Ministry for Primary Industries
Manatū Ahu Matua

CTO DECISION DOCUMENT – Measures for non-compliant consignment

A CTO direction is required to fulfil MPIs reporting requirements - section 27(3) of the Biosecurity Act.
This direction should not be attached to the PDF version of the permit; but must be retained in Piritahi for reporting purposes

Decision document and CTO direction to be signed by (highlight)**:**			
Director (PFE)	**Group Manager (PIE)**	**Team Manager**	**Senior Adviser** (not currently delegated)

Under what authority is the decision being made (highlight)**:**		
Appointed Chief Technical Officer – **Peter Thomson**	**Appointed Deputy Chief Technical Officer** – **Stephen Butcher**	**Delegated CTO authority** <insert position title>
Section of the Act the decision is being made under:	**27(1)(d)(iii)** – a chief technical officer has issued guidelines, or given directions, on measures, different from those in the standard, that may be applied to manage effectively risks of the kind arising from the non-compliance	
The Biosecurity Act can be viewed on the website:http://www.legislation.govt.nz/act/public/1993/0095/latest/DLM314623.html Delegations under the Biosecurity Act can be searched on the following website:http://kotahi.maf.govt.nz/do/policies/view/article/1169/delegations-and-authorisations - CTO delegations can be searched under the heading: Biosecurity (Chief Technical Officer		

Author:	Richard Lardner	**Team:**	PIE
Subject:	**CTOPlants: 2019029** Non-compliant *Malus* plants for planting from Australia	**Due date:**	
The unique CTO decision number can be found under the tab 'CTO27(1)(d)(iii)decisions' in the spreadsheet: 2015-16 Plants Non-compliance Log (s27 functional).xlsx			

Review steps	**Name**	**Team**	**Date**
Peer review	Jo Wilson	Plants & Pathways	02/08/2019; comments in Piritahi version of this document
	Barry Wards	Plant Product imports	11/08/2019; comments in Piritahi version of this document
Consultation with other MPI groups	Rebecca Easterbrook	Legal Services	15/08/2019; comments in Piritahi version
External			
Review and Team Manager sign out			
Group Manager sign out			
Director sign out			

Link to Word version of this Decision Document:

- This document will be stored in the Decisions Document library

https://piritahi.cohesion.net.nz/Sites/SAI/PP/PIM/DecisionDocuments/20190718%20CTO%20Decision%20Document%20PFR%20woody%20indicator%20testing%20C2015_249551.doc?web=1

Insert other relevant documents here, this may include:

Plant & Food Research report on woody indicator testing completed for consignment C2015/249551
https://piritahi.cohesion.net.nz/Sites/SAI/PP/PIM/PPNS/Food%20Crops/20190523%20PFR%20Malus%20Woody%20Indexing%20Report%2017795%20-%20Nicholas%20Amponsah%20-%20Woody%20indexing%20of%20a%20Malus%20budwood%20FINAL.PDF#search=PFR%20woody%20Nicholas

Insert copy of Decision Document here once signed

Signed copy
Decision document

CTOPlants 2019029
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CTO DECISION DOCUMENT

CTO Plants: 2019029 Non-compliant *Malus* plants for planting from Australia

ISSUE

Plant & Food Research will soon be seeking biosecurity clearance for a consignment of *Malus (*apple) plants for planting imported from Australia that are non-compliant with the requirements of the import health standard 155.02.06: *Importation of nursery stock* (the Standard).

A CTO decision is required under section 27(1)(d)(iii) of the Biosecurity Act 1993, to direct that measures, different to those required under the Standard, be applied to effectively manage the risks posed by non-compliant *Malus* plants for planting imported from Australia.

This is a one-off CTO decision, the outcome of which will be relevant only to the effective risk measures assessment for *Malus* plants for planting, imported under consignment C2015/249551, import permit number 2015056749 by Havelock North Fruit Co. Ltd. These plants are currently being held in post entry quarantine (PEQ) at the MPI PHEL Level 3B greenhouse; they were moved to this facility in May 2019 following the closure of the Plant & Food Research (PFR) Level 3B PEQ facility in Palmerston North.

BACKGROUND

In 2015, a consignment of three lines of *Malus* plants for planting was imported from Australia into the PFR Level 3B PEQ greenhouse at Palmerston North. The plants were imported from a non-approved source under option 3.2 of the *Malus* schedule of the Standard. Under this option, the imported plants must remain in a Level 3B PEQ greenhouse for a minimum period of 36 months, and all testing for regulated pests must be completed in New Zealand. A total of 20 plants derived from the three imported lines are currently being held in the MPI PHEL Level 3B PEQ facility.

One of the required tests is woody indicator testing. This is done (a) to verify that imported plants are free from four diseases of unknown aetiology, and (b) as a supplementary test to verify freedom from certain species of phytoplasma and viroid, as follows:

Table 1: Summary of organisms for which woody indexing is required under the *Malus* schedule of the Standard

Organism	Testing requirement
Apple dimple fruit viroid	Woody indexing ('Red Delicious') AND PCR
Apple fruit crinkle viroid	Woody indexing ('Golden Delicious') AND PCR
Apple scar skin viroid	Woody indexing ('Golden Delicious' and 'Red Delicious') AND PCR
'*Candidatus* Phytoplasma mali' (Apple proliferation phytoplasma)	Woody indexing ('Golden Delicious') AND nested PCR or real time PCR using universal phytoplasma primers
Apple dead spur agent	Woody indexing ('Golden Delicious' and 'Red Delicious')
Apple rough skin agent	Woody indexing ('Golden Delicious')
Apple russet wart agent	Woody indexing ('Golden Delicious')
Apple star crack agent	Woody indexing ('Golden Delicious' and 'Red Delicious')

The woody indicator testing was done by PFR at its MPI-accredited diagnostic facility in Palmerston North. All other required tests (PCR) were done by MPI at its PHEL MPI-accredited diagnostic facility in Auckland.

The woody indicator testing done by PFR does not comply with all requirements of the Standard because:

- Positive control plants did not all display the expected disease symptoms. The Standard states that "*Negative and positive control plants must be included; the positive control must develop the expected symptoms*".
- One of the three imported lines was not tested using both woody indicator cultivars ('*Golden Delicious*' and '*Red Delicious*'). This may not comply with the requirements of the Standard, and differs to the testing procedure agreed on in emails between MPI and PFR at the time of import.

PFR has done some additional testing of woody indicator plants to demonstrate that the testing done meets the intent of, and is equivalent to, the requirements of the Standard.

The consignment is due to be considered for biosecurity clearance in October 2019. As the testing does not comply with all requirements of the Standard, a CTO decision is needed to identify whether the testing that has been completed can be considered equivalent to that required under the Standard. This decision will be made under section 27(1)(d)(iii) of the Act.

ASSESSMENT

ISSUE 1: Positive control plants did not display the expected disease symptoms

The Standard states that "*Woody indexing relies on the development of fruit and bark symptoms on susceptible Malus cultivars which would only be expressed under field conditions (i.e. Level 1 post entry quarantine [PEQ]). Negative and positive control plants must be included; the positive control must develop the expected symptoms*".

PFR initially selected Apple green crinkle disease as the positive control. Four positive control plants (two Golden Delicious plants, and two Red Delicious) were inoculated with buds from a plant known to harbour Apple green crinkle disease. Symptoms of Apple green crinkle disease were only observed on one of the four originally grafted positive control plants, so this did not comply with the requirements of the Standard. PFR subsequently proposed also using Apple mosaic virus and Apple stem pitting virus as positive controls. This was because it was observed that symptoms of both of these diseases were also present in the plant that had originally been used to inoculate positive control plants. However, neither of these viruses induced symptoms on fruit or bark, as required by the Standard.

In the absence of symptoms on fruit and bark of all positive control plants, PFR did PCR testing and confirmed the presence of the three non-regulated viruses selected for use as positive controls, despite the absence of symptoms. It also did some additional grafting to show that the environmental conditions in Level 1 PEQ were conducive to expression of symptoms of Apple green crinkle disease. PFR has proposed that this methodology is equivalent to the requirements of the IHS.

MPI has assessed the testing done by PFR (Appendix 1). PFR's testing report is available in Piritahi here. As described in the following section, based on the MPI assessment, it is agreed that the testing done by Plant &

Food Research can be seen as effectively managing risks of non-compliance with the requirements of the Standard.

1. The testing done by PFR, using three non-regulated viruses as positive controls and including PCR testing in addition to growing season inspection to demonstrate successful disease transmission via woody indexing, is equivalent to the requirements of the IHS because:

    a. The combination of PCR testing and symptom observations provides the same or greater level of experimental control as is currently required under the IHS:

        i. Grafted buds (on positive control and test plants) 'took' successfully (i.e. grafted buds remained viable and some of the grafted buds developed shoots, leaves and fruit in subsequent growing seasons).
        ii. There is good evidence, based on PCR testing, that two non-regulated viruses (Apple mosaic virus and Apple stem pitting virus) were successfully transferred to all positive control plants. These viruses were present in donor trees used to establish positive controls. They were not detected in negative controls.
        iii. Symptoms consistent with those of Apple mosaic virus were observed on leaves of all four positive control plants, and not on negative controls. These symptoms were visible in both growing seasons in Level 1 PEQ.
        iv. Symptoms of *Apple green crinkle disease* were seen on one Golden Delicious positive control plant originally inoculated in 2015. PCR testing showed that Apple green crinkle associated virus (suspected to cause *Apple green crinkle disease*) was present in this plant.
        v. PCR testing also showed that Apple green crinkle associated virus may have been present in the second Golden Delicious positive control (although there was only very weak PCR amplification in this plant, meaning that the result was uncertain).
        vi. Symptoms of *Apple green crinkle disease* were also seen on large budsticks grafted onto Golden Delicious positive controls in 2018; this indicates that the environmental conditions in 2018 were conducive to the expression of disease symptoms on fruit.
        vii. No symptoms of *Apple green crinkle disease* were seen on Red Delicious positive control plants, and PCR testing did not detect Apple green crinkle associated virus in these plants. This supports published scientific evidence showing that Red Delicious (and some other red varieties) may not show symptoms of Apple green crinkle disease. This implies that *Apple green crinkle disease* may not be an appropriate disease to use as a positive control on Red Delicious (contrary to what is stated in the Standard; this will be re-considered when MPI reviews requirements for woody indicator testing for *Malus*).
        viii. One Golden Delicious positive control plant showed symptoms that can be attributed to grafting done when positive control plants were established in 2015. Both Golden Delicious positive control plants were re-grafted with large budsticks known to harbour *Apple green crinkle disease* in 2018. Symptoms on the second Golden Delicious positive control were due to this subsequent grafting, and fruit on growth from the originally grafted buds on the second plant did not show disease symptoms. This cannot be taken as reliable evidence that *Apple green crinkle disease* (or other diseases of unknown aetiology that induce symptoms on fruit/bark) would necessarily have been transferred to all positive controls under the conditions used to set up woody indicator testing in 2015 (where small buds were grafted onto trees). However, it does provide evidence

that environmental conditions were conducive to the expression of this disease on fruit of a susceptible cultivar.

ISSUE 2: One of the imported lines was not tested using both woody indicator cultivars

The Standard states that "*The unit for testing is an individual imported plantlet (imported in vitro) or cutting. Each plantlet or cutting must be labelled individually and tested separately, with the following exceptions:*" The exceptions relate to testing of composite samples (made up of material from more than one individual imported plantlet or cutting).

In the case of the current consignment, the phytosanitary certificate included an additional declaration certifying that the cuttings had all been taken from the same mother plant. Based on this, MPI allowed composite samples to be used for woody indicator testing. This means that buds taken from multiple plants undergoing PEQ could be grafted onto the same woody indicator test plant. However, at the time of import, PFR were advised by MPI that at least one bud from each plant in PFR must still be tested using woody indexing.

1. For one of the three imported plant lines, the plants in PEQ (plants 71P-2F and 71P-2P) do not meet the requirements of the IHS for woody indicator testing on Red Delicious and Golden Delicious respectively because:

    a. plants were either not set up on woody indicators or grafts failed to 'take'.

        i. When setting up woody indicator testing, no buds from Plant 71P-2F were grafted onto Golden Delicious, and the one bud from Plant 71P-2P that was grafted onto Red Delicious failed to take.

        ii. Five buds taken from different budsticks derived from the same mother plant in Australia were tested on Golden Delicious, and six buds on Red Delicious.

This matter is discussed further in the following sections, in particular "Risk management of diseases of unknown aetiology".

ASSESSMENT OF RISK MANAGEMENT MEASURES IN THE STANDARD

<u>Risk management of phytoplasmas and viroids</u>

Under the current version of the *Malus* schedule, woody indicator testing is required as a supplementary test to verify freedom from certain species of phytoplasma and viroid, and is the only test to verify freedom from four diseases of unknown aetiology. The Standard specifies that woody indicator testing must be done using a combination of Golden Delicious and Red Delicious (depending on the particular species of viroid and/or phytoplasma).

Using woody indicator testing to verify freedom from phytoplasmas and viroids is now seen as redundant. This is because our current judgement is that using PCR as the sole test method will achieve the required level of risk management for phytoplasmas and viroids, and MPI will undertake a process of amending the standard to clarify this, and to ensure risk management measures for these types of organism on *Malus* are aligned with requirements for other plant genera. Part of this consideration is that in the future, the CTO is likely to be

comfortable with removing the requirement for woody indicator testing for phytoplasmas and viroids because we believe risk is appropriately managed without doing woody indicator testing.

It is noted that MPI recently consulted on risk management measures for phytoplasmas and *Apple scar skin viroid* (one of the diseases for which woody indexing is required under the existing *Malus* schedule) as part of the consultation on the draft import health standard for *Prunus* plants for planting. PCR was proposed as being sufficient to verify that plants are free from these regulated pests. None of the submissions on the *Prunus* standard, including the submission from New Zealand Apples and Pears, disagreed with the proposal to use PCR as the sole testing method for these regulated pests.

Likewise, in the existing *Vitis* schedule, PCR testing is the sole method required to verify freedom from phytoplasmas (although it is noted that two sets of PCR testing are currently required).

In regards to using PCR as the sole method to verify pest freedom, Lia Liefting (Principal Scientist, Virology) provided the following advice:

For the detection of *Apple dimple fruit viroid*, *Apple fruit crinkle viroid*, *Apple scar skin viroid* and '*Candidatus* Phytoplasma mali', testing by PCR only will be sufficient for the following reasons:

1. The effectiveness of woody indexing for viroids can vary considerably depending on the isolate and environmental conditions.
2. For PCR detection, a range of different tissue types can be selected at the appropriate time of the year for testing compared to woody indexing where dormant material is used.
3. The viroids and phytoplasma have highly conserved regions in their genome sequences to which the PCR primers have been designed, ensuring that all isolates will be detected. The PCR primers are regularly checked against the sequences of all isolates in the public sequence database.

Based on the above, it is proposed that the *Malus* schedule of the Standard should be logged for future amendment, to remove the requirement for woody indicator testing to be used to help verify freedom from phytoplasmas and viroids.

<u>Risk management of diseases of unknown aetiology</u>

In contrast to testing for phytoplasmas and viroids, woody indicator testing and growing season inspection are the only diagnostic methods that can be used to verify that plants are free from diseases of unknown aetiology. The existing *Malus* schedule lists 19 diseases of unknown aetiology as regulated pests. Of these, four (Apple dead spur agent, Apple rough skin agent, Apple russet wart agent and Apple star crack agent) require woody indicator testing to verify their absence. Growing season inspection is used as the sole method to verify freedom from the other 15 diseases.

As part of the MPI "PEQ 2018" biosecurity response, the CTO considered whether there was enough information to support a review of the IHS regarding removal of measures for the diseases of unknown aetiology for *Malus* (and *Prunus*) species. The conclusion at that time was that "*The current knowledge of diseases of unknown aetiology suggests that de-regulating any of these diseases without further assessment may adversely impact New Zealand's apple and stonefruit industries*". Since then, MPI has re-assessed requirements for diseases of unknown aetiology of *Prunus* plants for planting and has proposed removing the requirement for woody indicator testing. However, no such assessment has been made for *Malus* (and, therefore, no similar proposal). Until such an assessment is done, it is proposed that the requirement for

woody indicator testing to manage risk associated with the four diseases of unknown aetiology of *Malus* should be retained. As such, all imported plant lines would need to meet relevant requirements of the Standard (or equivalent requirements) in relation to woody indicator testing. It is noted that in their submission on the draft standard for *Prunus* plants for planting, New Zealand Apples and Pears supported the proposal to remove the requirement for woody indicator testing to detect this type of disease (in *Prunus*) in cases where regulation is deemed necessary, and that they believed that the regulatory status of some of these diseases should be re-considered if they are "*rare, transmissible only by grafting, have a low biosecurity risk and pose no apparent commercial concern*". They also noted that they would support a comprehensive review of diseases of unknown aetiology during future review of *Malus* and *Pyrus* import health standards.

Golden Delicious is required as a woody indicator species for the four diseases of unknown aetiology for which woody indicator testing is required. Red Delicious is additionally required for Apple dead spur agent and Apple star crack agent. For each of the four diseases, the 2009 MPI *Import Risk Analysis: Viruses, Viroids, Phytoplasma, Bacteria and Diseases of Unknown Aetiology on Malus Nursery Stock from all Countries*[1] lists Golden Delicious as an appropriate indicator cultivar that may display symptoms if the disease is present. The risk analysis also states that indexing should be considered only a partially effective phytosanitary measure (presumably because little is known about the aetiology of these diseases). The risk analysis does not recommend Red Delicious as an indicator species for any of these four diseases. As such, it is not known why Red Delicious is listed in the Standard as a required indicator species for Apple dead spur agent and Apple star crack agent. The risk management proposal that was prepared when the standard was issued in 2011[2] does not state why these indicators are required. It is noted that the MPI testing manual for *Malus*[3] does state that Apple dead spur agent will induce symptoms on Red Delicious (and also Golden Delicious); however, no reference is given to support this statement.

Based on the above information, using one woody indicator cultivar (Golden Delicious) is likely to manage risk in accordance with the appropriate level of protection established for *Malus* when the Standard was the subject of public consultation in 2011. As such, it is recommended that the *Malus* schedule of the Standard should be logged for future amendment, to remove the requirement for woody indicator testing to be done using Red Delicious. If the CTO agrees with this recommendation, the plant that has only been tested using the cultivar Golden Delicious could be seen as meeting the intent of the Standard, whereas the plant that has only been tested on Red Delicious could not.

LEGAL

A CTO decision is required, under section 27(1)(d)(iii) of the Biosecurity Act, to give directions to the MPI Inspector that certain measures, different from those in the IHS, may be applied to manage the risks set out in the IHS to enable biosecurity clearance.

Section 27(1)(d)(iii) of the Biosecurity Act states:

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[1] Available at https://www.mpi.govt.nz/dmsdocument/2873-apple-malus-domestica-nursery-stock-micro-organisms-and-diseases-final-import-risk-analysis-july-2012.

[2] Available at https://piritahi.cohesion.net.nz/Sites/SAI/PP/PIM/PPNS/_layouts/15/WopiFrame.aspx?sourcedoc={F741DC26-744E-418B-8798-69FDA7857AB9}&file=2011-06%20Malus%20RMP%20Public%20consultation%20v1.0.doc&action=default&DefaultItemOpen=1.

[3] Available at https://www.mpi.govt.nz/dmsdocument/13642.

An inspector must not give a clearance for goods unless satisfied that a chief technical officer has issued guidelines, or given directions, on measures, different from those in the standard, that may be applied to manage effectively risks of the kind arising from the non-compliance.

DECISION

1. That the requirements described below, which have occurred on arrival in New Zealand whilst plants were held in post entry quarantine, are measures that effectively manage the risks of non-compliance with the requirements for woody indicator testing for diseases of unknown aetiology set out in the *Malus* schedule in the IHS 155.02.06: *Importation of nursery stock*.

   This part of the decision applies only to the following plants currently held in Level 3B post entry quarantine at the MPI PHEL post entry quarantine facility, from consignment C2015/249551: 71P-1F (2 plants), 71P-1K (2 plants), 71P-1L (4 plants), 71P-2P (4 plants), 71P-3K (3 plants) and 71P-3L (4 plants).

   Measures taken that have been applied to the above plants consist of the following:

   a) Demonstrating successful transmission of the non-regulated pest Apple mosaic virus to both Golden Delicious and Red Delicious positive control plants. This transmission is based on expression of symptoms of Apple mosaic virus on leaves of all positive control plants, and absence of symptoms in negative controls;

   b) Demonstrating successful transmission of the non-regulated pests Apple mosaic virus and Apple stem pitting virus to both Golden Delicious and Red Delicious positive control plants. This transmission is based on PCR testing to demonstrate the presence of these viruses in all positive control plants, and their absence from negative controls;

   c) Demonstrating successful transmission of Apple green crinkle disease to at least one positive control of Golden Delicious (based on symptoms being evident on fruit of one of the two Golden Delicious positive control plants established in 2015), with the presence of Apple green crinkle associated virus in this plant being confirmed by PCR;

   d) Demonstrating that environmental conditions were conducive to expression of symptoms of Apple green crinkle disease on fruit of Golden Delicious (based on grafting of large shoots to positive control plants in 2018, and expression of symptoms on one plant inoculated in 2015).

AGREE / DISAGREE

2. That plants from line 71P-2P, which have been tested using the measures proposed above, can also be seen as meeting the requirements of the standard, even though woody indicator testing for plants from this line was only done on Golden Delicious (i.e. they were not tested on Red Delicious). This is because there is no evidence in the MPI risk analysis about whether symptoms will be displayed on Red Delicious and it is not clear why this requirement is listed in the standard.

AGREE / DISAGREE

3. That plant line 71P-2F should not be considered eligible for biosecurity clearance because material from this plant has not been tested by woody indexing on Golden Delicious. This plant will either need to be tested on Golden Delicious as required under the standard, or reshipped or destroyed.

AGREE / DISAGREE

4. That risk from phytoplasmas and viroids on all plants from C2015/249551 has been appropriately managed by PCR testing alone, that has been completed as set out in the IHS.

AGREE / DISAGREE

5. That the standard should be logged for future amendment to remove the requirement for woody indicator testing to be used as a supplementary test for phytoplasmas and viroids of *Malus*, and to remove the requirement for Red Delicious to be used as a woody indicator cultivar when testing for diseases of unknown aetiology, pending the completion of a risk management proposal and public consultation.

AGREE / DISAGREE

RECOMMENDATION

It is recommended that you accept the proposal described below.

1. That a CTO direction is issued to the MPI Inspector to direct that specified plants from non-compliant consignment C2015/249551 may be considered for biosecurity clearance. This is because doing PCR testing to demonstrate successful disease transmission, and using two additional non-regulated viruses as positive controls, which are different measures to those specified in the *Malus* schedule of the import health standard 155.02.06: Importation of nursery stock, may be applied to effectively manage risks of the kind arising from the non-compliance. This direction will apply to all plants identified in paragraph (1) and paragraph (2) of the "Decision" section of this document.

AGREE / DISAGREE

Peter Thomson

Chief Technical Officer

Date:

CTO Direction to MPI Inspector
Biosecurity clearance of non-compliant consignment/s

CTO direction code for recording in Quantum: CTOPlants:2019029

Pursuant to section 27(1)(d)(iii) of the Biosecurity Act 1993 I, **Peter Thomson**, give the following directions for consignment C2015/249551 of *Malus* plants for planting imported from Australia, by Havelock North Fruit Co Ltd, to be considered for biosecurity clearance in accordance with the following measures, different from those required by the *Malus* schedule in the import health standard (IHS) 155.02.06: Importation of nursery stock:

This decision applies only to the following plants from the above consignment, currently held in Level 3B post entry quarantine at the MPI PHEL Level 3B post entry quarantine facility: 71P-1F (2 plants), 71P-1K (2 plants), 71P-1L (4 plants), 71P-2P (4 plants), 71P-3K (3 plants) and 71P-3L (4 plants).

- Demonstrating successful transmission of the non-regulated pest Apple mosaic virus to both Golden Delicious and Red Delicious positive control plants. This transmission is based on expression of symptoms of Apple mosaic virus on leaves of all positive control plants, and absence of symptoms in negative controls;

- Demonstrating successful transmission of the non-regulated pests Apple mosaic virus and Apple stem pitting virus to both Golden Delicious and Red Delicious positive control plants. This transmission is based on PCR testing to demonstrate the presence of these viruses in all positive control plants, and their absence from negative controls;

- Demonstrating successful transmission of Apple green crinkle disease to at least one positive control of Golden Delicious (based on symptoms being evident on fruit of one of the two Golden Delicious positive control plants established in 2015), with the presence of Apple green crinkle associated virus in this plant being confirmed by PCR;

- Demonstrating that environmental conditions were conducive to expression of symptoms of Apple green crinkle disease on fruit of Golden Delicious (based on grafting of large shoots to positive control plants in 2018, and expression of symptoms on one plant inoculated in 2015).

These measures have been applied on arrival in New Zealand, within a post entry quarantine transitional facility operated by Plant & Food Research Ltd at Palmerston North. This facility has now closed, and plants to be considered for biosecurity clearance are being held at the Level 3B post entry quarantine greenhouse in Auckland operated by the MPI Plant Health & Environment Laboratory.

All other relevant sections of the IHS 155.02.06: Importation of nursery stock for *Malus* plants for planting must be complied with.

This direction takes effect from the date of signing, and applies only to the specific plants identified above, from consignment C2015/249551.

Peter Thomson

Chief Technical Officer

Plant, Food & Environment Directorate

Date:

APPENDIX 1: Summary of woody indicator testing done by Plant & Food Research

ISSUE 1: Positive control plants did not display the expected disease symptoms

The standard states the following in regards to woody indicator testing:

> Woody indexing relies on the development of fruit and bark symptoms on susceptible *Malus* cultivars which would only be expressed under field conditions (i.e. Level 1 post entry quarantine [PEQ]). Negative and positive control plants must be included; the positive control must develop the expected symptoms (e.g. Apple green crinkle [non-regulated]).

Requirement: Positive controls are required to demonstrate disease transmission and symptom expression under the conditions used to establish and maintain woody indicator test plants. Apple green crinkle disease is listed in the standard as an example of a disease that could be used as a positive control for woody indicator testing and this disease was used by PFR.

Apple green crinkle disease is a disease of unknown aetiology that is present in New Zealand, and is non-regulated. There is now some evidence that this disease is caused by a virus that was identified in 2013 as Apple green crinkle associated virus.

Method: Bud inoculations for positive controls were done by PFR in 2015, on two Golden Delicious and two Red Delicious positive control plants. The buds for positive controls were taken from a field-grown plant in the Hawkes Bay that was known to show symptoms of Apple green crinkle disease (hereafter referred to as the "*donor plant*").

Results & Discussion: The donor plant showed symptoms typical of Apple green crinkle disease. All grafted buds 'took' indicating that grafts on positive control plants were successful. Negative controls (un-grafted plants of Golden Delicious and Red Delicious) were established at the same time.

Woody indicator test plants were established at the same time as positive and negative controls. Some grafted buds from each imported plant line took successfully (based on observations of survival of grafted buds made between five and 13 months after grafting; see Appendix 2 for a summary). Some additional grafting onto test plants was done in 2016 to try and ensure that all plants that would be released from post entry quarantine were tested by woody indicator testing.

All woody indicator plants were initially grafted and held in a Level 3B quarantine greenhouse under controlled environmental conditions. As required under the standard, plants were subsequently transferred to a Level 1 post entry quarantine facility and inspected for signs or symptoms of disease over two growing seasons.

In the first growing season in Level 1 post entry quarantine (i.e. 2017-2018) none of the positive control plants showed symptoms of *Apple green crinkle disease* (the disease selected for use as a positive control).

- Plant & Food Research believe that this is probably because insufficient time had elapsed to build enough disease titre to affect fruit development.

In the second growing season (2018-2019), fruit on one Golden Delicious replicate showed symptoms consistent with infection by *Apple green crinkle disease*. These symptoms were on a scion that had been grafted when woody indicator plants were originally established in 2015. The other Golden Delicious positive control also displayed symptoms of *Apple green crinkle disease* in the second growing season. However, symptoms on this tree were on large budsticks that had been additionally grafted onto the tree in September

2018 (see following paragraph). Neither of the Red Delicious positive control plants, or any of the negative controls, showed symptoms.

- Plant & Food Research believe that symptoms of *Apple green crinkle disease* were not displayed on Red Delicious because this disease may not be well expressed on red cultivars under New Zealand conditions. This conclusion is based on information in a 1974 report about *Apple green crinkle disease* in the New Zealand Journal of Agricultural Research.

Both Golden Delicious positive control plants were re-grafted with large budsticks taken from a symptomatic donor plant in the Hawkes Bay in September 2018.

- Plant & Food Research did this to verify that the environmental conditions in Palmerston North (where the indicator plants were being grown in Level 1 post entry quarantine) were conducive to symptom expression of Apple green crinkle disease. This is because cool spring temperatures are needed for *Apple green crinkle disease* symptoms to be expressed; Plant & Food Research were concerned that spring temperatures in Palmerston North may not be cool enough.

It is understood that as well as re-grafting the two Golden Delicious positive control plants with large budsticks, all four positive controls were also re-inoculated with smaller buds taken from the symptomatic plant in the Hawkes Bay. These grafts did not result in symptom expression. Red Delicious plants were not inoculated with large budsticks because there were no suitable sites on the positive control plants to do this style of grafting. The re-grafting done in 2018 shows that disease was successfully expressed under the conditions in Palmerston North in 2018. However, this does not prove that conditions under which original grafting was done (in 2015) were conducive to transmission of *Apple green crinkle disease* (or other diseases of unknown aetiology).

Under the standard, all positive control plants must display the expected symptoms. The plants do not meet this requirement, based on the use of *Apple green crinkle disease* as a positive control to verify disease transmission.

- However, Plant & Food Research noted that the donor plant material used to establish positive controls was also infected with two other non-regulated viruses (in addition to Apple green crinkle disease), namely Apple mosaic virus and Apple stem pitting virus. Similar to Apple green crinkle disease, these viruses are only known to be graft transmissible (for example based on information in the Crop Protection Compendium). This means that they are unlikely to be present in non-grafted material (for example such as negative controls). As such, the presence of these viruses in positive control plants may be able to be taken as further evidence that conditions under which positive controls were established were conducive to disease transfer to woody indicator test plants.

Symptoms of Apple mosaic virus were observed on leaves on all positive control plants (and also in trees from which positive control inoculum was obtained), but not in negative control plants. These symptoms were seen in both the 2017-2018 and the 2018-2019 growing seasons. No symptoms of Apple stem pitting virus were evident.

- As well as inspecting for visible symptoms of graft-transmissible viruses, Plant & Food Research also did PCR testing of positive control plants and donor plants to check for the presence of the three non-regulated graft transmissible viruses (i.e. Apple green crinkle associated virus [identified as a possible cause of Apple green crinkle disease], Apple mosaic virus and Apple stem pitting virus). In particular, Apple mosaic virus and Apple stem pitting virus were shown to be present in all donor and positive control plants. In contrast, Apple green crinkle associated virus was only reliably detected in one

Golden Delicious positive control plant (the plant that showed symptoms attributed to the original grafts done in 2015), and not in either Red Delicious plant.

- Some PCR testing of negative controls was also done, however this was mainly restricted to testing cambium and bark tissue (with two leaf samples also tested). None of the target viruses were detected in these samples. It is noted that a much wider range of tissues was tested in positive controls, and the viruses were generally more reliably detected in tissue types that were not tested in negative controls.

ISSUE 2: One of the imported lines was not tested using both woody indicator cultivars

Requirement: All imported plants must be tested by woody indicator testing. The unit for testing is an individual imported cutting, which must be tested separately unless MPI has given prior permission to combine samples taken from up to five plants for predetermined testing by graft indexing.

Method: Before the import permit for this consignment was issued, Plant & Food Research discussed the woody indexing protocol with the Plant Germplasm Team. They advised that the following procedure would be used when establishing plants in post entry quarantine and when doing woody indicator testing:

We are expecting to receive material whose phytosanitary certificate stipulates that the bud sticks in a particular lot were derived from the same mother plant. If the phytosanitary certificate stipulates this we propose to:

<u>Woody indexing</u>

Graft, by double budding, five rootstocks per accession. Each rootstock will be double budded from a single bud stick. This will result in

Plant 71P-1A derived from bud stick A in Lot 71P-1

Plant 71P-1B derived from bud stick B in Lot 71P-1

Plant 71P-1C derived from bud stick C in Lot 71P-1

Plant 71P-1D derived from bud stick D in Lot 71P-1

Plant 71P-1E derived from bud stick E in Lot 71P-1

The remainder of buds and Budwood from the bud sticks A, B, C, D &E will be combined and used as a composite sample for the woody indexing set up. The wooding indexing will consist of the grafting of buds and bark onto two replicate trees of `Red delicious' and `golden delicious' plus control trees (negative and positive). Each tree needs to be successfully grafted with a minimum of two buds and two pieces of bark.

Discussion: The plant germplasm team confirmed that the testing regime proposed by Plant & Food Research was acceptable and provided the following information (by email from Richard Lardner to Mary Horner at Plant & Food Research in 2015): *if the additional declaration regarding bud sticks being derived from the same mother plant is provided, samples can be combined to form a composite sample. However, we will still require that at least one bud and one piece of bark from each imported cutting is successfully grafted. Please also note that this adds some element of risk to the importer because if any symptoms were observed on indicators it would not be possible to identify which imported cutting was infected. As such, we would have to assume that all cuttings were infected.*

The key point in the confirmation provided by the plant germplasm team was the expectation that when doing woody indicator testing, MPI "… *will still require that at least one bud and one piece of bark from each imported cutting is successfully grafted"*[4]. This is also the requirement of the import health standard. For one of the plant lines, this is not the case, as described below:

The plant line of concern is line "71P-2". For this line, there are two plants undergoing post entry quarantine in a Level 3B greenhouse, plant "71P-2F" and "71P-2P". These are the plants that will ultimately be considered for biosecurity clearance. Plant "71P-2F" was generated using buds taken from one imported budstick, and plant "71P-2P" generated using buds from another imported budstick. Both budsticks were taken from the same mother plant in Australia. When setting up woody indicator testing, no buds from Plant 71P-2F were grafted onto Golden Delicious, and the one bud from Plant 71P-2P that was grafted onto Red Delicious failed to take. This means that Plant 71P-2F does not meet the requirements of the standard with regards to woody indicator testing on Golden Delicious (i.e. it has not been tested on that indicator species) and Plant 71P-2P does not meet the requirements with regards to woody indicator testing on Red Delicious.

It is noted that five buds taken from different budsticks derived from the same mother plant in Australia have been tested on Golden Delicious, and six buds on Red Delicious. However, this testing does not meet the requirements of the standard, which states that "*The unit for testing is an individual imported plantlet (imported in vitro) or cutting. Each plantlet or cutting must be labelled individually and tested separately, with the following exceptions:*" The exceptions referred to relate to testing of composite samples (made up of material from more than one individual imported plantlet or cutting). However even when composite sampling is allowed, the expectation is that some material from each imported plantlet or cutting that will be released into New Zealand must still be tested before plants can receive a biosecurity clearance. This is not the case for this plant line; as such it does not comply with the requirements of the standard.

The risk of not testing buds from plants that will ultimately be given a biosecurity clearance is that if a disease of unknown aetiology is unevenly distributed in the mother plant, it may not be present in the buds that were tested by woody indexing, but could be present in the plant that will receive a biosecurity clearance.

As noted in the "Assessment of risk management measures in the standard", the recommended indicator species for the four diseases of unknown aetiology for which woody indicator testing is required is Golden Delicious. One of the imported plants for which biosecurity clearance will be requested has been tested on Golden Delicious, and multiple other buds taken from the same mother plant (but not from the plant that will be released from post entry quarantine) have been tested on Red Delicious. this means that the testing that has been done on the plant successful grafted onto Golden Delicious (i.e. plant 71P-2P) may meet the intent of the standard with regards to woody indicator testing for the four diseases of unknown aetiology.

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[4] It is also noted that, based on information in the Plant & Food Research report summarising results of woody indicator testing, it is not clear if bark was grafted as well as buds. However, the import health standard does not state what type of material must be grafted onto indicator plants, and the testing manual does not specify if bark must be used. As such, this difference between what was initially proposed and the methodology that was used is not seen as significant.

Appendix 2 Summary of material grafted for woody indicator testing:

71P-1 – Imported plant Line 1

Fourteen budsticks imported, labelled 71P-1A, 71P-1B etc.

Purpose	**Replicate/id**	**Budsticks that were alive** (Grafted in October 2015, assessed March and Nov 2016)	**Supplementary budsticks that were alive** (Grafted in April 2016, assessed Nov 2016)
Test plant	E0736 (Golden Delicious)	1G, 1J, 1K, 1M	1G, 1L
Test plant	E0739 (Golden Delicious)	1F, 1L, 1M	1J
Test plant	E0722 (Red Delicious)	1F, 1J, 1L, 1M	
Test plant	E0745 (Red Delicious)	1G, 1J, 1K, 1M	1G
Release candidates (8 plants from both set 2 and set 2A)	n/a	1F (x2) 1K (x2) 1L (x 4)	n/a

71P-2 – Imported plant Line 2

Twenty budsticks imported, labelled 71P-2A, 71P-2B etc.

Purpose	**Replicate/id**	**Budsticks that were alive** (Grafted in October 2015, assessed March and Nov 2016)	**Supplementary budsticks that were alive** (Grafted in April 2016, assessed Nov 2016)	**Notes**
Test plant	E0732 (Golden Delicious)	2J, 2K, 2P, 2Y	2P	
Test plant	E0737 (*Red Delicious*)	2F, 2G, 2L, 2T	2F	

Test plant	E0723 (Red Delicious)	2F	2G, 2T, 2Y	
Test plant	E0752 (Red Delicious)	2J, 2K, 2Y	2F, 2Y	
Release candidates (5 plants from both set 2 and set 2A)	n/a	2F (x1) 2P (x4)	n/a	It looks like budstick P from plant 71P-2 was only successfully tested on Golden Delicious (one replicate). Plant 2F was not tested on Golden Delicious.

71P-3 – Imported plant Line 3

Eleven budsticks imported, labelled 71P-3A, 71P-3B etc.

Purpose	**Replicate/id**	**Budsticks that were alive** (Grafted in October 2015, assessed March and Nov 2016)	**Supplementary budsticks that were alive** (Grafted in April 2016, assessed Nov 2016)	**Notes**
Test plant	E0715 (Golden Delicious)	3G, 3H, 3J	3L	
Test plant	E0734 (Golden Delicious)	3G, 3H, 3L	3G, 3J, 3K	
Test plant	E0747 (Red Delicious)	3H, 3J	3G	
Test plant	E0749 (Red Delicious)	3G, 3J, 3K, 3L	n/a	
Release candidates (7 plants)	n/a	3K (x3) 3L (x4) (= set 2 and set 2A release candidates)	n/a	