arrival and while in PEQ is the most appropriate method at this stage. This testing only apply to cuttings of the genus <i>Citrus</i> . Refer to paragraphs 69-70 for further details.	CABI datasheet Harrington 2015
Helicobasidium mompa	Special conditions (section 2.2.1.9)	None.	<i>Helicobasidium mompa</i> is currently effectively managed under the special conditions (section 2.2.1.9) of the IHS.	IHS 155.02.06

Organism currently on the IHS and proposed new addition (in bracket)	Current phytosanitary measures	Proposed changes to IHS	Note	References
Elsinoë australis	Country freedom OR growing season inspection for symptom expression.	Replace current phytosanitary measures with plating on semi-selective medium and PCR as alternative tests.	Semi-selective medium and PCR are the most reliable detection method for <i>Elsinoë australis</i> (Sweet orange scab). The fungus affects only the fruit and the post entry quarantine period is too short for plants to produce fruit for symptom observation. Refer to paragraphs 69-70 for further details.	Chung 2011 EPPO datasheet Hyun <i>et al.</i> 2009
Eremothecium coryli (new)	None.	Add to pest list without any specific phytosanitary measure.	syn. Nematospora coryli There is insufficient evidence for having specific phytosanitary measures at present. MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this virus and will re-assess if the situation changes. Refer to paragraphs 71-73 for further details.	Miles <i>et al.</i> 2009 Shivas <i>et al.</i> 2005
Phyllosticta citricarpa	Country freedom OR growing season inspection for symptom expression.	Update name from <i>Guignardia citricarpa</i> to <i>Phyllosticta citricarpa</i> . Replace current phytosanitary measures with PCR.	Guignardia citricarpa is synonym of <i>Phyllosticta citricarpa</i> (Black spot). PCR is the most reliable detection method for this fungus. It affects mainly the fruit and the post entry quarantine period is too short for plants to produce fruit for symptom observation. Latent infections on leaves are also common. Refer to paragraphs 74-75 for further details.	CABI datasheet EPPO datasheet
Phytophthora ramorum	Special conditions (section 2.2.1.11)	Add the testing required for the special conditions of section 2.2.1.11: PCR from original cuttings and on actively growing plants while in PEQ.	PCR from original cuttings and on actively growing plants while in PEQ is currently the most appropriate method for detecting <i>Phytophthora ramorum</i> (Dr. M. Toome, Mycologist at the Plant Health and Environment Laboratory, MPI).	Hughes et al. 2006
Plenodomus tracheiphilus	Country freedom OR growing season inspection for symptom expression.	Update name from <i>Phoma tracheiphila</i> to <i>Plenodomus tracheiphilus.</i> Replace current phytosanitary measures with PCR.	Phoma tracheiphila is synonym of Plenodomus tracheiphilus (Mal socco). PCR is the most reliable detection method for this fungus. The disease may be latent for an extended period so symptoms may not have time to develop during post entry quarantine. Refer to paragraphs 76-77 for further details.	Cacciola & Magnano di San Lio 2007 Donovan <i>et al.</i> 2014
Liberibacters 'Candidatus Liberibacter	Country freedom OD graft	Update name from Liberobacter africanum to	Liberabastar of rison up is super up of (Candidatus Liberibastar of rison us)	CABI datasheet
africanus'	Country freedom OR graft- inoculated sweet oranges, orange pineapple, 18 to 25°C.	'Candidatus Liberibacter africanus'.	Liberobacter africanum is synonym of 'Candidatus Liberibacter africanus'. Woody indexing alone is not reliable enough to detect 'Ca. Liberibacter africanus' causing Huanglongbing, one of the most damaging disease of citrus. 'Candidatus Liberibacters' are temperature sensitive so plants must be grown in a temperature controlled glasshouse (already included in the IHS). Refer to paragraphs 78-80 for further details.	GISD 2014 Razi <i>et al.</i> 2012

Organism currently on the IHS and proposed new addition (in bracket)	Current phytosanitary measures	Proposed changes to IHS	Note	References
' <i>Candidatus</i> Liberibacter americanus' (new)	None.	Add to pest list. Add woody indexing (<i>Citrus sinensis</i> 'Pineapple' and <i>Citrus reticulata</i> 'Parson's special') AND PCR directly from candidate plants actively growing at 18-25°C for a minimum of 5 months.	<i>'Ca.</i> Liberibacter americanus' is associated with Huanglongbing disease, one of the most damaging disease of citrus. The candidate plants for biosecurity clearance must be tested by PCR after they have been grown at 18-25°C for a minimum of five months. Refer to paragraphs 81-83 for further details.	CABI datasheet Folimonova <i>et al</i> , 2009 GISD 2014 Razi <i>et al</i> . 2012 Teixeira <i>et al</i> . 2005
' <i>Candidatus</i> Liberibacter asiaticus'	Country freedom OR graft- inoculated sweet oranges, orange pineapple, 18 to 25°C.	Update name from Liberobacter asiaticum to 'Candidatus Liberibacter asiaticus'. Rename sweet oranges with Citrus sinensis 'Pineapple'. Replace orange pineapple with Citrus reticulata 'Parson's special'. Add PCR directly from candidate plants actively growing at 18-25°C for a minimum of 5 months, as a compulsory test.	Liberobacter asiaticum is synonym of 'Candidatus Liberibacter asiaticus'. Woody indexing alone is not reliable enough to detect 'Ca. Liberibacter asiaticus' causing Huanglongbing, one of the most damaging disease of citrus. 'Candidatus Liberibacters' are temperature sensitive so plants must be grown in a temperature controlled glasshouse (already included in the IHS). Refer to paragraphs 78-80 for further details.	CABI datasheet Folimonova et al. 2009 GISD 2014 Razi <i>et al.</i> 2012
'Candidatus Liberibacter caribbeanus' (new)	None.	Add to pest list without any specific phytosanitary measure.	There is insufficient evidence for having specific phytosanitary measures at present. Furthermore it can be indirectly detected using the same PCR for ' <i>Candidatus</i> Liberibacter asiaticus' (Huanglongbing) which is already on the pest list. MPI also propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this liberibacter and will re-assess if the situation changes. Refer to paragraphs 84-85 for further details.	Keremane <i>et al.</i> 2015
Phytoplasmas				
Australian citrus dieback	Country freedom OR other suitable test.	Move from diseases of unknown aetiology to phytoplasma category. Replace current phytosanitary measures with nested-conventional or real-time PCR using phytoplasma universal primers.	Australian citrus dieback has been associated with a phytoplasma in Australia. PCR is the best method of detection for phytopalsmas. Woody indexing is not an option as the phytoplasma is not easily graft transmissible. Refer to paragraphs 88-89 for further details.	Broadbent <i>et al.</i> 1976 Davis <i>et al.</i> 1997 Timmer <i>et al.</i> 2000
<i>'Candidatus</i> Phytoplasma asteris' (new)	None.	Add to pest list. Add nested-conventional or real-time PCR using phytoplasma universal primers as phytosanitary measures.	PCR is the most sensitive and reliable method of detection for ' <i>Candidatus</i> Phytoplasma asteris' (Huanglongbing-like disease). Refer to paragraphs 90-91 for further details.	Arratia-Castro <i>et al.</i> 2014 Chen <i>et al.</i> 2009 Liefting <i>et al.</i> 2011
' <i>Candidatus</i> Phytoplasma aurantifolia'	Country freedom OR graft inoculated lime. Grow indicators at cool temperatures 18 to 25°C.	Update name from <i>Candidatus phytoplasma</i> aurantifolia to 'Candidatus Phytoplasma aurantifolia'. Replace woody indexing with nested- conventional or real-time PCR using phytoplasma universal primers.	'Candidatus Phytoplasma aurantifolia' (Witches' broom) is often in low titre and unevenly distributed in the plant, making their detection by woody indexing unreliable. Furthermore the period of time for symptoms to develop is very variable, ranging from five months to <i>more</i> than two years. Refer to paragraphs 92-93 for further details.	EPPO standards PM7/61 Liefting <i>et al.</i> 2011 Ochoa-Corona & Ward 2010

Organism currently on the IHS and proposed	Current phytosanitary measures	Proposed changes to IHS	Note	References
new addition (in bracket)				
Huanglongbing- associated 16SrIX-A phytoplasma (new)	None.	Add to pest list. Add nested-conventional or real-time PCR using phytoplasma universal primers as phytosanitary measures.	PCR is the most sensitive and reliable method of detection for Huanglongbing - associated 16SrIX-A phytoplasma. Refer to paragraphs 94-95 for further details.	Liefting <i>et al.</i> 2011 Teixeira <i>et al.</i> 2008
Viroids				
Citrus bark cracking viroid	Country freedom OR sPAGE and PCR on graft inoculated citron extract. Grow citron at hot temperature 27 to 32°C.	Update name from Citrus viroid IV to <i>Citrus bark</i> <i>cracking viroid.</i> Remove sPAGE Keep woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special') but without other testing on indicator plants PCR directly from candidate plants actively growing at 28-32°C for a minimum of 3 months.	Citrus viroid IV is synonym of <i>Citrus bark cracking viroid</i> . PCR is more sensitive, less time consuming and more reliable than sPAGE. Viroid multiplication is best at warm temperatures thus the specified temperature range. Woody indexing is a valuable generic test for the detection of unknown viroids. Refer to paragraphs 97-99 for further details.	Barbosa <i>et al.</i> 2002 Duran-Vila <i>et al.</i> 1988 Hadidi <i>et al.</i> 2003 ICTV 2014
Citrus bent leaf viroid	Country freedom OR sPAGE and PCR on graft inoculated citron extract. Grow citron at hot temperature 27 to 32°C.	Update name from Citrus variable viroid and Citrus viroid I to <i>Citrus bent leaf viroid</i> . Remove sPAGE Keep woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special') but without other testing on indicator plants Add PCR directly from candidate plants actively growing at 28-32°C for a minimum of 3 months.	Citrus variable viroid initially called Citrus viroid Ib and Citrus viroid I are synonyms of <i>Citrus bent leaf viroid</i> . PCR is more sensitive, less time consuming and more reliable than sPAGE. Viroid multiplication is best at warm temperatures thus the specified temperature range. Woody indexing is a valuable generic test for the detection of unknown viroids. Refer to paragraphs 97-99 for further details.	Barbosa <i>et al.</i> 2002 Duran-Vila <i>et al.</i> 1988 Hadidi <i>et al.</i> 2003 ICTV 2014
Citrus dwarfing viroid	Country freedom OR sPAGE and PCR on graft inoculated citron extract. Grow citron at hot temperature 27 to 32°C.	Remove from pest list (named as Citrus viroid III and Dwarfing factor viroid).	<i>Citrus dwarfing viroid</i> (syn. Citrus viroid III, Dwarfing factor viroid) was reported from New Zealand in 2011 and has a non-regulated status.	BORIC MPI database ICTV 2014 Quemin <i>et al.</i> 2011
Citrus viroids (group I-IV)	Country freedom OR sPAGE and PCR on graft inoculated citron extract. Grow citron at hot temperature 27 to 32°C.	Remove from pest list.	These groups of viroids have been separated out; refer to <i>Citrus bent leaf viroid</i> for Citrus viroid I, <i>Hop stunt viroid</i> for Citrus viroid II, <i>Citrus dwarfing viroid</i> for Citrus viroid III and <i>Citrus bark cracking viroid</i> for Citrus viroid IV.	
Citrus viroid V (new)	None.	Add to pest list. Add PCR directly from candidate plants actively growing at 28-32°C for a minimum of 3 months AND woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special').	PCR is a reliable and specific method of detection for <i>Citrus viroid V</i> (CVd-V). Viroid multiplication is best at warm temperatures thus the specified temperature range. Woody indexing is a valuable generic test for the detection of unknown viroids. Refer to paragraphs 100-103 for further details.	Bani Hashemian <i>et</i> <i>al.</i> 2010 Barbosa <i>et al.</i> 2002 Serra <i>et al.</i> 2008a & 2008b
Citrus viroid VI (new)	None.	Add to pest list. Add PCR directly from candidate plants actively growing at 28-32°C for a minimum of 3 months AND woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special').	PCR is a reliable and specific method of detection for <i>Citrus viroid VI</i> (CVd-VI). Viroid multiplication is best at warm temperatures thus the specified temperature range. Woody indexing is a valuable generic test for the detection of unknown viroids. Refer to paragraphs 100-103 for further details.	Barbosa <i>et al.</i> 2002 Ito <i>et al.</i> 2001

Organism currently on the IHS and proposed new addition (in bracket)	Current phytosanitary measures	Proposed changes to IHS	Note	References
Citrus viroid VII (new)	None.	Add to pest list. Add PCR directly from candidate plants actively growing at 28-32°C for a minimum of 3 months AND Woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special')	PCR is a reliable and specific method of detection for <i>Citrus viroid VII</i> (CVd-VII). Viroid multiplication is best at warm temperatures thus the specified temperature range. Woody indexing is a valuable generic test for the detection of unknown viroids. Refer to paragraphs 104-105 for further details.	Chambers et al. 2016
<i>Hop stunt viroid</i> [citrus strain]	Country freedom OR sPAGE and PCR on graft inoculated citron extract. Grow citron at hot temperature 27 to 32°C.	Update name from Citrus viroid II, Xyloporosis viroid to <i>Hop stunt viroid</i> [citrus strain]. Remove sPAGE Keep woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special') but without other testing on indicator plants Add PCR directly from candidate plants actively growing at 28-32°C for a minimum of 3 months.	Citrus cachexia viroid, Citrus viroid II and Xyloporosis viroid are synonyms of <i>Hop stunt viroid</i> . PCR is more sensitive, less time consuming and more reliable than sPAGE. Viroid multiplication is best at warm temperatures thus the specified temperature range. Woody indexing is a valuable generic test for the detection of unknown viroids. Refer to paragraphs 97-99 for further details.	Barbosa <i>et al.</i> 2002 Hadidi <i>et al.</i> 2003 ICTV 2014 Reanwarakorn & Semancik 1999
Viruses				
Apple stem grooving virus	Country freedom OR graft inoculated Rusk citrange, rough lemon, <i>Citrus excelsa</i> , citrange (Troyer). Grow indicators at cool temperatures 18 to 25°C.	Remove from pest list (named as Citrus tatter leaf capillovirus)	Citrus tatter leaf virus is considered to be the same specie as <i>Apple stem grooving virus</i> (ASGV) by the International Committee on Taxonomy of Viruses. ASGV is present in New Zealand and has a non-regulated status. There are no evidence of strains of ASGV. Refer to paragraphs 106-108 for further details.	BORIC MPI database ICTV, 2014 Veerakone <i>et al.</i> , 2015 Yoshikawa <i>et al.</i> , 1993 Yoshikawa, 2000
Citrus chlorotic dwarf- associated virus	Country freedom OR graft inoculated rough lemon at cool temperatures 18 to 25°C.	Update name from Citrus chlorotic dwarf to <i>Citrus chlorotic dwarf-associated virus.</i> Replace woody indexing with PCR.	Citrus chlorotic dwarf is the disease caused by <i>Citrus chlorotic dwarf-associated virus</i> . PCR is more reliable and sensitive than woody indexing for the detection of <i>Citrus chlorotic dwarf-associated virus</i> . Refer to paragraphs 109-110 for further details.	Korkmaz <i>et al.</i> 1995 Loconsole <i>et al.</i> 2012 Timmer <i>et al.</i> 2002
Citrus variegation virus	Country freedom OR graft inoculated citron, sour orange, lemon, cidro etrog. Grow indicators at cool temperatures 18 to 25°C.	Update name from Citrus infectious variegation ilarvirus and Citrus infectious variegation ilarvirus [crinkly leaf strain] to <i>Citrus variegation virus</i> . Remove woody species sour orange and cidro etrog Rename citron with <i>Citrus medica</i> 'Arizona 861' and lemon with <i>Citrus limon</i> 'Eureka'. Add PCR test as an alternative test.	Citrus infectious variegation ilarvirus and Citrus infectious variegation ilarvirus [crinkly leaf strain] are synonyms of <i>Citrus variegation virus</i> . Minimum of 2 species for woody indexing; the best woody indicators for <i>Citrus variegation virus</i> (CVV) are citron (<i>Citrus medica</i> 'Arizona 861') and lemon (<i>Citrus limon</i> 'Eureka'). The virus can also be detected by PCR. Refer to paragraphs 111-112 for further details.	Barone <i>et al.</i> 2009 Bové <i>et al.</i> 2010 Frison & Taher 1991 Ochoa-Corona & Ward 2010 Roistacher 1991 Timmer <i>et al.</i> 2002
Citrus leaf rugose virus	Country freedom OR graft inoculated Mexican lime or sour orange. Grow indicators at cool temperatures 18 to 25°C.	Update name from Citrus leaf rugose ilarvirus to <i>Citrus leaf rugose virus.</i> Replace Mexican lime and sour orange with <i>Citrus limon</i> 'Eureka' and <i>Citrus medica</i> 'Arizona 861'. Add PCR as an alternative test.	Citrus leaf rugose ilarvirus is synonym of <i>Citrus leaf rugose virus</i> . Citron (<i>Citrus medica</i>) and Lemon (<i>Citrus limon</i>) are indicators as good as Mexican lime and sour orange; the species were changed to keep the overall number of indicator species to a minimum. The virus can also be detected by PCR. Refer to paragraphs 111-112 for further details.	Garnsey 1974 Roistacher 1991 Ochoa-Corona & Ward 2010 Timmer <i>et al.</i> 2002

Organism currently on the IHS and proposed new addition (in bracket)	Current phytosanitary measures	Proposed changes to IHS	Note	References
Citrus leathery leaf virus	Country freedom OR Rangpur lime. Grow indicators at cool temperatures 18 to 25°C.	Remove specific phytosanitary measures but retain the name on the pest list.	There is not enough evidence to justify retaining specific phytosanitary measures. Instead MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this virus and will re-assess if the situation changes. Refer to paragraph 113-114 for further details.	
Citrus leprosis virus C	Country freedom OR graft inoculated sweet orange. Grow indicators at cool temperatures 18 to 25°C.	Update name from Citrus leprosis rhabdovirus to <i>Citrus leprosis virus</i> C. Replace woody indexing with PCR.	Citrus leprosis rhabdovirus is synonym of <i>Citrus leprosis virus</i> C. PCR is more reliable and sensitive than woody indexing for the detection of <i>Citrus leprosis virus</i> C which is <i>not</i> easily graft transmissible. Refer to paragraphs 115-117 for further details.	Brlansky & Hartung 2013 Locali-Fabris 2006 Lovisolo 2001
Citrus leprosis virus cytoplasmic type 2 (new)	None.	Add to pest list. Add PCR as phytosanitary measure.	PCR is the only method suitable for the detection of <i>Citrus leprosis virus</i> cytoplasmic type 2. Refer to paragraphs 118-121 for further details.	Hartung <i>et al.</i> 2013 Roy <i>et al.</i> 2013& 2014 Timmer <i>et al.</i> 2000
Citrus leprosis virus nuclear type (new)	None.	Add to pest list. Add PCR as phytosanitary measure.	PCR is the only method suitable for the detection of <i>Citrus leprosis virus</i> nuclear type. Refer to paragraphs 118-121 for further details.	Hartung <i>et al.</i> 2013 Roy <i>et al.</i> 2013 & 2014 Timmer <i>et al.</i> 2000
Citrus necrotic spot virus (new)	None.	Add to pest list without any specific phytosanitary measure.	There is insufficient evidence for having specific phytosanitary measures at present. MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: http://biointel.org/) to gather any new information related to this virus and will re-assess if the situation changes. Refer to paragraph 122 for further details.	Cruz-Jaramillo <i>et al.</i> 2014 ICTV 2014
Citrus psorosis virus	Country freedom OR graft inoculated dweet tangor, sweet orange, mandarin (Parson's Special). Grow indicators at cool temperatures 18 to 25°C.	Remove from pest list (named as Citrus ringspot virus).	<i>Citrus psorosis virus</i> (syn. Citrus ringspot virus) was reported from New Zealand in 2011 and has a non-regulated status. Refer to paragraphs 123-124 for further details.	Achachi <i>et al.</i> 2014 BORIC MPI database Quemin <i>et al.</i> 2011 Velázquez <i>et al.</i> 2012
Citrus sudden death- associated virus (new)	None.	Add to pest list. Add PCR as phytosanitary measure.	<i>Citrus sudden death-associated virus</i> can be detected by PCR from symptomatic and asymptomatic material. PCR is more reliable and sensitive than woody indexing. Furthermore symptoms on woody indicators may take more than two years to develop. Refer to paragraphs 125-128 for further details.	Bassanezi <i>et al.</i> 2007 Maccheroni <i>et al.</i> 2005 Yamamoto <i>et</i> <i>al.</i> 2011
Citrus tristeza virus	Country freedom OR ELISA, graft inoculated Mexican lime, sour orange and Citrus excelsa. Grow indicators at cool temperatures 18 to 25°C.	Remove from pest list (named as Citrus tristeza closterovirus [strains not in New Zealand]).	All currently described <i>Citrus tristeza virus</i> strains are present in New Zealand and exist as complex mixtures of strains. Therefore, it is no longer appropriate to regulate this virus. Refer to paragraphs 129-130 for further details.	CABI datasheet Harper <i>et al.</i> 2010 Harper & Pearson 2015

Organism currently on the IHS and proposed new addition (in bracket)	Current phytosanitary measures	Proposed changes to IHS	Note	References
Citrus variegation virus	Country freedom OR graft inoculated citron, sour orange, lemon, cidro etrog. Grow indicators at cool temperatures 18 to 25°C.	Update name from Citrus infectious variegation ilarvirus and Citrus infectious variegation ilarvirus [crinkly leaf strain] to <i>Citrus variegation virus</i> . Remove woody species sour orange and cidro etrog Rename citron with <i>Citrus medica</i> 'Arizona 861' and lemon with <i>Citrus limon</i> 'Eureka'. Add PCR test as an alternative test.	Citrus infectious variegation ilarvirus and Citrus infectious variegation ilarvirus [crinkly leaf strain] are synonyms of <i>Citrus variegation virus</i> . Minimum of 2 species for woody indexing; the best woody indicators for <i>Citrus variegation virus</i> (CVV) are citron (<i>Citrus medica</i> 'Arizona 861') and lemon (<i>Citrus limon</i> 'Eureka'). The virus can also be detected by PCR. Refer to paragraphs 111-112 for further details.	Barone <i>et al.</i> 2009 Bové <i>et al.</i> 2010 Frison & Taher 1991 Ochoa-Corona & Ward 2010 Roistacher 1991 Timmer <i>et al.</i> 2002
Citrus yellow mosaic virus	Country freedom OR graft inoculated sweet orange, sour orange and citron.	Update name from Citrus yellow mosaic badnavirus and Indian citrus mosaic badnavirus to <i>Citrus yellow mosaic virus.</i> Replace woody indexing with PCR.	Citrus yellow mosaic badnavirus and Indian citrus mosaic badnavirus are synonyms of <i>Citrus yellow mosaic virus</i> . PCR is more reliable and sensitive than woody indexing for the detection of <i>Citrus</i> <i>yellow mosaic virus</i> (CYMV). Refer to paragraph 131 for further details.	Ahlawat <i>et al.</i> 1996 Aparna <i>et al.</i> 2002 Barman 2013 Timmer <i>et al.</i> 2000
Citrus yellow mottle virus	Country freedom OR other suitable test.	Remove specific phytosanitary measures but retain the name on the pest list.	There is not enough evidence to justify retaining specific phytosanitary measures. Instead MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this virus and will re-assess if the situation changes. Refer to paragraph 113-114 for further details.	Bové <i>et al.</i> 2010 Ushiyama <i>et al.</i> 1984
Citrus yellow vein clearing virus	Country freedom OR graft inoculated Mexican lime or sour orange. Grow indicators at cool temperatures 18 to 25°C.	Update name from Yellow vein clearing of lemon to <i>Citrus yellow vein clearing virus.</i> Replace woody indexing with PCR.	Yellow vein clearing of lemon is caused by <i>Citrus yellow vein clearing virus</i> (CYCV). PCR is more sensitive and reliable than woody indexing for the detection of CYCV. Refer to paragraph 131 for further details.	Chen <i>et al.</i> 2014 Timmer <i>et al.</i> 2000
Hibiscus green spot virus 2 (new)	None.	Add to pest list without any specific phytosanitary measure.	There is insufficient evidence for having specific phytosanitary measures at present. MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this virus and will re-assess if the situation changes. Refer to paragraph 122 for further details.	BORIC Melzer <i>et al.</i> 2012
Indian citrus ringspot virus (new)	None.	Add to pest list. Add PCR as phytosanitary measure.	PCR is the most reliable and sensitive method of detection for <i>Indian citrus ringspot virus</i> . Refer to paragraphs 132-134 for further details.	Milne <i>et al.</i> 2007 Rustici <i>et al.</i> 2000
Iranian citrus ringspot- associated virus (new)	None.	Add to pest list without any specific phytosanitary measure.	There is insufficient evidence for having specific phytosanitary measures at present. MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this virus and will re-assess if the situation changes. Refer to paragraph 122 for further details.	Sadeghi <i>et al.</i> 2016
Olive latent virus 1 (new)	None.	Add to pest list. Add PCR as phytosanitary measure.	PCR is more sensitive and reliable than woody and herbaceous indexing for the detection of <i>Olive latent virus</i> 1 (OLV-1). Refer to paragraphs 135-137 for further details.	Félix <i>et al.</i> 2007 Martelli 2013 Martelli <i>et al.</i> 1996

Organism currently on the IHS and proposed new addition (in bracket)	Current phytosanitary measures	Proposed changes to IHS	Note	References
Satsuma dwarf virus	Country freedom OR graft inoculated satsumas. Grow indicators at cool temperatures 18 to 25°C.	Update name from Citrus mosaic virus, Navel orange infectious mottling virus, Satsuma dwarf nepovirus and Satsuma dwarf nepovirus [Natsudaidai dwarf strain] to <i>Satsuma dwarf</i> <i>virus</i> . Remove satsumas and replace with <i>Citrus</i> <i>reticulata</i> 'Parson's special'and <i>Citrus limon</i> 'Eureka'. Add PCR as an alternative test.	Citrus mosaic virus, Navel orange infectious mottling virus, Satsuma dwarf nepovirus and Satsuma dwarf nepovirus [Natsudaidai dwarf strain] are synonyms of <i>Satsuma dwarf virus</i> . The number of woody indicator species is increased to two to have a better chance of detecting <i>Satsuma dwarf virus</i> (SDV), if it is present. Replace woody indicator satsumas (suitable indicator only for SDV) with mandarin (<i>C. reticulata</i>) and lemon (<i>C. limon</i>) which are also suitable hosts for SDV. SDV can also be detected by PCR. Refer to paragraph 111-112 for further details.	Frison & Taher 1991 Ochoa-Corona & Ward 2010 Roistacher 1991 Timmer <i>et al.</i> 2000 EPPO datasheet
Diseases of unknown aetiology				
Bud union	Country freedom OR other suitable test.	Update name from Bud union disease to Bud union. Remove specific phytosanitary measures but retain the name on the pest list.	There is not enough evidence to justify retaining specific phytosanitary measures. Instead MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this disease and will re-assess if the situation changes. Refer to paragraph 140 for further details.	Bové <i>et al.</i> 2010 Navarro <i>et al.</i> 1993 Roistacher 1991
Citrus blight	None (cuttings collected from blight free area). Inspect source tree after 2 years before releasing from quarantine.	Update name from Citrus blight disease to Citrus blight. Replace current phytosanitary measures with 'Growing season inspection of mother plants prior to production of cuttings/ tissue cultures for export.	Rewording of the current phytosanitary measure for Citrus blight. Inspections are conducted on mother plants prior to produce cuttings and/or tissue culture for export. Refer to paragraph 138 for further details.	Roistacher 1991 Derrick 2006 Brlansky & Wang 2014 Timmer & Brlansky 2006
Citrus fatal yellows	Country freedom OR graft inoculated <i>Citrus macrophylla</i> .	Remove specific phytosanitary measures but retain the name on the pest list.	There is not enough evidence to justify retaining specific phytosanitary measures. Instead MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this disease and will re-assess if the situation changes. Refer to paragraph 140 for further details.	Schneider 1984 & 1988
Citrus impietratura	Country freedom OR graft inoculated dweet tangor or sweet orange. Growth indicators at cool temperatures 18 to 25°C.	Update name from Citrus impietratura disease to Citrus impietratura. Remove specific phytosanitary measures but retain the name on the pest list.	There is not enough evidence to justify retaining specific phytosanitary measures. Instead MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this disease and will re-assess if the situation changes. Refer to paragraph 140 for further details.	Caruso <i>et al.</i> 1993 Roistacher 1991
Citrus rubbery wood disease	Country freedom OR graft inoculated sweet orange or lemon. Grow citron at hot temperature 27 to 32°C.	Move from phytoplasmas category to disease of unknown aetiology. Update name from Rubbery wood to Citrus rubbery wood disease. Remove specific phytosanitary measures but retain the name on the pest list.	The presence of phytoplasmas on citrus with Rubbery wood symptoms has never been confirmed (Lia Liefting, pers. com., Feb 2015). If it is a phytoplasma, it would be detected by the same PCR assay used for the other regulated phytoplasmas (see entries above). MPI also propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this disease and will re-assess if the situation changes. Refer to paragraphs 142 for further details.	Frison & Taher 1991 Timmer <i>et al.</i> 2000

Organism currently on the IHS and proposed new addition (in bracket)	Current phytosanitary measures	Proposed changes to IHS	Note	References
Citrus sunken vein	Country freedom OR other suitable test.	Update name from Citrus sunken vein disease to Citrus sunken vein. Remove phytosanitary measures but reatin the name on the pest list.	Instead MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this disease and will re-assess if the situation changes. Refer to paragraph 140 for further details.	Schneider 1988
Concave gum-blind pocket	Country freedom OR graft inoculated dweet tangor, sweet orange or Citrus excelsa. Grow indicators at cool temperatures 18 to 25°C.	Update name from blind pocket and concave gum to concave gum-blind pocket. Remove specific phytosanitary measures but retain the name on the pest list.	Concave gum and blind pocket have been combined together because they are caused by a similar, if not identical agent. There is not enough evidence to justify retaining specific phytosanitary measures. Instead MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this disease and will re-assess if the situation changes. Refer to paragraph 140 for further details.	Roistacher 1991 Roistacher & Bové 2009
Cristacortis	Country freedom OR graft inoculated dweet tangor, sweet orange or Citrus excelsa. Grow indicators at cool temperatures 18 to 25°C.	Remove specific phytosanitary measures but retain the name on the pest list.	There is not enough evidence to justify retaining specific phytosanitary measures. Instead MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this disease and will re-assess if the situation changes. Refer to paragraph 140 for further details.	Bové 2008 Roistacher 1991
Gum pocket	Country freedom OR graft inoculated dweet tangor, sweet orange or Citrus excelsa. Grow indicators at cool temperatures 18 to 25°C.	Remove specific phytosanitary measures but retain the name on the pest list.	There is not enough evidence to justify retaining specific phytosanitary measures. Instead MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this disease and will re-assess if the situation changes. Refer to paragraph 140 for further details.	Duran-Vila <i>et al.</i> 2002 Timmer <i>et al.</i> 2000 van Vuuren <i>et al.</i> 2005
Gummy bark	Country freedom OR SPAGE of graft inoculated citron extract. Grow citron at hot temperature 27 to 32°C.	Remove from pest list.	Citrus bark cracking viroid (syn. Citrus viroid IV) is the only candidate for the putative aetiology of gummy bark. Specific phytosanitary measures for this viroid are already in place (refer to Viroids section above). Refer to paragraph 140 for further details.	Bernad <i>et al.</i> 2005 Mohamed <i>et al.</i> 2009 Roistacher 1991
Kassala	Country freedom, cuttings collected from kassala free area.	Remove from pest list (named as Kassala disease).	Different viroids have been isolated from kassala diseased samples. Specific phytosanitary measures for viroids are already in place (refer to Viroids section above). Refer to paragraph 140 for further details.	Mohamed <i>et al.</i> 2009
Lemon sieve tube necrosis	Country freedom OR other suitable test.	Remove from pest list.	Lemon sieve tube necrosis is a physiological disorder. Refer to paragraph 139 for further details.	Roistacher 2012
Shell bark of lemons	Country freedom OR other suitable test.	Remove from pest list.	Different viroids have been isolated from Shell bark diseased samples. Specific phytosanitary measures for viroids are already in place (refer to Viroids section above). Refer to paragraph 140 for further details.	Timmer <i>et al</i> . 2000

Organism currently on the IHS and proposed new addition (in bracket)	Current phytosanitary measures	Proposed changes to IHS	Note	References
Zonate chlorosis	Country freedom, cuttings collected from kassala free area.	Remove specific phytosanitary measures but retain the name on the pest list.	There is not enough evidence to justify retaining specific phytosanitary measures. Instead MPI propose to set up the active monitoring of the International Biosecurity Intelligence System (IBIS: <u>http://biointel.org/</u>) to gather any new information related to this disease and will re-assess if the situation changes. Refer to paragraph 140 for further details.	

Table 2. Summary of Risk Assessment of pathogens found to be associated with citrus hosts since the last review of the *Citrus* schedule IHS nursery stock.

		Biology			Risk assessment				
Pathogens	Host/s	Transmission	Distribution	Reference	Regulatory status in NZ	Likelihood of entry & establishment in NZ ¹	Economic impacts in NZ ²	Economic impacts on <i>Citrus</i> and other crop production industries ³	Additional notes
Liberibacter		-					1		
' <i>Candidatus</i> Liberibacter americanus'	C. latifolia C. limonia C. reshni C. reticulata C. reticulata × C. sinensis C. sinensis C. sunki Murraya exotica M. paniculata Poncirus trifoliata × C. paradisi	Psyllid vector: <i>Diaphorina citri</i> Graft transmission Propagation material	Brazil	Teixeira <i>et</i> <i>al.</i> 2005	Regulated	Low likelihood of entry. Moderate to high likelihood of establishment.	Low (without a vector) to moderate (with a vector).	Immediate impact on exports of citrus fruit or nursery stock to some countries. Potential impact on citrus production for domestic and export sales (greater if vector present).	Impact will be much greater on the citrus industry if the vector, <i>Diaphorina citri</i> is present in New Zealand (currently absent and has a regulated status). There is no clear evidence of liberibacters being seed transmitted.
Phytoplasma									
								Highly uncertain impact.	
<i>'Candidatus</i> Phytoplasma asteris'	C. aurantifolia C. japonica C. maxima C. reticulata C. sinensis	Phloem-feeding insect vector? Graft transmission Propagation material	China Mexico	Arratia- Castro et al. 2014 Chen et al. 2009 Poghosyan et al. 2015	Unassigned	Low likelihood of entry. Moderate to high likelihood of establishment.	Very low to high. (uncertain)	Potential impact on citrus production for domestic and export sales. Potential imporant impact on other crops such as apple, grapevine, potato, tomato, onion.Potential severe impact on other industries such as seed or ornamental.	Impacts are highly uncertain because of the limited knowledge of potential vectors and of symptoms. Phloem-feeding leafhoppers vectors have been identified for other 16SrIX- B (which are reported to have low vector specificity).

		Biology			Risk assessment				
Pathogens	Host/s	Transmission	Distribution	Reference	Regulatory status in NZ	Likelihood of entry & establishment in NZ ¹	Economic impacts in NZ ²	Economic impacts on <i>Citrus</i> and other crop production industries ³	Additional notes
Huanglongbing- associated 16SrIX-A phytoplasma	C. sinensis	Leafhopper vector: <i>Scaphytopius</i> (<i>Convelinus</i>) <i>marginelineatus</i> ? Graft transmission Propagation material	Brazil	Teixeira <i>et</i> <i>al.</i> 2008	Unassigned	Low likelihood of entry. Moderate to high likelihood of establishment.	Low to moderate. (uncertain)	Highly uncertain impact. Potential impact on citrus production for domestic and export sales. Potential impact for other industries such as pea, if suitable vectors present.	Some uncertainty around symptoms caused by Huanglongbing-associated 16SrIX-A phytoplasmawhich has not yet been proven to cause Huanglongbing disease. Impacts are highly uncertain because of the limited knowledge of potential vectors (i.e. <i>Scaphytopius</i> (<i>Convelinus</i>) marginelineatus tested positive by PCR for phytoplasma but there are no transmission studies to confirm as being a vector). Impact will be much greater on the
Viroid									citrus industry if a suitable vector is present.
	Atalantia citroides C. australasica								
Citrus viroid V	C. australasica C. aurantium C. bergamia C. clementina C. latifolia C. limettioides C. limon C. madurensis C. paradisi × C. tangerina & hybrids C. sinensis C. tamuranua C. temple C. unshiu C. unshiu C. unshiu × C. sinensis Eremocitrus glauca Fortunella margarita	Graft transmission Mechanical transmission Propagation material	China Iran Japan Nepal Pakistan Spain Sultanate of Oman Turkey USA	Bani Hashemian <i>et al.</i> 2010 Serra <i>et al.</i> 2008a & 2008b Onelge & Yurtmen 2012	Unassigned	Low likelihood of entry. Moderate to high likelihood of establishment.	Low.	Potential impact on citrus production for domestic and export sales. May limit future rootstock options.	Effects of CVd-V alone likely to be subtle, perhaps moderate effects of tree size and yield. Potential for synergy in the presence of other citrus viroids resulting in a greater impact on citrus production. Synergy interactions has been demonstrated for <i>Citrus viroid V</i> with <i>Citrus dwarfing viroid</i> (non-regulated) and <i>Citrus bent leaf viroid</i> (regulated). Symptom expression more likely in warmer areas. Existing plantings affected through grafting or mechanical transmission.

		Biology			Risk assessment				
Pathogens	Host/s	Transmission	Distribution	Reference	Regulatory status in NZ	Likelihood of entry & establishment in NZ ¹	Economic impacts in NZ ²	Economic impacts on <i>Citrus</i> and other crop production industries ³	Additional notes
Citrus viroid VI	<i>C. sinensis</i> and hybrids <i>C. reticulata</i> and hybrids <i>Diospyros kaki</i>	Graft transmission Mechanical transmission Propagation material	Japan	Ito <i>et al.</i> 2001 & 2002 Nakauro & Nakano 2008 ?	Unassigned	Low likelihood of entry. Moderate to high likelihood of establishment.	Low.	Potential impact on citrus production for domestic and export sales. May limit future rootstock options. Potential impact on persimmon industry.	Effects of CVd-VI alone likely to be subtle, perhaps moderate effects on tree size and yield. Potential for synergy in the presence of other citrus viroids resulting in a greater impact on citus production. Symptom expression more likely in warmer regions. Existing plantings affected through grafting or mechanical transmission. A variant has been detected in persimmon but no information on any impact.
Virus								L	
Citrus sudden death- associated virus	C. aurantifolia C. jambhiri C. nobilis × C. deliciosa C. latifolia C. limettioides C. limonia C. medica C. reticulata C. sinensis C. sinensis × C. reticulata C. sunki C. volkameriana Poncirus trifoliata P. trifoliata × C. paradisi	Aerial vector? Graft transmission transmission ⁴ Propagation material	Brazil	Maccheroni <i>et al.</i> 2005 Yamamoto <i>et al.</i> 2011	Unassigned	Low likelihood of entry. Moderate to high likelihood of establishment.	Low.	Some impact on citrus production for domestic and export sales. May limit future rootstock options.	The virus is a very good marker for Citrus sudden death disease but not confirmed as the causal agent. Low likelihood of expression of the disease due to the low prevalence of susceptible scion/rootstock combinations in New Zealand. <i>Toxoptera citricida</i> which is widespread in New Zealand but vector regulated, is a vector of the disease.

		Biology			Risk assessment				
Pathogens	Host/s	Transmission	Distribution	Reference	Regulatory status in NZ	Likelihood of entry & establishment in NZ ¹	Economic impacts in NZ ²	Economic impacts on <i>Citrus</i> and other crop production industries ³	Additional notes
Indian citrus ringspot virus	C. aurantifolia C. jambhiri C. medica C. nobilis × C. deliciosa C. reticulata C. sinensis	Graft transmission transmission ⁴ Propagation material	India	Hoa and Ahlawat 2004 Milne <i>et al.</i> 2007 Rustici <i>et</i> <i>al.</i> 2000	Unassigned	Low likelihood of entry. Moderate likelihood of establishment.	Low.	Some impact on citrus production for domestic and export sales.	Disease expression depends on suitable climate and susceptible citrus species/varieties. Likely limited areas of suitable climate for disease expression. Kinnow mandarin and Mosambi sweet orange are the most susceptible hosts (reduce fruit yield and quality, and tree decline to plant death). No known natural vectors.
Olive latent virus 1	C. aurantifolia Citrus × paradisi C. limon C. sinensis Olea europaea Solanum Iycopersicum Tulipa sp.	Seed transmission? Root transmission? Soil transmission? Graft transmission Mechanical transmission ⁴ Propagation material	Africa Asia Europe Middle East	Félix <i>et al.</i> 2007 Martelli <i>et</i> <i>al.</i> 1996	Unassigned	Low likelihood of entry. Moderate to high likelihood of establishment.	Uncertain but considered to be low to moderate.	Potential impact on citrus production for domestic and export sales, but this is unclear. Potential impact (domestic and export production) on other crops such as tomato, tulip, olive.	Impact on yield and quality for citrus and olive production elsewhere is unclear. Seed transmission has been demonstrated on olive. Mechanically transmissible to many herbaceous species.

¹The likelihood of entry took into account the current IHS measures for *Citrus* nursery stock; the establishment of an organism in New Zealand considered factors such as host availability, means of transmission (e.g. grafting, vectors) and climate.

 $^{2}\mbox{The economic impact was considered at the level of the New Zealand economy as a whole.$

³Direct economic Impact to the New Zealand citrus industry, as well as to other industries that may be affected by a disease, based on the information available at the time of writing.

⁴Mechanical transmission to herbaceous host plants has only been demonstrated experimentally.

Type of nursery stock	Origin	Note ¹	Current Level of quarantine	New Level of quarantine
	Elizabeth Macarthur Agricultural Institute (EMAI), Australia (offshore MPI- accredited facility)	 EMAI conduct a number of tests on the mother-plants prior to export, reducing the risk of harbouring regulated pathogens (e.g. sPAGE for viroids, immunoassay for <i>Citrus tristeza virus</i>). Australia is free of a number of citrus regulated organisms such as liberbacters, <i>Xylella fastidiosa</i>, citrus leprosis viruses. Insecticides and miticides treatments prior to export to New Zealand. Visual inspections on arrival and whilst in post entry quarantine. 	Level 2 (with additional temperature control)	Level 2 (with additional temperature control)
	Other offshore MPI- accredited facilities	 A number of tests on the mother-plants have been performed prior to export, reducing the risk of harbouring regulated pathogens. Budwood and tissue culture imported from countries free of a number of important citrus pathogens (e.g. liberibacters, <i>xylella fastidiosa</i>, citrus leprosis viruses). Citrus tissue culture plants are grown in sterile conditions, eliminating the risk of insects and mites infections. Certains pathogens (i.e. fungi, viruses, viroids, phytoplasmas) may also be eliminated. Bacteria and fungi that are not readily dispersed by wind/aerosol action and fungi are unlikely to produce fruiting bodies in quarantine e.g. <i>Elsinoë australis, Phyllosticta citricarpa</i>. Insecticides and miticides treatments prior to export to New Zealand. Visual inspections on arrival and whilst in post entry quarantine. 	Level 2 (with additional temperature control)	Level 2 (with additional temperature control) OR Level 3A
Cutting OR Tissue culture	Non-accredited facilities	 Absence of testing history of the mother-plants and absence of additional declarations of freedom of regulated citrus organisms. Smallest vectors of citrus regulated organisms are <i>Brevipalpus</i> mites, the vectors of citrus leprosis viruses. Some species of mites are present in New Zealand and they can only be excluded by HEPA filtration. Citrus tissue culture plants are grown in sterile conditions, eliminating the risk of insects and mites infections. Certains pathogens (i.e. fungi, viruses, viroids, phytoplasmas) may also be eliminated. Bacteria and fungi that are not readily dispersed by wind/aerosol action and fungi are unlikely to produce fruiting bodies in quarantine e.g. <i>Elsinoë australis, Phyllosticta citricarpa</i>. Insecticides and miticides treatments prior to export to New Zealand. Visual inspections on arrival and whilst in post entry quarantine. 	Level 3	Level 3B
	Non-accredited facilities from countries free of citrus leprosis viruses	 Absence of testing history of the mother-plants. Addditional declaration of pest free area for the citrus leprosis viruses (i.e. <i>Citrus leprosis virus C, Citrus leprosis cytoplasmic type 2, Citrus leprosis virus</i> nuclear type) is endorsed on the phytosanitary certificate. Vectors of citrus regulated organisms other than the <i>Brevipalpus</i> mites (vectors of citrus leprosis viruses) are aphids, leafhoppers, mealybugs, psyllids, sharpshooters and whiteflies, all of which are efficiently excluded by 0.2 mm mesh size. Furthermore these vector species (with the exception of aphids) involved in transmitting the regulated citrus organisms are absent in New Zealand (Refer to Appendix 5 for details). Citrus tissue culture plants are grown in sterile conditions, eliminating the risk of insects and mites infections. Certains pathogens (i.e. fungi, viruses, viroids, phytoplasmas) may also be eliminated. Bacteria and fungi that are not readily dispersed by wind/aerosol action and fungi are unlikely to produce fruiting bodies in quarantine e.g. <i>Elsinoë australis, Phyllosticta citricarpa</i>. Insecticides and miticides treatments prior to export to New Zealand. Visual inspections on arrival and whilst in post entry quarantine. 	Level 3	Level 3A

Table 3. Assessment of the level of quarantine for citrus nursery stock.

¹Mode of transmission of citrus infecting pathogens can be found in Appendix 5. ²Tissue culture plants kept as tissue culture during the PEQ period must be held in a Level 3 tissue culture laboratory as set out in the revised PEQ standard for plants.

Table 4. Proposed period of quarantine for citrus nursery stock.

Type of nursery stock	Origin of material	Current minimum quarantine period	Proposed minimum quarantine period	Note
	Offshore MPI-accredited facilities: sourced from an insect-proof plant house	6 months	8 months	 Allow testing of candidate plants grown at different temperature for five months (liberibacters testing) followed by three months (viroids testing). Proposed quarantine period should be long enough to allow the completion of woody indexing (symptoms observations for up to seven months for liberibacters). Current minimum quarantine period of 6 months may be extended to 12 months to allow for testing to be completed.
Cutting	Offshore MPI-accredited facilities: sourced from an open field	16 months	16 months	 Current quarantine period is considered to appropriately manage the risks. A number of tests on the mother-plants have been performed prior to export, reducing the risk of harbouring regulated pathogens.
	Non-accredited facilities: sourced from an insect-proof plant house or from an open field	16 months	18 months	 The risks associated with materials sourced from non-accredited facilities is considered to be higher than material sourced from offshore MPI-accredited facilities because of absence of testing history of the mother-plants. 18 months quarantine allows the observation of plants for 2 seasons of active growth (2 complete spring and summer periods) for potential disease development.
Tissue	Offshore MPI-accredited facilities	6 months	8 months	 Allow testing of candidate plants grown at different temperature for five months (liberibacters testing) followed by three months (viroids testing). Proposed quarantine period should be long enough to allow the completion of woody indexing (symptoms observations for up to seven months for liberibacters). Current minimum quarantine period of 6 months may be extended to 12 months to allow for testing to be completed.
culture	Non-accredited facilities	16 months	12 months	 The risks associated with tissue culture plants sourced from non-accredited facilities is considered to be lower than cuttings sourced from non-accredited facilities but higher than tissue culture plants sourced from offshore MPI-accredited facilities because of the absence of testing history of the mother-plants. Citrus tissue culture plants are grown in sterile conditions, eliminating the risk of insects and mites infections as well as surface contaminants such as bacteria and fungi. Furthermore certains pathogens (i.e. fungi, viruses, viroids, phytoplasmas) may also be eliminated.

Proposed Citrus schedule in the Import Health Standard 155.02.06: Nursery Stock

Citrus

- **Note:** The entry conditions in this schedule only apply to species in the Plants Biosecurity Index listed under Import Specifications for Nursery Stock as "see 155.02.06 under *Citrus*", and are additional to those specified in Parts 1, 2 and 3 of the import health standard.
- **1.** Type of *Citrus* nursery stock approved for entry into New Zealand Cuttings; Plants in tissue culture
- 2. Approved countries All
- 3. Pests of *Citrus* Refer to the pest list.

4. Special conditions

The following special conditions must be met in addition to basic conditions in Part 1 and 2, and the Entry conditions in section 5 of this schedule:

- a. Conditions for *Ceratocystis fimbriata sensu lato* complex (strains not in New Zealand) (section 2.2.1.8)
 Note: Only applies to cuttings of members of the *Citrus* genus
- b. Conditions for *Helicobasidium mompa* (section 2.2.1.9) **Note**: Only applies to cuttings
- c. Conditions for *Phythophthora ramorum* (section 2.2.1.11) **Note**: Only applies to cuttings of members of the *Citrus* genus
- d. Conditions for *Xylella fastidiosa* (section 2.2.1.12) **Note**: Only applies to cuttings and tissue culture of members of the *Citrus* genus

5. Entry conditions

The entry conditions are applicable to *Citrus* cuttings and tissue culture from offshore MPI-accredited facilities and non-accredited facilities in any country.

An offshore accredited facility is a facility that has been accredited to the Standard PIT.OS.TRA.ACPQF to undertake phytosanitary activities. For *Citrus*, the accredited facility operator must also have an agreement with MPI on the phytosanitary measures to be undertaken for *Citrus*.

(i) *Documentation*

All *Citrus* nursery stock must be accompanied by:

- An **import permit** (requested and obtained prior to import); AND
- A completed **phytosanitary certificate** issued by the NPPO of the exporting country.

The exporting NPPO must endorse the required additional declarations on the

phytosanitary certificate for these to be considered equivalent to testing in MPI-accredited facilities or in post entry quarantine.

(ii) <u>Phytosanitary measures</u>

Before a phytosanitary certificate is to be issued, the exporting country NPPO must be satisfied that the following activities required by MPI have been undertaken.

The Citrus cuttings / plants in tissue culture [choose ONE option] have been:

inspected in accordance with appropriate official procedures and found to be free of any visually detectable regulated pests.

AND

- treated for regulated insects and mites as described in section 2.2.1.6 of the basic conditions of this Import Health Standard within 7 days prior to shipment [cuttings only]

AND

held in a manner to ensure that infestation/reinfestation does not occur following testing at the MPI-accredited facility, and certification.

The following phytosanitary measures are additional to the above and only apply to offshore MPI-accredited facilities:

The *Citrus* cuttings / plants in tissue culture [choose ONE option] have been:

- sourced from *either* mother plants that have been kept in insect-proof plant houses *or* from open ground mother plants [cuttings only, choose ONE option].

AND

- held and tested for/classified free from specified regulated pests as required in the agreement between MPI and the [name of the MPI-accredited facility].

(iii) Additional declarations to the phytosanitary certificate

If satisfied that the pre-shipment activities have been undertaken, the exporting country NPPO must confirm this by recording the treatments applied in the Disinfestation and/or Disinfection Treatment" section and by providing the following additional declarations to the phytosanitary certificate:

"The *Citrus* cuttings / plants in tissue culture [choose ONE option] have been:

- held in a manner to ensure infestation/reinfestation does not occur following inspection and testing at the accredited facility, and certification. "

The following additional declaration/s, endorsing the absence of fungi, viruses, viroids, phytoplasmas and disease of unknown aetiology listed in the Table 'Inspection, Testing and Treatment Measures for *Citrus*' will be considered equivalent to testing by the MPI-accredited facility or in post entry quarantine.

"The following organisms/diseases are not known to occur in the exporting country, [name of the country]: [list of names of fungi, viruses, viroids, phytoplasmas and disease of unknown aetiology]."

The following additional declarations are additional to the above and only apply to offshore MPI-accredited facilities:

"The *Citrus* cuttings / plants in tissue culture [choose ONE option] have been:

held and tested for/classified free from specified regulated pests as required in the

agreement between MPI and the [name of the MPI-accredited facility].

AND

- sourced from mother plants that have been kept in insect-proof plant houses *or* from open ground mother plants [cuttings only, choose ONE option].

AND

- held and tested for/classified free from specified regulated pests at the accredited facility as required in the agreement between MPI and the accredited facility operator."

AND

 held and tested for/classified free from specified regulated pests at the accredited facility as required in the agreement between MPI and the accredited facility operator. "

Guidance

• Additional declarations: Any missing additional declarations of country freedom and pest free areas for citrus regulated fungi, viruses, viroids, phytoplasmas and disease of unknown aetiology, would result in testing for the organism as specified in the testing table below.

6. Post entry quarantine

(i) *Level of and period in quarantine*

All *Citrus* nursery stock must be imported under permit into a quarantine facility accredited to standard PBC-NZ-TRA-PQCON *Specification for the registration of a plant quarantine or containment facility, and operator* for a minimum of quarantine period as defined in the table below.

Type of material	Type of facility	Location of material	Level of quarantine facility	Minimum period of quarantine
	Offshore MPI- accredited	Insect proof screenhouse	Level 2 with additional temperature control OR Level 3A	8 months
Cutting	facilities	Open field	Level 2 with additional temperature control OR Level 3A	16 months
	Non- accredited	Insect proof screenhouse	Level 3B	18 months
	facilities	Open field	Level 3B	18 months
Tissue	Offshore MPI- accredited facilities	Tissue culture laboratory	Level 2 with additional temperature control OR Level 3A	8 months
culture	Non- accredited facilities	Tissue culture laboratory	Level 3B	12 months

Alternatively:

Following 5 months at 18-25°C for Liberibacters testing and 3 months at 28-32°C for viroids testing, provided that the only remaining test is woody indexing, no regulated organisms have been detected and based on a direction from the Inspector, the plants can be moved to the next quarantine level down (i.e. L3A when they were in Level 3B or Level 2 with additional temperature when plants were in Level 3A) for the rest of the quarantine period. Upon completion of the quarantine period, the plants can be given biosecurity clearance.

- (ii) Inspection and testing requirements
 - The nursery stock will be grown in post-entry quarantine and will be inspected, treated and/or tested for regulated pests, at the expense of the importer.
 - For tissue cultures, the quarantine period begins when tissue cultures are deflasked into the quarantine greenhouse.
 - Plants must be grown at 18-25°C for a minimum of 5 months prior to conduct testing for liberibacters then at 28-32°C for a minimum of 3 months to conduct testing for viroids.

Guidance

- Level of quarantine: A lower level of quarantine may be appropriate depending on the country of origin of the nursery stock and whether some testing has been completed prior to export. For example if *Citrus* nursery stock (cutting or tissue culture) is imported from a non-accredited facility in a country free of citrus leprosis viruses and the additional declaration is endorsed on the phytosanitary certificate, the material may be improted into a Level 3A instead of Level 3B. This is because the vectors of citrus regulated organisms other than the *Brevipalpus* mites (vectors of citrus leprosis viruses) are aphids, leafhoppers, mealybugs, psyllids, sharpshooters and whiteflies, all of which are efficiently excluded by 0.2 mm mesh size (Level 3A) while the *Brevipalpus* mites can only be excluded in a facility with HEPA filtration (Level 3B).
- **Period in quarantine:** The number of months is an indicative minimum quarantine period and this period may be extended if material is slow growing, pests are detected, or further treatment/testing is required.
- **Temperature requirements:** The period in quarantine may be extended if the temperature requirements for liberibacters and viroids testing are not respected.

Pest List for Citrus

REGULATED PESTS (actionable)

Insect, mite, mollusc and spider

Check the MPI database, Biosecurity organisms register for imported commodities (BORIC) for regulatory status, <u>http://archive.mpi.govt.nz/applications/boric</u>

Fungus	
Ascomycota	
Botryosphaeriales	
Botryosphaeriaceae	
Macrophoma mantegazziana	-
Sphaeropsis tumefaciens	stem gall
Phyllostictaceae	
Phyllosticta citricarpa	citrus black spot, syn. Guignardia citricarpa
Capnodiales	
Capnodiaceae	
Capnodium citri	sooty mould
Mycosphaerellaceae	
Mycosphaerella horii	greasy spot
Mycosphaerella millegrana	syn. Cercospora microsora
Pseudocercospora angolensis	cercospora spot, syn. <i>Phaeoramularia</i> angolensis
Septoria limonum	-
Zasmidium citri	rind blotch, syn. Mycosphaerella citri
Diaporthales	
Diaporthaceae	
Diaporthe rudis	phomopsis canker
Diaporthe cytosporella	rot, syn. Phomopsis cytosporella
Erysiphales	
Erysiphaceae	
Fibroidium tingitaninum	powdery mildew, syn. Oidium tingitaninum
Helicobasidiales	
Helicobasidiaceae	
Helicobasidium mompa	
Helotiales	
Dermateaceae	Closer arium falijaala C falijaalum
Colletotrichum coccodes	Gloesporium foliicola, G. foliicolum
Hypocreales Clavicipitaceae	
Aschersonia placenta [Animals Biosecurity]	
Ophiocordycipitaceae	-
Hirsutella thompsonii [Animals Biosecurity]	
Microascales	-
<i>Ceratocystis fimbriata sensu lato</i> complex	Ceratocystis blight
Myriangiales	Ceratorysus onght
Elsinoaceae	
Elsinoë australis	sweet orange scab
Pleosporales	sweet orange seas
Didymellaceae	
Plenodomus tracheiphilus	mal secco, syn. Phoma tracheiphila
Pleosporaceae	
Alternaria pellucida	_
Alternaria scorzonerae	syn. Alternaria linicola
Stemphylium rosarium	-
Saccharomycetales	
Eremotheciaceae	
Eremothecium coryli	dry rot, syn. Nematospora coryli
Basidiomycota	
Boletales	
Coniophoraceae	

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Coniophora eremophila	brown wood rot
Septobasidiales Septobasidiaceae	
Septobasidium pseudopedicellatum	felt fungus
Glomeromycota	Terr Tungus
Glomerales	
Glomeraceae	
Claroideoglomus etunicatum [Animals Biosecurity]	syn. Glomus etunicatum
Oomycota Domano ang log	
Peronosporales Peronosporaceae	
Phytophthora ramorum	sudden oak death
Mitosporic Fungi (Coelomycetes)	
Sphaeropsidales	
Sphaerioidaceae	
Phoma erratica var. mikan	-
Bacterium	
Mollicutes	
Entomoplasmatales	
Spiroplasmataceae	
Spiroplasma citri	citrus stubborn
Proteobacteria Xanthomonadales	
Xanthomonadaceae	
Xanthomonas citri subsp. citri	citrus canker
Xanthomonas fuscans subsp. aurantifolii	-
Xanthomonas alfalfae subsp. citrumelonis	citrus bacterial spot
Xylella fastidiosa	citrus variegated chlorosis, Pierce's disease
Liberibacter	
Alphaproteobacteria	
Rhizobiales	
Rhizobiaceae	·, · · · ·
<i>Candidatus</i> Liberibacter africanus' <i>Candidatus</i> Liberibacter asiaticus'	citrus greening bacterium citrus greening bacterium
<i>Candidatus</i> Liberibacter astaticus'	citrus greening bacterium
'Candidatus Liberibacter caribbeanus'	citrus greening bacterium
Directoryland	
Phytoplasma Acholeplasmatales	
Acholeplasmataceae	
<i>Candidatus</i> Phytoplasma asteris'	Chinese Huanglongbing disease-associated
• •	phytoplasma, Mexican Huanglongbing disease-
	associated phytoplasma
<i>Candidatus</i> Phytoplasma aurantifolia'	witches' broom disease of lime
Not described	HLB-associated 16SrIX-A phytoplasma, Brazilian Huanglongbing disease-associated
	phytoplasma
Not described	Australian citrus dieback disease
T 74 4 T	
Viroid Pospiviroidae	
Apscaviroid	
Citrus bent leaf viroid	citrus viroid I, citrus variable viroid
Citrus viroid V	-
Citrus viroid VI	citrus viroid original sample
Citrus viroid VII	
Cocadviroid	citrus viroid IV
Citrus bark cracking viroid Hostuviroid	
Hop stunt viroid - citrus	citrus cachexia viroid, citrus viroid II, citrus
	xyloporosis viroid

Virus	
Alphaflexiviridae	
Mandarivirus	
Citrus yellow vein clearing virus	yellow vein clearing of lemon
Indian citrus ringspot virus	-
Bromoviridae	
Narvirus	
Citrus variegation virus	citrus infectious variegation virus, crinkly leaf strain
Citrus leaf rugose virus	-
Caulimovoridae	
Badnavirus	
Citrus yellow mosaic virus	Indian citrus mosaic virus
Geminiviridae	
Unassigned	
Citrus chlorotic dwarf-associated virus	citrus chlorotic dwarf
Secoviridae	
Sadwavirus	
Satsuma dwarf virus	citrus mosaic virus, Natsudaidai dwarf strain, navel orange infectious mottling virus
Tombusviridae	
Alphanecrovirus	
Olive latent virus 1	-
Unassigned	
Cilevirus	
Citrus leprosis virus C	-
Citrus leprosis virus cytoplasmic type 2	-
Dichorhabdovirus	
Citrus leprosis virus nuclear type	-
Higrevirus	
Hibiscus green spot virus 2	-
Unassigned	
Citrus leathery leaf virus	-
Citrus necrotic spot virus	-
Citrus yellow mottle virus	-
Iranian citrus ringspot-associated virus	-
Tymoviridae	
Marafivirus	
Citrus sudden death-associated virus	-
Disease of unknown aetiology	
Citrus blight	-

Inspection, Testing and Treatment Measures for Citrus

ORGANISM TYPES	MPI ACCEPTABLE METHODS							
Insects	Visual inspection (Refer to section 5(ii) of the Citrus schedule) AND approved							
	insecticide treatments (Refer to section 2.2.1.6 of the basic conditions).							
Mites	Visual inspection (Refer to section 5(ii) of the Citrus schedule) AND approved							
	miticide treatments (Refer to section 2.2.1.6 of the basic conditions).							
Fungus	All cuttings must be dipped in 1% sodium hypochlorite for 2 minutes upon arrival							
	in the post entry quarantine facility.							
	Growing season inspection in PEQ for symptom expression.							
Ceratocystis fimbriata	Plating from original budstick and on actively growing plants while in PEQ (any							
	suspect C. fimbriata will be confirmed by PCR) [only apply to cuttings of the							
	genus <i>Citrus</i>]. Refer to section 2.2.1.8 of the basic conditions.							
Elsinoë australis	PCR OR plating on semi-selective medium.							
Phyllosticta citricarpa	PCR.							
Helicobasidium mompa	Refer to section 2.2.1.9 of the basic conditions [only apply to cuttings].							
Plenodomus tracheiphilus	PCR.							
Phytophthora ramorum	PCR from original budstick and on actively growing plants while in PEQ [only							
	apply to cuttings of the genus <i>Citrus</i>]. Refer to section 2.2.1.11 of the basic							
	conditions.							
Bacterium	All cuttings must be dipped in 1% sodium hypochlorite for 2 minutes upon arrival							
	in the post entry quarantine facility.							
Spiroplasma citri	PCR.							
Xanthomonas citri subsp. citri	PCR.							
Xanthomonas fuscans subsp.	Growing season inspection in PEQ for symptom expression.							
aurantifolii								
Xanthomonas alfalfae subsp.	Growing season inspection in PEQ for symptom expression.							
citrumelonis								
Xylella fastidiosa	PCR (two sets, samples to be collected at least four weeks apart while in PEQ).							
	Refer to section 2.2.1.12 of the basic conditions.							
Liberibacter								
'Candidatus Liberibacter	PCR after candidate plants actively growing at 18-25°C for at least 5 months AND							
africanus'	woody indexing (Citrus sinensis 'Pineapple' and Citrus reticulata 'Parson's							
	special').							
'Candidatus Liberibacter	PCR after candidate plants actively growing at 18-25°C for at least 5 months AND							
americanus'	woody indexing (Citrus sinensis 'Pineapple' and Citrus reticulata 'Parson's							
	special').							
'Candidatus Liberibacter	PCR after candidate plants actively growing at 18-25°C for at least 5 months AND							
asiaticus'	woody indexing (Citrus sinensis 'Pineapple' and Citrus reticulata 'Parson's							
	special').							
'Candidatus Liberibacter	PCR after candidate plants actively growing at 18-25°C for at least 5 months AND							
caribbeanus'	woody indexing (Citrus sinensis 'Pineapple' and Citrus reticulata 'Parson's							
	special').							
Phytoplasma								
Australian citrus dieback	Nested-conventional or real-time PCR using phytoplasma universal primers							
'Candidatus Phytoplasma	Nested-conventional or real-time PCR using phytoplasma universal primers.							
aurantifolia'								
'Candidatus Phytoplasma asteris'	Nested-conventional or real-time PCR using phytoplasma universal primers.							
HLB-associated 16SrIX-A	Nested-conventional or real-time PCR using phytoplasma universal primers.							
phytoplasma								
Virus								
Citrus leaf rugose virus	PCR OR woody indexing (Citrus limon 'Eureka' and Citrus medica 'Arizona							
	861').							
Citrus leprosis virus C	PCR.							
Citrus leprosis virus cytoplasmic	PCR.							
type 2								
Citrus leprosis virus nuclear type	PCR.							
	DCD							
Citrus sudden death-associated	PCR.							

ORGANISM TYPES	MPI ACCEPTABLE METHODS					
Citrus variegation virus	PCR OR woody indexing (<i>Citrus limon</i> 'Eureka' and <i>Citrus medica</i> 'Arizona					
~	861').					
Citrus yellow mosaic virus	PCR.					
Citrus yellow vein clearing virus	PCR.					
Indian citrus ringspot virus	PCR.					
Olive latent virus 1	PCR.					
Satsuma dwarf virus	PCR OR woody indexing (<i>Citrus limon</i> 'Eureka' and <i>Citrus reticulata</i> 'Parson's special').					
Viroid						
Citrus bark cracking viroid	PCR after candidate plants actively growing at 28-32°C for at least 3 months AND woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special')					
Citrus bent leaf viroid	PCR after candidate plants actively growing at 28-32°C for at least 3 months AND woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special')					
Citrus viroid V	PCR after candidate plants actively growing at 28-32°C for at least 3 months AND woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special')					
Citrus viroid VI	PCR after candidate plants actively growing at 28-32°C for at least 3 months AND woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special')					
Citrus viroid VII	PCR after candidate plants actively growing at 28-32°C for at least 3 months AND woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special')					
Hop stunt viroid [citrus strain]	PCR after candidate plants actively growing at 28-32°C for at least 3 months AND woody indexing (<i>Citrus medica</i> 'Arizona 861' and <i>Citrus reticulata</i> 'Parson's special')					
Disease of unknown aetiology						
Citrus blight	Growing season inspection of mother plants prior to production of cuttings/ tissue cultures for export.					

Guidance:

- **Country freedom and pest free areas declarations**: These endorsments are only for regulated fungi, viruses, viroids, phytoplasmas and disease of unknown aetiology listed in the Table 'Inspection, Testing and Treatment Measures for *Citrus*'. The exporting NPPO must endorse the additional declarations on the phytosanitary certificate to be considered equivalent to testing in MPI-accredited facilities or in post entry quarantine.
- **Controls**: Positive and negative controls must be included for each test.
- Unit: The unit for testing is defined in section 2.3.2.1.
- **Testing methods:** The recommended woody indexing and PCR protocols (including primers) can be found at the following link: <u>http://www.biosecurity.govt.nz/files/regs/imports/plants/high-value-crops/citrus-testing-manual.pdf</u>. The term PCR includes conventional (simplex or duplex) as well as real-time PCR unless it is specified (e.g. phytoplasmas). Recommended sample collection and time of testing are also provided in the manual. Note the specific temperature range for liberibacters and viroids in the Table.
- Other internationally recognised testing methods including woody indicator species may be accepted by MPI with prior notification.

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^{60 •} Risk Management Proposal: Citrus schedule of the IHS nursery stock

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Appendix 1: Taxonomy and regulatory status of the *Citrus* pest list

The taxonomy and the regulatory status of the Citrus pests are presented in tables as follows:

Table 1.1: Taxonomy and regulatory status of bacteria

Table 1.2: Taxonomy and regulatory status of fungi

Table 1.3: Taxonomy and regulatory status of liberibacters

 Table 1.4: Taxonomy and regulatory status of phytoplasmas

 Table 1.5: Taxonomy and regulatory status of viroids

Table 1.6: Taxonomy and regulatory status of viruses

Table 1.7: Taxonomy and regulatory status of diseases of unknown aetiology

Table 1.8: Taxonomy and regulatory status of insects, mites, spiders, molluscs (note: only the changes are listed)

MPI propose to have all the pathogens with undetermined status to be regulated based on the proposed phytosanitary measures (refer to section 'Phytosanitary measures' for details).

Genus and Species	Family	Order	Class	Status ¹	Proposed changes to the pest list for Citrus ²
Burkholderia cepacia	Pseudomonadaceae	Burkholderiales	Betaproteobacteria	Non-regulated	Remove from pest list – present in New Zealand
Spiroplasma citri	Spiroplasmataceae	Entomoplasmatales	Mollicutes	Regulated	Update taxonomy
Xanthomonas axonopodis pv. citri (syn)	Xanthomonadaceae	Xanthomonadales	Gammaproteobacteria	Regulated	Update taxonomy and name to Xanthomonas citri subsp. citri
Xanthomonas campestris pv. aurantifolii (syn)	Xanthomonadaceae	Xanthomonadales	Gammaproteobacteria	Regulated	Update taxonomy and name to Xanthomonas fuscans subsp. aurantifolii
Xanthomonas campestris pv. citrumelo (syn)	Xanthomonadaceae	Xanthomonadales	Gammaproteobacteria	Regulated	Update taxonomy and name to Xanthomonas alfalfae subsp. citrumelonis
Xylella fastidiosa	Xanthomonadaceae	Xanthomonadales	Gammaproteobacteria	Regulated	Include Citrus variegated chlorosis as the common name and update taxonomy
Xylella fastidiosa pv. citri	Xanthomonadaceae	Xanthomonadales	Gammaproteobacteria	Regulated	Remove from pest list – refer to Xylella fastidiosa

Table 1.1. Update on taxonomy (in blue) and regulatory status (in green) of bacteria.

¹Reference for status: BORIC, MPI database.

²Reference for taxonomy: GISD (2014) Global Invasive Species Database, <u>http://www.issg.org/database/welcome/</u> (accessed 21 May 2015); Ngā Harore o Aotearoa – New Zealand Fungi Databases, <u>http://nzfungi2.landcareresearch.co.nz/</u> (accessed 03 June 2015).

Genus and Species	Family	Order	Class	Status ¹	Proposed changes to the pest list for <i>Citrus</i> ²
Alternaria limicola	Pleosporaceae	Pleosporales	Ascomycota	Regulated	Update taxonomy and name to Alternaria scorzonerae
Alternaria pellucida	Pleosporaceae	Pleosporales	Ascomycota	Regulated	Update taxonomy
Aschersonia placenta [Animals Biosecurity]	Clavicipitaceae	Hypocreales	Ascomycota	Regulated	Update taxonomy
Aureobasidium spp.	Saccotheciaceae	Dothideales	Ascomycota	Non-regulated	Remove from pest list - Aureobasidium pullulans, the only Aureobasidium species associated with citrus is present in New Zealand.
Capnodium citri	Capnodiaceae	Capnodiales	Ascomycota	Regulated	Update taxonomy
Cercospora microsora (syn)	Mycosphaerellaceae	Capnodiales	Ascomycota	Regulated	Update taxonomy and name to Mycosphaerella millegrana
Coniophora eremophila	Coniophoraceae	Boletales	Basidiomycota	Regulated	No changes
Debaryomyces hansenii	Saccharomycetaceae	Saccharomycetales	Ascomycota	Non-regulated	Remove from pest list – present in New Zealand
Diaporthe rudis (anamorph Phomopsis rudis)	Diaporthaceae	Diaporthales	Ascomycota	Regulated	Update taxonomy
Didymosphaeria spp.	Didymosphaeriaceae	Pleosporales	Ascomycota	Regulated	Remove from pest list – none of the <i>Didymosphaeria</i> species are associated with citrus
Elsinoë australis	Elsinoaceae	Myriangiales	Ascomycota	Regulated	Update taxonomy
Eremothecium coryli	Eremotheciaceae	Saccharomycetales	Ascomycota	Regulated	Addition to pest list – synonym: Nematospora coryli
Galactomyces citri-aurantii (anamorph Geotrichum citri-aurantii)	Dipodacaceae	Saccharomycetales	Ascomycota	Non-regulated	Remove from pest list – present in New Zealand (current name is Dipodascus reessii)
Gloeosporium foliicolum (syn)	Dermateaceae	Helotiales	Ascomycota	Non-regulated	Remove from pest list – synonym of <i>Colletotrichum coccodes</i> (syn <i>Gloeosporium foliicola, G. foliicolum</i>) which is present in New Zealand.
Glomus etunicatum [Animals Biosecurity]	Glomeraceae	Glomerales	Glomeromycota	Regulated	Update taxonomy and name to Claroideoglomus etunicatum
Guignardia citricarpa (anamorph Phyllosticta citricarpa) [black spot strain]	Phyllostictaceae	Botryosphaeriales	Ascomycota	Regulated	Update taxonomy and name to <i>Phyllosticta citricarpa</i>
Hirsutella thompsonii [Animals Biosecurity]	Ophiocordycipitaceae	Hypocreales	Ascomycota	Regulated	Update taxonomy
Isaria sp. [Animals Biosecurity]	Cordycipitaceae	Hypocreales	Ascomycota	Regulated	Remove from pest list – none of the <i>Isaria</i> species are associated with citrus
Macrophoma mantegazziana	Botryosphaeriaceae	Botryosphaeriales	Ascomycota	Regulated	Update taxonomy
Mycosphaerella citri (anamorph Stenella citri-grisea)	Mycosphaerellaceae	Capnodiales	Ascomycota	Regulated	Update taxonomy and name to Zasmidium citri
Mycosphaerella horii	Mycosphaerellaceae	Capnodiales	Ascomycota	Regulated	Update taxonomy
Oidium tingitaninum	Erysiphaceae	Erysiphales	Ascomycota	Regulated	Update taxonomy and name to Fibroidium tingitanium
Phaeoramularia angolensis (syn)	Mycosphaerellaceae	Capnodiales	Ascomycota	Regulated	Update taxonomy and name to Pseudocercospora angolensis
Phoma erratica var. mikan	Sphaerioidaceae	Sphaeropsidales	Mitosporic Fungi (Coelomycetes)	Regulated	No changes
Phoma tracheiphila (syn.)	Didymellaceae	Pleosporales	Ascomycota	Regulated	Update taxonomy and name to Plenodomus tracheiphilus

Table 1.2. Update on taxonomy (in blue) and regulatory status (in green) of fungi.

Genus and Species	Family	Order	Class	Status ¹	Proposed changes to the pest list for <i>Citrus</i> ²
Phomopsis spp.	Diaporthaceae	Diaporthales	Ascomycota	Regulated	Update taxonomy and name to <i>Diaporthe cytosporella</i> (syn. <i>Phomopsis cytosporella</i>), the only regulated <i>Phomopsis</i> species associated with <i>Citrus</i>
Rhytidhysteron rufulum	Patellariaceae	Patellariales	Ascomycota	Non-regulated	Remove from pest list – present in New Zealand
Septobasidium pseudopedicellatum	Septobasidiaceae	Septobasidiales	Basidiomycota	Regulated	No changes
Septoria spp.	Mycosphaerellaceae	Capnodiales	Ascomycota	Regulated	Update taxonomy and name to <i>Septoria limonum</i> , the only regulated <i>Septoria</i> species associated with <i>Citrus</i>
Sphaceloma fawcettii var. scabiosae	Elsinoaceae	Myriangiales	Ascomycota	Non-regulated	Remove from pest list – present in New Zealand (current name is <i>Elsinoë fawcettii</i>)
Sphaeropsis tumefaciens	Botryosphaeriaceae	Botryosphaeriales	Ascomycota	Regulated	Update taxonomy
Sporobolomyces roseus	Microbotryomycetes	Sporidiobolales	Basidiomycota	Non-regulated	Remove from pest list - present in New Zealand
Stemphylium rosarium	Pleosporaceae	Pleosporales	Ascomycota	Regulated	Update taxonomy
Stenella sp.	Mycosphaerellaceae	Capnodiales	Ascomycota	Regulated	Remove from pest list - Stenella citrigrisea (anamorph), the only Stenella species associated with citrus, is already on the pest list as Mycosphaerella citri
Syncephalastrum racemosum	Syncephalastraceae	Mucorales	Zygomycota	Non-regulated	Remove from pest list – present in New Zealand
Ulocladium obovoideum	Pleosporaceae	Pleosporales	Ascomycota	Non-regulated	Remove from pest list – present in New Zealand (current name is Alternaria obovoidea)

¹Reference for status: BORIC, MPI database.

²Reference for taxonomy: Farr, D.F., & Rossman, A.Y. (2006) Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA, <u>http://nt.ars-grin.gov/fungaldatabases/</u> (accessed March 2016); Ngā Harore o Aotearoa – New Zealand Fungi Databases, <u>http://nzfungi2.landcareresearch.co.nz/</u> (accessed March 2016); Species Fungorum database, <u>http://www.indexfungorum.org/names/names.asp</u> (accessed March 2016).

Table 1.3. Update on taxonomy (in blue) and regulatory status (in green) of liberibacters.

Genus and Species	Family	Order	Class	Status ¹	Proposed changes to the pest list for Citrus ²
'Candidatus Liberibacter americanus'	Rhizobiaceae	Rhizobiales	Alphaproteobacteria	Regulated	Addition to pest list as 'Candidatus Liberibacter americanus' and update taxonomy
'Candidatus Liberibacter caribbeanus'	Rhizobiaceae	Rhizobiales	Alphaproteobacteria	Undetermined	Addition to pest list
Liberobacter africanum	Rhizobiaceae	Rhizobiales	Alphaproteobacteria	Regulated	Update taxonomy and name to 'Candidatus Liberibacter africanus'
Liberobacter asiaticum	Rhizobiaceae	Rhizobiales	Alphaproteobacteria	Regulated	Update taxonomy and name to 'Candidatus Liberibacter asiaticus'
Liberobacter sp.	Rhizobiaceae	Rhizobiales	Alphaproteobacteria	Regulated	Remove from pest list - covered by the specific species entries for liberibacters (above)

Genus and Species	Family	Order	Class	Common name	16Sr group- subgroup	Status ¹	Proposed changes to the pest list for <i>Citrus</i> ²
Not described	Acholeplasmataceae	Acholeplasmatales	Mollicutes	Huanglongbing -associated 16SrIX-A phytoplasma, Brazilian Huanglongbing disease- associated phytoplasma	16SrIX-A	Undetermined	Addition to pest list
'Candidatus Phytoplasma asteris'	Acholeplasmataceae	Acholeplasmatales	Mollicutes	Chinese Huanglongbing disease-associated phytoplasma Mexican Huanglongbing disease-associated phytoplasma	16SrI-B	Undetermined	Addition to pest list
' <i>Candidatus</i> Phytoplasma aurantifolia'	Acholeplasmataceae	Acholeplasmatales	Mollicutes	Witches'-broom disease of lime	16SrII-B	Regulated	No changes
Not described	Acholeplasmataceae	Acholeplasmatales	Mollicutes	Citrus rubbery wood	Unknown	Regulated	Update name and move to pest list of diseases of unknown aetiology

Table 1.4. Update on taxonomy (in blue) and regulatory status of phytoplasmas.

¹Reference for status: BORIC, MPI database.

²Reference for taxonomy: http://www.bacterio.net/-candidatus.html.

Species	Genus	Family	Status1 Proposed changes to the pest list for Citrus2		
Citrus cachexia viroid (syn)	Hostuviroid	Pospiviroidae	Strains regulated Update taxonomy and name to <i>Hop stunt viroid</i> – citrus (synonym: citrus cachexia viroid)		
Citrus variable viroid (syn)	Apscaviroid	Pospiviroidae	Regulated Update taxonomy and name to Citrus bent leaf viroid (synonym: Citrus viroid I)		
Citrus viroids (groups I-IV)	Not applicable	Pospiviroidae	Remove from pest list - refer to separate entries (below)		
Citrus viroid I (syn)	Apscaviroid	Pospiviroidae	Regulated Remove from pest list - refer to citrus variable viroid above (synonym of <i>Citrus bent leaf viroid</i>)		
Citrus viroid II (syn)	Hostuviroid	Pospiviroidae	Strains regulated	Remove from pest list - refer to citrus cachexia viroid above (synonym of Hop stunt viroid)	
Citrus viroid III (syn)	Apscaviroid	Pospiviroidae	Non-regulated	Remove from pest list - present in New Zealand, now named <i>Citrus dwarfing viroid</i> (Quemin <i>et al.,</i> 2011)	
Citrus viroid IV (syn)	Cocadviroid	Pospiviroidae	Regulated Update taxonomy and name to <i>Citrus bark cracking viroid</i> (synonym: Citrus viroid IV)		
Citrus viroid V	Apscaviroid	Pospiviroidae	Undetermined	Addition to pest list	
Citrus viroid VI	Apscaviroid	Pospiviroidae	Undetermined	Addition to pest list	
Citrus viroid VII	Apscaviroid	Pospiviroidae	Undetermined	Addition to pest list	
Dwarfing factor viroid	Apscaviroid	Pospiviroidae	Non-regulated	Remove from pest list – synonym of citrus viroid III which is present in New Zealand (Quemin <i>et al.</i> , 2011)	
Xyloporosis viroid (syn)	Hostuviroid	Pospiviroidae	Strains regulated	Remove from pest list -isolate of Hop stunt viroid	

¹Reference for status: BORIC, MPI database.

²Reference for taxonomy: International Committee on Taxonomy of Viruses, <u>http://www.ictvonline.org/</u> (accessed 21 May 2015).

Species	Genus	Family	Status ¹	Proposed changes to the pest list for <i>Citrus</i> ²
Citrus chlorotic dwarf	Undetermined	Geminiviridae	Regulated	Update taxonomy and name to Citrus chlorotic dwarf-associated virus
Citrus infectious variegation ilarvirus (syn)	Ilarvirus	Bromoviridae	Regulated	Update taxonomy and name to Citrus variegation virus
Citrus infectious variegation ilarvirus [crinkly leaf strain] (syn)	llarvirus	Bromoviridae	Regulated	Remove from pest list – synonym of Citrus variegation virus
Citrus leaf rugose ilarvirus	Ilarvirus	Bromoviridae	Regulated	Update taxonomy and name to Citrus leaf rugose virus
Citrus leathery leaf virus	Unassigned	Unassigned	Regulated	Update taxonomy
Citrus leprosis rhabdovirus (syn)	Cilevirus	Unassigned	Regulated	Update taxonomy and name to Citrus leprosis virus C
Citrus leprosis virus cytoplasmic type 2	Cilevirus	Unassigned	Undetermined	Addition to pest list
Citrus leprosis virus nuclear type	Dichorhavirus	Unassigned	Undetermined	Addition to pest list
Citrus mosaic virus (syn)	Sadwavirus	Secoviridae	Regulated	Remove from pest list – synonym of Satsuma dwarf virus
Citrus necrotic spot virus	Unassigned	Unassigned	Undetermined	Addition to pest list
Citrus ringspot virus (syn)	Unassigned	Ophioviridae	Non-regulated	Remove from pest list - present in New Zealand, now named <i>Citrus psorosis virus</i> (Quemin <i>et al.</i> , 2011)
Citrus sudden death-associated virus	Marafivirus	Tymoviridae	Undetermined	Addition to pest list
Citrus tatter leaf capillovirus (syn)	Capillovirus	Betaflexiviridae	Regulated	Remove from pest list and change status to non-regulated – It is the same specie as <i>Apple stem grooving virus</i> which is present in New Zealand and there is no evidence of strains of this virus (ICTV, 2014; Veerakone <i>et al.</i> , 2015; Dr Bénédicte Lebas, PHEL, pers. coms, March 2016).
Citrus tristeza closterovirus [strains not in New Zealand]	Closterovirus	Closteroviridae	Strains regulated	Remove from pest list and change status to non-regulated– all strains present in New Zealand (Harper and Pearson, 2015)
Citrus yellow mosaic badnavirus	Badnavirus	Caulimoviridae	Regulated	Update taxonomy and name to Citrus yellow mosaic virus
Citrus yellow mottle virus	Unassigned	Unassigned	Regulated	Update taxonomy
Hibiscus green spot virus 2	Unassigned	Higrevirus	Undetermined	Addition to pest list
Indian citrus mosaic badnavirus (syn)	Badnavirus	Caulimoviridae	Regulated	Remove from pest list - synonym of Citrus yellow mosaic virus
Indian citrus ringspot virus	Mandarivirus	Alphaflexiviridae	Undetermined	Addition to pest list
Iranian citrus ringspot-associated virus	Rhabdoviridae	Cytorhabdovirus	Undetermined	Addition to pest list
Navel orange infectious mottling virus (syn)	Sadwavirus	Secoviridae	Regulated	Remove from pest list – synonym of Satsuma dwarf virus
Satsuma dwarf nepovirus	Sadwavirus	Secoviridae	Regulated	Update taxonomy and name to Satsuma dwarf virus
Satsuma dwarf nepovirus [Natsudaidai dwarf strain] (syn)	Sadwavirus	Secoviridae	Regulated	Remove from pest list – synonym of Satsuma dwarf virus
Yellow vein clearing of lemon	Mandarivirus	Alphaflexiviridae	Regulated	Update taxonomy and name to Citrus yellow vein clearing virus

Table 1.6. Update on taxonomy (in blue) and regulatory status (in green) of viruses.

¹Reference for status: BORIC, MPI database.

²Reference for taxonomy: International Committee on Taxonomy of Viruses, <u>http://www.ictvonline.org/</u> (accessed 21 May 2015).

Disease name	Status ¹	Proposed changes to the pest list for Citrus
Australian citrus dieback	Regulated	Move to phytoplasmas (genus/species: not described)
Blind pocket	Regulated	Remove from pest list – renamed as concave gum - blind pocket (refer to entry for concave gum)
Bud union disease	Regulated	Update name to Bud union diseases (Roistacher 1991)
Citrus blight disease	Regulated	Update name to Citrus blight (Roistacher 1991)
Citrus fatal yellows	Regulated	No changes
Citrus impietratura disease	Regulated	No changes
Citrus sunken vein disease	Regulated	No changes
Concave gum	Regulated	Update name to Concave gum-blind pocket (Roistacher 1991)
Cristacortis	Regulated	No changes
Gum pocket	Regulated	Remove from pest list – likely to be caused by one or more viroids (e.g. CVd-II variants or CVd-III variants)) which are already listed on the Citrus pest list
Gummy bark	Regulated	Remove from pest list – caused by Citrus bark cracking viroid (syn. CVd-IV) which is already listed on the Citrus pest list
Kassala disease	Regulated	Remove from pest list – likely to be caused by one or more viroids which are already listed on the Citrus pest list (or present in New Zealand)
Lemon sieve tube necrosis	Regulated	Remove from pest list – it is a non-transmissible disease, but rather a physiological disorder (Roistacher 2012)
Shell bark of lemons	Regulated	Remove from pest list – likely to be caused by one or more viroids which are already listed on the Citrus pest list (or present in New Zealand)
Zonate chlorosis	Regulated	No changes

Table 1.7. Update on taxonomy (in blue) and regulatory status (in green) of diseases of unknown aetiology.

¹Reference for status: BORIC, MPI database.

Table 1.8. Update on taxonomy (in blue) and regulatory status (in green) of insects, mites, spiders and molluscs.

Genus and species	Family	Order	Class	Insect/Mite	Status ¹	Proposed changes to the pest list for Citrus ²
Allococcus spp.	Pseudococcidae	Homoptera	Insecta	Insect	Regulated	Update name to <i>Delottococcus euphorbiae</i> (syn. <i>Allococcus euphorbiae</i> , the only regulated <i>Allococcus</i> species associated with citrus
Archips rosanus (syn.)	Tortricidae	Lepidoptera	Insecta	Insect	Regulated	Update name to Archips rosana
Archipsocus sp.	Archipsocidae	Psocoptera	Insecta	Insect	Non-regulated	Remove from pest list – none of the Archipsocus species are regulated
Artipus sp.	Curculionidae	Coleoptera	Insecta	Insect	Regulated	Update name to Artipus floridanus, the only regulated Artipus species associated with citrus
Austropeplus sp.	Miridae	Hemiptera	Insecta	Insect	Non-regulated	Remove from pest list – none of the Austropeplus species are regulated
Caedicia sp.	Tettigoniidae	Orthoptera	Insecta	Insect	Regulated	Update name to Caedicia strenua, the only regulated Caedicia species associated with citrus
Cosmophyllum pallidulum	Tettigoniidae	Orthoptera	Insecta	Insect	Regulated	Update taxonomy
Cryptophlebia leucotreta (syn.)	Tortricidae	Lepidoptera	Insecta	Insect	Regulated	Update name to Thaumatotibia leucotreta
Halyomorpha mista (syn)	Pentatomidae	Hemiptera	Insecta	Insect	Regulated	Update name to Halyomorpha halys

Genus and species	Family	Order	Class	Insect/Mite	Status ¹	Proposed changes to the pest list for <i>Citrus</i> ²
Homalodisca coagulata (syn.)	Cicadellidae	Homoptera	Insecta	Insect	Regulated	Update name to Homalodisca vitripennis
Lepidosaphes beckii	Diaspididae	Homoptera	Insecta	Insect	Non-regulated	Remove from pest list - present in New Zealand
Nipaecoccus vastator (syn.)	Pseudococcidae	Homoptera	Insecta	Insect	Regulated	Remove from pest list – synonym of Nipaecoccus viridis which is already on the pest list
Prepodes sp.	Curculionidae	Coleoptera	Insecta	Insect	Non-regulated	Remove from pest list – none of the Prepodes species are regulated
Pseudococcus citriculus (syn)	Pseudococcidae	Homoptera	Insecta	Insect	Regulated	Update name to Pseudococcus cryptus
<i>Salagena</i> sp.	Scolytidae	Coleoptera	Insecta	Insect	Non-regulated	Remove from pest list – none of the Salagena species are regulated
Scolypopa sp.	Ricaniidae	Homoptera	Insecta	Insect	Non-regulated	Remove from pest list – none of the Scolypopa species are regulated
Signiphora flavella	Signiphoridae	Hymenoptera	Insecta	Insect	Non-regulated	Remove from pest list - present in New Zealand
Siphoninus phillyreae	Aleyrodidae	Homoptera	Insecta	Insect	Non-regulated	Remove from pest list - present in New Zealand
Stethorus histrio [Animals Biosecurity]	Coccinellidae	Coleoptera	Insecta	Insect	Non-regulated	Remove from pest list - present in New Zealand
Stethorus punctata picipes [Animals Biosecurity] (syn)	Coccinellidae	Coleoptera	Insecta	Insect	Regulated	Update name to Stethorus punctum subsp. picipes
Thriphleps thripoborus [Animals Biosecurity] (syn)	Anthocoridae	Hemiptera	Insecta	Insect	Regulated	Remove from pest list – synonym of Orius thripoborus which is already on the pest list
Trisopsis sp.	Cecidomyiidae	Diptera	Insecta	Insect	Non-regulated	Remove from pest list – none of the <i>Trisopsis</i> species are regulated
<i>Tambinia</i> sp.	Tropiduchidae	Homoptera	Insecta	Insect	Non-regulated	Remove from pest list – none of the Tambinia species are regulated
Tuckerella ornata	Tuckerellidae	Acarina	Arachnida	Mite	Regulated	Update taxonomy
Xylomyges curialis (syn.)	Noctuidae	Lepidoptera	Insecta	Insect	Regulated	Update name to Egira curialis

¹Reference for status: BORIC, MPI database.

²Note: only taxonomy and status changes is listed; there was no changes for spiders and molluscs. Reference for taxonomy: Index to Organism Names, <u>http://www.organismnames.com/</u> (accessed May 2015)

Appendix 2: Background information on diagnostic techniques

Some background information on the testing measures that are currently listed on the IHS *Citrus* schedule with their advantages and disadvantages, is provided below.

Shoot-tip grafting bioassay

- 1. Shoot tip grafting bioassay is a technique for grafting the small apical shoot of a plant to the top of a decapitated seedling grown *in vitro*. Pathogens are often eliminated through this process. Another advantage is the plants are growm in sterilised environment, eliminating the risk of bacteria and fungi contamination.
- 2. The main disadvantages include some cultivars are very difficult or fail to establish in tissue culture. Tissue culture is also time consuming and labour intensive.

Detached leaf bioassay

- 3. The detached leaf bioassay consist of using susceptible citrus hosts to which a suspension of the macerated tissue to be tested is added and incubated at an appropriate temperature. Symptoms may take several days to develop; for example, 4-12 days for *Xanthomonas citri* subsp. *citri* (ISPM27/DP6, 2014).
- 4. The test is not specific to a particular pathogen but will provide some indication based on symptom type produced and also if it is a pathogenic bacteria or not.

Semi-selective medium technique

- 5. The semi-selective medium technique requires the use of chemicals such as fungicide, antibiotic for the selective growth of a pathogen (bacteria or fungi) on agar plate at an appropriate temperature.
- 6. This technique allows the differentiation of some pathogens (e.g. *Elsinoë australis*). However it is time consuming and costly. The pathogens may also take time to grow or may not grow if it is in insufficent concentration.

Biological indexing

- 7. Biological indexing is a test in which plant pathogens, if present, are transmitted to sensitive indicator plants which develop characteristic symptoms. These indicator plants can be woody or herbaceous plants.
- 8. Woody indexing is by grafting while herbaceous indexing is by mechanical means. Indicator plants are observed for symptom development for up to 4 weeks for herbaceous plants and up to several years for woody plants. Success of biological indexing depends on type of tissue, host species, environmental factors, procedure, pathogen titre and its distribution in the plant.
- 9. Biological indexing has the advantages of being a generic test which can detect multiple pathogens at a time. Woody indexing can potentially detect any graft transmissible pathogens while herbaceous indexing can detect a broad range of viruses. However any symptomatic indicators would require additional testing for confirmation of the identity of the pathogen as mixed-infection may occur.
- 10. The main disadvantages are the requirements of specific greenhouse conditions necessary for an accurate assay. Woody indexing is time consuming and labor intensive, this may result in the delay in getting access to new cultivars with potentially additional quarantine cost.
- 11. Transmission to woody indicators can sometimes be difficult because of the pathogen being in low titre or unevenly distributed in the plant (e.g. *Spiroplasma citri*) or not easily

transmissible (e.g. *Citrus leprosis virus C, Xanthomonas citri* subsp. *citri*, viroids). Symptoms may only be transient (e.g. *Citrus viroid V, Citrus viroid VI*) or symptom development on woody indicator may take several years to develop (e.g. *'Candidatus* Phytoplasma aurantifolia', Citrus blight, *Citrus sudden death-associated virus*).

ELISA (serology)

- 12. Enzyme-linked immunosorbent assay (ELISA) is a serology test using antibodies (antigens) which have been developed specifically against pathogens (e.g. bacteria, virus). Plant tissue is ground in buffer solution and incubated with antiserum to specific pathogens in a plastic microtiter plate.
- 13. ELISA is a fast, inexpensive, and sensitive technique compare to bioligcal indexing, also a large number of samples which may be bulked can be processed in a short period of time. It only takes two days to complete an ELISA test but samples must be collected during specific seasons to obtain accurate results. Success of ELISA depends on type of tissue, host species, pathogen titre and its distribution in the plant.
- 14. ELISA is not sensitive enough to detect the pathogen at very low concentration and it has a lower sensitivity than PCR (e.g. *Citrus tristeza virus*: Mathews *et al.*, 1996; *Xylella fastidiosa*: Li *et al.*, 2013).

PCR (molecular)

- 15. Polymerase chain reaction (PCR) is a molecular test which specifically detects a small part of the nucleic acid sequence of a particular organism. It has been extensively used for the diagnostic of pests and diseases of human, animals and plants. It is very sensitive, specific, reliable and fast to perform.
- 16. The conventional PCR requires the use of gel electrophoresis to visualise the results. The nested-PCR is similar to conventional PCR but with an additional PCR run to increase the sensitivity of the test; for example it is used for the detection of Phytoplasmas. The real-time PCR allows the results to be viewed in real-time. Both methods require nucleic acid extraction.
- 17. The conventional PCR (within 4-5 hours) is longer to perform than real-time PCR (within 2 hours). Real-time PCR is often even more sensitive than conventional PCR. The success of the PCR depends on several factors such as the quality and quantity of the nucleic acid extract, the choice of primers, the type of sample. PCR may sometimes be too specific and unknown divergent isolates/strains of a pathogen may not be detected.
- 18. PCR is more sensitive than any other tests currently on the *Citrus* schedule (i.e. plating, culturing, sPAGE, ELISA and biological indexing). For example, PCR has been demonstrated to be very sensitive, fast and more reliable than graft indexing and culturing for *Spiroplasma citri* (Yokomi *et al.*, 2008). The high sensitivity of PCR often allows the detection of a pathogen from assymptomatic tissue; for example *Plenodomus tracheiphilus* (Balmas *et al.*, 2005).
- 19. PCR has been successfully developed for the detection of bacteria (e.g. *Xylella fastidiosa*, Li *et al.*, 2013), fungi (e.g. *Elsinoë australis*, Hyun *et al.*, 2009), phytoplasmas (e.g. all phytoplasmas, Liefting *et al.*, 2011), viroids (e.g. *Hop stunt viroid*, Hadidi *et al.*, 2003) and viruses (e.g. *Citrus leprosis virus C*, Locali *et al.*, 2003).

sPAGE (molecular)

- 20. Sequential polyacrylamide gel electrophoresis (sPAGE) uses the molecular properties of viroid particle RNA to produce a band which migrates through a gel according to its size when an electric current is applied. Bands are then visualised after staining (Duran-Vila *et al.*, 1988).
- 21. sPAGE is a generic method used for the detection of viroids. Viroids of similar size may not be distinguished. This method is time consuming and not as sensitive as PCR.

Appendix 3: Background information on the type of organisms infecting *Citrus*

Some background information on the type of organisms infecting citrus is provided below.

BACTERIA

- 1. Bacteria are microscopic living organisms that can be found on plant surfaces or inside the plants. Any plant part can be a source of bacterial inoculum. Dissemination of plant pathogenic bacteria is easy; it commonly occurs by windblown soil and sand particles that cause plant wounding. Machinery, clothing, packing material and water can also disseminate bacteria, as can insects and birds (Vidaver and Lambrecht, 2004).
- 2. Bacteria cause many serious diseases of plants throughout the world. The most common symptoms are spots on leaves or fruit, blights or readening of tissue on leaves, stems or tree trunks, and rots of any part of the plant and sometimes plant death. They can also cause large plant abnormalities, such as galls or distorted plant parts as well as wilting (Vidaver and Lambrecht, 2004).
- 3. Plant bacterium are irregularly distributed in host tissues. They are often occurring in a latent form or take sometimes for symptoms to develop (Li *et al.*, 2013; Timmer *et al.*, 2000). Therefore sensitive and reliable detection methods are required.

LIBERIBACTERS

- 4. Liberibacters are Gram-negative bacterium, restricted to the phloem. They have never been obtained in culture and had to be characterized by molecular techniques. Liberibacters are mainly transmitted by infected propagated materials and by phloem-feeding insect such as psyllids (CABI datasheet; Teixeira *et al.*, 2005; Timmer *et al.*, 2005).
- 5. Liberibacters can cause yellow shoot in citrus or induce phytoplasmas like-symptoms on Solanaceous species (e.g. stunting, short internodes). They are irregularly distributed in host tissues and may occur in a latent form or symptoms may take time to develop (CABI datasheet; Teixeira *et al.*, 2005; Timmer *et al.*, 2005).

FUNGI

- 6. Fungi are living organisms that often reproduce by spores and have a body. Spores may be spread long distances by air or water, or they may be soil borne. Some fungi obtain their nutrients from a living host others from dead plants. Some fungi are decomposers, parasites or pathogens of other organisms, and others are beneficial partners. They can grow in a wide range of habitats, including extreme environments (Carris *et al.*, 2012).
- 7. Fungi are pathogens of many cultivated plants causing extensive damage and losses to agriculture and forestry. Symptoms cause by fungi include canker, leaf spots (e.g. necrosis), rot, rust and wilting. Rust fungi attack a wide range of plants. Some fungi colonize the surface of the plants (e.g. rust pathogens) while other fungi infect through the natural plant openings (e.g. leaf spot pathogens), the roots or wounds (e.g. vascular wilt pathogens) (Carris *et al.*, 2012).

PHYTOPLASMAS

- 8. Phytoplasmas are non-culturable microorganisms that invade the plant phloem. They are transmitted by vegetative propagation and grafting. Phytoplasmas are also vectored by phloem-feeding insects such as leafhoppers, planthoppers and psyllids (Bertaccini *et al.*, 2014).
- 9. Phytoplasmas are associated with diseases infecting a wide range of plant species, including many economically important vegetable and fruits crops. Typical symptoms include virescence/phyllody, sterility of flowers, witches' broom and stunting (Bertaccini *et al.*, 2014).

VIROIDS

- 10. Viroids are the smallest plant pathogens that usually invade the whole plant. Viroids are mainly transmitted by vegetative propagation and grafting. Most viroids are transmitted mechanically, by seed and pollen but rarely by aphid vectors.
- 11. Viroids infect a limited host range, causing a number of economically important plant diseases. The symptoms caused by viroids are generally similar to those caused by viruses (refer to section 'Virus' below). The best preventative measures for viroids is the use of certified viroid-free propagating materials (Hadidi *et al.*, 2003).

VIRUSES

- 12. Viruses are very small infectious agent that can only live inside their hosts inducing localized or systemic infections. Viruses are mainly transmitted by vegetative propagation and grafting. A number of vectors have been reported to transmit viruses, including aphids, bugs, beetles, leafhoppers, planthoppers, thrips and whiteflies. Some viruses are also transmissible by contact, seed or pollen (Gergerich and Dolja, 2006).
- 13. Viruses induce a wide range of symptoms on leaf, flower, fruit and stems of plants including chlorosis, mosaic, necrosis, discoloration, deformation, stunting, stem pitting and grooving (Gergerich and Dolja, 2006). Virus diseases can reduce the crop quality and yield. There are virtually no antiviral compounds available to cure plants with viral diseases, the best preventative measures is the use of certified virus-free propagating materials.

Appendix 4: Definitions of the Level of quarantine

- The new standard for post entry quarantine (PEQ) of plants can be view here: <u>Post Entry</u> <u>Quarantine for Plants - Facilities Standard</u>. It has four levels of quarantine which allow plants to be held in a level of quarantine that better reflects the level of risk. The definition of the different levels of quarantine is provided in Table 4.1.
- 2. The implementation periods for the new PEQ standard are as follows:
 - a) Existing facilities must comply with all operational requirements of the revised standard within 12 months of the commencement date (i.e. before 1st March 2017);
 - b) Existing facilities that are active (i.e. are holding consignments in quarantine) at the date of commencement (1st March 2016) must comply with all physical/structural requirements of the revised standard a maximum of 12 months after the clearance of plant material held in a facility on 1st March 2016. This is to allow any changes that may affect the structural integrity of a facility to be made without compromising biosecurity. However, it is MPI's preference that any physical/structural changes should be made as soon as practicable if this can be done without affecting the integrity of the facility.

Level of quarantine	Types of quarantine pest potentially associated with the host plant	Operational measures that may apply	Glasshouse requirements
Level 1 (L1) open field	Graft transmitted organisms (viruses, viroids or diseases of unknown aetiology) with no other means of transmission. Some other organisms if risk can be managed by a combination of operational and/or physical measures.	Approved countries only. Offshore assurances (e.g. growing season inspection, freedom from regulated pests, phytosanitary certification). Import permit. Treatment (e.g. for insects, mites, nematodes or fungi). Inspections on arrival and in PEQ. Quarantine pest unlikely to be associated with the plant parts being imported or only graft transmissible pathogens. Testing for specified quarantine pests completed offshore and/or preliminary quarantine in New Zealand completed in a higher level of facility.	Clearly delineated site Minimum isolation requirements Signs at main entrance and corners
Level 2 (L2)	All organisms contained within L1 PEQ as well as: Insects, or insect vectors excluded by 0.6 mm mesh size (e.g. <i>Liriomyza</i> spp., some aphids). Soil or water borne pests or diseases, or organisms vectored by such pests (e.g. <i>Pratylenchus convallariae</i> , <i>Grapevine fanleaf virus</i>). Bacteria and fungi that are not aerially dispersed.	Approved countries. Offshore assurances (e.g. growing season inspection, freedom from regulated pests, phytosanitary certification). Import permit required. Treatment on arrival (e.g. for insects, mites, nematodes or fungi). Inspections on arrival and in PEQ. Material obtained from MPI accredited facilities with all relevant testing done offshore.	Glass, polycarbonate twin skin polyfilm Anteroom with hand washing facilities 1m wide buffer strip to surround all facilities

Table 4-1. Definitions of the four Levels of quarantine in the revised PEQ standard.

Level of	Types of quarantine pest potentially	Operational measures that may	Glasshouse
quarantine	associated with the host plant	apply	requirements
	Some other organisms if risk can be		
	managed by a combination of		
	operational and/or physical measures.		
Level 3A	All organisms contained within a L1 or	Approved countries.	Glass, polycarbonate,
(L3A)	L2 facility as well as:	Offshore assurances (e.g. growing	other rigid material
	Insects excluded by 0.2 mm mesh	season inspection, freedom from	Vents screened by 0.2
	size; aphids, whiteflies, most thrips,	regulated pests, phytosanitary	mm stainless steel
	some mites (e.g. Green peach aphid,	certification.	mesh
	Melon aphid, Sweetpotato whitefly).	Import permit).	Mechanical
	Organisms transmitted by a vector	Treatment on arrival (e.g. for insects,	heatin/colling system
	capable of being excluded by 0.2 mm	mites, nematodes or fungi).	Plumbed-in hand basin
	mesh size, including some bacteria,	Inspections on arrival and whilst in	with hand free
	phytoplasmas, viroids and viruses e.g.	PEQ.	mechanism
	Plum pox virus (based on Myzus	Additional declarations (e.g. growing	Benches of metal or
	persicae as the vector, Liberibacter	season inspection, freedom from	similar inert material
	asiaticum (based on Diaphorina citri as	regulated pests).	
	the vector).	Material from accredited facilities.	
	Mechanically transmitted viruses or	Specific testing for high risk	
	viroids, e.g. Hop stunt viroid.	organisms.	
	Bacteria and fungi that are not readily	Specific growth requirements (e.g.	
	dispersed by wind/aerosol action	minimum growth temperature or day	
	and/or unlikely to produce fruiting	length).	
	bodies in quarantine e.g. Elsinoë		
	australis, Phyllosticta citricapra (syn.		
	Guignardia citricarpa).		
	Some other organisms if risk can be		
	managed by a combination of		
	operational and/or physical measures.		
Level 3B	All organisms contained within a L1,	Approved countries.	Glass, polycarbonate
(L3B)	L2 or L3A facility as well as:	Import permit.	No mesh/openings
	Insects and mites excluded by HEPA	Phytosanitary certificate.	Mechanically ventilated
	filtration.	Treatment on arrival (e.g. for insects,	HEPA filtration
	Organisms transmitted by a vector	mites, nematodes or fungi).	Negative air pressure
	excluded by HEPA filtration (e.g.	Growing season inspection in PEQ	
	eriophyid mites which vector Peach	(by facility operator and MPI	
	mosaic virus and Cherry mottle leaf	Inspector).	
	virus).	Specific testing for high risk	
	Bacteria and fungi that are aerially	organisms.	
	dispersed.		

Appendix 5: Mode of transmission of *Citrus* pests with specific phytosanitary requirements

The mode of transmission of pests with specific phytosanitary measures in the *Citrus* schedule, was used for the assessment of the level of quarantine for citrus nursery stock. Refer to section 'Level of post entry quarantine' for details (refer to paragraphs 143-147).

Note: *Citrus tristeza virus* (CTV) is proposed to be non-regulated (refer to paragraphs 129-130 for details), thus it has not been included in the Table below. CTV is transmitted by aphids including *Aphis gossypii*, *A. spiraecola, Toxoptera aurantii* and *T. citricida* which are present in New Zealand but vector regulated (BORIC). These aphids are bigger than 0.2 mm.

Pests with	Transmission	Note	Reference
phytosanitary measures			
Insects	Not applicable	Insects are currently effectively managed under the basic conditions (i.e. pesticide treatments) and under the <i>Citrus</i> schedule (i.e. pre-export phytosanitary inspection) set out in the IHS.	IHS 155.02.06
Mites	Not applicable	Mites are currently effectively managed under the basic conditions (i.e. pesticide treatments) set out in the IHS.	IHS 155.02.06
Bacteria			
Spiroplasma citri	Leafhopper vector: Circulifer tenellus, Neoaliturus haematoceps, Scaphytopius nitridus, S. acutus delongi Graft transmission Propagation material	These leafhoppers (absent in New Zealand) are bigger than 0.6 mm. Leafhopper vectors feed on a wide host range including field crops, fruit trees, ornamentals, wild plants and weeds. Best temperature range for <i>S. citri</i> to multiply and symptoms to develop is 28-35°C.	Timmer <i>et al.</i> 2000 EPPO datasheet
Xanthomonas citri subsp. citri	Rain Propagation material	Syn. Xanthomonas axonopodis pv. citri Optimal temperature: 28-30°C Best condition of dissemination is rain Can survive many years on woody branches	CABI datasheet Timmer <i>et al.</i> 2000
Xanthomonas fuscans subsp. aurantifolii	Rain Propagation material	Syn Xanthomonas campestris pv. aurantifolii Optimal temperature: 28-30°C Best condition of dissemination is rain Can survive many years on woody branches	Timmer <i>et al.</i> 2000
Xanthomonas alfalfae subsp. citrumelonis	Rain Propagation material	Syn Xanthomonas campestris pv. citrumelo Optimal temperature: 28-30°C Best condition of dissemination is rain Can survive many years on woody branches	Timmer <i>et al.</i> 2000
Xylella fastidiosa	Sharpshooter insects (sub- family Cicadellidae) Spittle bugs (sub-family Cercopidae) Graft transmission Propagation material	These sharpshooters and spittle bugs (absent in New Zealand) are bigger than 0.6 mm. Most likely transmitted from wild, generally symptomless hosts to cultivated hosts. Disease can be latent for nine to 12 months	Timmer <i>et al.</i> 2000
Fungi			
Elsinoë australis	Spores disperse by rain or water splash Propagation material	Infection takes place between 14°C and 25°C.	Chung 2011 EPPO datasheet
Eremothecium coryli	Sap-sucking pentatomid (Hemiptera) insects Propagation material?	Syn. Nematospora coryli Pentatomid insects (eg. spined citrus bug, Biprorulus bibax, absent in New Zealand; green vegetable bug, Nezara viridula present in New Zealand) are bigger than 0.6 mm. Localised fruit disease	Fawcett 1929 Miles <i>et al.</i> 2009 Shivas <i>et al.</i> 2005
Phyllosticta citricarpa	Spores disperse by wind or water Survive in dead leaves Propagation material	Syn. <i>Guignardia citricarpa</i> Disease infection best develop at 24-25°C. Ascospores only infect fruits and can take 1-4 days to germinate.	CABI datasheet EPPO datasheet

Pests with phytosanitary	Transmission	Note	Reference
measures			
Plenodomus tracheiphilus	Spores disperse by water (rain splash, wind-blown rain, overhead irrigation) Survive in soil within infected twigs Propagation material	Syn. <i>Phoma tracheiphila</i> Disease infection best develop at 20-25°C.	Donovan <i>et al.</i> 2014
Liberibacters			
'Candidatus Liberibacter africanus'	Psyllid vector: <i>Trioza erytreae</i> Graft transmission Propagation material	Syn. Liberobacter africanum This psyllid (absent in New Zealand) is bigger than 0.6 mm. Psyllid can fly up to 1.5 km. Optimal temperature for psyllid development: 24-28°C. Best temperature range for Liberibacters to multiply and symptoms to develop is 18-25°C.	CABI datasheet Teixeira <i>et al.</i> 2005 Timmer <i>et al.</i> 2000
'Candidatus Liberibacter americanus'	Psyllid vector: <i>Diaphorina citri</i> Graft transmission Propagation material	This psyllid (absent in New Zealand) is bigger than 0.6 mm. Optimal temperature for psyllid development: 24-28°C. Best temperature range for Liberibacters to multiply and symptoms to develop is 18-25°C.	Teixeira <i>et al.</i> 2005 Timmer <i>et al.</i> 2000
'Candidatus Liberibacter asiaticus'	Psyllid vector: <i>Diaphorina citri</i> Graft transmission Propagation material	Syn. Liberobacter asiaticum This psyllid (absent in New Zealand) is bigger than 0.6 mm. Optimal temperature for psyllid development: 24-28°C. Best temperature range for Liberibacters to multiply and symptoms to develop is 18-25°C.	CABI datasheet Teixeira <i>et al.</i> 2005 Timmer <i>et al.</i> 2000
Phytoplasmas			
Australian citrus dieback	Graft transmission (with difficulty) Propagation material	No known vector.	Broadbent <i>et al.</i> 1976 Timmer <i>et al.</i> 2000
Candidatus phytoplasma aurantifolia	Leafhopper vector: <i>Hishimonus</i> <i>phycitis</i> Graft transmission Propagation material	This leafhopper (absent in New Zealand) is bigger than 0.6 mm. Symptom expression requires warm temperature (30°C).	Timmer <i>et al.</i> 2000
<i>'Candidatus</i> Phytoplasma asteris'	Phioem-feeding insect vector? Graft transmission Propagation material	No vectors have been reported yet for this phytoplasma. However, it belongs to a group of phytoplasmas that are transmitted by phloem-feeding vectors such as leafhoppers.	Arratia-Castro et al. 2014 Chen et al. 2009
HLB- associated 16SrIX-A phytoplasma (new)	Leafhopper vector: Scaphytopius marginelineatus? Graft transmission Propagation material	This leafhopper is a likely vector (absent in New Zealand) that is bigger than 0.6 mm.	Marques <i>et al.</i> 2012
Viroids			
Citrus bark cracking viroid	Graft transmission Propagation material	Syn. Citrus viroid IV. Viroid multiplication is best between 27-32°C temperatures. No vector reported.	Hadidi et al. 2003
Citrus bent leaf viroid	Graft transmission Propagation material	Syn. Citrus viroid I, Citrus variable viroid. Viroid multiplication is best between 27-32°C temperatures. No vector reported.	Hadidi et al. 2003
Citrus viroid V	Graft transmission Mechanical transmission Propagation material	Viroid multiplication is best between 27-32°C temperatures. No vector reported.	Serra <i>et al.</i> 2008a & 2008b
Citrus viroid VI	Graft transmission Mechanical transmission Propagation material	Viroid multiplication is best between 27-32°C temperatures. No vector reported.	Ito <i>et al.</i> 2001 & 2002
Citrus viroid VII	Graft transmission Propagation material	Viroid multiplication is best between 27-32°C temperatures. No vector reported.	Chambers <i>et al.</i> 2016 Hadidi <i>et al.</i> 2003
Hop stunt viroid [citrus strain]	Graft transmission Propagation material	Syn. Citrus viroid II, Citrus cachexia viroid, Xyloporosis viroid. Viroid multiplication is best between 27-32°C temperatures. No vector reported.	Hadidi <i>et al.</i> 2003
Viruses			
Apple stem grooving virus	Graft transmission Mechanical transmission Propagation material	Syn. Citrus tatter leaf virus. No vector reported.	EPPO datasheet Trimmer <i>et al.</i> 2000
Citrus chlorotic dwarf- associated virus	Whitefly vector: <i>Parabemisia</i> <i>myricae</i> Graft transmission Propagation material	This whitefly (absent in New Zealand) is bigger than 0.6 mm. Symptoms develop at 20-25°C but become stronger at 30- 35°C.	Loconsole <i>et al.</i> 2012

Pests with phytosanitary	Transmission	Note	Reference
measures			
Citrus leaf rugose virus	Graft transmission Mechanical transmission Propagation material	Best temperature range for symptom development: 24-27°C max. day and 18-21°C min. night.	Frison & Taher 1991 Roistacher 1991 Timmer <i>et al.</i> 2000
Citrus leprosis virus C	Mite vector: <i>Brevipalpus</i> sp. Graft transmission (with difficulty) Mechanical transmission Propagation material	Syn. Citrus leprosis virus. Some <i>Brevipalpus</i> sp. are present in New Zealand but vector regulated. The width of <i>Brevipalpus</i> sp. is 150 µm, which can only be contained or excluded in HEPA filtered facility.	Locali-Fabris 2006 Lovisolo 2001
<i>Citrus leprosis virus</i> cytoplasmic type 2	Mite vector: <i>Brevipalpus</i> sp.? Graft transmission Propagation material	There is no information about vector transmission for <i>Citrus leprosis virus</i> cytoplasmic type 2. However, because the other two closely related leproviruses are transmitted by the mite vector, it is possible that this virus may also be transmitted by the same way.	Roy <i>et al.</i> 2013& 2014
<i>Citrus leprosis virus</i> nuclear type	Mite vector: <i>Brevipalpus</i> sp. Graft transmission Propagation material	Some <i>Brevipalpus</i> sp. are present in New Zealand but vector regulated. The width of <i>Brevipalpus</i> sp. is 150 µm, which can only be contained or excluded in HEPA filtered facility.	Roy <i>et al.</i> 2013 & 2014 Timmer <i>et al.</i> 2000
Citrus sudden death- associated virus	Aerial vector? Graft transmission Mechanical transmission Propagation material	The virus was detected on aphids and leafhoppers but no transmission studies have been conducted. Aphids are bigger than 0.2 mm and leafhoppers are bigger than 0.6 mm.	Bassanezi <i>et al.</i> 2007 Maccheroni <i>et al.</i> 2005 Yamamoto <i>et al.</i> 2011
Citrus variegation virus	Graft transmission Mechanical transmission Propagation material	Syn. Citrus infectious variegation ilarvirus. No vector reported.	Frison & Taher 1991 Roistacher 1991 Timmer <i>et al.</i> 2000
Citrus yellow mosaic virus	Mealybug vector: <i>Planococcus</i> <i>citri</i> Graft transmission Mechanical transmission Propagation material	The virus was transmitted experimentally by <i>Planococcus citri</i> (present in New Zealand but vector regulated). The mealybug is bigger than 0.6 mm.	Trimmer <i>et al.</i> 2000
Citrus yellow vein clearing virus	Graft transmission Mechanical transmission Propagation material	Syn. Yellow vein clearing of lemon. No vector reported.	Chen <i>et al.</i> 2014 Trimmer <i>et al.</i> 2000
Indian citrus ringspot virus	Graft transmission Mechanical transmission Propagation material	No vector reported.	Milne <i>et al.</i> 2007 Rustici <i>et al.</i> 2000
Olive latent virus 1	Graft transmission Mechanical transmission (with difficulty) Propagation material	No vector reported. The virus is seed and pollen transmissible in olive but it has not been demonstrated for citrus.	Félix <i>et al.</i> 2007 Martelli 2013 Roistacher 1991
Satsuma dwarf virus	Graft transmission Mechanical transmission Propagation material	No vector reported.	Roistacher 1991 Trimmer <i>et al.</i> 2000
Diseases of unknown aetiology			
Citrus blight disease	Graft transmission Propagation material?	No vector reported. The causal agent seems to be restricted to the roots, nursery stock budwood and tissue culture are unlikley to be infected.	Roistacher 1991 Timmer & Brlansky 2006