**Approved arrangements for nursery stock and tissue cultures from countries with a higher risk of *Xylella fastidiosa* (Pierce’s disease)**

Department of Agriculture and Water Resources (DAWR) 14 December 2015

These requirements are for arrangements approved under Australia’s emergency measures for *Xylella fastidiosa* and related *Xylella* species. Pierce’s disease of grapevines is caused by *Xylella fastidiosa*. The requirements apply to nursery stock and tissue cultures of plants exported from high risk countries as indicated in the BICON alert1. These requirements do not apply to imports of true botanical seeds. This document should be read in conjunction with the emergency measures indicated in the BICON [alert](https://bicon.agriculture.gov.au/BiconWeb4.0/ViewElement/Element/Alert?elementPk=230789)1.

The arrangements are intended to provide guidance to the National Plant Protection Organisation (NPPO) of the exporting country, which will ensure that the requirements in the approved arrangements are met. Several other parties, including the grower and testing laboratory will need to work with the NPPO to do this.

This document describes the overarching systems and processes to ensure that nursery stock that is produced for export to Australia is grown, tested and confirmed free from infection by *X. fastidiosa*. The roles and responsibilities of the key parties involved in the arrangements are also described, as are the requirements for sampling and testing. The exporter and the testing laboratory should verify Australia’s requirements before testing is commenced. The department retains the right to monitor the arrangements by auditing and by sampling and testing consignments after they arrive in Australia.

***The arrangements***

The arrangements require the following elements:

1. Propagation, growth, testing, certification and export under the authority of the NPPO of the exporting country

2. The facility where plants are grown is insect-proof so that it excludes all insects of the suborder Auchenorrhyncha (leafhoppers, froghoppers, sharpshooters, spittlebugs and treehoppers)

3. Plants for export to Australia are grown for their entire life in the facility regardless of the propagation technique used (e.g. plants grown from seed, grown vegetatively or grown in tissue culture).

4. All the mother plants are tested by the approved protocol (Appendix 1)

* + nursery stock – the mother plants that are the immediate source from which the nursery stock plant lot was propagated are tested
  + tissue cultures –the mother tissue cultures that are the immediate source from which the nursery stock plant lot was propagated are tested

5. The mother plants have been grown under the arrangement, in the facility, for 12 months before they are tested (Diagram 1)

* + nursery stock – the mother plants are protected in the facility for 12 months before testing
  + tissue cultures – the mother tissue culture line is protected and propagated in the facility for 12 months before testing

6. Prior to export, an official sample is drawn from the plant lot and tested according to the approved protocolfor *X. fastidiosa* in Appendix 1. Appendix 1 contains details of sampling and sample size

7. Plants are packed and packaged to prevent infection by *X. fastidiosa*

8. Phytosanitary certificates issued by the NPPO with additional declarations including information that enables tracing of plant lines for export to Australia to test results and the facility in which the lines were grown.

***Roles and responsibilities***

**The NPPO**

The NPPO will provide oversight of the arrangements to ensure that they meet Australia’s requirements. It is responsible for approving facilities or any independent entities that approve facilities on its behalf. The NPPO may be requested by the Australian Government DAWR to provide records relevant to the approval and management of the arrangements.

The NPPO or an entity acting under the authority of the NPPO will:

* Inspect the production facility to confirm that:
* phytosanitary requirements are met
* the facility is insect proof
* the facility is constructed and equipped to achieve requirements
* records are maintained by the producer or the facility
* Approve the facility under the arrangement
* audit the facility and records
* Approve sampling of mother plants by the grower for testing or take samples of mother plants
* Take official samples of the plant lot for testing
* Maintain records of:
* approved facilities
* audits of facilities
* phytosanitary practices within facilities
* laboratory tests for *X. fastidiosa*
* Certify that plants exported to Australia are free from *X. fastidiosa* as shown by the testing. This will be based on evidence of systems that confirm:
* plants were kept in the registered facility throughout their life, from propagation to export
* testing has been undertaken by a competent laboratory and laboratory reports have been sighted
* Phytosanitary Certificates must include:
* additional declarations that indicate the status of the plant lot
* the facility approval code/number
* the laboratory report code/number

**The grower**

The grower will ensure that:

* phytosanitary conditions for Australia are met
* plants are appropriately tested
* provide samples of mother plants for testing, if approved by NPPO
* the facility is free from *X. fastidiosa*
* the facility is insect-proof

**The grower (responsibilities continued)**

* complete records are maintained of :
* plant lot identifying numbers or codes
* the parentage of the exported plant lots
* dates that plants are introduced to the facility
* mother plants, i.e. plants that are the immediate propagation source from which plant lots were propagated
* pathogens detected in plants in the facility
* arthropods found in the facility
* any plant material destroyed and the reason for the destruction
* All plants destined for export to Australia are transported in insect-proof closed containers or packaging

**The testing laboratory**

The testing laboratory is approved by the NPPO as competent to undertake the testing required by Australia, using the prescribed testing methods (Appendix 1).

It will:

* use the approved test protocol(Appendix 1)
* record the plant lots or mother plants that are tested and the number of samples tested
* provide the evidence of tests, results and operating processes to the NPPO, as required.

***References***

1. BICON, Australian Biosecurity Import Conditions (2015), Alerts – Further notification of emergency quarantine measures for plant pathogen *Xylella fastidiosa*, The Australian Department of Agriculture and Water Resources, 5 November 2015. <https://bicon.agriculture.gov.au/BiconWeb4.0/Home/Notice/>
2. Commission Implementing Decision (EU) 2015/789 of 18 May 2015 as regards measures to prevent the introduction into and the spread within the Union of *Xylella fastidiosa* (Wells *et al*.) (notified under document C(2015) 3415). European Commission

Diagram 1

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**Appendix 1**

This appendix provides the requirements for PCR testing under Australia’s emergency measures for *Xylella fastidiosa* and related *Xylella* species. It should be read in conjunction with the emergency measures indicated in the BICON [alert](https://bicon.agriculture.gov.au/BiconWeb4.0/ViewElement/Element/Alert?elementPk=230789). This document is current on the date of issue. The exporter and the testing laboratory should verify Australia’s requirements before testing is commenced.

The testing will be required for tissue cultures and nursery stock, including budwood, cuttings, rooted plants, bulbs and corms. Testing will be permitted off-shore by NPPO approved laboratories under circumstances indicated in the alert, and testing will be required on-arrival in Australia if plant lots are not certified in a manner consistent with the emergency measures. The department retains the right to monitor the arrangements by auditing, sampling and testing consignments after they arrive in Australia. Testing will use the PCR protocols outlined below.

During a transitional phase until 1 February 2016, the department will accept testing to detect four of the sub-species and will not specify which PCR tests should be done. From 1 February 2016 the department will require a particular combination of PCR tests so that five of the six sub-species can be reliably detected. An update will be issued once test methods for the sixth sub-species, tashke, have been reviewed.

Testing should be undertaken when the bacteria are most likely to be detected - which is when leaves are mature, before senescence, and typically from late summer or in autumn for perennials.

There are six sub-species of *X. fastidiosa*: *fastidiosa*, *multiplex*, *pauca, sandyi, tashke* and pear leaf scorch (PLS).

***PCR tests***

**19 November 2015 to 1 February 2016 (Transition phase)**

Australia will accept PCR tests that will detect *X. fastidiosa* subspp. *fastidiosa, multiplex, pauca* and *sandyi*

**1 February 2016 onwards**

Australia will require PCR tests that will detect *X. fastidiosa* subspp. *fastidiosa, multiplex, pauca, sandyi* and PLS

PCR testing will include:

* the rimM gene sequence real-time PCR test of Harper *et al*. (2010)1,

AND

* the conventional PCR of Minsavage *et al*. (1994)2 or an equivalent PCR that detects *X. fastidiosa* subsp. pear leaf scorch (PLS)

***Material to be tested***

* all the mother plants will be tested for *X. fastidiosa*, i.e. the mother tissue cultures or the mother plants that are the immediate source from which the nursery stock plant lot was propagated

AND

* prior to export (up to 8 weeks before), an official sample will be drawn from the plant lot and will be tested for *X. fastidiosa*
* mother plants or mother tissue cultures will have been grown under the arrangement, within the facility, for 12 months before testing

***Samples from plants***

* when testing the sample from the plant lot, the sample size (number of units) will be will be set according to Table 1 in ISPM 313 and will be sufficient to detect, with a 95% confidence level, that *X. fastidiosa* is not present in more than 0.5% (level of detection) of each lot, with the units defined as individual plants.
* two tissue samples per unit will be tested from tissue cultures, bulbs and corms
* three tissue samples per unit will be tested from nursery stock plants and cuttings
* samples will include mid-ribs of leaves, if the plant has leaves
* if the material lacks leaves, then living tissue with vascular structures will be sampled

Note: a unit is a tissue culture plantlet, nursery stock plant, a bulb or a corm.

***Bulking of samples for testing***

* DNA extracted from up to 10 samples may be tested in a single PCR as a pool or batch, where a sample is defined as a single piece of tissue
* samples from different species should not be pooled

***Positive controls***

* house-keeping gene positive controls will be run for each batch of tests and the positive controls will show that the DNA was extracted successfully
* house-keeping positive controls will be run for each different plant species

***Record keeping and certification***

* the laboratory will record the plant lots and mother plants that are tested and the number of samples tested
* NPPO will see the laboratory report and retain a copy
* the identifying code or number of the laboratory report will be provided on the Phytosanitary Certificate

***References***

1. Harper, S. J., Ward, L. I., and Clover, G. R. G. (2010). Development of LAMP and real-time PCR methods for the rapid detection of Xylella fastidiosa for quarantine and field applications. Phytopathology 100:1282-1288.
2. Minsavage, G.V., Thompson, C.M., Hopkins, D.L., Leite, M.V.B.C. and Stall, R.E. (1994) Development of a Polymerase Chain Reaction protocol for detection of Xylella fastidiosa in plant tissue. Phytopathology 84: 456-461.
3. ISPM 31 in International Standards for Phytosanitary Methods, No. 1 to 32 (2009), the Secretariat of the International Plant Protection Convention, Food and Agriculture Organization of the United Nations, Rome. pp. 401–420. https://www.ippc.int/en/publications/588/