

# Contingency plan for Pierce's disease and other diseases caused by *Xylella fastidiosa*

Queensland Department of Agriculture and Fisheries  
Nursery & Garden Industry Australia  
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**Acknowledgements**

This contingency plan is an update of the 2011 “Threat specific contingency plan for Pierce’s disease (*Xylella fastidiosa*)” which was prepared by John McDonald (on behalf of NGIA) and PHA. The current version was prepared by Lindy Coates, Andrew Manners (Queensland Department of Agriculture and Fisheries) and John McDonald (NGIA).

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# 1 Purpose and background of this contingency plan

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This contingency plan is designed to enhance Australia's capacity to respond to and manage an incursion of Pierce's disease, and other diseases caused by *Xylella fastidiosa*, with special emphasis on production nurseries. The contingency plan specifically focuses on the pathogen, but recognises that the introduction, spread and economic impact of the disease will depend strongly on the presence of one of its main vectors, the glassy winged sharpshooter (*Homalodisca vitripennis*). A separate contingency plan has previously been prepared by Plant Health Australia (2009) for glassy winged sharpshooter (GWSS), and is referenced where appropriate in this contingency plan.

As this contingency plan was developed specifically for the Nursery and Garden Industry Australia (NGIA), it is focused on production nurseries covered by this association. In the event of an incursion, operations not covered by the NGIA (e.g. retail outlets) will not be eligible for Owner Reimbursement Costs, as defined in the Emergency Plant Pest Response Deed, if affected by actions carried out under an approved Response Plan.

This contingency plan is an update of the 2011 "Threat specific contingency plan for Pierce's disease (*Xylella fastidiosa*)", which was prepared by John McDonald (on behalf of NGIA) and PHA. Key references used in the preparation of the current plan include the National Diagnostic Protocol for Pierce's disease, *Xylella fastidiosa* (Luck *et al.* 2010), a review of incursion preparedness for *X. fastidiosa* and *H. vitripennis* in Australia (Rathe *et al.* 2012b), and a range of EPPO resources which have become available as a result of recent *X. fastidiosa* incursions in Italy and France. Information on pest biology, host range, distribution, symptoms and disease management is given.

## 2 Australian nursery industry

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The Australian nursery industry is a significant horticultural sector with a combined supply chain (production to retail/grower) valued at more than \$6 billion dollars annually. The industry employs approximately 45,000 people spread over more than 20,000 small to medium sized businesses, including production nurseries and retail outlets. The industry is located predominantly along the Australian coastline, and in major inland regions servicing urban and production horticulture.

Nursery production adds significant value to Australia's primary industry's sector annually, contributing more than \$2 billion to the national economy. Nursery production is a highly diverse industry, providing a critical service to the broader horticultural sector, valued at \$14 billion within Australia (Table 1).

**Table 1.** Nursery production supply sectors within Australian horticulture

Production nursery	Horticultural market	Economic value
Container stock <sup>1</sup>	Ornamental/urban horticulture	\$2 billion retail value
Foliage plants <sup>1</sup>	Interior-scapes	\$87 million industry
Seedling stock <sup>2</sup>	Vegetable growers	\$3.3 billion industry
Forestry stock <sup>3</sup>	Plantation timber	\$1.7 billion industry
Fruit and nut tree stock <sup>2</sup>	Orchardists	\$5.2 billion industry
Landscape stock <sup>1</sup>	Domestic & commercial projects	\$2 billion industry
Plug and tube stock <sup>4</sup>	Cut flower	\$319 million industry
Revegetation stock	Farmers, government, landcare groups	\$109 million industry
Mine revegetation	Mine site rehabilitation	Value unknown

### 3 Impact of Pierce's disease and other diseases caused by *Xylella fastidiosa*

A wide range of crops (e.g. grape, various stone fruit, citrus, almond, coffee, olive, blueberry, avocado), ornamentals (e.g. oleander), forest trees, grasses and weeds can be affected by *Xylella fastidiosa*, some of which can carry the disease without symptoms. Symptoms vary according to host, extent of colonisation and other factors, but typically include those associated with water stress, such as drying, scorching, chlorosis, dwarfing and wilting of foliage, defoliation and dieback. Plant death may occur in some hosts (EPPO 2016a).

A 2012 review article on incursion preparedness for *Xylella fastidiosa* and the vector *Homalodisca vitripennis* (glassy-winged sharpshooter) suggests that the Australian environment is suitable for the establishment of *H. vitripennis*, and that a number of common Australian native plant species are likely to act as hosts of *X. fastidiosa* should the pathogen gain entry into Australia (Rathe *et al.* 2012b). In the United States, Pierce's disease has had a major economic impact on the Californian grape, citrus and nursery industries, with annual costs associated with the disease estimated at \$USD104 million (Tumber *et al.* 2013). These costs include direct disease losses (e.g. death and decline of vines/trees), as well as efforts to mitigate damage. The main burden of the compliance costs (i.e. shipping protocol measures such as inspections, pesticide sprays and quarantines) in California has been borne by the nursery industry, and have been estimated at \$USD 91 million between 1999 and

<sup>1</sup> Data sourced from Market Monitor

<sup>2</sup> Data sourced from Horticultural Handbook 2004

<sup>3</sup> Data sourced from ABARE 2005

<sup>4</sup> Data sourced from industry

2010. Luck *et al.* (2001) indicated that Pierce's disease could be as serious in Australia as it has been in California.

While *X. fastidiosa* was for many years confined to the Americas, there are now detections in Asia and Europe (EPPO 2016a). The first European detection was in Italy in 2013 on olive (EPPO 2016a), where it is now causing serious damage (death is occurring in all trees infected by the bacterium). It has also been detected on numerous other host plants (mainly ornamentals) in Italy, and on the ornamental plant species, *Polygala myrtifolia*, in France. The pathogen is currently under eradication in both countries.

## 4 Eradication decision support matrix

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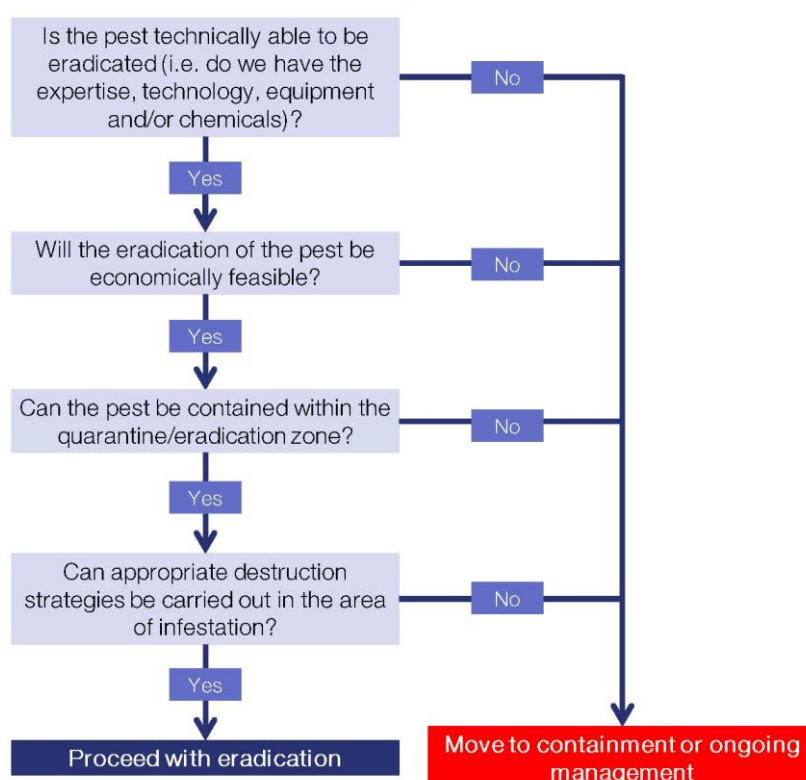
Production nurseries are important as pathways for the potential entry and spread of Pierce's disease and other diseases caused by *Xylella fastidiosa*. Following an outbreak of Pierce's disease, the response needs to be clearly explained, decisive, coordinated and rapidly implemented. Initially it will be assumed that eradication of Pierce's disease is possible; containment will be the second option. Containment measures will be based on the biology of the pathogen and its vectors, and the institutional and commercial structures in place for the management of plant disease outbreaks.



The decision matrix to aid in the decision between eradication and containment is shown in Figure 1 and Table 2.

**Fig. 1.** Decision outline for the response to an exotic pest incursion and a summary of the basis on which each decision could be made.

<b>Basis for technical feasibility:</b> <ul style="list-style-type: none"> <li>○ Early detection</li> <li>○ Confined space/restricted area of dispersal</li> <li>○ Known distribution of host plants</li> <li>○ Effective, reliable, quick detection method</li> <li>○ Support from industries, businesses and communities involved.</li> </ul>
<b>Basis for economic feasibility:</b> <ul style="list-style-type: none"> <li>○ Value of crop destroyed by uncontrolled pest is more than cost of controlling the pest</li> <li>○ Value of environmental amenity (native species lost) vs cost or loss of other amenity (loss of native insects due to spraying in native forests etc)</li> </ul>
<b>Basis for quarantine containment:</b> <ul style="list-style-type: none"> <li>○ Legislation to create a pest quarantine area (PQA)</li> <li>○ Resources to maintain the PQA, inspection points, staffing, detection equipment, diagnostics</li> <li>○ Support of industry and community to make the PQA work</li> </ul>
<b>Basis for destruction/control strategies required:</b> <ul style="list-style-type: none"> <li>○ How much destruction and or control measures are industry and individuals prepared to undertake?</li> <li>○ What level of destruction is technically feasible?</li> <li>○ Do the benefits of destruction outweigh the problems created?</li> </ul>
<b>What would containment or ongoing management look like?</b> <ul style="list-style-type: none"> <li>○ Is containment feasible?</li> <li>○ What would ongoing management really mean?</li> <li>○ Many similar features to eradication, but at less intense / restrictive levels.</li> </ul>



**Table 2.** Factors considered in determining whether eradication or alternative action will be taken for an EPP incident from PLANTPLAN (Plant Health Australia, 2016 Table 2).

a) the capability to accurately diagnose or identify the EPP.
b) the effectiveness of recommended control technique options, which are likely to be the most cost-effective in eradicating the EPP.
c) the ability to remove or destroy all EPPs present by the recommended control techniques.
d) the ability to remove the EPP at a faster rate than it can propagate until proof of freedom can be achieved.
e) the recommended control techniques are publicly acceptable (taking into consideration cultural and social values, humaneness, public health impacts, non-target impacts and environmental impacts)
f) whether Emergency Containment measures have been put in place by the Lead Agency(s).
g) whether there are controls methods, commonly employed for endemic pests and diseases, that may limit or prevent the establishment or impact of the EPP.
h) any legislative impediments to undertaking an emergency response.
i) the resources e.g. chemicals, personnel etc. required to undertake an emergency response are accessible or available.
j) the ability to delimit the known area of infestation.
k) the ability to identify the pathway for entry into, and trace the spread of the EPP within Australia.
l) the ability to determine whether the likelihood of further introductions is sufficiently low.
m) the dispersal ability of the EPP (that is, whether the EPP is capable of rapid spread over large distances).
n) the capability to detect the EPP at very low densities for the purpose of declaring freedom, and that all sites affected by the EPP have or can be found.
o) the ability to put in place surveillance activities to confirm Proof of Freedom for sites possibly infested by the EPP.
p) whether community consultation activities have or will be undertaken.

## 5 Pest information/status

### 5.1 Pest details

**Scientific name:** *Xylella fastidiosa* (various subspecies).

**Common names of diseases caused by *Xylella fastidiosa* in major hosts:** Pierce's disease of grape; plum leaf scald; phony disease of peach; citrus variegated chlorosis; leaf scorch disease (pecan, pear, almond, coffee, elm, sycamore, oleander, maple, oak, purple-leafed plum, mulberry); olive quick decline syndrome; dwarf lucerne; sweetgum dieback.

### 5.2 Biology

Pierce's disease of grapevines was first discovered in 1892 in California, and is now a damaging pest in southern parts of the United States, Mexico and Central America. The disease is caused by the xylem-limited bacterium *Xylella fastidiosa* (Wells *et al.* 1987), which is also the causal agent of a range of similar diseases in other host species. These include leaf scorch of oak, oleander, elm, sycamore and maple (Hearon *et al.* 1980), sweetgum dieback and leaf scorch of purple-leafed plum (Hernandez-Martinez *et al.* 2009) and diseases of agriculturally important crops such as peach, plum, pear, coffee, lucerne, citrus, almond, pecan and olive (Hopkins 1989; Leu and Su 1993; de Lima *et al.* 1998; Carlucci *et al.* 2013). Diseases caused by *X. fastidiosa* are no longer confined to the Americas – they are also present in Taiwan, Italy, France, Iran, Turkey, Lebanon, Kosovo, and unconfirmed in India and Morocco (DAWR 2016).

In grapevine, Pierce's disease is a lethal disease killing vines outright by blocking the transport of water and soluble mineral nutrients in xylem tissue. The plant can die within 1-2 years of the initial infection date. The disease and the vector can persist all year round (Luck *et al.* 2010).

A generic life cycle of the pathogen in shade trees is depicted in Figure 2. The bacteria proliferate in the xylem vessels of susceptible hosts, and notably, are maintained or can multiply in wild hosts. Survival of the bacteria depends strongly on winter climate, as persistence in plants over winter is limited by cold conditions (Purcell 1980). The bacterium is sensitive to dry conditions, such as those found in many seeds, but despite this, seed transmission of the bacteria is known to occur in citrus (Li *et al.* 1973; CABI 2016). *X. fastidiosa* can also persist in the gut of vector insects indefinitely, with the ability to multiply in the foregut (Janse and Obradovic 2010). In particular, the presence of vectors that overwinter as adults (as opposed to eggs or nymphs) appears to be a major factor in disease prevalence, as these vectors have the capacity to establish early season infections (Purcell 1997).

Several molecular studies have shown that distinct groups or clusters of *X. fastidiosa* exist (e.g. Chen *et al.* 1995, Pooler *et al.* 1995, Henderson *et al.* 2001). Schaad *et al.* (2004a) described three subspecies of *X. fastidiosa* based on genetic and phenotypic evidence, namely subsp. *piercei*; subsp. *multiplex* and subsp. *pauca*. Subspecies *piercei* was subsequently renamed as subsp. *fastidiosa* due to a naming error (Schaad *et al.* 2004b). Schuenzel *et al.* (2005) further classified a group of oleander leaf scorch isolates as a separate subspecies, *X. fastidiosa* subsp. *sandyi*. A further subspecies, *X. fastidiosa* subsp. *tashke*, was proposed for isolates from infected chitalpa (*Chitalpa tashkentensis*) trees in the U.S. (Randall *et al.*, 2009). Subspecies *fastidiosa*, *multiplex*, *pauca* and *sandyi* are currently accepted taxa, but subspecies *tashke* is still pending full acceptance (EFSA 2013).

For each of the four currently accepted subspecies, some of the major hosts which have been reported include (from Janse and Obradovic 2010 unless otherwise referenced):

- (i) *Xylella fastidiosa* subsp. *fastidiosa* – grapevine, almond, lucerne, maple and other hosts.
- (ii) *X. fastidiosa* subsp. *multiplex* – peach, plum, elm, pigeon grape, sycamore, almond and other hosts.

(iii) *X. fastidiosa* subsp. *pauca*, - citrus, coffee (Janse and Obradovic, 2010; Jacques *et al.*, 2015), olive (EPPO 2016a) and other hosts.

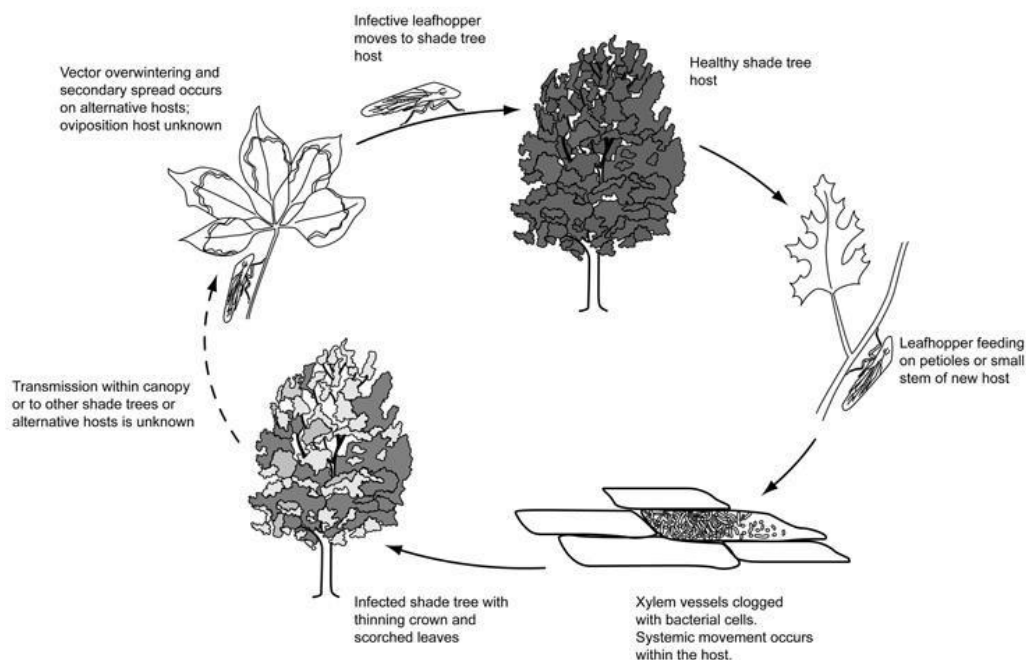
(iv) *X. fastidiosa* subsp. *sandyi* – oleander and other hosts.

Despite these classifications, the relationship between strains and hosts appears complex and is still not fully understood and is further complicated by the existence of pathovars (within plant-host strains) (Schuenzel *et al.* 2005). For example, some pathovars causing almond leaf scorch can also cause Pierce's disease in grapes, yet other pathovars are limited to causing disease symptoms only in almonds (Hendson *et al.* 2001). However, the sequencing of the *X. fastidiosa* genome (Simpson *et al.* 2000) and subsequent sequencing of various strains of *X. fastidiosa* should improve understanding.

The geographical distribution of the four *X. fastidiosa* subspecies in relation to a number of important hosts is listed in Table 3, although it is recognised that new detections in other countries may not be captured in this summary.

**Table 3:** Important susceptible plants and geographic distribution of subspecies of *X. fastidiosa* - from European Food Safety Authority (EFSA) 2013.

Subspecies	Geographic distribution	Important susceptible plants
<i>fastidiosa</i>	Central and North America, Taiwan	grapevines, citrus, coffee, almond
<i>pauca</i> <sup>5</sup>	Brazil, Paraguay, Argentina	citrus, coffee, almond, peach, plum, oak,
<i>multiplex</i>	United States, Brazil	blueberry, pecan, etc.
<i>sandyi</i>	United States	oleander



**Figure 2:** Disease cycle of *X. fastidiosa* in shade trees (from Gould and Lashomb 2007).

<sup>5</sup> Now also present (and under eradication) in Italy (olive, various ornamentals) and France (*Polygala myrtifolia*)

## 5.3 Dispersal

Pierce's disease can be transmitted and dispersed by graft transmission and by propagative material (Smith *et al.* 1997). Seed transmission and spread from tree to tree has been reported in citrus (Laranjeira *et al.* 1998; Li *et al.* 2003), but these mechanisms of spread are not common for other species. Other research has shown the disease is not transmitted by contaminated pruning shears (Varela 2000).

Dispersal typically occurs through using infected grafting material or by insect vectors which include nearly all sucking insects that feed predominantly on xylem fluid (Purcell 1989). The most common vector species in North America are Leafhoppers (Cicadellidae) in the subfamily Cicadellinae (sharpshooters) and spittle bugs or froghoppers (Cercopidae). The bacteria adhere to the mouthparts and are released directly when the insect feeds again. Few live cells are needed for effective transmission (Purcell *et al.* 1979; Hill and Purcell 1995). Transmission is usually from wild, generally symptomless hosts to cultivated hosts (grapevines, peaches) rather than between cultivated hosts, though the latter can occur.

Once acquired, the bacteria can persist in the gut of vectors indefinitely (Janse and Obradovic 2010), which then can transmit the disease in the subsequent season. The vector identified as the greatest threat to Australia is the Glassy winged sharpshooter (GWSS), which has a wide host range, flies long distances and unlike other vectors, often feeds directly on stems rather than leaves or extremities so pruning is not a viable control measure. For information on dispersal of the GWSS, refer to the Threat specific contingency plan for GWSS prepared for the NGIA (Plant Health Australia 2009).

Plant parts able to carry *X. fastidiosa* in trade/transport are: bulbs, tubers, corms, rhizomes, flowers, inflorescences, cones, calyx, fruits (including pods), leaves, roots, seedlings, micropropagated plants, stems (above ground), shoots, trunks, branches and true seeds (including grain) (CABI 2016). In all cases the pathogen is borne internally, not visible to naked eye but usually visible under light microscope

Plant parts not known to carry the pest in trade/transport are: bark, growing medium accompanying plants and wood (CABI 2016).

Vectors can also be carried internationally on plants or plant products (usually as viable egg masses on plants), which is a major concern to Australia because no vectors are known to exist in Australia at present.

Australia has no record of Pierce's disease or Glassy winged sharpshooter.

## 5.4 Affected hosts

### 5.4.1 Host range

*Xylella fastidiosa* has an extremely wide host range. There are currently over 350 different plant host species known from 204 genera and 75 different families (EFSA 2016). Of these, 269 species were reported to be associated with natural infections, and 194 species were recorded from experimental infections. The majority of host species listed are wild hosts (e.g. wild grasses, sedges, various shrubs and trees) on which no leaf scorch symptoms are observed. Some of the main commercially important hosts include grapevine, citrus, almond, peach, coffee, oleander and olive, although the disease has been reported in a number of other crops (e.g. Asian pear, avocado, blueberry, Japanese plum, pecan, plum, sour cherry) and ornamental species (e.g. elm, sycamore, maple, oak, red mulberry). Current *X. fastidiosa* host listings are available from EFSA<sup>6</sup> and CABI<sup>7</sup> publications.

<sup>6</sup> <http://www.efsa.europa.eu/fr/efsajournal/pub/4378>

<sup>7</sup> <http://www.cabi.org/isc/datasheet/57195>

It is worth noting that the host range of vectors, in particular the GWSS, will have a bearing on the spread of the disease. Hosts of the GWSS are listed in the GWSS contingency plan.

## 5.4.2 Current geographic distribution

Diseases caused by *X. fastidiosa* were for many years confined to the Americas, with the exception of pear leaf scorch which occurs in Taiwan (Leu and Su 1993). *X. fastidiosa* now also occurs in Italy, France, Iran, Turkey, Lebanon, Kosovo, with unconfirmed detections in India and Morocco (EPPO 2016a; DAWR 2016).

The geographic distribution of Pierce's disease appears to be related to the ability of the bacteria to survive winter temperatures (Varela 2000). In general the disease is less prevalent where winter temperatures are colder. Wet winters also promote survival of vector populations and favour disease spread in regions with dry summers.

As winter weather conditions in Australia are not as severe as those in the USA, the effects of winter are likely to favour survival of the bacterium in Australia (Luck *et al.* 2010).

The current geographic distribution of the pathogen, based on all hosts, is given below (from EPPO 2016a):

**EPPO region:** France (first detected in Corsica, and then also in Alpes-Maritimes and Var, under eradication), Italy (introduced in Puglia, under eradication).

**Asia:** Iran, Taiwan (introduced, first found in Asian pears and then in grapevine).

**North America:** Canada (Ontario), Mexico, USA (Alabama, Arizona, Arkansas, California, Delaware, District of Columbia, Florida, Georgia, Indiana, Kentucky, Louisiana, Maryland, Mississippi, Missouri, Montana, Nebraska, New Jersey, New Mexico, New York, North Carolina, Oklahoma, Oregon, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, Washington, West Virginia).

**South America:** Argentina, Brazil (Bahia, Espirito Santo, Goias, Minas Gerais, Parana, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, Sao Paulo, Sergipe), Costa Rica, Paraguay, Venezuela.

A recent factsheet published by DAWR (2016) also indicated the presence of *X. fastidiosa* in the Caribbean, Turkey, Lebanon and Kosovo, with unconfirmed reports of the pathogen in India and Morocco. CABI (2016) provide a detailed distribution table for *X. fastidiosa*.

## 5.4.3 Symptoms

Symptom development depends on the rate and extent of colonisation of the xylem vessels of the host. The symptoms produced are usually those associated with water stress, and vary with the host plant. Symptoms typically include leaf scorch, veinal chlorosis, wilt and dwarfing. In some hosts (e.g. olive), plant death can occur. Refer to the nursery **factsheet on diseases caused by *Xylella fastidiosa*** (bacterial leaf scorch) at the NGIA website.

### 5.4.3.1 Pierce's disease of grapevines

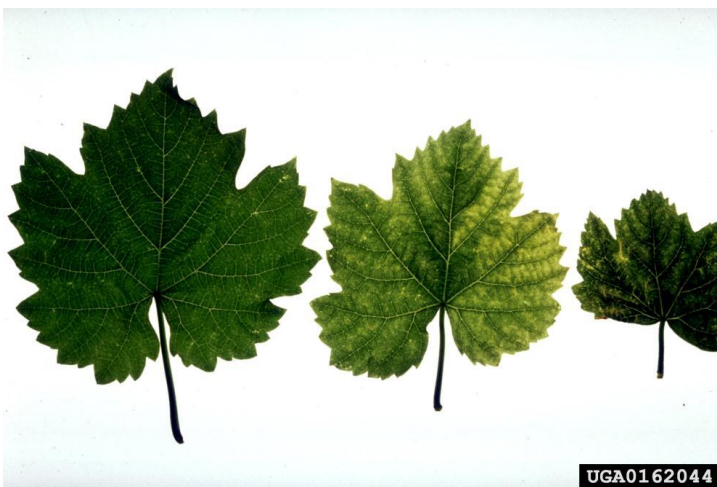
Leaf scorch is the most characteristic symptom of primary infection, with early signs including sudden drying of parts of green leaves, which then turn necrotic with adjacent tissues turning yellow or red (Figure 3). Scorched leaves may shrivel and drop, leaving bare petioles attached to stems. Diseased stems often mature irregularly, with patches of brown and green tissue. In later years, infected plants develop late and produce stunted chlorotic shoots. Chronically infected plants may have small, distorted leaves with interveinal chlorosis (Figure 4) and shoots with shortened internodes (CABI 2016). Highly susceptible cultivars rarely survive more than 2-3 years while tolerant cultivars may survive chronic infection for more than 5 years (Goodwin and Purcell 1992).



It can take four to five months for the symptoms to appear, with only one or two canes showing symptoms in year 1. With young vines the symptoms appear more quickly covering the entire vine in a single season (Varela *et al.* 2001).



**Figure 3:** Leaf symptoms in the field include yellowing and reddening of leaf tissue. Image courtesy of ENSA-Montpellier Archive, Ecole nationale supérieure agronomique de Montpellier, Bugwood.org



**Figure 4:** Leaf symptoms of Pierce's disease (right) on Chardonnay grape compared to healthy leaf (left). Image courtesy of Alex. H. Purcell, University of California - Berkeley, Bugwood.org

#### 5.4.3.2 Phony peach disease

Young shoots are stunted with greener, denser foliage than healthy trees (CABI 2016). The shortening of internodes is accompanied by increased development of lateral branches that grow horizontally or droop (Janse and Obradovic 2010). Leaves and flowers appear early, and leaves remain on the tree longer than on healthy trees. Trees are not generally killed, but suffer fruit yield losses and are susceptible to attack from insects and other diseases.

#### 5.4.3.3 Citrus variegated chlorosis

Typical symptoms on trees up to 10 years of age include foliar chlorosis resembling zinc deficiency with interveinal chlorosis (Figure 5); symptoms in older trees appear as a few diseased branches. As the leaves mature, small, light-brown, slightly raised gummy lesions (becoming dark-brown or even necrotic) appear on the underside, directly opposite the yellow chlorotic areas on the upper side. Newly affected trees show sectoring of symptoms, whereas trees which have been affected for a period of time show variegated chlorosis throughout the canopy. Affected trees show stunting and slow growth rate; twigs and branches die back and the canopy thins, but affected trees do not die (CABI 2016). Trees may also wilt. Fruit are smaller (Figure 6) with a hard rind and higher sugar content (CABI 2016).



**Figure 5:** Leaf interveinal chlorosis caused by Citrus variegated chlorosis disease. Image courtesy of Alex. H. Purcell, University of California, Bugwood.org





**Figure 6:** Fruit are smaller, and small raised lesions appear on the underside of leaves. Image courtesy of Alex. H. Purcell, University of California, Bugwood.org

#### 5.4.3.4 Other leaf scorch diseases caused by *X. fastidiosa*

'Scorching' or bronzing of the leaf margins is the classic early symptom of diseases caused by *X. fastidiosa* (Figures 7 & 8). The bronzing may intensify (Figure 9) and become water soaked before browning and drying (Janse and Obradovic 2010). Symptoms usually appear on just a few branches but later spread to cover the entire plant. Depending on the plant, dieback, stunting, fruit distortion or plant death may occur.



**Figure 7:** Oleander leaf scorch symptoms. Image courtesy of Jack Kelly Clark, University of California Statewide IPM Program.





**Figure 8:** Bronzing of oak leaves caused by *X. fastidiosa*.  
Image courtesy of Randy Cyr, Greentree, Bugwood.org



**Figure 9:** Bronzing intensifies over time (leaf from American Sycamore).  
Image courtesy of Theodor D. Leininger, USDA Forest Service, Bugwood.org

## 5.5 Diagnostic information

An endorsed National Diagnostic Protocol (NDP) for Pierce's Disease, *Xylella fastidiosa* is currently available (Luck *et al.* 2010). This protocol describes morphological, biochemical and molecular methods for the positive identification of *X. fastidiosa*. A revision of this 2010 NDP is currently in the final stages, due to be released in the near future.

A draft EPPO diagnostic protocol (EPPO 2016b) is also available<sup>8</sup>, which provides the latest detailed information (and relevant references) on the detection and identification of *X. fastidiosa*, including:

- Symptoms in a range of important hosts (including lucerne, almond, blueberry, ornamental trees, citrus, coffee, olive, grape, peach, plum, oleander)
- Sample collection and preparation (including both symptomatic and asymptomatic tissue)
- Serological tests such as ELISA (enzyme-linked immunosorbent assay), IF (indirect immunofluorescence test) and DTBIA (direct tissue blot immunoassay)
- Molecular tests test including conventional PCR, real time PCR, loop mediated isothermal amplification (LAMP)
- Electron microscopy
- Colony and cell morphology
- Pathogenicity tests
- Bioassay (tobacco)

The protocol recommends that once a pure culture of *X. fastidiosa* is obtained, "identification should be performed using at least two different tests, based on different biological principles or targeting two different parts of the gene for molecular tests".

Flow diagrams for the diagnostic procedure for *X. fastidiosa* on both symptomatic and asymptomatic plant material are also provided in the EPPO document.

For a list of diagnostic facilities and advisory services that can be utilised in the event of an incursion, see Section 11.2.

### 5.5.1 Morphological methods

*X. fastidiosa* is a Gram-negative, slow growing rod-shaped bacterium that lacks flagella for motility and is strictly aerobic (Janse and Obradovic 2010). Bacterial cells typically possess a rippled (undulating) cell wall and terminal fimbriae (surface structures, shorter than flagella, that help to anchor the cells together in the xylem stream) (Gould and Lashomb 2007).

Even from symptomatic plants, *X. fastidiosa* can be difficult to isolate and grow in pure culture (EPPO 2016b). As the name suggests, *X. fastidiosa* has fastidious nutrient requirements and grows only on selective media to form small colonies that appear white to yellow (Gould and Lashomb 2007). Specialised media has been developed for isolating and growing the bacterium (Luck *et al.* 2002 & 2010). The use of at least two different media types is recommended (EPPO 2016b). If bacterial colonies are isolated and have similar growth characteristics and morphology to *X. fastidiosa* on at least one media type, the isolation is considered to be positive. The presumptive identification of *X. fastidiosa* must then be confirmed by serological and/or molecular tests.

### 5.5.2 Serological methods

Loconsole *et al.* (2014) compared and validated diagnostic protocols based on ELISA and conventional PCR for *X. fastidiosa* detection in olive samples in Italy. This was done using an inter-laboratory ring test in which three accredited laboratories participated. They found that both procedures were equally effective, but suggested that ELISA may be more suitable for large scale monitoring of *X. fastidiosa* due to simplicity of sample preparation.

The EPPO diagnostic protocol (EPPO 2016b) describes the use of serological tests for the identification of *X. fastidiosa*, including ELISA, IF and DTBIA.

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<sup>8</sup> [https://www.eppo.int/QUARANTINE/special\\_topics/Xylella\\_fastidiosa/16-21486%20REV%20EPPDP%20XF%20PM7-24%20final.pdf](https://www.eppo.int/QUARANTINE/special_topics/Xylella_fastidiosa/16-21486%20REV%20EPPDP%20XF%20PM7-24%20final.pdf)

### 5.5.3 Molecular methods

Molecular methods for the detection and identification of *X. fastidiosa*, and the identification of subspecies, have been detailed in the EPPO diagnostic protocol (EPPO 2016b). In summary, the following methods are described:

- Conventional PCR (Minsavage *et al.* 1994) – suitable for the detection and identification of *X. fastidiosa*
- Real-time PCR (Francis *et al.* 2006) - suitable for the detection and identification of *X. fastidiosa*
- Real-time PCR (Harper *et al.* 2010; erratum 2013) - suitable for the detection and identification of *X. fastidiosa*
- Real-time LAMP (Harper *et al.* 2010; erratum 2013) - suitable for the detection of *X. fastidiosa* in host plants and insects
- PCR for MLST (Yuan *et al.* 2010) – suitable for the identification of *X. fastidiosa* subsp. *fastidiosa*, *multiplex*, *pauca* and *sandyi* from DNA of pure bacterial culture or plant extract
- Conventional simplex PCR (Hernandez-Martinez *et al.* 2006) – suitable for subspecies determination in-plant and identification of *Xylella fastidiosa* subsp. *fastidiosa*, *multiplex* and *sandyi* isolates
- Conventional multiplex PCR (Hernandez-Martinez *et al.* 2006) – mainly used for the identification of *Xylella fastidiosa* subsp. *fastidiosa*, *multiplex* and *sandyi* isolates from DNA of pure bacterial culture. It may be used for subspecies determination in-plant but has not been validated
- Conventional PCR (Pooler and Hartung 1995) – suitable for the detection and identification of *Xylella fastidiosa* subsp. *pauca*

### 5.5.4 Pathogenicity tests

Pathogenicity tests can also be used in the determination of subspecies, although it was noted that verification of pathogenicity of *X. fastidiosa* is sometimes difficult and can take several months (EPPO 2016b). The EPPO diagnostic protocol provides general guidance on pathogenicity testing for *X. fastidiosa*,

### 5.5.5 Bioassay on tobacco plants

Methods for conducting tobacco bioassay tests to support identification of *X. fastidiosa* subspecies, based on Francis *et al.* 2008, are described in the EPPO diagnostic protocol. The method has not been tested for all subspecies.

## 6 Pest risk assessment

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*X. fastidiosa* and its vector GWSS are not known to be present in Australia, but both pests have the potential for establishment of spread and economic consequences in Australia, and therefore meet the criteria for a quarantine pest.

The risk assessments in this section focus on the major pathways identified for the potential introduction of *X. fastidiosa*. Unlike most other pests, the risk of establishment and spread will depend both on the commodity on which it enters Australia, and also whether or not the vector is present. Much of the data on the risk of entry, probability of establishment, probability of spread has been sourced on *X. fastidiosa* from the 'Final IRA report: Stone fruit from California, Idaho, Oregon and Washington (2010) and the 'Report on Pierce's disease and the Glassy winged sharpshooter' more

specifically with reference to importing grapes from the USA (Scott and De Barro 2000). More recent information has been obtained from The European Food Safety Authority (2013). For further information on the phytosanitary risk of *X. fastidiosa* with the vector GWSS, refer to the Contingency plan developed for NGIA (Plant Health Australia 2009).

## 6.1 Entry of the pathogen with a vector

All sucking insects that feed on xylem fluid are potential vectors of *X. fastidiosa*, with all known vectors limited to the Homoptera suborder (Purcell 1999). Insects currently known to be capable of transmitting *X. fastidiosa* all belong to the spittlebug/ froghopper family (Cercopidae) and the 'sharpshooter' subfamily. GWSS is a major vector for *X. fastidiosa*.

Xylem feeding insects acquire the bacterium from infected hosts. The bacterium adheres to and is retained in the foregut of the vector where it multiplies and from which it is transmitted to new hosts almost immediately (Purcell and Hopkins 1996) with virulence maintained throughout the life of adult vectors (Redak *et al.* 2004). In the case of sharpshooter nymphs, inoculum is lost at each moult (Almeida *et al.* 2013).

### 6.1.1 Entry potential

**Rating: Medium**

The most likely pathway of entry for GWSS is as a hitchhiker on plant material (particularly on imported nursery stock) and transport machinery. Evidence suggests that the leafhopper entered California in nursery stock as eggs, which are difficult to detect. Since then the agriculture quarantine inspections have frequently intercepted leafhopper specimens.

While table grape imports into Australia could be a potential entry pathway for sharpshooter, AQIS (2010, as cited in EFSA 2013) considered this not to be epidemiologically significant because eggs are not laid on grape clusters; sharpshooter vectors are easily disturbed and unlikely to occur on harvested grape clusters as hitchhikers; and the concentration of *Xylella fastidiosa* in grape clusters is very low.

The risk of entry of GWSS into Australia is **medium**. Given the reasonable likelihood that the vectors that enter may also be harbouring *X. fastidiosa*, the entry potential of the pathogen in the presence of the vector is also **medium**.

### 6.1.2 Establishment potential

**Rating: Medium**

The wide host range of GWSS together with suitable environmental conditions, would allow for the establishment of GWSS in many regions of Australia. Luck *et al.* (2001) concluded that conditions suitable for GWSS currently exist in south-east Queensland, eastern New South Wales, the majority of Victoria and Tasmania, south-eastern South Australia and south-western Western Australia. However, cold stress would be expected to exclude the pathogen from Tasmania and some areas of Victoria (Hoddle 2004). Drought stress would most likely exclude GWSS from the interior of Australia, with the exception of irrigated areas such as Mildura (Rathe *et al.* 2012b). It has been predicted that incursion severity would be greatest in tropical and subtropical northern regions of Australia where conditions are favourable for both GWSS and *X. fastidiosa* establishment (Rathe *et al.* 2012b).

Overall, the likelihood of GWSS establishment in Australia following entry, and therefore the likelihood of establishment of *X. fastidiosa*, is considered **medium**.



### 6.1.3 Spread potential

#### **Rating: High**

GWSS adults are strong flyers allowing rapid movement of the insect. In addition, all life stages can move on machinery, equipment and plant material. These factors combined with the wide distribution of suitable host species results in a **high** spread potential for GWSS.

The wide host range of *X. fastidiosa* and lack of latent period and retention of the pathogen in the gut of vectors result in a **high** spread potential for the pathogen in the presence of the vector.

### 6.1.4 Economic impact

#### **Rating: High**

The pathogen has a high economic impact on grapevines in southern USA (Hopkins 2005) and on a range of other agricultural and amenity plants in North and South America (Schaad *et al.* 2004). Current outbreaks in Europe are also causing significant damage to olive crops. Australian climatic conditions that favour pathogen survival (e.g. milder winters), wide host range and lack of chemical/physical control methods or plant resistance to the pathogen suggest that economic impact of the pathogen in Australia would be **high**.

### 6.1.5 Environmental impact

#### **Rating: Medium**

The pathogen is damaging to a number of ornamental and amenity trees in the USA (Schaad *et al.* 2004), many of which are found in parks and gardens in Australia (oaks, sycamores, maples, elm, oleander). *X. fastidiosa* could also impact on a number of Australian native plant species. Other potential environmental effects would be the increased use of pesticides.

### 6.1.6 Overall risk

#### **Rating: Medium-High**

Based on the individual ratings above, the combined overall risk is considered to be **medium-high**.

## 6.2 Entry of the pathogen in the absence of a vector

### 6.2.1 Entry potential

#### **Rating: Low**

Fruit, wood (not for propagation purposes), seeds, cut flowers and ornamental foliage are all considered to be minor entry pathways for *X. fastidiosa* (EFSA 2013). It was noted that grape clusters showing Pierce's disease symptoms were unlikely to be harvested and exported, and that the survival of *X. fastidiosa* would be low under normal in-transit cold storage regimes for fruit clusters. Furthermore the likelihood of inoculum bearing clusters being fed upon by potential Australian vectors was considered to be extremely low (AQIS 2010). An import risk analysis for stone fruit drew similar conclusions (Biosecurity Australia 2010).

The major pathway for entry of *X. fastidiosa* is thought to be the trade and movement of plants for planting (excluding seed) (EFSA 2013). *X. fastidiosa* has a very wide host range, and in many hosts, the disease can be asymptomatic. However, given there are strict post entry quarantine requirements

in place for the importation of nursery stock, the entry potential for the pathogen in the absence of a known vector is considered to be **low**.

## 6.2.2 Establishment potential

**Rating: Medium**

If *X. fastidiosa* were distributed in a viable state to a suitable host it could establish in Australia given the wide range of hosts spread throughout the country. Rathe *et al.* (2012a) found that *X. fastidiosa* could be re-isolated from four out of twelve Australian native plant species artificially inoculated with the pathogen in California. These four species (*Leptospermum laevigatum*, *Swainsona gaeligifolia*, *Grevillea alpina*, and *Hakea petiolaris*) all have a wide geographic distribution in Australia.

*X. fastidiosa* proliferates in the USA in environments with warm conditions and mild winters and with such similar climates, *X. fastidiosa* could establish in Australia. *X. fastidiosa* is sensitive to cold and with Australia's winters less severe than those in North America, the Australian environments may allow for growth of the bacterium throughout the year. Rathe *et al.* (2012b) concluded that much of Queensland, coastal Northern Territory, New South Wales, South Australia and southern Western Australia would be suitable for *X. fastidiosa* establishment, although cold stress would be expected to exclude the pathogen from Tasmania and some areas of Victoria (Hoddle 2004).

*X. fastidiosa* reproduces inside its hosts by cell division, doubling in population in less than 48 hours (Hopkins 1989). This short generation time suggests there would be potential for genetic variation leading to adaption to new environments.

Based on this information, the establishment potential for *X. fastidiosa* is considered to be **medium**.

## 6.2.3 Spread potential

**Rating: Low**

With the warmer conditions and milder winters in Australia compared with the USA, *X. fastidiosa* would be expected to spread more easily all year round. The broad host range of *X. fastidiosa* includes many host weeds, crops and native plants present in Australia, and suggests the pathogen could have many potential hosts within close proximity to an infection allowing spread to occur readily.

While the spread of *X. fastidiosa* in the absence of a vector would be expected to be much lower than in the presence of a vector, movement of infected plants (and propagative material) is still considered to be a major pathway for the long distance dispersal of *X. fastidiosa* (EFSA 2013).

Information on the presence of the pathogen in fruit and seeds and the capacity of vectors to penetrate xylem in infected fruits is limited. Li *et al.* (2003) showed the presence of *X. fastidiosa* in the seeds of sweet orange, and demonstrated its ability to be transmitted from seeds to seedlings. However the study was conducted on only one species out of the wide host range, and the experiment was stopped soon after germination (EFSA 2013).

It is also unknown the extent to which Australian native froghoppers and sharpshooters would vector the disease (Rathe *et al.* 2012).

Spread potential in the absence of a vector is considered **low**.

## 6.2.4 Economic impact

**Rating: Medium-Unknown**

It is extremely difficult to predict the economic impact of *X. fastidiosa* in the absence of any known vectors. If Australian native insects were capable of vectoring the disease it is most likely that the epidemiology would be similar to that observed in Californian riparian environments prior to the introduction of the GWSS, where the disease can be managed and losses can be kept to manageable levels (Merriman et al. 2001). If native insects were able to vector the disease the economic impact may be more severe.

### 6.2.5 Environmental impact

**Rating: Medium**

The pathogen is damaging to a number of ornamental and amenity trees in the USA (Schaad *et al.* 2004), many of which are found in parks and gardens in Australia (oaks, sycamores, maples, elm, oleander). *X. fastidiosa* could also impact on a number of Australian native plant species. Other potential environmental effects would be the increased use of pesticides.

### 6.2.6 Overall risk

**Rating: Low-Medium**

Based on the individual ratings above, the combined overall risk is considered to be **low-medium**.

## 7 Surveillance and collection of samples

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Information provided in the following sections provides a framework for the development of early detection and delimiting surveys for Pierce's disease and other diseases caused by *X. fastidiosa*.

### 7.1 Surveillance

Detection and delimiting surveys are required to delimit the extent of the outbreak, ensuring areas free of the pest retain market access and appropriate quarantine zones are established.

Where *X. fastidiosa* is found in a production nursery that is in close proximity to potential plants (including weeds), periodically inspect nearby hosts for symptoms caused by *X. fastidiosa* (leaf scorching) by examining leaves closely and looking for symptoms. Infected sources within a production nursery may provide an opportunity for *X. fastidiosa* to spread outside the production nursery. With the vector GWSS, *X. fastidiosa* would be spread more rapidly.

Leaf scorching is the most typical symptom across the range of hosts that show symptoms. Agricultural inspectors and other production nursery visitors should avoid moving infested plant material between production nurseries. Shoes, tools and vehicle tyres should be thoroughly washed of soil and then sanitised with a registered disinfectant to reduce spread of soil-borne diseases that may be present at the survey area.

#### 7.1.1 Technical information for planning surveys

When developing surveys for *X. fastidiosa* presence and/or distribution, the following characteristics of the pest provide the basic biological knowledge that impact on the survey strategy:

- *X. fastidiosa* (and the GWSS vector) have a wide host range and share many of the same hosts



- Leaf scorch symptoms may look similar to other abiotic or biotic stress symptoms
- *X. fastidiosa* can be asymptomatic in many hosts
- Host species in Australia are likely to be numerous and widely dispersed
- Movement of *X. fastidiosa* can occur by human assistance through the transfer of nursery stock or with the GWSS vector by flight
- The risk of pest movement on machinery, equipment and personal effects is high
- Production nursery greenhouses and significant proportions of Australia have favourable climatic conditions for the spread and establishment of *X. fastidiosa* (and its vector)
- As the *X. fastidiosa* vector spreads readily in a greenhouse or production nursery environment the tracing of plant material from one nursery to another needs to be taken into consideration

### 7.1.2 Surveys for early detection of an incursion in a production nursery

The success of an eradication response to a *X. fastidiosa* incursion in a production nursery is more likely following early detection of the pest before it has had the opportunity to disperse to a wide area. This is especially so if the vector GWSS was present. It is therefore necessary to consider pathways and plan surveys accordingly: see the contingency plan for the Glassy winged sharpshooter (Plant Health Australia 2009) for information on surveys in an incursion of the vector). Important points to consider when developing early detection surveys for *X. fastidiosa* in production nurseries are:

- Systematic and careful inspection of crops and propagative plant material is essential to prevent introduction of the *X. fastidiosa* pathogen and limit its spread within and from contaminated outdoor and greenhouse production areas. Early detection of the pathogen (and if the vector is present), while at low levels, will provide the best chance of eradication
- An inspector must be trained to recognise *X. fastidiosa* disease symptoms (see Section 5.4.3), and other similar disorders for comparison
- Awareness information should be targeted at people who are in regular close contact with potential hosts in high risk areas or movement vectors (e.g. production nursery operators)
- Should the presence of *X. fastidiosa* be detected in Australia and movement of potential host material is permitted, any new host material entering nurseries from suspected areas of infection should be quarantined prior to distribution throughout the property to allow for visual inspection or testing for the presence of the pest

### 7.1.3 Delimiting surveys in the event of an incursion

- In the event of an incursion, delimiting surveys are essential to inform the decision-making process
- The size of the survey area will depend on the size of the infested area and the severity of the infection, as well prevailing winds and movement of plant material during the period prior to detection. Other considerations are for example, movement of people or plant material equipment as a result of trace-forward and trace-backs
- If vectors are present, they can readily spread by flying long distances or by being transported on infested plants
- Initial surveys should be carried out in 2 km radius of the initial detection in the absence of GWSS. If GWSS, or other vectors, are present, a larger area should be surveyed, focusing on

high risk areas close IPs. The exact radius to be surveyed will depend on the biology of the vector present, the type of environment in the area, extent of host plants present, etc.

- All potential host species should be surveyed, with particular attention paid to the species in which the pest was initially detected (refer to Section 5.4.1 for current host lists).
- In addition to inspection of possible host plants, material should be collected for diagnostic purposes, including asymptomatic host plants (EPPO 2016). Complete destruction should not occur until sufficient material has been collected for diagnostic purposes.
- If the incursion is in a populated area, publication and distribution of information sheets and appeals for public assistance may be helpful

## 7.2 Collection of samples

Protocols for the collection, transport and diagnosis of suspect Emergency Plant Pests (EPPs) must follow PLANTPLAN (Plant Health Australia 2016). Any personnel collecting samples for assessment should notify the diagnostic laboratory prior to submitting samples to ensure expertise is available to undertake the diagnosis.

The following sampling procedure (for grapevine) is based on information contained in the National Diagnostic Protocol for Pierce's Disease, *Xylella fastidiosa* (Luck *et al* 2010). Detailed sample collection procedures for a range of hosts are also outlined in the draft EPPO diagnostic protocol (EPPO 2016b).

### 7.2.1 Sampling procedures for grapevine

Grapevine samples should ideally be collected late summer to autumn. In chronically infected vines, bacteria do not move into the new season's growth until the middle of the summer. Leaves attached to the cane generally give the most reliable result.

Collect leaf material showing symptoms of *X. fastidiosa* infection which is attached to the cane. From each suspect plant collect 4-5 canes. The most optimum tissue to sample for the detection of *X. fastidiosa* is the mid-rib and petiole from symptomatic leaves. Select five leaves from affected canes and treat as one sample (Luck *et al.* 2010).

Samples should be treated in a manner that allows them to arrive at the laboratory in a fresh, well preserved state.

Wrap the cane samples in damp newspaper and place inside a sealed plastic bag. All sample containers should be clearly labelled with the name, address and contact phone number of both the sending and receiving officers. In addition, containers should be clearly labelled in accordance with the requirements of PLANTPLAN (Plant Health Australia 2016; Appendix 3). Containers should then be carefully sealed to prevent loss, contamination or tampering of samples. The Chief Plant Health Manager will select the preferred laboratory. Additional labelling includes the identification of plant species/parts affected, location of affected plant (where available include GPS reading) as well as symptoms and an image if available. Send samples to ensure chain of custody.

Precaution: overheating or desiccation of samples prior to despatch should be prevented.

## 7.3 Stakeholder engagement

It is recommended that factsheets for all relevant industries be developed and made available to growers and other key stakeholders. A number of factsheets are already available including for the

**production nursery, grape and almond** industries. Additional factsheets may become available over time.

Groups that should be engaged following a detection include:

- Local councils/main road authorities that may have roadside host plants, e.g. oleander
- Parks and garden organisations, e.g. botanic gardens, national/state parks
- Relevant community groups, e.g. groups that maintain community gardens.
- Industry groups:
  - o Nursery and Garden Industry Australia (NGIA), state NGI's; production nurseries and retail outlets
  - o Host industry groups (e.g. Wine Grape Growers Australia, Australian Table Grape Association, Citrus Australia, Summerfruit Australia, Almond Board Australia, Australian Olive Association, Australian Nashi Growers' Association, Australian Subtropical Coffee Association, Australian Blueberry Growers' Association, Avocados Australia)

### 7.3.1 Activities for ongoing general surveillance following a detection

Undertake General Surveillance elements for Pierce's disease and other diseases caused by *X. fastidiosa*. To establish effective General Surveillance in Australia, several elements require additional support. The following is recommended to address gaps in these elements:

- Awareness material on state DPI websites.
- Inclusion of awareness material for PHA industry members.
- Inclusion of regulations to limit movement of plant material and equipment for jurisdictions with proof of freedom.
- Establish dedicated Australian web resource(s) as a repository of information for the public, affected plant industries and transport industries.

## 8 Course of action – immediate response to a detection

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For a range of specifically designed procedures for the emergency response to a pest incursion and a general communication strategy refer to PLANTPLAN (Plant Health Australia 2016).

### 8.1 Tracing

Trace backs and trace forwards are essential for delimiting survey activities following an initial detection. Trace backs attempt to determine the source of the infection whereas trace forwards further define potential spread of and dissemination of the infection. There are many potential sources of trace backs/trace forwards. These are summarized to assist in the investigations to locate potential populations of *X. fastidiosa*. However, not all of these will be relevant to all scenarios so one must

determine the importance of certain lines of investigation on a case by case basis. In any case, trace backs and trace forwards will identify movement linked to IPs, CPs and SPs.

### 8.1.1 Trace backs

Investigate where the infected material may have been purchased or obtained, this may include (not an exhaustive list):

- Retail nursery, weekend or road-side market, or internet sale
- Production nursery – trace back to mother stock plants
- Staff, visitors, etc., both domestic & international
- Legal or illegal importation of plant material
- Items of equipment, machinery and vehicles which have been shared between properties (e.g. storage and transport bins)

Trace back plant movements should focus on stock that was received within twelve months of the detection, or longer if deemed necessary.

Where a vector is present, it is critical to survey host plants in the near vicinity of the infested premises.

### 8.1.2 Trace forwards

- Local movement of vectors to other host plants. Leafhoppers generally have a short flight range - about 100m for GWSS (Blackmer *et al.* 2004). However, leafhoppers can be transported by wind over long distances (EFSA 2013).
- Long distance movement of plants via sale of plants:
  - At production nurseries there should be records of where consignments of plants have been sold. Sales of all host plants should be investigated from the last 6 months, or longer if deemed necessary.
  - At retail outlets, markets etc. – this will cause the scope of residential surveillance to be widened substantially.

For both trace forward and trace back plant movements, the critical period could be longer than the stated time periods, as symptoms may take longer than this to appear. This period of time should, of course, be modified based on the individual circumstances of the detection. However, an initial period of six months for trace forward and twelve months for trace back is suggested as a suitable compromise between scientific rigour and the practicalities of responding to a detection.

## 8.2 Quarantine and movement controls

Consult PLANTPLAN (Plant Health Australia 2016) for administrative details and procedures.

### 8.2.1 Quarantine priorities

Plant material and growing media/soil at the site of infestation to be subject to movement restrictions.

Machinery, equipment, vehicles and disposable equipment in contact with infested plant material or growing media/soil, or present in close proximity to the site of infestation to be subject to movement restrictions.

### 8.2.2 Movement controls

- Movement controls need to be put in place to minimise the potential for transport of the pest, and this will apply to all plant material, growing media and other items within the quarantined area.
- Movement of people, vehicles, equipment and plant material, from and to affected properties or areas, must be controlled to ensure that the pest is not moved off-property. Movement controls can be achieved through the following, however specific measures must be endorsed in the Response Plan:
  - Signage to indicate quarantine area and restricted movement into and within these zones
  - Fenced, barricaded or locked entry to quarantine areas
  - Movement of equipment, machinery, plant material or growing media/soil by permit only. Therefore, all non-essential operations in the area or on the property should cease
  - Where no dwellings are located within these areas, strong movement controls should be enforced
  - Where dwellings and places of business are included within the Restricted and Control Areas movement restrictions are more difficult to enforce, however limitation of contact with infested plants should be enforced
  - If a production nursery is situated within the Restricted Area, all nursery trading in host and non-host material must cease and no material may be removed from the site without permission, due to the high likelihood of pest spread. Movement restrictions would be imposed on both host and non-host material
  - Residents should be advised on measures to minimise the inadvertent transport of vectors, should the pathogen and vector both be present
  - Clothing and footwear worn at the infested site should either be double-bagged prior to removal for decontamination or should not leave the site until thoroughly disinfected, washed and cleaned
  - Plant material or plant products must not be removed from the site unless part of an approved disposal procedure
  - All machinery and equipment should be thoroughly cleaned down with a high pressure cleaner or by scrubbing with a detergent/degreaser, followed by application of an appropriate disinfectant, prior to leaving the affected area. Machinery should be inspected for the presence of insects and if found, treatment with insecticide may be required. The clean down procedure should be carried out on a hard surface, preferably a designated wash-down area, to avoid mud being re-collected from the affected site onto the machine. When using high pressure water, care should be taken to contain all plant material and mud dislodged during the cleaning process

## 8.3 Zoning

The size of each quarantine area will be determined by a number of factors, including the location of the incursion, the time of year, climatic conditions and the proximity of the infested property to other infested properties. The size of the zones will be determined by the consultative committee and agreed by the National Management Group during the production of the response plan. Immediately after an initial detection the zones in the following sections should be identified.

For private residences, in the first phases of a suspected incursion, government agencies in each jurisdiction will attempt to work with residents to gain permission to access premises for the purposes of surveillance or eradication. Once confirmation of an incursion has occurred (i.e. validation diagnosis has been made), legislation in most jurisdictions provides greater powers to access premises. For private residences, access may be possible to backyards and surrounds but entry into houses is limited without invitation from the resident.

If denied access, confirmatory diagnosis may be required in most jurisdictions before being able to enter premises or conduct treatments. For these reasons, eradication or management programs requiring establishment of treatment zones or restricted areas must be coupled with communication programs to achieve best outcomes.

### 8.3.1 Destruction/treatment zone

The size of the destruction zone (i.e. zone in which the pest and all host material is destroyed) will depend on the ability of the pest to spread, distribution of the pest (as determined by delimiting surveys), time of season (and part of the pest life cycle being targeted) and factors which may contribute to the pest spreading.

All host plants should be destroyed after the level of infestation has been established. The delimiting survey will determine whether or not neighbouring plants are infested and need to be destroyed. Non-host plant material within this zone may be destroyed, based on recommendations in the Response Plan. The Destruction Zone may be defined as contiguous areas associated with the same management practices as, or in contact with, the infested area (i.e. the entire production nursery, property or area if spread could have occurred prior to the infection being identified).

Particular care needs to be taken to ensure that plant material (including non-hosts) is not moved into surrounding areas.

### 8.3.2 Restricted area

The Restricted Area is defined as the zone immediately around the infected premises and suspected infected premises. The Restricted Area is established following initial surveys that confirm the presence of the pest. The Restricted Area will be subject to intense surveillance and movement control with movement out of the Restricted Area to be prohibited and movement into the Restricted Area to occur by permit only. Multiple Restricted Areas may be required within a Control Area.

### 8.3.3 Control area

The Control Area is defined as all areas affected within the incursion. The Control Area comprises the Restricted Area, all infected premises and all suspected infected premises and will be defined as the minimum area necessary to prevent spread of the pest from the Quarantine Zone. The Control Area will also be used to regulate movement of all susceptible plant species to allow trace back, trace forward and epidemiological studies to be completed.

## 8.4 Destruction strategy

### 8.4.1 Destruction protocols

General protocols:

- No plant material should be removed from the infested area unless part of the disposal procedure
- Disposable equipment, infested plant material or growing media/soil should be disposed of by autoclaving, high temperature incineration or deep burial
- Any equipment removed from the site for disposal should be double-bagged
- Machinery used in destruction processes need to be thoroughly washed, preferably using a detergent or farm degreaser, followed by application of an appropriate disinfectant.

## 8.4.2 Availability of control measures

Preventative measures are of critical importance to the management of diseases caused by *X. fastidiosa*, as there are currently no chemical curative treatments available for the pathogen (Janse and Obradovic 2010). Quarantine and phytosanitary procedures to exclude the pathogen is the first line of defence, but must be supported by other strategies such as the use of resistant varieties (if available), cultural and hygiene practices, and vector control. The challenges with control of *X. fastidiosa* relate to the wide host range of the pathogen, the numerous insect vectors, the extensive international trade of plant material, the large number of symptomless hosts and the latent nature of the *X. fastidiosa* diseases (Janse and Obradovic 2010).

### 8.4.2.1 General procedures for control

- Keep traffic out of affected areas and minimise movement in adjacent areas
- Adopt best-practice property hygiene procedures to retard the spread of the pest between glasshouses, fields and adjacent properties
- After surveys are completed, and permission has been obtained from the Chief Plant Health Manager, destruction of the infested plant material is an effective control
- On-going surveillance of infected areas to ensure the pest is eradicated
- Do not use any material from infected plants for propagation

### 8.4.2.2 Quarantine exclusion and phytosanitary measures

Quarantine exclusion is the first line of defence against the introduction of *X. fastidiosa* into Australia. As diseases caused by *X. fastidiosa* are extremely difficult to control, it is recommended that importation of planting material from known hosts is severely restricted from countries where the pathogen is present. The DAWR has recently amended emergency quarantine measures for *X. fastidiosa* in relation to the importation of plant material into Australia, including nursery stock, tissue cultures and corms and bulbs (DAWR 2016b). In summary, the following measures apply to plant tissue cultures and nursery stock that are hosts of *X. fastidiosa*, and are applied in addition to current import requirements:

- nursery stock and plant material coming from countries or regions where *X. fastidiosa* occurs will need to be tested offshore and certified as being free from *X. fastidiosa* by the government of the exporting country
- an approved arrangement that ensures the health of plants will need to be in place for off-shore testing and certification of nursery stock from high risk countries.
- material that does not meet the above requirements may be held and tested in an approved post entry quarantine facility for 12 months or nursery stock material may be hot water treated, followed by standard post entry quarantine screening arrangements.

DAWR provide specific information on these requirements (e.g. in relation to PCR testing and post-entry quarantine) on their website <http://www.agriculture.gov.au/import/goods/plant-products/how-to-import-plants/xylella/notification-amended-emergency-quarantine-measures>

With recent incursions of *X. fastidiosa* in Italy and France, phytosanitary procedures for the inspection of plant consignments and places of production have been updated in Europe (EPPO 2016c; 2016d).

In California, approximately 70% of the 12,000 licensed nurseries are located in GWSS-infested areas (Tumber *et al.* 2014). Those nurseries that choose to send consignments to non-infested areas must



comply with approved shipping protocols, which can be very expensive for nursery operators. These measures include inspections, pesticide applications and quarantines.

#### 8.4.2.3 Chemical control

There is currently no curative chemical treatment available for the control of *X. fastidiosa*.

#### 8.4.2.4 Cultural control

- Monitoring for the presence of Pierce's disease, identification of the disease through diagnostics, roguing of infected plants (and neighbouring symptomless plants), and pruning of infected branches are important strategies for the reduction of *X. fastidiosa* inoculum in the USA (Appel *et al.* 2011; EFSA 2015)
- Literature from the USA has shown the use of resistant or tolerant cultivars is an effective control for Pierce's disease in areas at high risk for development of the disease (University of California at Berkley 2015).
- Identification and removal of alternative hosts in and around production areas may help to reduce inoculum sources for the disease (Appel *et al.* 2011). Furthermore, management guidelines for riparian vegetation have been developed in California to address the issue of alternative hosts of *X. fastidiosa* (and vector breeding hosts) growing in these zones<sup>9</sup>. Vineyards in California are commonly established near streams and rivers.
- Maintenance of optimal plant health, including crop load management, is a recommended strategy for the management of Pierce's disease in the USA (Appel *et al.* 2011).

#### 8.4.2.5 Vector control

Details of vector control are covered in the GWSS contingency plan (PHA, 2009). However, a summary of the key strategies is outlined below:

- Should there be an incursion GWSS and *X. fastidiosa* into Australia, the application of insecticides such as neonicotinoids (e.g. imidacloprid) and repellents would be the first line of defence (Rathe *et al.* 2012b). A combination of soil and foliar applied insecticides, and repellents, are used in California for sharpshooter control (University of California at Berkley 2015).
- Application of insecticides to areas surrounding vineyards can be effective providing grapevine cultivars are not highly susceptible or very young (less than 3 years old).
- While there is also potential for the use of native Australian parasitoids as biocontrol agents against GWSS (should they be shown to be effective), Rathe *et al.* (2012b) noted that a more likely scenario would be the importation, quarantine screening, release and establishment of *Gonatocerus ashmeadi*, the most common natural enemy of GWSS in three US states (California, Louisiana and Florida) (Triapitsyn *et al.* 1998). Rathe *et al.* (2012b) recommended that proactive screening of *G. ashmeadi* should be undertaken so that it could be approved for release in advance of GWSS establishment in Australia.

<sup>9</sup> <https://nature.berkeley.edu/xylella/control/PDNorthCoast/info.htm>



- In Texas and other eastern US states, recommendations for vector control in vineyards include application of neonicotinoids, creating buffer zones around production areas (where perennial trees and shrubs are removed), mowing within production areas, and vector monitoring using sticky traps. Site selection for new plantings, including avoidance of planting crops near native perennial vegetation, is also considered in some detail (Appel *et al.* 2011).
- Application of kaolin (a non-toxic clay product used for reduction of plant transpiration), has been shown to repel GWSS and reduce Pierce's disease by up to 50% in field trials on grape (Tubajika *et al.* 2003), although can leave unsightly residues on fruit (University of California at Berkley 2015).

### 8.4.3 Decontamination protocols

Machinery, equipment and vehicles in contact with infested plant material or growing media/soil, or present within the Quarantine Area, should be first washed to remove plant material and growing media/soil using high pressure water or scrubbing with a detergent/degreaser, followed by application of an appropriate disinfectant (e.g. quaternary ammonium compound) in a designated wash down area. When using high pressure water, care should be taken not to spread plant material. High pressure water should be used in wash down areas which meet the following guidelines:

- Located away from crops or sensitive vegetation
- Readily accessible with clear signage
- Access to fresh water and power
- Mud free, including entry and exit points (e.g. gravel, concrete or rubber matting)
- Gently sloped to drain effluent away
- Effluent must not enter water courses or water bodies
- Allow adequate space to move larger vehicles
- Away from hazards such as power lines
- Waste water, growing media/soil or plant residues should be contained
- Disposable overalls and rubber boots should be worn when handling infested plant material or growing media/soil in the field. Boots, clothes and shoes in contact with infested plant material or growing media/soil should be disinfected at the site or double-bagged to remove for cleaning
- Skin and hair in contact with infested plant material or growing media/soil should be washed

Procedures for the sterilisation of plant containers and growing media are provided within the BioSecure HACCP Guidelines, however, in the event of a *X. fastidiosa* incursion, additional or modified procedures may be required for the destruction of the pest. Any sterilisation procedure must be approved for use in the endorsed Response Plan.

### 8.4.4 Priorities

- Confirm the presence of the pest
- Limit movement of people and prevent movement of vehicles and equipment through affected areas

- Stop the movement of any plant material that may be infested with the pest
- Determine the strategy for the eradication/decontamination of the pest and infested host material
- Determine the extent of infestation through survey and plant material trace back and trace forward which would be assessed on a case by case basis and included within the response plan

#### 8.4.5 Plants, by-products and waste processing

- Any growing media/soil or infected plant material removed from the infected site should be destroyed by (enclosed) high temperature incineration, autoclaving or deep burial
- As the pest can be spread with plant material, plant debris from the destruction zone must be carefully handled and transported
- Infested areas or production nursery yards should remain free of susceptible host plants until the area has been shown to be free from the pathogen (and/or vector)

#### 8.4.6 Disposal issues

- Particular care must be taken to minimise the transfer of infected plant material from the area
- Host material including leaf litter should be collected and incinerated or double bagged and deep buried in an approved site

## 9 Recommendations for preparedness activities

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Plant Health Australia coordinated a National Xylella Preparedness Workshop held in Melbourne on 1 June 2016, funded by the Australian Government Department of Agriculture and Water Resources (PHA 2016). The workshop was attended by participants from industry, government and research agencies, including representatives from New Zealand, for the purpose of:

- Enhancing identification, awareness and coordination of national preparedness activities for *Xylella fastidiosa* (Xylella) for key stakeholders.
- Enhancing understanding by workshop participants of the impacts of Xylella.
- Assessing the current status of biosecurity preparedness activities, identification of gaps in preparedness efforts and plans for how these gaps might be addressed.
- Outlining future areas of investment for Xylella biosecurity preparedness.

Workshop outcomes included an assessment of Australia's current preparedness for Xylella, as well as priorities for future Xylella biosecurity preparedness activities. These outcomes are listed below (reproduced from <http://www.planthealthaustralia.com.au/about-us/events/xylella-preparedness-workshop/national-xylella-preparedness-workshop-outcomes/>)

## 9.1 Assessment of current preparedness for Xylella

Participants were asked to consider current preparedness activities for Xylella and its vectors, and other preparedness and risk mitigation activities currently undertaken that could be expanded to cover the pest. The following key areas were identified:

**Diagnostics capacity and capability** – Experience from other countries has shown that Xylella is a complex pathogen with a wide host range and a large number of vectors. There is a need to improve Australia's capacity to test to the sub-species level and ensure tests are effective for Australian hosts, consider 'surge' capacity to test large numbers of samples that may be experienced in the event of an incursion, develop rapid field tests and ensure diagnostic tests are available for the vectors of Xylella.

**Communication and awareness** – Improving awareness of the significance and impact of Xylella amongst plant industries should be undertaken through development of support material such as websites, fact sheets and industry newsletters. Consideration could also be given to improving awareness in other groups such as travellers, environmental groups, researchers and government staff. Coordination of material would be useful to ensure consistent messaging is being delivered.

**Planning and preparedness** – Activities such as development of a cross-industry pest contingency plan, delivery of a simulation exercise, development of a regional containment plan, ensuring that all affected industries are signed to the EPPRD, and ensuring Xylella is included within biosecurity plans for potentially affected industries will assist in Australia's preparedness for Xylella.

**Research, development and extension** – Nationally coordinated R&D was identified for the vectors (including native insect species), hosts (including Australian native species), asymptomatic hosts, potential economic impact, resistant cultivars, strain specific host ranges and pathway analyses.

**Surveillance** – To confirm Australia's status for Xylella, and to improve our likelihood of early detection of Xylella should it enter Australia, improved surveillance for the pathogen and its vectors is required. This should include:

- At the border – surveying potential hosts for the presence of potential vectors in the vicinity of high risk points of entry, including quarantine approved premises;
- Post-border – specific surveys targeting potential hosts. Surveillance programmes including specific surveys for the pathogen in high risk hosts. General surveillance programs that increase awareness of Xylella symptoms and reporting mechanisms for industry and communities.

**Control and eradication** — Preparedness activities that provide information for control of the vector and the pathogen are required. These could include review of pesticides and preparation of emergency permits for vectors, and improved knowledge of potential eradication strategies and distances of buffer and quarantine zones. Preparedness information is also required on management options in the event that Xylella is not technically feasible to eradicate. This could include strategies that slow the spread or minimise the impact of Xylella, by identifying management priorities and potential movement control requirements.

## 9.2 Priorities for future Xylella biosecurity preparedness activities

The workshop considered a range of preparedness activities based on potential impact and ease of implementation. Key areas that were determined to be of highest priority and would result in the highest impact were as follows:

### Awareness

Development of awareness material suitable for multiple audiences was identified as a high priority that would achieve considerable impact. Types of audiences included government staff, R&D providers, industry and growers, the public and biosecurity inspectors.

Information should be provided on the impact of Xylella, what to look for and how to report suspected samples. Training within industry and government in identification, surveillance and reporting was also seen as part of awareness activities.

### Incursion simulation exercise

Given the large number of industries and jurisdictions that could be involved in the event of a detection of Xylella in Australia, a simulation exercise that assisted with preparedness for a response was seen as a high priority. A simulation exercise should involve industry and government and will assist with improving capacity and capability, planning and coordination and identifying any gaps in preparedness.

### Host and vector identification

Improved knowledge and understanding of Xylella and its vectors is required. A review of potential vectors (endemic and exotic species) anticipated to be of most importance under Australian conditions was identified as a high priority, as well as monitoring any changes in pest status of both the pathogen and vectors in affected countries. A review of plant species (including Australian) known to be hosts/infected from affected countries was also identified as important.

### Surveillance and diagnostic capacity

There is a need to undertake assessment of current diagnostic capacity & capability as well as surge capacity requirements should large numbers of samples need to be processed. A review of the current national diagnostic protocol to address any issues with diagnostics in a range of hosts is required.

A nationally coordinated surveillance strategy and protocol for Xylella is needed to confirm and support Australia's plant health status, including whether surveillance should focus on early warning or proof of freedom, and to improve Australia's capacity for early detection of Xylella.

## Regional containment

As part of preparedness activities, improved knowledge is needed to gain a better understanding of regional containment requirements should an incursion of *Xylella* be deemed not technically feasible to eradicate. Planning is needed on measures that may be required such as the size of a host free buffer zone, control options, surveillance requirements, determination of the potential role of asymptomatic hosts and risk pathways for spread within Australia.

These outcomes will be considered by governments and industry in the context of future preparedness investment, with some activities to address these priorities already in progress.

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# 11 Appendices

## 11.1 Important nursery contacts

It is important to note that the Industry Development Officers (IDOs) change from time to time. Therefore, the current list may become out of date relatively quickly. For this reason, one can always refer to the NGIA website for the latest details for the NGI for each state and territory. In addition, some states may have more than one IDO, the below list are important contacts who may then direct you to the most appropriate person.

Northern Territory Website: <a href="http://www.ngia.com.au/Category;jsessionid=57FAB91F4A656937462E3F34A910B531?Action=View&amp;Category_id=266">http://www.ngia.com.au/Category;jsessionid=57FAB91F4A656937462E3F34A910B531?Action=View&amp;Category_id=266</a> NT Farmers Representative NGINT PO Box 348 Palmerston NT 0831 Ph: 08 8983 3233 Fax: 08 8983 3244 Email: <a href="mailto:ngint@ntha.com.au">ngint@ntha.com.au</a>	Western Australia Website: <a href="http://www.ngia.com.au/Category?Action=View&amp;Category_id=308">http://www.ngia.com.au/Category?Action=View&amp;Category_id=308</a> Executive Officer NGIWA Ph: 0410 714 207 Email: <a href="mailto:reception@ngiwa.com.au">reception@ngiwa.com.au</a>
South Australia Website: <a href="http://www.ngisa.com.au">www.ngisa.com.au</a> Executive Officer NGISA Fax: 08 8372 6833 Ph: 08 8271 1012 Email: <a href="mailto:info@ngisa.com.au">info@ngisa.com.au</a> 505 Fullarton Rd (Gate A) Netherby SA 5062	NSW and ACT Web: <a href="http://www.ngina.com.au">www.ngina.com.au</a> Executive Officer NGINA 344-348 Annangrove Road (PO Box 3013) Rouse Hill NSW 2155 Ph: 02 9679 1472 Fax: 02 9679 1655 Email: <a href="mailto:info@ngina.com.au">info@ngina.com.au</a>
Queensland Website: <a href="http://www.ngiq.asn.au">www.ngiq.asn.au</a> Executive Officer NGIQ PO Box 345 SALISBURY QLD 4107 Ph: 07 3277 7900 Fax: +61 07 3277 7109 Email: <a href="mailto:info@ngiq.asn.au">info@ngiq.asn.au</a>	Victoria Website: <a href="http://www.ngiv.com.au">www.ngiv.com.au</a> Executive Officer NGIV PO Box 2280 Wattletree Road LPO East Malvern Victoria 3145 Ph.: 03 9576 0599 Fax: 03 9576 0431 Email: <a href="mailto:ngiv@ngiv.com.au">ngiv@ngiv.com.au</a>
Tasmania Website: <a href="http://www.ngia.com.au/Category?Action=View&amp;Category_id=307">http://www.ngia.com.au/Category?Action=View&amp;Category_id=307</a> President NGIT PO Box 3009 Rosny Park Tasmania 7018 Email: <a href="mailto:president@ngitas.com.au">president@ngitas.com.au</a>	Australia Website: <a href="http://www.ngia.com.au">http://www.ngia.com.au</a> Chief Executive Officer, NGIA Ph: 02 8861 5107 Fax: 02 9659 3449 Email: <a href="mailto:info@ngia.com.au">info@ngia.com.au</a>

## 11.2 Resources and facilities – diagnostic service facilities in Australia

The below diagnostic facilities should be contacted prior to sending any samples to ensure that necessary equipment and reagents to complete all tests required.

Facility	State	Details
Crop Health Services	VIC	AgriBio Specimen Reception Main Loading Dock, 5 Ring Road La Trobe University, Bundoora VIC 3083 Ph: 03 9032 7515; Fax: 03 9032 7064
DPI New South Wales – Elizabeth Macarthur Agricultural Institute	NSW	Woodbridge Road Menangle NSW 2568 PMB 8 Camden NSW 2570 Ph: 02 4640 6327; Fax: 02 4640 6428
SARDI Plant Research Centre – Waite Main Building, Waite Research Precinct	SA	Hartley Grove Urrbrae SA 5064 Ph: 08 8303 9400; Fax: 08 8303 9403
Biosecurity Queensland	QLD	DAF Ecosciences Precinct Dutton Park Q 4102 Ph: 07 3255 4378; Fax: 07 3844 4529
Department of Agriculture and Food, Western Australia (AGWEST) Plant Laboratories	WA	3 Baron-Hay Court South Perth WA 6151 Ph: 08 9368 3721; Fax: 08 9474 2658
Department of Primary Industry and Fisheries	NT	Department of Primary Industry and Fisheries Plant Industries Division BAL building, Berrimah Farm, Makagon Road, Berrimah NT 0828 Ph: 08 8999 2261; Fax: 08 8999 2312