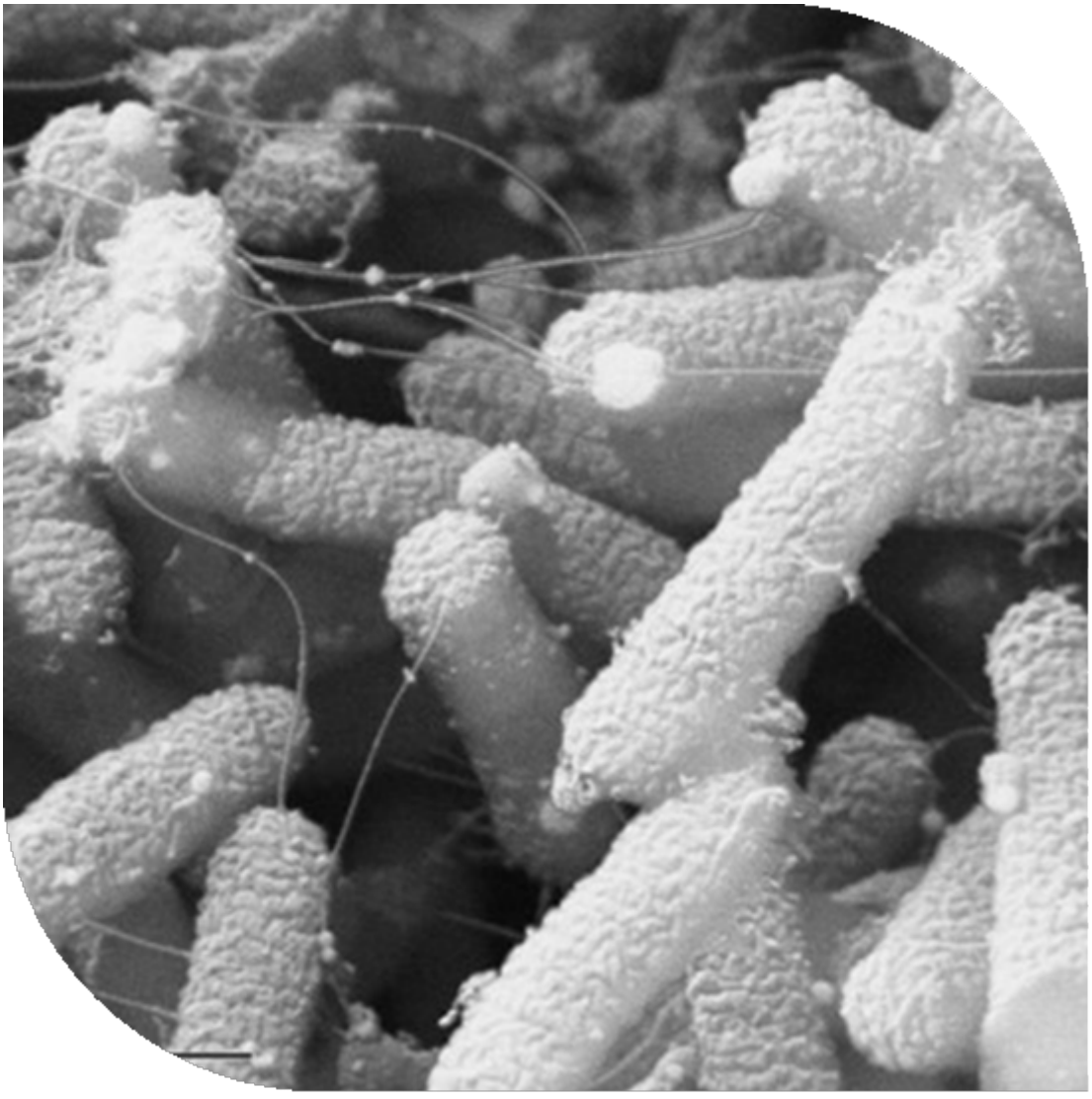
Australian Government
Department of Agriculture, Fisheries and Forestry

Draft pest risk analysis for bacterial pathogens in the genus *Xylella*

December 2022



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Acknowledgement of Country

We acknowledge the Traditional Custodians of Australia and their continuing connection to land and sea, waters, environment and community. We pay our respects to the Traditional Custodians of the lands we live and work on, their culture, and their Elders past and present.

Stakeholder submissions on draft reports

This draft report has been issued to give all interested parties an opportunity to comment on relevant technical biosecurity issues, with supporting rationale. A final report will then be produced taking into consideration any comments received.

Submissions should be sent to the Department of Agriculture, Fisheries and Forestry following the conditions specified within the related Biosecurity Advice, which is available at: agriculture.gov.au/biosecurity-trade/policy/risk-analysis/memos.

Cover image

Xylella fastidiosa, © 2001 University of California Berkeley, Electron Microscopy Laboratory, em-lab.berkeley.edu/EML/images/SEM-Gallery1/pages/XyellaBacteria.htm.

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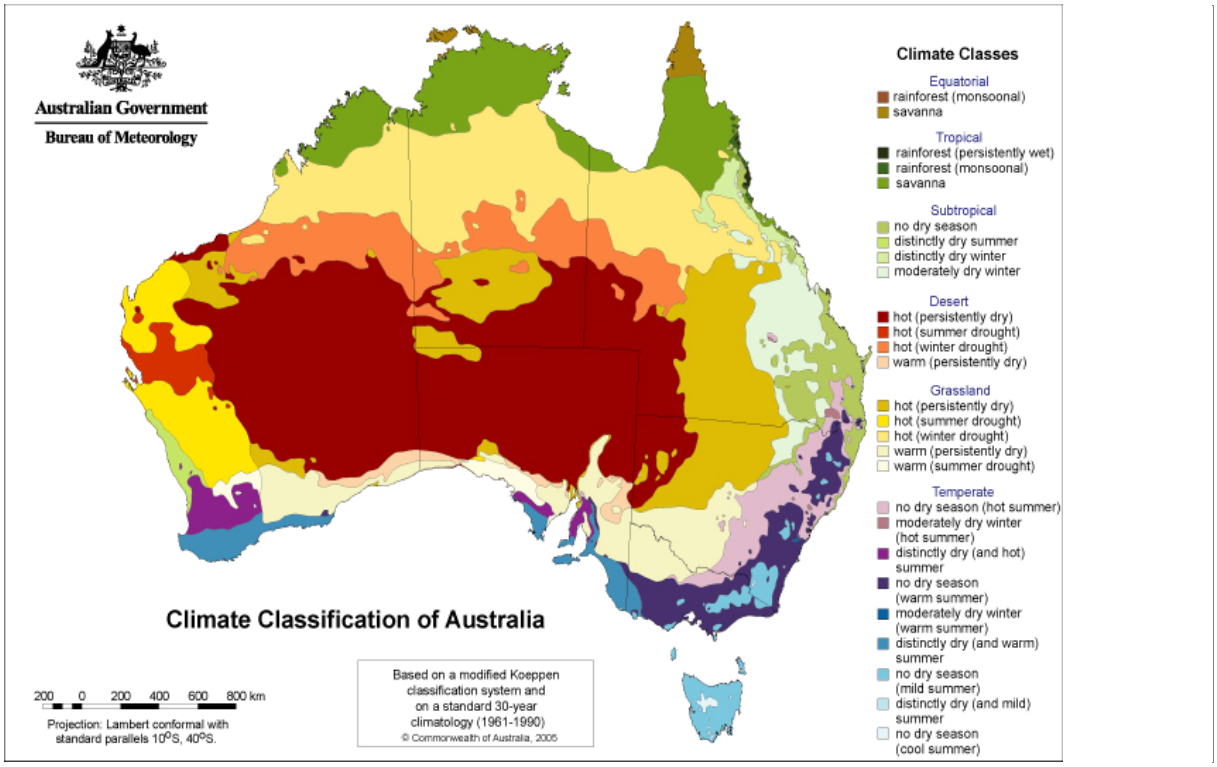


Summary

The Australian Government Department of Agriculture, Fisheries and Forestry (the department) initiated this pest risk analysis (PRA) in response to the introduction of emergency measures to manage the bacterium *Xylella fastidiosa* Wells et al. 1987 associated with the trade in commercially produced plants for planting (live plants, referred to in this PRA as nursery stock) and seeds for sowing. No *Xylella* species is known to occur in Australia and no known *Xylella* vectoring insects are present in Australia.

Xylella fastidiosa is one of the most significant emerging plant pests worldwide causing a broad spectrum of diseases across a wide range of horticulturally important host plants. A second species in this genus has also been described, *Xylella taiwanensis* Su et al. 2016, identified as the cause of pear leaf scorch in Taiwan. Both *Xylella* species have similar biologies—the bacteria are transmitted from host to host by xylem-feeding insects in the sub-order Auchenorrhyncha (Hemiptera), commonly known as leafhoppers and sharpshooters, and seed to seedling transmission of *Xylella* has been confirmed in *Carya illinoinensis* (pecan). Infected seeds will generally be asymptomatic and infected plants may show delayed symptom expression or be asymptomatic, presenting great risk when host seeds and live plants are imported into Australia.

Xylella is reported to cause hundreds of millions of dollars in production losses and high financial costs associated with attempts to manage the disease and its insect vectors in countries where it is present. In addition, some Australian native plants ubiquitous across the Australian landscape are known to be susceptible to the pest overseas. This makes *Xylella fastidiosa* the highest ranked pest threat to Australian horticultural and plant-based industries, and the environment. Australia introduced emergency measures to manage these risks in 2015, and revised these measures in 2016, 2019, 2020, 2021 and 2022.

The International Plant Protection Convention (IPPC) and the 'World Trade Organisation Agreement on the Application of Sanitary and Phytosanitary Measures' (SPS Agreement) require that phytosanitary measures against the introduction of new pests be technically justified. The IPPC's International Standards for Phytosanitary Measures (ISPM 1) states that countries may take appropriate emergency action on a pest posing a potential threat to its territories; however, it requires that the action be evaluated as soon as possible to justify the continuance of the action. This PRA meets Australia's international obligations to review the emergency phytosanitary measures for *Xylella fastidiosa* and the later recognised species *X. taiwanensis* as currently applied to imported nursery stock of over 20 000 species of plants, and to seeds for sowing for *Carya* spp.

This draft report presents a pest risk assessment for *Xylella* species arriving in Australia on the nursery stock and seeds for sowing pathways. The department does not consider fruit as a pathway for the transmission of *Xylella* because this is not supported by scientific literature. Relevant information about the epidemiology of the bacteria, their expanding plant host ranges, and the known insect vector species and their plant hosts, including case studies of 4 well known vector insects, is presented.

This draft report also proposes a range of risk management measures that target *Xylella* species with the aim of preventing the bacteria's entry into Australia. The proposed measures are differentiated according to the assessed *Xylella* status of the country of origin, the host status of the plant taxonomic grouping, any offshore measures applied, and the form of the commodity

imported: non-tissue culture (rooted plants, cuttings, budwood, corms and bulbs), tissue culture or seed. Proposed requirements include mandatory laboratory testing for host nursery stock from countries/regions where *Xylella* is known to be present and seeds for sowing from any source location, as well as operational systems, and/or a period of Post Entry Quarantine (PEQ) in Australia for disease screening to confirm the imported material can be released.

Together these measures mitigate the risks posed by *Xylella* pests associated with imports of nursery stock and seeds for sowing to a level that achieves the appropriate level of protection for Australia.

The proposed measures are largely consistent with the current emergency measures, but some amendments are indicated. These proposed amendments include changing the taxonomic level of plant regulation from the current target at plant family level to a proposal for regulating at plant genus level. That is, current regulation includes all plants within a family that has one or more species confirmed as a natural host of *Xylella* spp., and the proposal would instead regulate all plants within a genus of plants that has one or more species confirmed as a natural host of *Xylella* spp. Three experimental host plant genera are included as they have strong associations as host plants of competent *Xylella* vector species. Amendments are also proposed to strengthen the regulation of imported tissue culture pathways. Laboratory test reports for the material that is tested offshore would be required. A program of assurance and verification of selected imported nursery stock and tissue cultures, including by conducting molecular testing for *Xylella* spp., is also proposed.

The emergency measures will remain in place until the PRA is finalised following stakeholder consultation on the draft report and consideration of the comments received.

This draft report has been published on the department's website to allow interested parties to provide comments and submissions within the consultation period.

1 Introduction

1.1 Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, as well as those agricultural industries that are relatively free from serious pests.

The risk analysis process is an important part of Australia's biosecurity policy development. It enables the Australian Government to formally consider the level of biosecurity risk that may be associated with proposals to import goods into Australia. If the biosecurity risks do not achieve the appropriate level of protection (ALOP) for Australia, risk management measures are proposed to reduce the risks to an acceptable level. If the risks cannot be reduced to an acceptable level, the goods will not be imported into Australia until suitable measures are identified or developed.

Successive Australian governments have maintained a stringent, but not a zero risk, approach to the management of biosecurity risks. This approach is expressed in terms of the ALOP for Australia, which is defined in the *Biosecurity Act 2015* as providing a high level of protection aimed at reducing risk to a very low level, but not to zero.

Australia's risk analyses are undertaken by the Department of Agriculture, Fisheries and Forestry (the department) using technical and scientific experts in relevant fields and involve consultation with stakeholders at various stages during the process.

Risk analyses may take the form of a biosecurity import risk analysis (BIRA) or a review of biosecurity import requirements (such as scientific review of existing policy and import conditions, pest-specific assessments, weed risk assessments, biological control agent assessments or scientific advice).

Further information about Australia's biosecurity framework is provided in the *Biosecurity Import Risk Analysis Guidelines 2016* located on the department's website at agriculture.gov.au/biosecurity-trade/policy/risk-analysis/guidelines.

1.2 This risk analysis

1.2.1 Background

The International Plant Protection Convention (IPPC) and the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary measures (SPS Agreement) requires emergency phytosanitary measures against the introduction of new pests to be technically justified. Australia initially notified trading partners of the implementation of emergency phytosanitary measures to manage the risk of *Xylella fastidiosa* Wells et al. 1987 entering Australia with imported nursery stock through a World Trade Organisation Sanitary and Phytosanitary (WTO SPS) notification (G/SPS/N/AUS/376) on 9 November 2015.

The department is undertaking this pest risk analysis (PRA) to meet Australia's obligations under the IPPC and the International Standard for Phytosanitary Measures (ISPM) No. 1 (FAO 2016). This PRA reviews the existing emergency phytosanitary measures for imported nursery stock and seeds for sowing.

1.2.2 Scope

The scope of this PRA is analysis of the biosecurity risk caused by the introduction into Australia of bacteria in the genus *Xylella* in association with imported commercially produced nursery stock, and seeds for sowing (planting).

The department defines nursery stock as all live plants or plant material, other than fruit or seeds, imported for the purposes of propagation or planting. This material includes budwood, bulbils, bulbs, corms, cuttings, grafting wood, leaves, plants, rhizomes, roots, seedlings, slips, stems, tissue cultures and tubers (DAFF 2022a) (Biosecurity Import Conditions (BICON) system: bicon.agriculture.gov.au/BiconWeb4.0/). The department defines plant tissue cultures as undifferentiated or partially differentiated plant cellular materials derived from living plant tissue and maintained on or in artificial substrates under *in vitro* or other laboratory conditions.

The PRA incorporates:

- a pest risk assessment for member species of the genus *Xylella*, namely *X. fastidiosa* (including the subspecies *fastidiosa*, *multiplex*, and '*pauca*') and *X. taiwanensis* Su et al. 2016 (the department's acceptance of *X. fastidiosa* subspecies terminology is explained in Section 2.1)
- an assessment of the biosecurity risk of introducing *Xylella* bacteria through the:
  - imported commercially produced nursery stock and associated insect vector pathway from all trading partners
  - imported commercially produced seeds for sowing pathway from all trading partners.
- an overview of the insect vectors of *Xylella* potentially associated with imported nursery stock
- a review and evaluation of existing risk management measures and emergency measures for nursery stock imports and seeds for sowing imports
- proposals for additional and/or amended risk management measures where appropriate.

As discussed in Section 1.2.3, Australia requires mandatory treatments to manage arthropod risks on imported nursery stock (non-tissue culture only). This mandatory treatment is not required for the tissue culture pathway, as tissue culture is considered an effective method for excluding arthropod risks. Consequently, this draft report does not include pest risk assessments for the recognised insect vectors of *Xylella*. The entry pathways for fruit and cut flowers are also not included. Further information on potential transmission pathways, and the department's consideration of these, is provided in Section 2.6.

1.2.3 Existing policy

Australia's regulation of imported nursery stock

The department's standard nursery stock import conditions are determined on the basis of the country of origin, species of plant and the growth form being imported. Standard import conditions for all nursery stock include requirements for an import permit issued by the department, inspection and phytosanitary certification by the exporting authority's National Plant Protection Organisation (NPPO), appropriate taxonomic identification, freedom from soil and other contaminating matter, and phytosanitary inspection on arrival. With a few exceptions that mostly relate to department-approved high health sources of plants and tissue cultures, nursery stock is also subject to mandatory treatment to manage arthropod risks (non-tissue

culture only), and post-entry isolation, growth and disease screening to verify the material does not harbour detectable quarantine pests and diseases. Details of these standard conditions are provided in Section 4.1.1.

Australia's regulation of imported seeds for sowing

Seeds of many species can be imported from all sources under the department's standard seeds for sowing import conditions. In summary, the seeds must be clearly identified by species name, free from contaminating matter and weed seeds. Depending on the species of seed and country of origin, seeds may also be subject to an insecticidal treatment. Details of these standard conditions are provided in Section 4.2.1.

History of regulation of *Xylella* plant hosts for Australia

Australia has had phytosanitary measures in place to manage the potential association of *X. fastidiosa* with imported plant materials for about 50 years. These historic measures have included those coordinated by the then Plant Quarantine of the Department of Health (Ikin 1973) for *X. fastidiosa* ('Pierce's disease') in grapevine nursery stock, and for imported peach and nectarine nursery stock.

In the 1980s, the department extended phytosanitary measures to manage risks of *X. fastidiosa* in a greater number of imported plant species, including *Citrus* spp. (for 'citrus variegated chlorosis' disease) and other clonal vegetatively propagated nursery stock. The import conditions were further extended in 2009 to a total of 188 known agricultural and ornamental plant hosts of *X. fastidiosa*.

The department became aware of a further change in biosecurity risk following reported disease outbreaks of *X. fastidiosa* in Italy in 2013 and in France in 2015. These outbreaks highlighted the potential for *X. fastidiosa* to be transported to and become established in new areas through international trade of asymptomatically infected plants, and to be further transmitted by both recognised and previously unrecognised insect vectors, including leafhoppers and sharpshooters (Cicadellidae) and spittlebugs (Aphrophoridae).

In response to an expanding documented host range of *X. fastidiosa* among economically important plants and increasing evidence of global spread in commonly traded plant hosts, Australia implemented emergency measures in 2015 (DAWE 2020b). The measures were intended to strengthen the import conditions put in place for *X. fastidiosa* in 2009, to further reduce the likelihood of entry of *X. fastidiosa* and related *Xylella* species. The changes to import conditions for nursery stock included extension to cover host plant tissue cultures, rooted plants, cuttings, budwood, and some corms and bulbs of 89 plant families known to have one or more member species confirmed as natural hosts of *X. fastidiosa*. These emergency measures were implemented in two phases, with the first phase commencing on 19 November 2015 for identified high risk countries/regions [that is, the Americas (including the Caribbean), Europe, India, Iran, Lebanon, Taiwan and Türkiye (formerly referred to as Turkey)]. High risk countries/regions were defined as those that had reported *Xylella*, as well as their associated trading blocs (such as the European Union), and areas where *Xylella* is known to be native (the Americas and Caribbean). The second phase followed on 19 January 2016 for other low risk countries/regions (that is, all countries/regions not listed as being of high risk).

Following a notification of the presence of *X. fastidiosa* in Israel on 25 June 2019 (EPPO 2019; Plant Protection and Inspection Services 2019), Australia communicated that it had extended the list of recognised high risk countries/regions for *X. fastidiosa* to include Israel, effective 6 July 2019 (through notification G/SPS/N/AUS/376/Add.1 (WTO 2019)).

Since this time, the emergency measures have been extended to account for increases in the confirmed plant host range of *X. fastidiosa* on nursery stock on 4 occasions, and on seeds for sowing on one occasion:

- 3 August 2020, emergency measures were extended to 9 additional plant families due to documented changes in the bacterium's host range. Six of the families (Cannaceae, Gesneriaceae, Resedaceae, Scrophuliriaceae, Strelitiaceae and Tamaricaceae) have confirmed natural host plant species of *Xylella* (references for these associations are given in Appendix D for plant hosts). Three of the plant families (Polemoniaceae, Simmondsiaceae and Linaceae) contain experimental hosts of *Xylella* but have strong associations with the known competent insect vectors of *Xylella—Philaneaus spumarius* and/or *Homalodisca vitripennis* (Black 2010; Wistrom & Purcell 2005).
- 1 June 2021, emergency measures were extended to 7 additional plant families due to documented changes in the bacterium's natural host range (DGAV 2020; Groenteman et al. 2015). These families were the Araucariaceae, Argophyllaceae, Athyriaceae, Corynocarpaceae, Dennstaedtiaceae, Haloragaceae and Violaceae.
- 15 November 2021, emergency measures were extended to the plant family Hypericaceae based on additional information on the bacterium's natural host range (DGAV 2022).
- 20 May 2022, Australia notified of emergency measures taken for *Carya* spp. seeds for sowing, on confirmation of seed transmission in pecan (Cervantes et al. 2022).

The emergency measures currently applying to nursery stock and seeds for sowing for *X. fastidiosa* and related *Xylella* species are described in 'Notification of amended emergency quarantine measures for plant pathogen *Xylella fastidiosa*' (DAFF 2022b) and a summary of these is provided in Chapter 4. These measures are in addition to the standard nursery stock and seed import conditions applying to individual plant species.

Australia's regulatory policy

The *Biosecurity Act 2015* (Biosecurity Act) and its subordinate legislation provides the legal basis for preventing or controlling the entry of plants and plant products including nursery stock and seeds into Australia, and for managing the biosecurity risk arising from nursery stock and seed consignments, including the pests associated with those consignments, after they arrive in Australia.

Domestic arrangements

The Australian Government is responsible for regulating the movement of goods, such as plants and plant products, into and out of Australia. State and territory governments, however, are responsible for plant health controls within their individual jurisdiction. Legislation relating to resource management or plant health may be used by state and territory government agencies to control interstate movement of plants and plant products. Interstate movement conditions may apply once plants and plant products have been cleared by Australian Government biosecurity officers. An importer is responsible for identifying and complying with all requirements.

1.2.4 Contaminating pests

In addition to the *Xylella* spp. that are assessed in this risk analysis, other organisms may arrive with the imported commodities. These organisms may include pests considered not to be associated with the nursery stock or seeds for sowing pathway, pests of other crops, or predators and parasitoids of arthropods. The department considers these organisms to be contaminating pests ('contaminants') that could pose sanitary (to human or animal life or health) or phytosanitary (to plant life or health) risks. These risks are identified and addressed using existing operational procedures that require an inspection of all consignments during processing and preparation for export. Consignments will also undergo another inspection on arrival in Australia. The department will investigate whether any pest identified through import verification processes may be of biosecurity concern to Australia and may thus require remedial action.

1.2.5 Consultation

Prior to implementing the emergency measures, the department communicated with commercial industry stakeholders and with other NPPOs. The department also published information on specific elements of its regulation of nursery stock and seeds for sowing for *Xylella* spp. through industry alerts and on its website. A summarised chronology of consultation follows.

22 October 2015—the department held a teleconference with the former Nursery and Garden Industry Australia (now known as Greenlife Australia) to discuss the proposed emergency measures for imported nursery stock of confirmed natural *X. fastidiosa* hosts.

28 October 2015—the department held a teleconference with domestic nursery stock industry stakeholders to discuss the proposed emergency measures.

30 October 2015—the department published information on its website (Industry Advice Notice 88–2015). Alerts were also published on the department's closing (ICON) and replacement (BICON) import conditions databases (Public Quarantine Alert PQA1069). These notices explained the reasons for introducing emergency measures and advised that further details would be published on ICON/BICON prior to implementation.

3 November 2015—the department emailed individual import permit holders to advise of the notifications published on 30 October 2015.

5 November 2015—the department published detailed information about the emergency measures on its website (Industry Advice Notice 95–2015), and through alerts in ICON and BICON (Public Quarantine Alert PQA1071). These notifications advised that the measures to be implemented comprised requirements for offshore testing and government certification of freedom from *Xylella fastidiosa* for nursery stock confirmed to be natural hosts of the bacterium, or a mandatory growth period and subsequent testing in Post Entry Quarantine (PEQ) in Australia or mandatory hot water treatment of nursery stock material. Lists of host families and high risk countries/regions were included, with advice that the measures would be introduced in two phases to minimise trade disruption:

- **Phase 1**: measures for high risk countries/regions (all countries in the Americas including the Caribbean, all countries/regions in Europe, India, Iran, Lebanon, Taiwan and Türkiye (formerly referred to as Turkey)) would be implemented on 19 November 2015.

- **Phase 2**: measures for low risk countries/regions (all other countries/regions not listed as high risk) would be implemented on 19 January 2016.

The department also emailed individual permit holders to advise that specific details of the phytosanitary measures, host and country lists, and arrangements for material in transit to Australia were available.

9 November 2015—Australia notified trading partners of the emergency measures through a WTO SPS notification (G/SPS/N/AUS/376) (WTO 2015).

10 November 2015—in response to questions from industry, the department emailed individual permit holders to provide additional information on the host plant list and a flow-chart to assist them in determining which imports were subject to the emergency measures.

13 January 2016—the department published information on its website and through a BICON alert notifying industry of an amendment to the emergency measures to more clearly define the targeted bacterial species (that is, *X. fastidiosa* and all subspecies), and to update the wording required on phytosanitary certificates. This notice detailed the requirements for overseas production arrangements for nursery stock to be approved by that country's NPPO (known as an 'approved arrangement'), and the Polymerase Chain Reaction (PCR) testing protocols to be used under those approved arrangements. The notice also announced a delay in implementing arrangements for affected bulbs produced under the Bloembollenkeuringsdienst (BKD) scheme in the Netherlands.

30 March 2016—as a result of information provided by the Netherlands NPPO, the department issued a BICON alert advising the postponement of introduction of emergency measures on specified bulbs (*Narcissus* spp., *Hyacinthus* spp. and *Hippeastrum* spp.) produced and certified under the BKD scheme in the Netherlands.

2 August 2018—the department publicly announced the commencement of the *Xylella* PRA on its website. Interested stakeholders were invited to register with the department's on-line subscription service to receive notifications relating to the *Xylella* PRA and other plant biosecurity topics.

6 September 2018—the inaugural meeting was held of the Imported Nursery Stock Regulation Working Group (no longer active), formed by the department to promote engagement across sectors on nursery stock biosecurity risks and their effective and efficient management. Group members represented the department, Plant Health Australia, state and territory governments, the nursery production industry and importers. The meeting discussed issues of relevance, including progress of the *Xylella* PRA and expected consultation dates.

18 July 2019—following a notification on 25 June 2019 of the presence of *X. fastidiosa* in Israel, Australia communicated, through notification G/SPS/N/AUS/376/Add.1 (WTO 2019), that it had extended the list of *Xylella* spp. high risk countries/regions to include Israel, effective 6 July 2019.

3 August 2020—based on additional information on the host range of *Xylella* bacteria and of two competent vector insect species, Australia communicated that emergency measures for *Xylella* were extended to 9 additional plant families, being the Cannaceae, Gesneriaceae, Linaceae, Polemoniaceae, Resedaceae, Scrophulariaceae, Simmondsiaceae, Strelitziaceae and

Tamaricaceae through notification G/SPS/N/AUS/376/Add.2 (WTO 2020). The department contacted individual importers of these plant families, informing them of the change, and issued a BICON notification about the same.

1 June 2021—based on additional information on the host range of *Xylella* bacteria, Australia communicated that emergency measures for *Xylella* were extended to 7 additional plant families, being the Araucariaceae, Argophyllaceae, Athyriaceae, Corynocarpaceae, Dennstaedtiaceae, Halagoraceae and Violaceae through notification G/SPS/N/AUS/376/Add.3 (WTO 2020). The department contacted individual importers of these plant families, informing them of the change, and issued a BICON notification about the same.

15 November 2021— based on additional information on the host range of *Xylella* bacteria, Australia communicated that emergency measures for *Xylella* were extended to the plant family Hypericaceae through notification G/SPS/N/AUS/376/Add.4 (WTO 2021). The department contacted individual importers of this plant family, informing them of the change, and issued a BICON notification about the same.

18 May 2022—the department convened and met with representatives from the Australian Nut Industry Council, Almond Board of Australia, Hort Innovation and several growers, importers and propagators of pecan nursery stock to discuss planned introduction of emergency measures for *Carya* spp. seeds for sowing. The meeting discussed the newly published scientific evidence of *Xylella* transmission in pecan seed, the potential impact *Xylella* could have if introduced to Australia, and sources of pecan seed used for propagating root stock for pecan plantations.

20 May 2022—based on confirmation of seed transmission of *Xylella fastidiosa* in pecan seed, Australia communicated that emergency measures were being applied to all *Carya* spp. seeds for sowing through notification G/SPS/N/AUS/538 (WTO 2022). The department contacted individual importers of this plant genus, informing them of the change, and issued a BICON notification about the same.

1.2.6 Overview of this pest risk analysis

A PRA is 'the process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it'. A pest is ‘any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products’ (FAO 2022). This definition is also applied in the *Biosecurity Act 2015*.

The department conducted this PRA in accordance with Australia’s method for pest risk analysis (Appendix A), which is consistent with the ISPMs, including ISPM 2: *Framework for pest risk analysis* (FAO 2019a) and ISPM 11: *Pest risk analysis for quarantine pests* (FAO 2019b), and the SPS Agreement (WTO 1995).

A summary of the process used by the department to conduct a risk analysis is provided in Appendix A: Method for pest risk analysis, and the process flow for this is depicted in Figure 1.1.

The PRA was conducted in the following 3 consecutive stages:

1) Initiation—identification of:
   - the pathway being assessed in the risk analysis

- the pest(s) that have potential to be associated with the pathway and are of biosecurity concern and should be considered for analysis in relation to the identified PRA area.

2) Pest risk assessment—this was conducted in 2 sequential steps:

   2a. Pest categorisation: examination of each pest identified in stage 1 to determine whether they are a quarantine pest and require further pest risk assessment.

   2b. Further pest risk assessment: evaluation of the likelihood of the introduction (entry and establishment), spread and the magnitude of the potential consequences of the quarantine pest(s). The combination of the likelihoods and consequences gives an overall estimate of the biosecurity risk of the pest, known as the unrestricted risk estimate (URE).

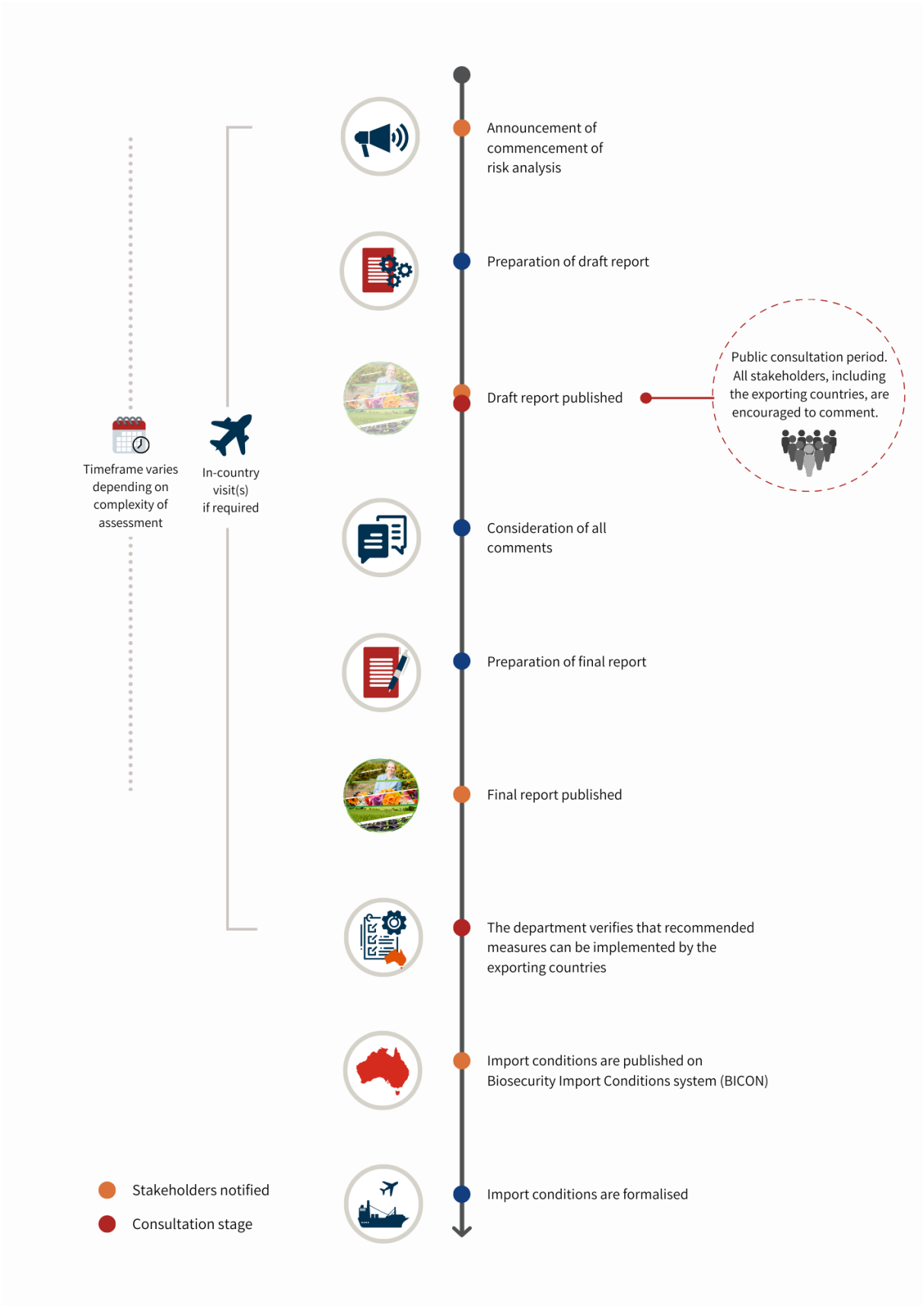
3) Pest risk management—the process of identifying and proposing/recommending required phytosanitary measures to reduce the biosecurity risk to achieve the ALOP for Australia where the URE is determined as not achieving the ALOP for Australia. Restricted risk is estimated with these phytosanitary measure(s) applied.

A phytosanitary measure is 'any legislation, regulation or official procedure having the purpose to prevent the introduction or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests' (FAO 2022).

For further information on the:

- method for PRA: see Appendix A
- terms used in this risk analysis see: Glossary, acronyms and abbreviations at the end of this report
- pathway being assessed in this risk analysis: see section 1.2.2
- initiation and pest categorisation: see Appendix B
- pest risk assessments for pests identified in Appendix B as requiring further pest risk assessment: see Chapter 3
- risk management measures for pests assessed in Chapter 3 as not achieving the ALOP for Australia: see Chapter 4.

Figure 1.1 Process flow diagram for conducting a risk analysis



1.2.7 Next steps

The department has notified the registered stakeholders and the WTO-Secretariat about the release of this draft report.

This draft report gives stakeholders an opportunity to comment on the department's review and proposed measures, and to draw attention to any scientific, technical or other gaps in the data, or misinterpretations or errors.

The department will consider submissions received on the draft report and may consult further with stakeholders. The department will revise the report as appropriate and then prepare a final report, taking into account stakeholder comments.

The final report will be published on the department website along with a notice advising stakeholders of the release. The department will also notify registered stakeholders and the WTO Secretariat about the release of the final report. Publication of the final report represents the end of the risk analysis process.

The biosecurity requirements recommended in the final report will form the basis of the conditions published on BICON, and for any import permits subsequently issued.

2 *Xylella* as a plant pathogen

This chapter introduces the members of the genus *Xylella*, including descriptions of their taxonomy, morphological traits, biology, host range, distribution, and symptoms of the plant diseases with which they are associated. The chapter also contains information about insect vectors and case studies involving 4 well-known vectors of *Xylella*. In addition, the chapter contains information on pathways of transmission, diagnosis and treatment.

2.1 Taxonomy

The genus *Xylella* is a member of the bacterial family Xanthomonadaceae, in the order Xanthomonadales (ITIS 2019). Genera most closely related to the genus *Xylella* are *Xanthomonas* and *Stenotrophomonas* (Comas et al. 2006; Naushad 2015; NCBI 2020; Wells et al. 1987), members of which are known plant and water-borne pathogens, respectively. In 2020, a new taxonomic placement for the genus *Xylella* was proposed, in the Family Lysobacteraceae of the Order Lysobacterales (Parte 2020). This PRA continues to use the earlier taxonomic placement of Xanthomonadaceae, as the new placement is not yet in common use.

The genus *Xylella* Wells et al. (1987) was initially described as containing a single bacterial species, *X. fastidiosa*, which is the recognised cause of serious diseases in a number of plant species (Wells et al. 1987).

The severity of *Xylella*-related diseases in many economically important horticultural crops and industries has led to extensive molecular characterisation of its members, and subsequent recognition of up to 6 subspecies of *X. fastidiosa* and associated disease-host relationships. Those subspecies are *X. fastidiosa* subsp. *fastidiosa* Schaad et al. 2009, *X. f.* subsp. *multiplex* Schaad et al. 2009 (Euzeby 2009), '*X. f.* subsp. *pauca*' (Schaad et al. 2004) (quotation marks indicating a currently unofficial taxonomic status), '*X. f.* subsp. *morus*' (Nunney et al. 2014c), '*X. f.* subsp. *sandyi*' (Schuenzel et al. 2005) and '*X. f.* subsp. *tashke*' (Randall et al. 2009).

Simpson et al. (2000) sequenced the genome of *X. fastidiosa*, providing a basis for analyses of genotypic relationships within the species. Two major intra-specific groupings have subsequently and consistently been demonstrated by phylogenetic analyses of sequenced genotypes, with '*X. f.* subsp. *pauca*' comprising an identified 'Group 1', and *X. f.* subsp. *multiplex* and *X. f.* subsp. *fastidiosa* a 'Group 2' (Cella et al. 2018; Coletta-Filho et al. 2017; Denance et al. 2017; Hernandez-Martinez et al. 2007; Marcelletti & Scortichini 2016; Nunney 2013; Vanhove et al. 2019). A recent comprehensive analysis of sequenced genotypes by Cella et al. (2018) also supported the observations of Marcelletti and Scortichini (2016).

With further sequencing, it is becoming more evident that some of the proposed subspecies are likely to be inter-subspecific homologous recombinants. For example, '*Xylella f.* subsp. *morus*' is likely to be a recombinant form of *X. f.* subsp. *multiplex* originating from the USA, and '*X. f.* subsp. *sandyi*' a recombinant of *X. f.* subsp. *fastidiosa*, probably introduced into the USA from central America (Nunney et al. 2014b; Nunney, Stouthamer & Bromley 2016, 2020). Subspecies of *X. fastidiosa* have high genetic diversity, with genetic variation and recombination potentially achieved via transfer of DNA through the process of conjugation (Burbank & Van Horn 2017). Plasmid transfer between *Xylella* genotypes to form recombinants may occur if a host is infected with multiple genotypes of *Xylella* and large aggregates of bacteria are present (Burbank & Van Horn 2017). Bi-directional gene flow through genetic recombination has been shown when

genotypes of different subspecies of *X. fastidiosa* come into contact as a result of human-mediated movement of infected plant material, or through polyphagous xylem-feeding insect vectors that acquire and transmit multiple *Xylella* subspecies or genotypes (Coletta-Filho et al. 2017; Nunes et al. 2003; Nunney et al. 2012). The ready occurrence of inter-subspecific recombination, as evidenced by transfer of virulence between *X. f.* subsp. *fastidiosa* and *X. f.* subsp. *multiplex*, was demonstrated by Kandel et al. (2017).

The causal agent of pear leaf scorch disease, first identified in Taiwan in 1993, was originally also designated as a strain of *Xylella fastidiosa* (Leu & Su 1993), but based on distinct differences in phenotypic and genotypic characteristics and fatty acid profiling, it was subsequently designated as a new species, *Xylella taiwanensis* sp. nov. (Su et al. 2016).

This PRA, therefore, recognises the existence of 2 species in the genus, *Xylella taiwanensis* and *X. fastidiosa*, and 3 subspecies of *X. fastidiosa* namely, *X. f.* subsp. *fastidiosa*, *X. f.* subsp. *multiplex* and '*X. f*. subsp. *pauca'*. References to '*X. f.* subsp. *sandyi'*, '*X. f.* subsp. *morus'* and '*X. f.* subsp. *tashke'* elsewhere in this draft report are identified as *X. f.* subsp. *fastidiosa* ('*sandyi'*), *X. f*. subsp. *fastidiosa* ('*morus*') and '*X. f.* subsp. *fastidiosa* ('*tashke'*).

2.2 Biology

Xylella members are gram-negative, rod-shaped, non-flagellated, strictly aerobic plant pathogenic bacteria (Janse & Obradovic 2010). *Xylella* bacteria are xylem-limited, moving passively and actively in the plant's xylem vessels by means of twitching motility, which allows the bacteria to migrate to distal tissues, systemically infecting the plant (Landa et al. 2022).

The xylem environment has a highly fluctuating negative pressure, a low oxygen and nutrient content (Landa et al. 2022), is a habitat sheltered from changes in the external environment (Gerlin et al. 2020) and consists mainly of non-living tissue (Roper, Castro & Ingel 2019). *Xylella* bacteria have adapted to this niche environment and are unable to utilise high nutrition, even if provided (Gerlin et al. 2020). Consequently, *Xylella* disease symptoms may take months or even years to express (Zecharia et al. 2022).

Xylella bacteria are not independently capable of entering plant hosts (Purcell 1995). Host infection requires either an insect vector for inoculation into host plant tissue, natural root grafting by plants growing in close proximity, or mechanical transmission through propagation; new evidence from Cervantes et al. (2022), confirmed *Xylella* seed transmission in *Carya illinoinensis* (pecan). Although not considered a very efficient transmission method, Pierce's Disease of grapevines has been experimentally transmitted by pruning shears from an infected shoot to a healthy shoot (Krell et al. 2007). An overview of vectors of *Xylella* and case studies of selected vector species are provided in Section 2.3, and all transmission methods are discussed further in Section 2.6.

Once in the xylem, the bacteria multiply and move within the plant host. Earlier theories suggested that dense bacterial colonisation causes occlusion of the xylem vessels, inhibiting water flow and resulting in associated *Xylella* disease symptoms (Li et al. 2007). However, the bacteria's irregular distribution within plant stems, petioles and leaves (Hopkins 1981), non-systemic infections where the bacteria does not move beyond the inoculation site (Wistrom & Purcell 2005), and the occurrence of asymptomatic infection in many host species (Almeida & Nunney 2015) made understanding this theory of the bacteria's pathology difficult.

Developments in research now suggest that more complex interactions between the bacteria and host plants are occurring, with *Xylella* bacteria constructing intricate biofilms to restrict their own movement within a host, delaying colonisation and symptom expression, while also actively misdirecting the host immune response away from its invasion. It is theorised that the bacteria maintain a balance between parasitism and commensalism to ensure its chance of survival in a susceptible host, as well as its probability of insect transmission to a new host before killing the current host plant (Gerlin et al. 2020; Landa et al. 2022; Roper, Castro & Ingel 2019). This theory better explains why *X. fastidiosa* can multiply in most plants, but, with influence of the environment, does not always move beyond the inoculation site, resulting in non-systemic infections (Wistrom & Purcell 2005). The irregular distribution within a host plant, and asymptomatic infections in many host species (Almeida & Nunney 2015), can complicate the detection of *Xylella* bacteria.

Interactions between the strains of *X. fastidiosa* subspecies, their sequence types (STs), plant hosts, and vector species are complex. Sequence types are defined based on sequence analysis of 7 housekeeping genes, and to date 89 STs have been identified (Landa et al. 2022). Individual STs appear to be associated with a limited number of host plants (Sicard et al. 2018), although data for all combinations of hosts x ST is incomplete and focused on commodity crops. As an example, ST1 has been found to infect 60 plant species, while ST54 to ST60 all have a single host reported (EFSA 2022b). A ST in one country will be expected to infect the same host range in a new country. Insect vectors can transmit all *X. fastidiosa* genotypes without specificity (Sicard et al. 2018).

This PRA does not discuss the subspecies and strain differences in detail, as all members of the genus *Xylella* are absent from Australia.

2.3 Insect vectors

There are currently over 120 insect species confirmed as able to acquire and carry *Xylella*, with 75 of these also proven capable of transmission and considered vectors (information presented in Appendix C: *Xylella* vectors and preferred plant hosts). An additional 100+ species have been either implicated in transmission, or are considered to be potential vectors, but lack transmission studies for confirmation (species without confirmation are not presented).

Insect vectors of *Xylella* have been confirmed across all global regions: Africa; Asia; North America, Central and South America; Oceania and Europe (EFSA Panel on Plant Health et al. 2019b). All known *Xylella* vectors are from the hemipteran sub-order Auchenorrhyncha, which contains xylem-feeding leafhoppers, sharpshooters, tree hoppers and cicadas. Within this sub-order, *Xylella* vectors are found across numerous families and/or superfamilies including the Aphrophoridae, Cicadellidae, Cercopoidea, Membracoidea, Clastopteridae and Cicadidae (EFSA Panel on Plant Health et al. 2019b)(references for individual associations are provided in Appendix C). None of the known vector insects are present in Australia. However, the sub-order is well represented in Australia by a large number of endemic and introduced species (See Section 2.3.2).

Insect vectors acquire *Xylella* bacteria when ingesting xylem sap from infected host plants. Once ingested, *Xylella* bacteria colonise the cuticular surface of the insect vector foregut, where they form a biofilm and proliferate (Almeida & Purcell 2006; de Mello Varani et al. 2008; Janse & Obradovic 2010). The ability of *Xylella* bacteria to metabolise chitin is a necessary component in

the persistent colonisation of insect vectors (Landa et al. 2022), as the bacteria parasitically exploit the vector cuticle as a substrate for multiplication, with a resultant negative impact on the vector. *Xylella* bacteria also encode proteins that induce behavioural changes in the vector that enhance transmission (Cornara et al. 2020).

When the insect moves to another plant, the feeding mechanisms of ingestion and egestion can transfer some of the *Xylella* bacteria to the new plant host (Backus et al. 2015; Killiny & Almeida 2014). All insects that can feed on xylem sap are considered to have the potential to be vectors of *Xylella* (Purcell & Frazier 1985); these include species that only occasionally, through requirement or accident, feed on xylem (Almeida & Nunney 2015; Chuche, Sauvion & Thiéry 2017). The European Food Safety Authority (EFSA) Panel on Plant Health has also considered that all xylem-feeding insects in Europe are potential vectors of *Xylella* (EFSA Panel on Plant Health 2015b). The number of bacteria required to cause infection in a new host is small (less than 200 viable bacterial cells) (Almeida et al. 2005; Redak et al. 2004). Vector transmission can occur without a latent period, and this post-acquisition transmission is driven by free *Xylella* cells acquired from the host plant, but not yet established as a biofilm in the vector foregut (Beal 2021).

Immature insects lose infective bacteria through the processes of developmental moulting and associated expulsion of foregut contents (Hopkins, Thompson & Wichman 1995); however, adults that acquire *Xylella* bacteria remain infectious for the remainder of their life (Almeida et al. 2005). Due to the limited mobility of most insect nymphs, adult insects are considered the main mechanism of natural spread of the bacteria within a region (EFSA PLH Panel et al. 2018), while long-distance distribution has always been attributed to movement of the bacteria within infected plant material (EFSA Panel on Plant Health et al. 2019b). Lago et al. (2020b) cited examples of vector species being captured at heights up to 200m above ground, determined that *Neophilaenus campestris* can fly more than 2 km in 5 weeks, and presented a case for even weakly flying species being capable of reaching low-level jet winds and achieving long-distance passive migration.

Xylem sap feeding insects are frequently polyphagous, apparently feeding across many host plant species in order to obtain sufficient nutrients from nutrient-poor xylem sap (Andersen, Brodbeck & Mizell 1992; Novotny 1994). There is no evidence of vector-pathogen specificity for transmission of *Xylella* bacteria (Almeida et al. 2005), with some insect vectors having been shown to carry and transmit multiple subspecies of *X. fastidiosa* to multiple host plants (Almeida & Nunney 2015) (see Appendix C for examples).

From the current knowledge of the feeding biologies of *Xylella* insect vectors, the potential plant host list is over 1500 plant species from 179 plant families (Appendix C). Major *Xylella* insect vectors such as *Philaenus spumarius* (L.) (meadow spittlebug) and *Homalodisca vitripennis* (Germar) (glassy-winged sharpshooter) are highly polyphagous, as shown by the extensive numbers of plant hosts reported (European Commission 2019). For example, 598 host plants are recorded for *H. vitripennis* (Appendix C) (Andersen, Brodbeck & Mizell 1992; Mizell et al. 2015).

In addition to the xylem-feeding Auchenorrhyncha, phloem feeders can be in contact with xylem vessels and may acquire the bacterium. The phloem feeder *Euscelis lineolatus* has been reported

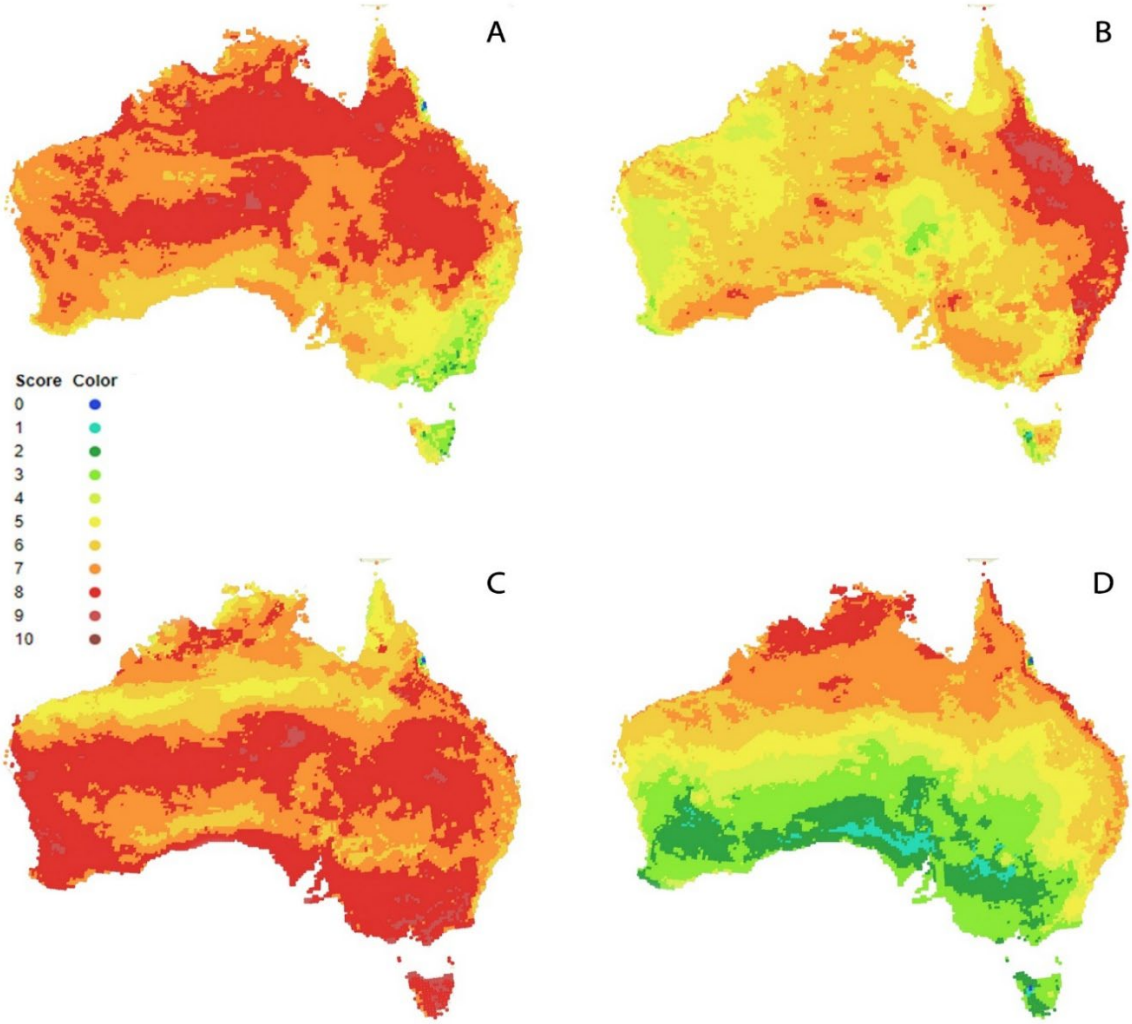
carrying *X. fastidiosa* in Italy (Elbeaino et al. 2014). However, transmission has yet to be successfully demonstrated by a phloem feeder (Antonatos et al. 2020).

2.3.1 Case studies

Biological and behavioural characteristics of insect vectors, including distances moved, feeding habits, population densities, numbers of generations per year, and number of plant hosts, are integral factors in determining capacity to spread *Xylella* bacteria. Intrinsically, these factors are also linked to environmental and habitat conditions. Thus, with an effective vector and appropriate circumstances, *Xylella* species can have significant impacts on horticultural and agricultural industries around the world. Four of the most well-known insect vectors of *Xylella* are discussed below, with brief details of their key characteristics and the roles they play in the spread of *Xylella* in different regions globally.

Australian environmental conditions are likely suitable for exotic insect vectors of *Xylella* to establish, should these pests remain undetected on arrival in Australia and be distributed across the country. Figure 2.1 depicts the department's analysis of predicted environmental suitability for the establishment of *Philaenus spumarius*, *Kolla paulula*, *Homalodisca vitripennis* and *Bucephalagonia xanthophis*. This modelling shows that much of the Australian environment would be suitable for the establishment of one or more of exotic vector species.

Figure 2.1 Predicted environmental suitability for establishment of (A) *Homalodisca vitripennis*, (B) *Bucephalogonia xanthophis*, (C) *Philaenus spumarius*, and (D) *Kolla paulula* in Australia



Source: Modelling conducted by the department, using temperature and rainfall data from known areas of vector distribution using Climatch v1.0 (ABARES 2020).
Notes: Climate data used for modelling included arid environments supplemented by irrigation. Lower ratings indicate lesser predicted suitability for vector colonisation and persistence (zones with a rating of 5-10 are areas of concern).

Philaenus spumarius

Philaenus spumarius (Linnaeus 1758) (Hemiptera: Aphrophoridae), known as the common spittlebug or meadow spittlebug, was first identified as a vector of *X. fastidiosa* in 1950 in the USA, and is known to be capable of transmitting genotypes of all 3 *X. fastidiosa* subspecies (DeLong & Severin 1950). *P. spumarius* has been identified as the main vector of *X. fastidiosa* in Europe (Cornara et al. 2017).

P. spumarius is native to Türkiye and Iran, and has spread to Europe, Asia, USA (including Hawaii), Canada, north-west Africa and Nigeria (CABI 2022a; Ejere & Okpara 2010). It has also established in New Zealand, with the first recorded collection being from Palmerston North in 1960 (Archibald, Cox & Deitz 1979). Its habitat preference is open land and open forests, and it is rarely found in very wet or very hot/dry habitats (Halkka et al. 1967; Yurtsever 2000).

Climatch v2.0 (ABARES 2020) modelling of temperature and rainfall data (Figure 2.1) predicts *P. spumarius* has the potential to establish in 99% of Australia.

P. spumarius is highly polyphagous (CABI 2022a), with the largest recorded plant host range of all currently identified *Xylella* vectors (Appendix C), comprising more than 1,000 plant species, and increasing with each new area of introduction (Cornara, Bosco & Fereres 2018). This spittlebug is a recognised economic pest in strawberry, alfalfa and clover crops and ornamental nursery plants (Halkka et al. 1967; Weaver & King 1954), and is considered responsible for disease spread in olives and other plant hosts (Cornara et al. 2017). The broad plant host range of this vector ensures a plentiful range of available food species should it establish in Australia.

P. spumarius populations generally complete one generation per year, which includes an over-wintering egg stage (Halkka et al. 1967; Hasbroucq et al. 2017; Yurtsever 2000). The ability to over-winter provides a tolerance of a wide range of environmental conditions, and also increases the likelihood of importation and survival on imported nursery stock. A single incursion of undetected eggs laid on leaves or stems of host plants imported from England was believed to have allowed entry and then establishment of *P. spumarius* in New Zealand (Archibald, Cox & Deitz 1979; Hamilton 1979; Hamilton & Morales 1992). *P. spumarius* are not good flyers and, unless carried by the wind, cannot fly long distances (Albre, Carrasco & Gibernau 2021).

Kolla paulula

Kolla paulula (Walker, 1858) (Hemiptera: Cicadellidae) is a leafhopper first identified as a vector of *X. taiwanensis* in pear orchards (Leu & Su 1993) and of *X. f.* subsp. *fastidiosa* in grapevines (Lin & Chang 2012; Su et al. 2013; Su et al. 2012; Tuan et al. 2016), both in Taiwan.

K. paulula is native to much of Asia including areas in India, China, Taiwan, Japan, the Philippines, the Malay Peninsula, Sri Lanka, Indonesia, Nepal, Thailand, Cambodia, Vietnam and Myanmar (Fletcher, 2022; McKamey 2007; Metcalf 1965). In Taiwan it is found in low to medium altitude areas, with preferred habitat being wooded and weedy areas at the edges of orchards in cool and dry areas (Shih et al. 2013). Climatch v2.0 (ABARES 2020) modelling of temperature and rainfall data (Figure 2.1) predicts *K. paulula* has the potential to establish in 54.9% of Australia.

The host plants of *K. paulula* are species in the families Asteraceae, Moraceae, Commelinaceae and Convolvulaceae (Deng 2014; Shih et al. 2009; Shih et al. 2013), including *Vitis* spp., *Mikania micrantha*, *Bidens pilosa* var. *radiata* (synonym of *Bidens alba* (Royal Botanic Gardens 2022)), *Ageratum houstonianum*, and *Commelina diffusa* (Cornara et al. 2019), all of which are present in Australia (ABRS 2022b; ALA 2022; Royal Botanic Gardens 2022), and all but the final 2 are confirmed natural host plants of *Xylella*. The full host range of *K. paulula* is expected to be wider due to limited research on the insect.

The generation time of *K. paulula* is between 62-94 days, and 1.2 to 1.5 times longer during autumn and winter (Shih et al. 2013). For defence, they mostly rely on agility, by jumping and flying (EFSA Panel on Plant Health et al. 2019b), although flying distance is not recorded.

Homalodisca vitripennis

Homalodisca vitripennis (Germar, 1821) (Hemiptera: Cicadellidae), commonly known as the glassy–winged sharpshooter (GWSS), was recognised as a vector of *Xylella* after it was introduced into California (Cornara et al. 2019) in the 1990s. *H. vitripennis* is the main vector of *Xylella* in the USA (EFSA Panel on Plant Health et al. 2019b), and has been reported vectoring *X. f.* subsp. *fastidiosa* (Redak et al. 2004), *X. f.* subsp. *multiplex* (Krugner et al. 2014), and '*X. f.* subsp. *pauca'* experimentally (Almeida & Nunney 2015).

Originating from tropical areas in south-eastern USA to north-eastern Mexico (Hoddle 2004), *H. vitripennis* has a history of incursions and successful establishments in California (1998) (Almeida 2007; De Leon, Jones & Morgan 2004), French Polynesia (1999)(Hoddle 2004) including the Marquesas Islands (Nuku Hiva in 2004) and the Austral Islands (Tubuai and Rurutu in 2005), Hawaii, USA (2004) (Hoover 2004), Rapa Nui, Chile (2005), and the Cook Islands (2007)(EPPO 2009). Increases in the distribution of *H. vitripennis* across areas of the USA have been facilitated by the establishment of extensive irrigated orchards and gardens, which provide habitats in otherwise typically arid areas that would not be suitable for GWSS (Hoddle 2004). Climatch v2.0 (ABARES 2020) modelling of temperature and rainfall data (Figure 2.1) predicts *H. vitripennis* has the potential to establish in 96.3% of Australia.

H. vitripennis is a highly polyphagous, xylem sap feeding leafhopper which prefers to feed on plant stems, trunks, branches and leaf petioles (Novotny 1994; Redak et al. 2004). Its host plant range is large, with over 500 recorded species (Andersen, Brodbeck & Mizell 1992; Mizell et al. 2015)(Appendix C) from 67 plant families (CABI 2022a), including economically significant hosts such as citrus, grapes and *Prunus* spp., as well as ornamental and amenity plants such as crepe myrtle and oleander. It is also known to be able to feed on Australian natives such as *Acacia cowleana, Correa pulchella, Eremophila divaricata, Eucalyptus wandoo, Hakea laurina, Leptospermum laevigatum, Melaleuca lateritia, Swainsona galegifolia,* and *Phormium tenax* (Bruening et al. 2014; Groenteman et al. 2015; Rathe et al. 2014).

H. vitripennis is a high-volume feeder, ingesting 100 to 300 times its dry body weight in xylem sap per day (Brodbeck, Mizell & Andersen 1993); this attribute increases the likelihood of acquiring *Xylella* from infected plants, in addition to increasing the chance of acquiring multiple subspecies or genotypes if present within the feeding plant community. Adult *H. vitripennis* insects are strong flyers, and able to disperse over relatively long distances, thereby also enhancing their potential for spreading the bacterium (Bruening et al. 2014; Conklin & Mizell 2016; Subcommittee on Plant Health Diagnostic Standards 2013). *H. vitripennis* reproduction varies with environmental conditions. In California, 2 generations per year are observed (Blua, Phillips & Redak 1999); however, under more favourable conditions this can extend to up to 8 generations per year (Grandgirard et al. 2006).

Bucephalagonia xanthophis

Bucephalogonia xanthophis (Berg, 1879) (Hemiptera: Cicadellidae) is a polyphagous sharpshooter that was first discovered to be a vector of *Xylella* during an epidemic of plum leaf scald disease in Brazil in 1991-92 (Hickel, Ducroquet & Leite 2001). It has been reported vectoring *X. f.* subsp. *multiplex* (Kleina et al. 2020) and '*X. f.* subsp. *pauca*' (Esteves et al. 2019).

While entomological survey data is relatively limited, *B. xanthophis* is known to be present in neotropical areas of South America including Argentina, Bolivia, Brazil and Paraguay (Bezerra-

Silva et al. 2012; de Coll et al. 2000; Dellape, Bouvet & Paradell 2013; Marucci, Cavichioli & Zucchi 2002; Paradell et al. 2012; Yamamoto & Paiva 2014; Yamamoto et al. 2002). Climatch v2.0 (ABARES 2020) modelling of temperature and rainfall data (Figure 2.1) predicts *B. xanthophis* has the potential to establish in 93.1% of Australia, spreading in the predominately tropical areas of northern Australia.

B. xanthophis is associated with 17 different plant species across 8 families, including important plant crops such as coffee, citrus, grapes, plum and canola, in addition to weedy and endemic Brazilian plants (de Coll et al. 2000; Hickel, Ducroquet & Leite 2001; Marucci et al. 2003; Paris et al. 2012; Ringenberg et al. 2010). However, as noted for other insect vectors, it is likely that the currently documented host range of this insect underestimates its full feeding potential.

The feeding habits of *B. xanthophis* have made this relatively small sharpshooter an important vector in the spread of *Xylella* in Brazil. *B. xanthophis* is often present in high numbers in the grassy understorey of orchards, native forests surrounding citrus and coffee groves, and to a lesser extent, in the canopy of citrus orchards (Dellape, Bouvet & Paradell 2013; Giustolin et al. 2009; Lopes et al. 2008). While found feeding in all these areas, the insects prefer stems of young shoots of citrus plants (De Miranda et al. 2008; Yamamoto et al. 2002). As with all vectors, the feeding habits of this insect vector not only facilitate the spread of the bacteria but also create a *Xylella* reservoir which can be maintained within native plants and weed hosts in areas surrounding the orchards (Lopes et al. 2003). Specific flying distances are not available; however, species within the subfamily Cicadellinae have highly mobile adults (EFSA Panel on Plant Health et al. 2019b).

2.3.2 Potential insect vectors of *Xylella* in Australia

All the known insect vectors of *Xylella* bacteria are species of the hemipteran sub-order Auchenorrhyncha, and none of these species are currently recorded in Australia. However, the establishment and spread of competent exotic vectors is not a prerequisite for a *Xylella* spp. outbreak, as when the bacteria has been introduced into new regions, local species of xylem-feeding insects are known to have been found to be competent vectors (discussed further in this section).

Australia has 1,489 recorded species of insects within 549 genera in the sub-order Auchenorrhyncha. Australian native insects which primarily feed on xylem sap are known from the families Cercopoidea (40 species), Cicadoidea (310 species) and the Cicadomorpha subfamily Cicadellinae (13 species) (ABRS 2020, 2022a; Fletcher, 2022; Rathe 2012). These xylem-feeding insects are present throughout Australia, with Queensland and New South Wales having the highest representation. High numbers of xylem-feeding insect species in an area is considered to increase the likelihood of at least one species being a suitable insect vector of *Xylella* bacteria (Redak et al. 2004).

Five genera present in Australian (*Kolla*, *Lepyronia*, *Aphrophora*, *Erythroneura*, and *Typhlocyba*) (ABRS 2022a) contain species not present in Australia proven to be vectors (Appendix C). While the known vector species are not present in Australia, the related Australian species may share the ability to vector *Xylella*.

As all recognised *Xylella* insect vectors are exotic to Australia, it is currently impossible to predict transmission efficiencies and the potential roles native insects would play if *Xylella* were

to establish in Australia. Furthermore, although the Australian Auchenorrhyncha fauna is taxonomically well-characterised, information about feeding habits, host range and other attributes of potential importance for the spread of *Xylella* is relatively limited. There is little information recording whether native Australian Auchenorrhyncha feed on commercial plantings such as grapevines, or pecans. It is more likely that these species feed on native Australian plants, of which a number are known to be confirmed natural *Xylella* hosts (discussed in Section 2.4.1).

The EFSA Panel on Plant Health considered all xylem-feeding insects in Europe to be potential vectors of *Xylella* (EFSA Panel on Plant Health 2015b). In 2019, EFSA considered it not possible to predict with any accuracy the likelihood of persistence and multiplication of *Xylella* in any species in the potential insect vector families (EFSA Panel on Plant Health et al. 2019b). It is known that new vectoring insect species have been identified once *Xylella* moves into new regions, and that this has the potential for causing increased disease problems (Cornara et al. 2019). It is considered that Pierce's disease of grapevine in Taiwan originated from bacterial strains in the United States, but its spread occurred by vectors native to Taiwan (Su et al. 2013). Continuing studies, both in Europe (Cavalieri et al. 2019; Desprez-Loustau et al. 2021; Lester et al. 2020) and other regions, (for example the research by Müller et al. (2021) that identified 3 locally occurring sharpshooters as vectors of *Xylella fastidiosa* subsp. *multiplex* in Brazil), aim to identify and strengthen the current understanding and impacts of competent xylem-feeding insect vectors.

The ongoing identification of competent insect vectors across diverse geographical regions, and of different insect species not previously coexisting with the bacterium but which, once introduced to, are able to act as vectors of *Xylella*, indicates that xylem-feeding insects present throughout Australia may demonstrate this same vectoring capability. In addition, important vectors may not only potentially be able to transmit several isolates belonging to different *X. fastidiosa* subspecies (Almeida & Nunney 2015; Nunney et al. 2014a), but will likely display host–plant polyphagy (Andersen, Brodbeck & Mizell 1992; Novotny 1994). This will strongly influence the range of wild and cultivated plant species that will potentially be exposed to *Xylella* within the Australian environment. The extent of this risk with Australian native Auchenorrhyncha is unquantified. However, to date in:

- the European Union, the only xylem-feeding insect identified as being capable of both acquiring and transmitting the bacterium to a new host plant under natural conditions is *Philaenus spumarius* (Desprez-Loustau et al. 2021; EFSA 2020).
- South America the research has established a broader view, with multiple vectors reported to be capable of transmission, with variable efficiency depending on the pathosystem (Lopes 2017).

In Europe the lack of knowledge about available insect vectors and their interactions with host plants hindered the application of effective containment strategies (Cornara et al. 2021), and currently Australia is faced with a similar information gap. Research conducted as a collaboration between Hort Innovation and Wine Australia, through the Plant Biosecurity Research Initiative, is taking a first step in assessing insects in Australia that could act as potential vectors if *Xylella* were to arrive (Hort Innovation 2018). The research is focusing on

insects associated with at-risk crops such as grape, citrus, cherry and olive plantations (Agriculture Victoria 2020).

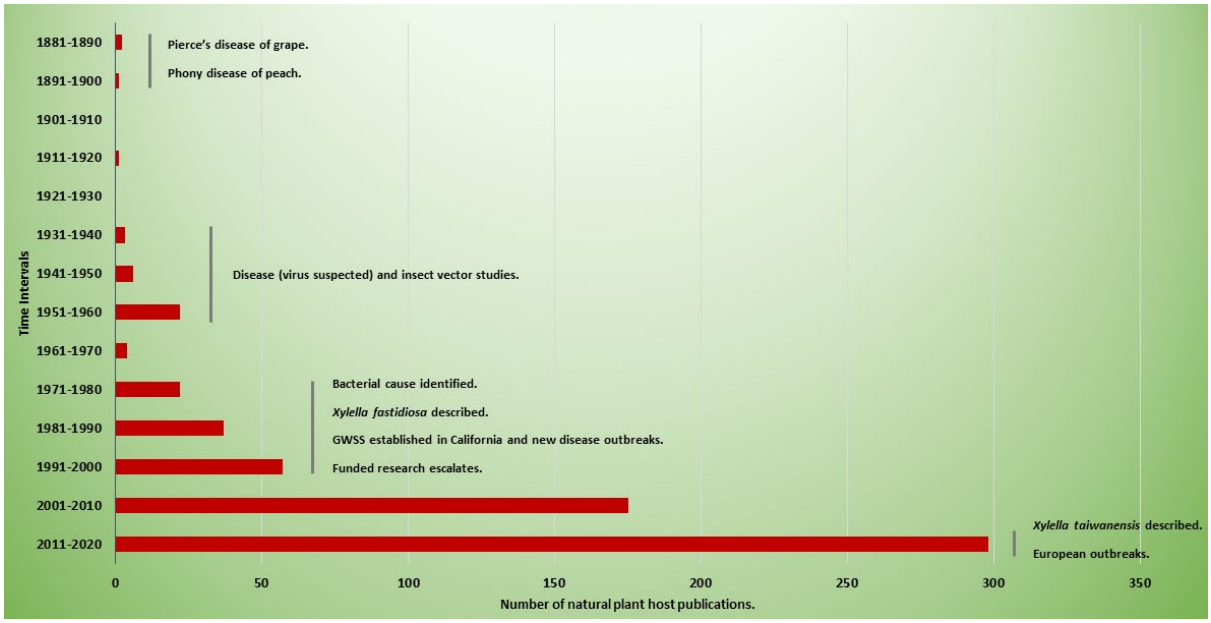
2.4 Plant host range

Xylella has a wide host range, and reports of the number of plant host species can vary. For example, the latest *Xylella* plant host database from EFSA (2022b) gives a total of 664 host plant species from 88 families. Recent *Xylella* publications defer to the EFSA plant host database for the current number of host species, with variability in the number based around the age of the database update being cited (for example: (CABI 2022b; Inspector-General of Biosecurity 2022; Landa et al. 2022). The lower estimate by EPPO (2022) still draws on EFSA plus additional sources, as well as grouping host output as a mixture of family or genus, in addition to species level.

The department assessed published literature and recent host plant lists from Europe (CABI 2022b; European Food Safety Authority 2022), and identified 103 plant families containing member species confirmed to be natural hosts of *Xylella* species. The department identified an additional three plant families which are experimental hosts of the bacteria but have strong associations as prefer food plants of competent vector species. The full host list identified by the department, and associated scientific references, is provided in Appendix D: *Xylella* plant hosts.

One of the difficulties in regulating plants to exclude entry of *Xylella* spp. into a country is the growing number of additional plant hosts continually being identified. Figure 2.2 depicts the number of scientific publications about *Xylella* hosts since 1881, when the disease was described and 2020. This shows an increase in the number of scientific publications about confirmed *Xylella* plant hosts, particularly since the 1980s when *X. fastidiosa* was formally named, and in the 2000s and 2010s in response to technological advancements and global disease spread.

Figure 2.2 Increase in the number of scientific publications about *Xylella* plant hosts, from 1881 to 2020



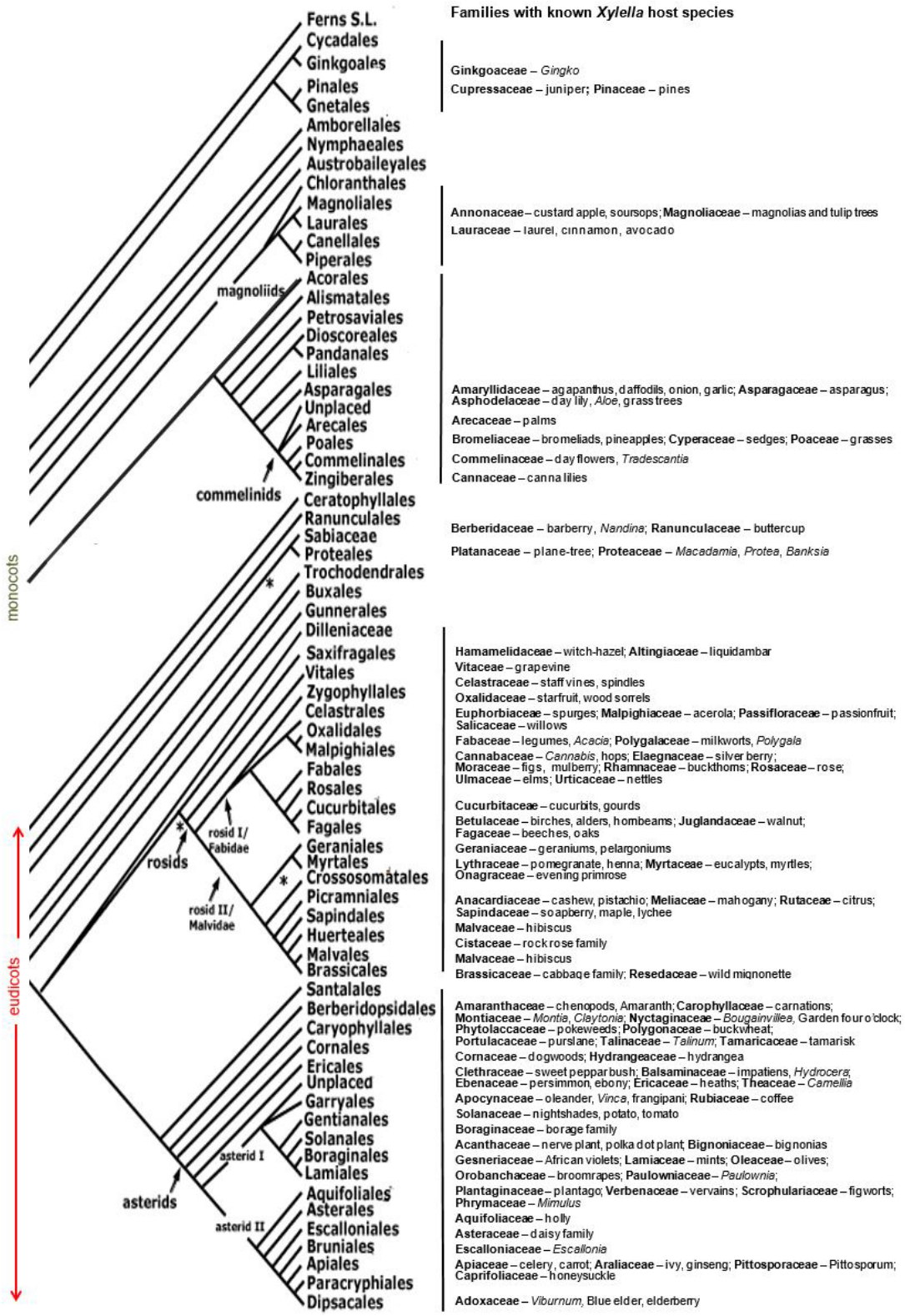
Source: Departmental analysis of scientific publications that report *Xylella* host plants, taken from the host list supplied in Appendix D, and based on detection of *Xylella* within plant tissues (whether disease symptoms were present or absent).
Note: Publications were plotted in 10-year intervals. Key events and research have been aligned with these year intervals.

Angiosperm plants appear to have a broadly-based susceptibility to infection with *X. fastidiosa*. This phenomenon is illustrated in Figure 2.3, by identification of the recorded distribution of plant hosts against an alignment of inferred plant phylogenetic relationships. In addition, numerous species of plants have been identified as being capable of hosting infections with multiple subspecies of *X. fastidiosa*, thus also providing opportunities for genetic recombination (as discussed in Section 2.1), and potential sources of mixed inocula for vector transmission. Hafi et al. (2021) estimated the economic and environmental impacts of one or more subspecies of *X. fastisiosa* entering and establishing in Australia, surmising that all susceptible crop species could be affected by any of the *X.f*. subspecies.

In the face of such a recognised generality of susceptibilities, Australian regulation of *Xylella* through emergency measures has been based on the consideration that where one species in a family is a confirmed natural host, all members of the family are likely to share that susceptibility.

Consistent with this understanding, all species within 89 plant families were regulated by Australia in 2015 under emergency phytosanitary measures to safeguard plant-based industries and the environment from the threat posed by *X. fastidiosa* and related *Xylella* species. Since that time numbers of confirmed hosts have continued to increase, and Australia's extension of emergency measures for *Xylella* to additional plant families is detailed in Section 1.2.3. Currently Australia regulates 106 plant families.

Figure 2.3 Distribution of confirmed *Xylella* hosts across phylogenetic plant groups



Source: Phylogenetic tree adapted from Angiosperm Phylogeny Website (Stevens 2020). Selection of families with confirmed *Xylella* host species taken from departmental analysis of plant hosts (Appendix D).

2.4.1 *Xylella* and potential Australian native plant susceptibility

There is an increasing awareness of the potential susceptibilities of species of Australian native plants to *X. fastidiosa*. Use of these species for amenity and garden plantings overseas has resulted in their exposure to both *X. fastidiosa* and its vectors.

From field and laboratory studies conducted overseas, Australian native species have been identified as among the confirmed natural plant hosts for the *Xylella* pathogen. Amongst those are some species iconic in the Australian landscape, including *Acacia saligna, Dodonaea viscosa, Westringia fruticosa* (Saponari et al. 2019), and *Eucalyptus globulus* (Rost, Matthews & Chatelet 2007). Table 2.1 presents information on the confirmed native Australian natural host plant species of *Xylella* spp.

Table 2.1 Australian native plants confirmed as natural hosts of *Xylella* spp.

Family	Australian native plant hosts
Asparagaceae	*Cordyline* spp.
Asphodelaceae	*Phormium tenax*
Cucurbitaceae	*Diplocyclos palmatus*
Cyperaceae	*Gahnia* spp.
Euphorbiaceae	*Mallotus paniculatus*
Fabaceae	*Acacia cultriformis*
	Acacia dealbata
	Acacia longifolia
	Acacia saligna
	Acacia melanoxylon
	Indigofera hirsuta
Lamiaceae	*Westringia fruticosa*
	Westringia glabra
Myrtaceae	*Callistemon citrina*
	Eucalyptus camaldulensis
	Eucalyptus globulus
	Eucalyptus spp.
	Eugenia myrtifolia
	Leptospermum laevigatum
Pittosporaceae	*Pittosporum undulatum*
Proteaceae	*Grevillea juniperina*
	Macadamia spp.
Rhamnaceae	*Pomaderris prunifolia*
Rubiaceae	*Coprosma baueri*
Sapindaceae	*Dodonaea viscosa*
Scrophulariaceae	*Eremophila maculata*
	Myoporum insulare
Violaceae	*Melicytus ramiflorus*

Source: More information is provided in Appendix D.
Note: When a species has not been identified, all species within a genus are broadly considered.

A limited body of work has been conducted on the experimental susceptibility of Australian native plant species to *Xylella*. For example, Rathé et al. (2012) inoculated 12 different Australian native plant species with *X. fastidiosa* and found that *Hakea petiolaris, Grevillea alpina, Leptospermum laevigatum*, and *Swainsona galegifolia* could be experimentally infected. This same research did not succeed in infecting *Eremophila maculata*, which has been recorded as a natural host by the European Commission (2019). These differences could be attributed to the subspecies of *Xylella* present and do indicate the potential unpredictability of Australian native plant susceptibility.

Research has also been initiated by New Zealand, studying perennial native New Zealand plantings in California as sentinel plants for infection by *Xylella* (Groenteman et al. 2015). The continuing research, as part of the New Zealand Better Border Biosecurity program to study the association between New Zealand native plants, *Xylella* and vectors of the bacteria (Manaaki Whenua Landcare Research 2021), is showing an increasing number of New Zealand native sentinel plant species infected with *Xylella*; however, not all infected plants present disease symptoms (Manaaki Whenua Landcare Research 2021). A number of the plant species being investigated are also native to Australia.

Symptoms of *Xylella* infection in Australian native plants include minor foliar leaf scorch of *Hakea petiolaris* through to major defoliation and leaf discolouration in *Swainsona galegifolia* (Rathé et al. 2012), extensive chlorosis and desiccation of leaves of *Westringia fruticosa* (Saponari et al. 2014a) and death of *Acacia saligna* (CNR-Institute for Sustainable Plant Protection 2017). Figure 2.4 provides some photos of Australian native plants affected by *Xylella* overseas. For example, *Acacia saligna*, planted in Apulia (Italy) as an amenity tree, has been shown to be heavily impacted by *Xylella* bacteria. *Grevillea juniperina*, a threatened species in NSW (NSW OEH 2019), and a confirmed host of '*X. f.* subsp. *pauca'* (Boscia 2016) is seriously affected by *Xylella*. '*X. f.* subsp. *pauca'* also causes extensive chlorosis and desiccation of leaves in *Westringia fruticosa*, which is commonly planted in gardens and public places in the eastern states of Australia (Saponari et al. 2014a).

In addition, certain related native plant species, while not known to be hosts of the bacterium, are known to be preferred hosts of known *Xylella* vector species overseas. Rathe et al. (2014) noted that *Acacia cowleana*, *Eucalyptus divaricata*, *Hakea laurina*, *Leptospermum laevigatum* and *Swainsona galegifolia* are among the Australian native plant species identified as being capable of sustaining all life stages of the insect vector *Homalodisca vitripennis*. This situation would provide a feeding and breeding resource for this exotic vector (should it become established in Australia) and accentuate greater environmental consequences from *Xylella* within the Australian environment.

Figure 2.4 *Xylella*-induced disease in Australian native species in Europe

Source: CNR-Institute for Sustainable Plant Protection (2017).
Notes: (a) *Acacia saligna* showing symptoms in August 2014, (b) advanced rapid decline in March 2016, (c) *Westringia fruticosa*; (d) and (e) *Grevillea juniperina* showing symptoms in Apulia 03-01-17 (f) *Dodonaea viscosa purpurea*.

A lack of evidence currently exists across several parameters relating to the susceptibility of Australian native plant species to *Xylella*. These parameters include the level of susceptibility of individual native plant species and cultivars to infection from the bacterium, as few studies on the susceptibility of Australian native plant species have yet been conducted. While much work has been conducted on the threat of *Xylella* to agriculture and horticulture, comparatively little is known of its impacts in native forests and plantations. Desprez-Loustau et al. (2021) state that no serious impacts of *Xylella* presence have been reported in forests in their native range, but also that the impact cannot be predicted from what is known in other areas, for example the devastating impact on olives in Italy compared to minimal impact on olive trees in California.

As the impact and spread of *Xylella* is so dependent on vectors, these vector-related factors encompass other parameters where information is lacking. For example, studies on the ability of Australian native insects to acquire and transmit *Xylella* are underway (documented in the National *Xylella* Action Plan 2019-2029 and updated in the implementation summary (Department of Agriculture 2019b) (also discussed in Section 2.3.2).

Information is also lacking about the feeding behaviour and host preferences of exotic insect vectors (if they were to establish in Australia), the expanse of suitable climes within the Australian environment to enable persistence of both the bacterium and suitable insect vectors, and the level and intensity of predation towards any such introduced insect vectors. This current lack of evidence means that the extent and magnitude of the predicted Australian native plant host range is unknown. What is known is that new plant hosts of *Xylella* are likely to be identified in native plant flora that have not previously been exposed to the bacteria due to geographic isolation (Groenteman et al. 2015).

2.5 Geographical distribution

2.5.1 Origins

Xylella fastidiosa is considered to be native to the Americas, with each of the unique subspecies having developed in geographical isolation within this region (Nunney et al. 2014c). *Xylella fastidiosa* subsp. *fastidiosa* is considered native to Central America (Nunney et al. 2014c; Nunney et al. 2010), *X. f.* subsp. *multiplex* native to temperate or subtropical North America (Nunney et al. 2010), and '*X. f.* subsp. *pauca'* native to South America (Nunney et al. 2014c; Nunney et al. 2010).

Schuenzel et al. (2005) proposed that *X. f.* subsp. *fastidiosa* ('*sandyi'*) evolved in North America; however, the close genetic association of the genotype with *X. f.* subsp. *fastidiosa* makes an origin of Central America more likely (Nunney et al. 2014c). The ancestry of *X. f.* subsp. *fastidiosa* ('*morus*') is believed to be mixed, with its genomic sequence partly derived from *X. f.* subsp. *fastidiosa* (native to Central America) and partly from *X. f.* subsp. *multiplex* (native to the USA), potentially as a result of inter-subspecific homologous recombination (Nunney et al. 2014c). The lack of genetic diversity within the genomic sequence of *X. f.* subsp. *fastidiosa* ('*morus*') suggests it may be a relatively recently evolved subspecies, consistent with the first reportings of its associated disease in mulberries in the 1980s (Kostka et al. 1986). *Xylella* f. subsp. *fastidiosa* ('*tashke'*) is believed to be native to south-western USA (New Mexico, Arizona and California), where it was isolated from the ornamental landscape plant *Chitalpa tashkentensis* (Randall et al. 2009).

The more recently described *X. taiwanensis* Su et al. (2016), associated with pear leaf scorch disease since the 1990s, has Asian origins, and to date has only been reported infecting *Pyrus pyrifolia* in Taiwan (Su et al. 2016).

2.5.2 Spread of *Xylella*

The first reports during the late 1800s of outbreaks of disease caused by an unknown pathogen described severely affected orchards of peach (*Prunus persica*) in Georgia, USA and exotic European grape (*Vitis vinifera*) vineyards in California (Janse & Obradovic 2010). Pierce (1892), during his investigations of this 'California vine disease', postulated the agent of the disease to be of bacterial nature, but during the following decades the causal agent continued to be accepted as a virus (Esau 1948; Hewitt 1958; Winkler 1949). Confirmation that a bacterial agent was the cause of the disease in grapes occurred in 1978 (Davis, Purcell & Thompson 1978); 9 years later, Wells et al. (1987) described the genus *Xylella* and assigned the species name *Xylella fastidiosa* to the organism.

Records of the spread of *Xylella* species became more frequent with expanding knowledge of the pathogen. Janse and Obradovic (2010) provided an historical summary of disease occurrence and spread of *Xylella* in the Americas before the European epidemics. Table 2.2 provides a timeline of first recognition of *Xylella*-induced diseases by country or region from 1890 to 2022 and their current recognised status. This information is presented in a different format in Appendix D, where the plant host, geographic location, and reference is provided.

Table 2.2 Timeline of global recognition of *Xylella* induced disease by country and/or region

Year	Location	Disease	Status at 30 October 2022[1]
1882	USA (California)	Pierce's disease	Present
1975	Argentina	Plum leaf scald, almond leaf scald	Present
1978	Brazil	Plum leaf scald	Present
1978	Paraguay	Plum leaf scald	Present
1980	Costa Rica	Pierce's disease	Present
1980	Mexico	Pierce's disease	Present
1985	Venezuela	Pierce's disease	Present
1987	India	Almond leaf scorch	Unreliable record
1992	Canada	Bacterial leaf scorch	Present
1993	Taiwan	Pear leaf scorch	Present
1998	Uruguay	Citrus variegated chlorosis	Present
1998	Kosovo (former Serbia, Yugoslavia)	Pierce's disease	Invalid record
2001	China	Pierce's disease	Eradicated[2]
2005	Türkiye (formerly referred to as Turkey)	Almond leaf scorch	Invalid record

2013	Italy	Olive quick decline	Present
2013	Iran	Almond leaf scorch	Present
2015	Corsica, France	Bacterial leaf scorch	Present
2015	Puerto Rico	Coffee leaf scorch	Present
2016	France, mainland	Bacterial leaf scorch	Present
2016	Balearic Islands, Spain	Bacterial leaf scorch	Present
2017	Spain, mainland	Almond leaf scorch	Present
2019	Portugal	Bacterial leaf scorch	Present
2019	Israel	Almond leaf scorch	Present
2022	Lebanon	Almond leaf scorch	Invalid record

Source: See Appendix D for *Xylella* host list and associated references.
Note: **1**. Status at 30 October 2022 taken from EPPO Global Database (gd.eppo.int/taxon/XYLEFA/distribution).
2: EPPO Global database does not mention. Reference taken from European Commission webpage for country declarations (food.ec.europa.eu/plants/plant-health-and-biosecurity/legislation/control-measures/xylella-fastidiosa/declarations-xylella-fastidiosa_en)

The Americas have experienced disease outbreaks from genotypes that were once isolated. For example, a single genotype of *X. f.* subsp. *fastidiosa* introduced into the USA from Central America is believed to be the cause of disease outbreaks in Mexico and North America (Nunney et al. 2014b; Nunney et al. 2010). Other studies propose that '*X. f.* subsp. *pauca*' was introduced from South America into Central America (Coletta-Filho et al. 2017; Nunney et al. 2012), and that *X. f.* subsp. *multiplex* moved from North America into South America (Coletta-Filho et al. 2017; Nunney et al. 2012).

Important changes in the geographical distribution of *Xylella* include the bacterias' entry into Europe and Asia (EPPO, 2016b), which conclusively illustrate that *Xylella*-induced disease is a serious global biosecurity concern. The spread of *Xylella*-induced leaf scorch diseases from the Americas to Europe via the movement of nursery stock had devastating results. First reports of a major outbreak of '*X. f.* subsp. *pauca*' in Europe were in olive groves from Apulia, Italy during October 2013 (Saponari et al. 2013). The disease severely affected olives causing rapid decline and death within 2 years of infection and became known as Olive Quick Decline Syndrome (OQDS). Surveys of the early spatial distribution pattern of disease in olive trees suggested that spread was occurring with the aid of an insect vector, which was later identified as the meadow spittlebug (*Philaenus spumarius*) (Elbeaino et al. 2014; Martelli 2014; Saponari et al. 2014b; White et al. 2017). The disease subsequently spread through the majority of olive trees in the Lecce province (EFSA Panel on Plant Health 2015b), with up to one million olive trees over approximately 10,000 hectares affected. The substantial damage to olive production and the impact on the local economy heightened concerns about *X. fastidiosa* in other olive producing nations around the Mediterranean (Abbott 2016; Bleve et al. 2016; Bosso et al. 2016).

The emergence of bacterial leaf scorch in myrtle plants on the island of Corsica, France in 2015 (RSI 2015) (Appendix D) and in cherry and oleander in the Balearic Islands, Spain in 2016 (Administración de la Comunidad Autónoma & Consejería de Medio Ambiente 2017; Denance et

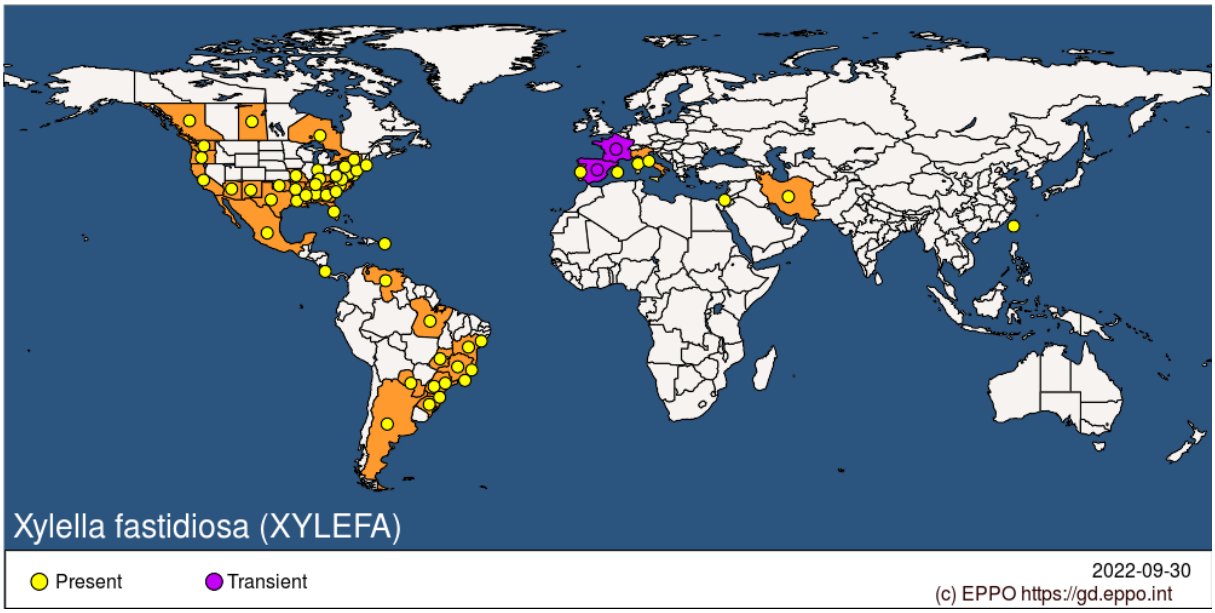
al. ; Olmo et al. 2017) (Appendix D) confirmed that *X. fastidiosa* had established in those areas (POnTE 2017). Iran also confirmed the presence of *X. fastidiosa* in samples of grapes and almonds from 7 provinces in 2011–2012 (Amanifar et al. 2014).

In 2001, Pierce's disease was identified in Shaanxi, Shanxi and Hebei Provinces in mainland China, notably in Californian red globe grape cultivars imported from the USA (Chu 2001, 2002). Area freedom for *Xylella* was later declared by China in 2015 (Li 2015). This declaration was still maintained in 2021 (Department of Animal and Plant Quarantine 2021).

Leaf scorching in grapes, observed in 2002 across the major grape production fields of central Taiwan, was also confirmed as Pierce's disease caused by *X. f.* subsp. *fastidiosa* (Su et al. 2013). In contrast, in 1993 the symptoms of leaf scorch on pear trees in low altitude areas in Taiwan was found to be caused by a novel species, *X. taiwanensis* (Su et al. 2016). The status of *X. taiwanensis* in neighbouring Asian countries is presently unknown.

As illustrated in Figure 2.5, the current accepted global distribution of *Xylella fastidiosa* includes the Americas, Europe, the Middle East and Asia (EPPO 2022).

Figure 2.5 Current global distribution of *Xylella fastidiosa*



Source: EPPO (2022)
Note: Both *Xylella fastidiosa* and *X. taiwanensis* are present in Taiwan (Su et al. 2013; Su et al. 2016).

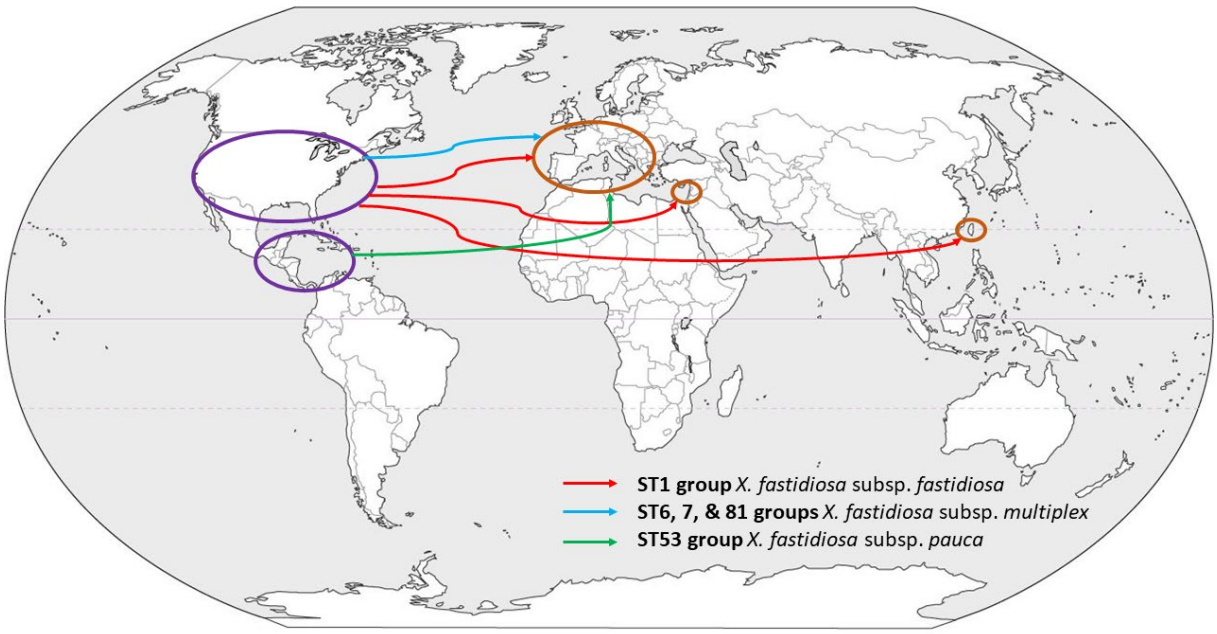
2.5.3 Interceptions and unconfirmed reports

Interception data from Europe have revealed the movement of *Xylella* species in imported nursery stock (Simpson 2016) (Appendix D also presents this information). Interceptions of *Xylella* by the European Union and Switzerland have been reported from coffee plants (EUROPHYT 2014), *Mandevilla sanderi* (EUROPHYT 2015), *Pelargonium* x *hortorum* (EUROPHYT 2016), walnut from California (EUROPHYT 2017), and blackberry (EUROPHYT 2018b) and raspberry (EUROPHYT 2018a) from the USA. Interceptions have also been reported in coffee plants infected with *Xylella* spp. by France (Legendre et al. 2017; Legendre et al. 2014), Italy (EPPO 2020; Giampetruzzi et al. 2015), Germany (EPPO 2020), the United Kingdom (Forest Research 2022) and Switzerland (EPPO 2020).

Imported raspberry root material containing *Xylella* was intercepted by the European Union from a USA region declared as a pest free area (EFSA PLH Panel et al. 2018), and infected *Polygala myrtifolia* was found in a nursery in Almeria, Spain (Monago 2018). A notable interception of *Xylella* in 1,500 walnut plants from California intended for planting in Spain (EUROPHYT 2017) raised serious concerns for Australia regarding the ongoing import of infected nursery stock from high risk *Xylella* countries/regions. Introduction of hosts with asymptomatic infections is of particular biosecurity concern for Australia with respect to nursery stock.

The EFSA PLH Panel et al. (2018) used genomic sequence types (ST) to plot the global occurrence of *X. f.* subsp. *multiplex* and outbreaks of subspecies of *X. fastidiosa* across Europe. Mapping movement of some representative genotypes identified as sequence types in grape (*X. f.* subsp. *fastidiosa* ST1), almond and olive (*X. f.* subsp. *multiplex* ST6 and ST7), and coffee and olive (‘*X. f.* subsp. *pauca’* ST53) linked the incidence of intercepted plants and new areas of *Xylella* infection to origins (Figure 2.6).

Figure 2.6 Global movement of selected *Xylella fastidiosa* genotypes with nursery stock



Source: (EFSA 2022a).
Note: Based on *Xylella fastidiosa* Multi Locus Sequence Typing (MLST) database Nunney, Stouthamer and Bromley (2020) and EFSA (2022a). The movement of imported nursery stock has allowed the spread of *X. f.* subsp. *fastidiosa* (ST1), *X. f.* subsp. *multiplex* (ST6 and ST7), and ‘*X. f.* subsp. *pauca’* (ST53) from the Americas into new territory, resulting in disease outbreaks.

There are several countries where the presence of *Xylella* could be considered uncertain, and Australia’s emergency measures include these as high risk countries for the purpose of nursery stock trade. These countries are:

- India—Jindal and Sharma (1987) published on the first detection of almond leaf scorch symptoms in almond orchards in Solan, in the state of Himachal Pradesh and stated this was caused by a ‘xylem limited fastidious walled bacteria’ (this report occurred prior to the naming of *X. fastidiosa* by Wells in 1987). Several publications after this date have repeated this

information (Gupta & Sharma 1998; Jindal & Sharma 1987; Verma & Sharma 1999). EPPO considers this an unreliable record, as the identification was not conducted using modern PCR methods (EPPO 2021)

- Lebanon—Temsah, Hanna and Saad (2015) published a first report of *X. fastidiosa* causing oleander leaf scorch in Beirut. Habib et al. (2016) reported results of general surveillance and re testing of the same oleander plants sampled in 2015 and stated that all samples were free from *X. fastidiosa*. In 2018, surveillance of likely host plants in Lebanon was also reported to find no presence of *X. fastidiosa* (Kubaa et al. 2019). EPPO considers that *Xylella* is absent from Lebanon, on the basis that the 2015 testing produced false positives (Choueiri 2017; EPPO 2021; Habib et al. 2016; Temsah, Hanna & Saad 2015). On 29 August 2022, Lebanon published an IPPC pest report notification stating that *X. f. fastidiosa* has been detected in multiple hosts in the south of the country, and that eradication and further surveys were being undertaken. This notice was subsequently removed from the IPPC's pest reports website (www.ippc.int/en/countries/lebanon/pestreports/) with no explanation.

- Türkiye—Güldür et al. (2005) published a first report of almond leaf scorch caused by *X. fastidiosa* in southern Türkiye. Türkiye's NPPO has attested that those results are not confirmed and that annual surveys for *Xylella* conducted since 2014 have not detected *Xylella* (General Directorate of Food and Control 2016, 2020). EPPO also considers that *Xylella* is absent from Türkiye (EPPO 2021).

- Kosovo—Berisha et al. (1998) published a report of *X. fastidiosa* being isolated from grapevine material in a region near the Albanian border. EPPO states that the authors did not confirm the reports, and that this record is invalid (EPPO 2021).

The department will write to the respective NPPOs to request clarification about the status of *Xylella* spp. in their countries and assess this information before considering changing the current Australian high risk country/region list. Included in this group is the United Kingdom, as up until January 2020, the United Kingdom was part of the trading bloc that is the European Union, and unregulated nursery stock movements occurred between those bloc countries.

2.6 Potential transmission pathways

The main potential pathway for introduction of *Xylella* spp. into new regions is considered to be the movement of infected plant material through trade, particularly of plants intended for planting, such as nursery stock (EFSA 2013). Other identified potential pathways for introduction of *Xylella* spp. are infected xylem-feeding insect vectors, seeds, fruit, and cut flowers and foliage. The following section discusses these pathways and their applicability in the Australian context. The department will continue to monitor new evidence and reserves the right to broaden *Xylella* risk management measures to additional pathways as required.

2.6.1 Nursery stock (exclusive of tissue culture)

Nursery stock has been recognised as one of the 2 major risk pathways for introduction of *X. fastidiosa*, through transport of infected plant material into geographic areas with vector species present; the second risk pathway is introduction of vector species carrying the pathogen (EFSA Panel on Plant Health 2015b).

Xylella bacteria inhabit xylem tissue of infected plants (de Mello Varani et al. 2008; Meng et al. 2005), allowing its survival in living plant material during transport and storage (Jacques et al. 2016; Martelli 2016), and movement of infected planting material contributes to disease spread over long distances and internationally (Almeida & Nunney 2015; CABI 2020; EFSA PLH Panel et al. 2019).

Lòpez-Fernàndez et al. (2017) expressed concerns about movements of large numbers of untested *Xylella* host plants, from countries where *Xylella* bacteria is known to be present (especially into EU countries), particularly as long latency or delayed disease expression of *Xylella*-induced disease can occur. As an example, Lòpez-Fernàndez et al. (2017) noted that more than 35,000 t of potted plants were imported yearly into the European Union for the period 2010–2014 from the known high risk countries of Costa Rica, Guatemala and Honduras. Pathways mapping movement of infected plants to areas of new disease outbreaks have been determined using molecular techniques and specific genomic profiles (Coletta-Filho et al. 2017; EFSA 2018) (Figure 2.6) and detected incidences in imported nursery stock (Appendix D).

Large volumes of nursery stock are imported into Australia for industry and private use, and Australia's emergency measures require plants from the 106 host plant families and from high risk countries/regions to be hot water treated or enter government PEQ for specific *Xylella* testing. To date there have been no detections of *Xylella* spp. on any of this material.

The form the plant takes in growing is also not a determinant of *Xylella* host status. For example, some plants that form true bulbs in the botanical sense (a short stem with fleshy leaves or leaf bases that function as food storage organs during dormancy) are confirmed natural *Xylella* hosts (such as *Allium*) whereas other genera of bulb forming plants such as *Narcissus*, *Hippeastrum* and *Tulipa* are not known to be *Xylella* hosts (see Appendix D for details and references). The ability of the bulb to become dormant does not remove the risk of *Xylella* bacteria being present in xylem tissue during dormancy.

2.6.2 Plant tissue culture

Plant tissue culture, as a distinct form of propagative material, is discussed in this section as there are uncertainties about whether *Xylella* can be transmitted through this pathway.

Plant tissue culture is a broad term that covers numerous systems and approaches, all with a number of commonalities. The term applies to the rearing of any part of a plant (from cells, tissues, and organs, up to whole plants) in an artificial media of defined physical and chemical conditions, in aseptic conditions, and under controlled environments (Loyola-Vargas & Ochoa-Alejo 2018; Phillips & Garda 2019; Thorpe 2007). The process of tissue culturing is used for multiple purposes, including ongoing, clonal multiplication of plant material, to bypass natural barriers that prevent plant survival, or as a means to rapidly generate genetic variability for various breeding outcomes. The source of cell material with which to commence cultures is dependent on characteristics or limitations of the plant species (Loyola-Vargas & Ochoa-Alejo 2018), and multiple methods of multiplication are available once a plant species is established in culture.

Plant tissue culture is also used to rapidly propagate plants in bulk, and the majority of nursery stock imported into Australia comes in the form of tissue culture (Inspector-General of

Biosecurity 2022). For example, in 2021, 87% of all nursery stock consignments imported into Australia were classed as tissue culture.

Plant tissue culture is frequently used in the plant biosecurity environment as a technique to reduce the risk of introduction of pests of biosecurity concern, including those organisms associated with soil. However, to date, there are uncertainties as to whether *Xylella* can be transmitted through tissue culture. What is known is that:

- In the 2013 pest risk assessment for importation of grapevine (*Vitis* species) propagative material into Australia (DAFF 2013), the department assessed that tissue cultured *Vitis* spp. was a pathway for entry for *Xylella fastidiosa* and that imported tissue culture must be subject to mandatory on-arrival inspection for *X. fastidiosa*, mandatory growth for a minimum of 12 months in a government PEQ facility and mandatory PCR testing for *X. fastidiosa* before release from biosecurity control.
- Plant tissue culture may be considered by some as an aseptic micropropagative technique for mass production of clean, disease-free plants. However, it is scientifically recognised (Kalużna et al. 2013; Leifert & Cassells 2001; Orlikowska, Nowak & Reed 2017; Reed & Tanprasert 1995) that microbial contamination, including by bacteria (Thomas 2010), of plant tissue culture can occur.
- While evidence for transmission of *Xylella* through tissue culture is currently lacking, there is evidence for transmission of other xanthomonads, the same taxonomic family as *Xylella*, via tissue culture through infected mother plants. For example, Norman and Alvarez (1994) provided evidence of transmission by tissue culture of *Xanthomonas campestris pv. dieffenbachiae* (now *X. phaseoli* pv. *dieffenbachiae* (Cottyn, Constantin & Maes 2018)) through asymptomatically infected *Anthurium andraeanum* plantlets.
- An example of another xylem-limited fastidious bacteria, *Leifsonia xyli* subsp. *xyli* (Lxx), is also a worthwhile comparison. Similar to *Xylella*, Lxx is difficult to culture and to detect, but unlike *Xylella*, Lxx also colonises parenchyma and leaf bundle sheath cells, is not insect vectored, and has only sugarcane as a host (Garcia et al. 2021). Meristem tissue culture is increasingly used in sugarcane production as a means of disease control, and with Lxx management, resistance breeding has been deemed ineffective, leaving clean planting material as the preferred option. With the use of meristem culture, fields planted in the USA had greatly reduced disease incidence, which is suitable as a management strategy, but they were not free of disease (Bhuiyan, Eglinton & Magarey 2021), indicating a lack of suitability as a sole biosecurity solution.
- Certain types of tissue culturing may have lower pathogen risks. For example, sourcing cells from the meristem of plants, known as meristem culture, is a known technique for excluding certain plant pathogens (notably viruses and viroids) from plant material. *Xylella* bacteria are xylem-limited, and the plant vascular tissues do not extend into the shoot apical tissue (the meristem) (Thorpe 2007). However, vasculature extends into the base of the leaf/flower primordia (Bradamante, Mittelsten Scheid & Incarbone 2021), which are included in the excised section of some forms of meristem culture.
- Tissue cultured material may also appear pathogen free as pathogen levels may be below the threshold for detection in *in vitro* material (Cassells 2011).
- Some bacteria, including xanthomonads, are not strongly visually expressed on specific plant media (Leifert & Cassells 2001), and therefore may escape detection during screening processes. For example, during isolation studies of *Xylella fastidiosa* from symptomatic leaf samples of pistachio plants, Amanifar, Babaei and Mohammadi (2019) reported that even when using a specifically formulated culture medium for *Xylella*, bacterial colonies of *Xylella* required microscopic technique and were not observed until 10 to 17 days after plating.

Gerlin et al. (2020) also emphasised the slow growth rate of *Xylella*. Other pathogenic agents may be present at the same time in samples and may hinder the detection of *Xylella* (EFSA Panel on Plant Health 2015b).

This evidence of the presence of various bacteria within micropropagated plant material supports the assessment that *Xylella*, which is known to be irregularly distributed within plant stems, petioles and leaves, with seasonal variability also observed (Hopkins 1981), has the potential to be inadvertently transferred from the parent plant with the tissue culture propagule.

Additionally, in the pest risk assessment for *Xylella fastidiosa* conducted by the EFSA (EFSA Panel on Plant Health 2015b) for plants for planting, the Panel noted that, in the absence of scientific data on *in vitro* plants as a pathway for *X. fastidiosa*:

– *"in vitro* plants, unless originating from countries with appropriate certification schemes, present similar risk to other plants for planting. The bacterium grows in the xylem and is difficult to cultivate in artificial media; thus, it could easily pass undetected through the *in vitro* production processes."

The recent Inspector-General of Biosecurity report (Inspector-General of Biosecurity 2022), detailed the effectiveness of preventative biosecurity arrangements to mitigate the risk of entry into Australia of *Xylella fastidiosa*. It also acknowledged the critical gap in international knowledge about *Xylella* spp. potentially transported via tissue culture, and that this gap in knowledge may contribute to viability of tissue culture as a pathway for entry of this pest into Australia via plant propagative material. The Inspector-General of Biosecurity report noted that the cryptic characteristics of *Xylella* spp. mean that onshore monitoring of imported plant hosts for *Xylella fastidiosa* infection is necessary, and that routine monitoring of imported *Xylella* spp. host materials using PCR testing is essential to check the effectiveness of offshore risk mitigation steps.

Departmental processes in place through the *Xylella* emergency measures differentiate between tissue cultures of plant species belonging to the 106 plant families regulated as *Xylella* hosts and the origin country or region of these. If host plant tissue cultures are:

- from high *Xylella* risk countries/regions, a phytosanitary certificate is required with the additional declaration that all tissue cultures of the consignment were derived from mother tissue cultures that were tested by PCR and found free of *Xylella fastidiosa* as indicated on a laboratory test report

- from low *Xylella* risk countries/regions, a phytosanitary certificate is required with the additional declaration that the tissue cultures in the consignment were derived from plants and tissue cultures that were grown only in 'name of country', which is free from *Xylella fastidiosa*.

2.6.3 Vectors

All known *Xylella* vectors are from the hemipteran sub-order Auchenorrhyncha, which contains xylem-feeding leafhoppers, sharpshooters, tree hoppers and cicadas. Within this sub-order, *Xylella* vectors are found across numerous families and/or superfamilies including the Aphrophoridae, Cicadellidae, Cercopoidea, Membracoidea, Clastopteridae and Cicadidae (EFSA

Panel on Plant Health et al. 2019b) (references for individual associations are provided in Appendix C).

Infected xylem-feeding insect vectors from the subfamily Cicadellinae (sharpshooters) and the superfamily Cercopoidea (spittlebugs) represent an identified potential pathway for the introduction of *Xylella* bacteria into new geographic regions (EFSA 2013). Natural dispersal of *Xylella*-infected insect vectors is possible on a local scale; however, in the absence of human intervention, long-distance dispersal of infected insects is very unlikely (EFSA 2013). EFSA Panel on Plant Health et al. (2019b) considered that the entry of infected vectors into Europe was moderately likely because of the association of the insect with nursery stock pathways. There is also a documented example of a vector being introduced via air travel—*Homalodisca vitripennis* has established on a number of islands in the Pacific region. Air travel from Tahiti subsequently introduced *H. vitripennis* to Easter Island, and these insects have also been intercepted on planes in Japan as well as in Cairns, Australia (Rathe 2012).

Australia's standard import conditions for the nursery stock pathway includes a mandatory treatment to manage arthropod risks (non-tissue culture only) (discussed in Section 1.2.3), so the entry of an infected vector via this pathway is unlikely.

None of the known *Xylella* vector insects overseas are present in Australia, but some of these species occasionally arrive in Australia as contaminating pests on or in shipping containers (Stanaway et al. 2001) and infrequently on cut flower consignments and nursery stock. Departmental analysis of records of the interceptions of insects within the taxonomic groups mentioned above found 25 detections on nursery stock, with only 8 of those thought to be associated with nursery stock exported from a *Xylella* country/region over a 15-year period (2000 to 2015). These data do not record whether the insects were vectoring *Xylella*, however these interceptions are recorded prior to nursery stock undergoing a mandatory treatment to manage arthropods. In addition, more recent analysis by the department conducted for the Final Pest Risk Analysis for Cut Flower and Foliage Imports-Part 2 found that only one known competent vector species (the meadow spittlebug *Philaenus spumarius*) was intercepted on the cut flower and foliage pathway in the 20-year period between 2000 and 2019 (DAWE 2021). The risk of vector entry into Australia, although confirmed, is considered to be low (Rathé et al. 2015; Stanaway et al. 2001), and departmental analysis of insect interceptions confirms this assessment.

2.6.4 Seed

Historically, few studies have focussed on transmission of *Xylella* through seeds, and of those studies conducted, the conclusions have been inconsistent.

Cross-generational transfer, or vertical transmission, of bacteria via seed is recognised (Darrasse et al. 2007; Frank, Guzman & Shay 2017; Shahzad et al. 2018). Internal infection of seed by bacteria from the parent plant is considered to occur either via movement of the bacteria through the vascular system (Compant et al. 2011), via the shoot apical meristem that differentiates into the reproductive organs (Frank, Guzman & Shay 2017) or via pollen (Frank, Guzman & Shay 2017) and the floral pathway (Compant et al. 2011; Darrasse et al. 2007; Frank, Guzman & Shay 2017). Darrasse et al. (2007) reported that the bacterial population size associated with the seed influences successful vertical transmission of bacteria to the seedling.

Laranjeira, Pompeu and Palazzo (2000) found no seed transmission of '*X. f.* subsp. *pauca'* in orange. However Li et al. (2003) reported transmission of *Xylella* through seeds to seedlings of sweet orange. More recent studies with citrus supported the conclusion that *X. fastidiosa* is not seed-transmissible (Coletta-Filho et al. 2014; Cordeiro et al. 2014; Hartung et al. 2014), even from orange fruits displaying typical symptoms of *Xylella* infection (Cordeiro et al. 2014). EFSA Panel on Plant Health (2015b) considered the *Xylella* transmission pathway on seeds as unlikely, with high uncertainty related to the lack of extensive studies. It is suggested that annual or biennial plant species have a lower risk for vertical transmission of *Xylella* through infected seed in comparison to perennials because of a shorter life cycle and therefore lower chance of encountering an insect vector (EFSA 2015). No evidence has been found for vertical seed transmission of '*X. f.* subsp. *pauca'* in olive (Altamura et al. 2019).

Pecan

Carya illinoinensis (pecan) was first reported as a *Xylella* host in 1998 (Sanderlin 1998), and widely since that time in the USA (Alabama, Arizona, California, Georgia, Indiana, Louisiana, Mississippi, New Mexico, North Carolina & Texas). Some pecan cultivars produce strong disease symptoms while other cultivars are susceptible or somewhat tolerant (there being no resistant cultivars) to *Xylella* strains from the subspecies *multiplex*. Pecan trees can lose limbs to dieback caused by *Xylella* and susceptible cultivars slowly decline but are eventually removed before complete death (Bock et al. 2018; Sanderlin 1998). Hilton (2017) found that 9 *Carya* species in addition to pecan and hybrids in the National Collection of Genetic Resources for Pecans and Hickories were infected with *Xylella*.

In 2016, the presence of *Xylella* in seeds from multiple cultivars of *Carya illinoinensis* was identified (Cervantes et al. 2016), with more recent confirmation of vertical transmission of *Xylella* from pecan seed to the germinated seedling (Cervantes et al. 2022). In response to these findings, the department extended emergency measures against *Xylella fastidiosa* and related *Xylella* species to *Carya* spp. seeds for sowing, effective 20 May 2022 (also discussed in Section 1.2.3). This introduced a mandatory requirement that imported *Carya* spp. seeds for sowing must be grown for a minimum of 12 months at a government PEQ facility. Before release from biosecurity control, the plants must be tested by PCR and found free from *Xylella* species.

2.6.5 Fruit

Transmission of *Xylella* from infected fruits is deemed unlikely (EFSA Panel on Plant Health 2015b). *Xylella* has been detected in citrus fruit (Li et al. 2003), however no further analysis was conducted and transmission by vectors from infected fruit was not verified. Purcell and Saunders (1995) demonstrated that 2 vectors (*Graphocephala atropunctata* and *Draeculacephala minerva*) were not able to transmit *Xylella* after feeding on infected grape clusters. Previous policy considerations by the department have determined that the risks of table grapes and stone fruit as sources of inoculum for *Xylella* are extremely low (Biosecurity Australia 2009, 2010).

2.6.6 Cut flowers and foliage

This section discusses the risk of *Xylella*-infected plant material being imported as cut flowers and foliage, as distinct from the risk of infected vectors arriving with imported cut flowers and foliage (discussed in Section 2.6.3).

Transmission of *Xylella* through the cut flower and foliage trade is considered unlikely, but with high uncertainty due to a lack of studies, and cut flowers and foliage are not expected to be attractive to xylem fluid feeders (EFSA Panel on Plant Health 2015b). Current Australian import conditions for cut flowers and foliage require a devitalisation treatment of propagable flower and foliage types to minimise the ability to grow any imports that could carry internal pathogens such as *Xylella*. In addition, all consignments are inspected for arthropods and treated if any are found. The department is conducting a risk analysis on the pathogens associated with the imported cut flower and foliage pathway, and this issue will be examined in more detail in that separate work (see the department's website for information www.agriculture.gov.au/biosecurity-trade/policy/risk-analysis/plant/cut-flowers#part-3--bacteria-viruses-and-fungi).

2.7 Diagnosis

2.7.1 Disease symptom expression

A variety of biological and ecological factors have been implicated in the expression of *Xylella*-related disease symptoms. This includes the species, subspecies and genotype identities of the infecting *Xylella*, host-plant interactions and specificities, seasonal and environmental influences (Almeida & Nunney 2015) and the plant's physiological and developmental stage (Garcia et al. 2012). For example, *X. f.* subsp. *fastidiosa* causes Pierce's disease (Californian vine disease, Anaheim disease) in grape (*Vitis vinifera* L.), but also leaf scorch in peach (*Prunus persica* (L.) Batch) and coffee plants (*Coffea* L. spp.) (Legendre et al. 2014; Nunney et al. 2010). Almond leaf scorch in almond (*Prunus dulcis* (Mill.) D.A.Webb) may be caused by either *X. f.* subsp. *fastidiosa* or *X. f.* subsp. *multiplex*, while leaf scorch in olive (*Olea* L.) in California and Spain (Coletta-Filho et al. 2016; EPPO 2017; Krugner, Johnson & Chen 2010) is caused by *X. f.* subsp. *multiplex*.

The devastating disease in olive groves in Apulia, Italy, known as olive quick decline syndrome (OQDS), is caused by '*X. f.* subsp. *pauca*' which also causes citrus variegated chlorosis in citrus throughout South America, and 'crespera' symptoms in coffee plants (EPPO 2016a, b). Oleander street trees (*Nerium oleander* L.) in the US developed oleander leaf scorch when infected with *X. f.* subsp. *fastidiosa* ('*sandyi*'), while disease in mulberry (*Morus* L. spp.) was reported to be caused by *X. f.* subsp. *fastidiosa* ('*morus*') (Nunney et al. 2014c). However, similar leaf scorch disease symptoms in pear (*Pyrus pyrifolia* (Burm.f.) Nakai) in Taiwan are due to infection by the species *X. taiwanensis* (Su et al. 2016). A detailed compilation of the global reports of the various *Xylella* taxa and their associated diseases in different host species is provided in Appendix D.

In overview, symptoms commonly observed as a consequence of *Xylella* infection include leaf chlorosis, leaf wilting, leaf scorching or scalding, defoliation, stunted growth, reduced fruit size, twig and branch dieback, re-sprouting and decline. The XF-ACTORS project website provides a compilation of disease images associated with the presence of *X. fastidiosa* (XF-ACTORS 2018).

Disease symptom expression may be delayed for several months following initial infection by *Xylella*, and plants may appear symptomless but have infections (Almeida & Nunney 2015). The period of latency varies depending on factors such as host species, cultivar susceptibility, and age of the plant. A longer asymptomatic period in olive, compared to grapevine or citrus, corresponds with a slower colonisation of the host plant (Saponari et al. 2017). Asymptomatic plant hosts are a possible source of inoculum for xylem sap-feeding insects to transfer and spread *Xylella* bacteria (Jacques et al. 2016; Martelli 2016), and the propagation of such

asymptomatically infected nursery stock could provide an unintentional distribution of bacterial populations.

Symptom expression can be inhibited by other members of the host plant microbiome. While interactions between *Xylella* bacteria and the host microbiome are complex, they are also bidirectional. Infection with *X. fastidiosa* can exert deleterious effects on the microbiome, as well as microbiome members being able to inhibit disease symptoms, although the mechanisms are not understood (Landa et al. 2022).

2.7.2 Impacts of delayed diagnosis of *Xylella*-induced disease

Detection of *Xylella* species can be difficult, particularly when plants with infections are asymptomatic (Jacques et al. 2016), and some tests have been reported to produce false negative results (EFSA Panel on Plant Health 2015b; Sicard et al. 2019). False negatives can occur, for example, when plant tissue is selected from a plant that has an early infection, or from part of the plant that does not contain the bacteria (EFSA Panel on Plant Health 2015b). Disease caused by *Xylella* can be overlooked when symptoms are confused with those of other primary diseases, secondary fungal infections or drought stress. For example, citrus variegated chlorosis can be confused with symptoms of nutrient deficiency, or anthracnose and greasy spot diseases (Serrano et al. 2013). Delays in *Xylella* pathogen diagnosis contributed to major disease outbreaks in olives in Italy (Saponari et al. 2013), maple leaf scald of big leaf maple in Canada and Pacific Northwest USA (Omdal & Ramsey-Kroll 2012; Pscheidt & Ocamb 2018), pecan leaf scorch in the USA (Sanderlin 1998), and almond leaf scorch disease in Spain (Moralejo et al. 2020). A misdiagnosis of the disease agent causing leaf scorching and dieback in almond trees in the Balearic Islands (Crespi 2018; Olmo et al. 2015; ProMED 2009, 2011) resulted in *Xylella* infection being transmitted to more than 950,000 almond trees, of which 150,000 trees died (Crespi 2018).

EPPO has issued an alert on *X. fastidiosa* in pecans (*Carya illinoinensis* (Wangenh.) K.Koch), expressing a concern about potential for international distribution of infected pecan germplasm (EPPO 2018; Hilton et al. 2017). Pecan bacterial leaf scorch symptoms were observed in 2015 and 2016 across Arizona, New Mexico, California, and Texas, with recent testing of orchards and US germplasm collections for pecan and hickory confirming the presence of *X. fastidiosa* (Hilton et al. 2017) which led to a halt on the supply of graftwood (Grauke, Wood & Harris 2016). Purcell (2013) provides a detailed history of research and difficulties experienced in identifying causal agents and vectors for *Xylella* disease across numerous crops.

If *Xylella* were to enter Australia, be misdiagnosed such that detection was delayed, spread via horticultural practices, and then be transmitted by suitable native insect vectors, the opportunity for eradication or containment would most likely be significantly compromised. The introduction of the pandemic biotype of *Austropuccinia psidii* (myrtle rust) into Australia demonstrated issues that can arise with a delayed response to a pathogen capable of infecting both commercial and environmental plant species (Carnegie & Pegg 2018), such as could occur with a *Xylella* incursion.

2.7.3 Detection methodologies adopted within Australia

A National Diagnostic Protocol (NDP) for detection of *Xylella fastidiosa* was developed for Australia, of which the most recent version was released in 2010 (Subcommittee on Plant Health Diagnostics 2010). The NDP has become outdated on a number of fronts and was replaced as

part of the emergency measures for *Xylella* species. An updated NDP is due to be released in 2022 (Inspector-General of Biosecurity 2022).

Methodologies utilised have moved away from morphological, serological, or biochemical markers due to constraints around timeliness, variability of morphological traits, and the increasingly-required specificity provided by molecular testing.

The routine diagnostic methods for detecting *Xylella* species within a plant host in use by the department's PEQ laboratories are the conventional PCR of Minsavage et al. (1994) and the real-time PCR Harper, Ward and Clover (2010, erratum 2013). Of these, the test by Minsavage et al. (1994) will detect both *X. fastidiosa* and *X. taiwanensis*, and is currently recommended in the Australian NDP (FAO 2018; Subcommittee on Plant Health Diagnostics 2010), although European validation showed it failed to detect a number of American strains (EPPO Bulletin 2019). The real time PCR of (Harper, Ward & Clover 2010, erratum 2013) is also included as it has a higher sensitivity of detection, despite being unable to detect *X. taiwanensis*, and is recommended by EPPO (2019) over the real time PCR of Francis et al. (2006). Both PCR diagnostic methods in use by the department align with international diagnostic protocols outlined in ISPM 27 Annex 25 (FAO 2018), and both tests are used for each host plant, which meets the minimum requirement set by the ISPM.

2.7.4 Detection methodologies adopted outside Australia

While Australian PEQ diagnostics are based around PCR methods, a number of other approaches remain supported in other areas internationally, consistent with ISPM 27 Annex 25 (FAO 2018). In addition to molecular methods, detection via serological methods is described. Isolation methods are not recommended for detection, and molecular methods were advised for testing asymptomatic plant material (FAO 2018).

ISPM 27 Annex 25 sets the minimum requirements for identification, which includes positive results from 2 tests based on different biological principles or from 2 molecular tests that amplify different genetic loci. Bacterial colony morphology on selective media, biochemical and physiological characteristics, pathogenicity testing, and serological methods (such as ELISA) are considered suitable approaches to contribute towards identification, as well as the molecular methods previously described (FAO 2018).

ISPM 27 Annex 25 also sets recommended sampling requirements for plant tissue in symptomatic and asymptomatic plants, including sampling when the plant is actively growing and the amounts of tissue and locations of tissue to sample. This annex recommends sampling after warm periods (late summer to early autumn) to increase the probability of accurate bacterial detection. No specific advice on pooling of samples for testing is provided, except a recommendation that when testing pooled samples for symptomatic plants, the limit of detection for each test protocol should be confirmed. This is a different scenario to standard plant import pathways where plants will be asymptomatic.

For asymptomatic plants, evaluation of the minimum amount of plant tissue required to be sampled, and how many samples can be pooled is ongoing, and current recommendations differ dependent on plant species. For example, guidance provided in the EPPO diagnostic protocol for *X. fastidiosa* states that a minimum of 4 leaves per olive plant must be sampled and up to 225

plants can be pooled, whereas for coffee plants a minimum of 2 leaves must be sampled and up to 50 plants can be pooled (EPPO Bulletin 2019).

Under the department's current emergency measures, the pooling of samples (or 'bulking') for testing is permitted (https://www.agriculture.gov.au/biosecurity-trade/import/goods/plant-products/how-to-import-plants/xylella/notification-amended-emergency-quarantine-measures#bulking-of-samples-for-testing). In summary:

- 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.

Detection of *Xylella* bacteria within insect vectors is achieved via similar approaches as with plant infection, with some additional limitations. Serological tests are known to lack adequate sensitivity for detection within vectors (EPPO Bulletin 2019; FAO 2018). While PCR testing is the most sensitive testing approach when sampling from plant material, Italian research demonstrated that Fluorescence of Loop Primer Upon Self Dequenching-LAMP (FLOSLAMP) was a more sensitive and specific assay for use with insect vectors. This was compared to conventional PCR, real-time PCR, and conventional LAMP assays, and less dependent on the extraction method used (Incerti et al. 2020).

Novel detection methodologies are being developed, with detection of asymptomatic trees at a landscape level achieved using hyperspectral and thermal imagery captured via aircraft and analysed by modelling based on plant functional traits (Zarco-Tejada et al. 2018). The approach was able to detect *X. fastidiosa* symptoms earlier than standard visual inspections by plant pathologists, was ground-truthed by qPCR testing, and symptom development was confirmed with ongoing site visits. Further work developed the system with multiple pathogens and multiple hosts (Zarco-Tejada et al. 2021). The widespread adoption of agricultural drones by Australian agronomists suggests a similar methodology could be incorporated to delimit incursion expansiveness in orchards or forestry, with aircraft use for broader natural landscapes.

2.8 Treatment

There is no known successful treatment able to eliminate *Xylella* bacteria from plants (EFSA Panel on Plant Health (PLH) et al. 2019; EFSA PLH Panel 2016). The following section discusses the status of potential treatments to eliminate *Xylella* on the identified plant material import pathways.

Research has been conducted into treatments for *Xylella*-infected plants, and various studies have focussed on the application of trace elements:

- Historically, copper-containing compounds have been widely used as antimicrobial substances to limit the spread of plant pathogenic bacteria on fruit and vegetable crops (Voloudakis, Reignier & Cooksey 2005); however, copper resistance has been observed in xanthomonad bacteria (Bender et al. 1990; Cooksey et al. 1990; Heydarpanah et al. 2019). Rodrigues et al. (2008) observed differences in cell susceptibility to copper and suggested a copper resistance mechanism by biofilm cells of *X. fastidiosa*. Results by Machini and Oliverira-Brett (2021), using DNA-electrochemical biosensors, also suggested resistance of *X. fastidiosa* to copper.

- The use of a zinc/copper citric acid biocomplex, Dentamet®, which acts as a systemic bactericide, has been assessed as a foliar treatment against *Xylella* (Scortichini et al. 2018; Scortichini et al. 2021; Scortichini et al. 2019).
- Zinkicide® has been trialled as a soil drench against *Xylella* in tobacco and blueberry plants under greenhouse conditions (Shantharaj et al. 2022).
- More recently, Thidiazuron has been assessed for its antibacterial activity against *Xylella* (Catalano et al. 2022).

These treatments appear effective at controlling, but not eliminating the disease from the plant. The positive response of increased plant vigour in response to treatment applications is considered largely due to improving the plant's resilience to stress, rather than removal of the bacteria from the plant's vascular system. In addition, many of the experiments have been conducted *in vitro*, and thus limited in their confirmation of effectiveness *in vivo* under natural field conditions (EFSA PLH Panel 2016).

2.8.1 Hot water treatment for nursery stock

Hot water treatment (HWT) is considered to be a robust and reliable technique for eliminating life stages of many pests (insects, nematodes) and pathogens (phytoplasma, bacteria, fungi) in dormant plant propagation materials, including grapevine cuttings (CABI 2020; Goheen, Nyland & Lowe 1973). For example:

- Treatment at 50°C for 20 minutes has been in use for imported grapevine cuttings as an Australian quarantine treatment since the mid-1970s, and treatment at 50°C for 2 hours has been used for imported vegetative grasses since the mid-1980s.
- HWT to eliminate Grapevine flavescence dorée phytoplasma (FD) from planting materials is among the special requirements for the introduction and movement of *Vitis* species to protected zones in the EU (EFSA Panel on Plant Health 2015b).
- In Australia, HWT at 50˚C for 30 minutes or 54˚C for 5 minutes is a treatment requirement for Phylloxera (*Daktulosphaira vitifoliae*) when moving grapevine propagation material interstate (Victoria Department of Primary Industries 2007).

HWT has also been recommended as a treatment for *Xylella fastidiosa* infection in certain circumstances. A review by the European Food Safety Authority EFSA Panel on Plant Health (2015a) recommended treatment of 50°C for 45 minutes to eliminate phytoplasmas and *X. fastidiosa* from grapevine, noting that the temperature needed to be greater than or equal to 45°C for elimination of the bacterium, but below 60°C to prevent adverse effects on plant material (EFSA Panel on Plant Health 2015b). Sanderlin and Melanson (2008) recommended treatment of pecan scion wood (*Carya illinoinensis*) infected with *X. fastidiosa* by submersion of the propagation material in water at 46°C for 30 minutes. Complete elimination of *Xylella* bacteria from all pecan scions was not, however, achieved, as 0.7% of grafted trees remained infected; treatment at 50°C was found to result in some damage to one pecan cultivar.

However, it is not known with any certainty whether '*X. f.* subsp. *pauca*' or *X. taiwanensis* is susceptible to HWT, although EFSA Panel on Plant Health (2015b) considers the HWT requirement of 50°C for 45 minutes to be effective against subspecies of *X. fastidiosa*.

While HWT is proven to be useful in certain applications, particularly in grapevine, there is currently insufficient evidence to enable HWT to be used as an efficacious treatment against *X. taiwanensis* and for species of host plant material other than grapevine. In addition, HWT may

initiate a switch to fermentative respiration in plant material and may not be suitable for some plant types. Treatment rates designed to eliminate in-situ systemic pathogens may be at or above the level of tolerance for many plant species. Therefore, propagation material in poor condition, heat-sensitive cultivars, or plants of generally known sensitivity, may suffer adversely from the treatment.

2.8.2 Plant tissue culture

Specific to plant tissue culture, antimicrobials, including antibiotics such as streptomycin, chloramphenicol and penicillin, and more recently nano silver (Safavi et al. 2011; Salisu et al. 2014), have been applied during the plant tissue culture process to combat both epiphytic and endophytic bacteria. However, rather than eliminating the bacterial contaminants, the antimicrobial treatments may tend to be inhibitory in nature (Safavi et al. 2011; Salisu et al. 2014), allowing for persistence of low levels of bacterial contamination. The use of antibiotics against Gram-negative bacteria in plant tissue culture is extremely difficult or unsuccessful (Leifert & Cassells 2001). Bacteria may also develop resistance to the antimicrobial compounds (Safavi et al. 2011; Salisu et al. 2014).

Due to the inhibitory nature of these additives, the department recommends that no antimicrobials are used in tissue culture media for all plant tissue cultures imported into Australia, as these can mask visible signs of infection.

2.8.3 *Xylella* in seed

Evidence for seed transmission of *Xylella* was only recently confirmed in *Carya* spp. seed (Cervantes et al. 2022). As such, studies of effective treatments for elimination of *Xylella* from seed are limited. Consequently, and from a review of the literature, reliable and effective treatments for seed internally infected by *Xylella* are currently unavailable.

In considering a heat treatment for seed, it is well known that HWT of seed is a cheap and effective method for killing internal seed pathogens; however, the temperature and duration of treatment will depend on the crop and the pathogens affecting the seed (McGrath 2022). Some large seeded crops cannot be effectively disinfested with HWT as the temperature required to heat the entire seed would result in devitalisation of the seed itself (Higgins 2018).

In the absence of an effective treatment for seed internally infected by *Xylella*, the department implemented, as an emergency measure, a mandatory requirement that imported *Carya* spp. seeds for sowing must be grown for a minimum of 12 months at a government PEQ facility at Mickleham, Victoria. Before release from biosecurity control, the plants must be tested by PCR and found free from *Xylella* species.

2.8.4 Other/emerging technologies

Cold recovery

Cold recovery is a field phenomenon that has been tested in grapevine treatment trials against *X. f.* subsp. *fastidiosa*, which appears to be cold limited in distribution and response (Anas et al. 2008; Lieth et al. 2011; Purcell 1980). This process may be suitable as a treatment regime in some circumstances, noting however that *X. f.* subsp. *multiplex* is cold tolerant in its geographic distribution, while the effects, if any, on '*X. f.* subsp. *pauca*' and *X. taiwanensis* are unknown (Amanifar, Taghavi & Salehi 2016).

There is currently insufficient information to enable cold recovery to be used as a treatment to eliminate *Xylella* from nursery stock.

Irradiation

Hilton et al. (2021) studied a novel thermal treatment using microwave irradiation for the phytosanitation of *Xylella* in pecan graftwood. These researchers reported comparable efficacy of microwave radiation exposure for 6 seconds at 55° to 65°C to that of hot water treatment in 46°C water for 30 minutes (Sanderlin & Melanson 2008) on *Xylella*-infected pecan scions. As well as remediation of *Xylella* from pecan scions, the novel approach was proposed as offering a time- and cost-effective treatment (Hilton et al. 2021).

There is currently insufficient information to enable irradiation to be used as a treatment to eliminate *Xylella* from nursery stock.

Plasma-activated water

In awareness of the irreversible damage that can be caused to plants by heat, the toxic effects of chemicals, and safety issues (including potential DNA damage) associated with radiation, Ambrico et al. (2022) recently applied plasma-activated water techniques as a potential antimicrobial to inactivate *Xylella fastidiosa* cells. Using the '*X. f* subsp. *pauca'* strain "De Donno" ST53, Ambrico et al. (2022) reported a 15 minute treatment sufficient to destroy *Xylella* cells in liquid culture in *in-vitro* experiments.

There is currently insufficient information to enable plasma-activated water to be used as a treatment to eliminate *Xylella* from nursery stock.

3 Pest risk assessment for quarantine pests

Consistent with the IPPC and ISPM 1 (FAO 2016), this pest risk assessment was initiated to fulfil Australia's obligations to review the emergency phytosanitary measures introduced in November 2015 and revised in 2016, 2019, 2020, 2021 and 2022 (Section 1.2.3). Australia introduced emergency measures following reported disease outbreaks of *Xylella fastidiosa* in Italy in 2013 and in France in 2015. These outbreaks highlighted the potential for *X. fastidiosa* to be transported to and become established in new areas through international trade of asymptomatically infected plants, and to be further transmitted by recognised and previously unrecognised insect vectors, including leafhoppers and sharpshooters (Cicadellidae), and spittlebugs (Aphrophoridae). The emergency measures were revised when *Xylella* was reported from new countries, when new plant hosts were confirmed and when new transmission pathways (pecan seeds for sowing) were confirmed.

The likelihoods of entry have been assessed for 2 pathways—nursery stock (inclusive of tissue culture), and seeds for sowing.

The potential establishment, spread and consequences of the importation of *Xylella* spp. in Australia are not expected to be affected by the origin pathway of the bacteria. Therefore, a single assessment of these elements is presented in Section 3.3 and used to determine the unrestricted risk estimate for *Xylella* species (Section 3.4).

3.1 *Xylella* spp. associated with nursery stock

The risk scenario of biosecurity concern is *Xylella* sp. arriving in Australia through the trade in imported nursery stock. This scenario includes any infected vectors that may be imported in association with that nursery stock.

3.1.1 Likelihood of entry

The likelihood of entry is considered in 2 parts: the likelihood of importation and the likelihood of distribution, which consider pre-border and post-border issues, respectively.

Likelihood of importation

The likelihood that *Xylella* species will arrive in Australia in a viable state with the importation of nursery stock is assessed as: **High**.

The following information provides supporting evidence for this assessment.

Members of the genus *Xylella* collectively have a wide host plant range, making it likely that an infected host plant will be imported:

- The confirmed *Xylella* host plant range comprises members of 356 genera belonging to 106 plant families of horticultural, ornamental, and Australian native plants (Appendix D).
- Large volumes of nursery stock are imported into Australia for industry and private use. In the 5-year period between 2017 and 2021, 8,645 consignments of imported nursery stock arrived in Australia (Inspector-General of Biosecurity 2022).
- Many confirmed *Xylella* host plant species are permitted species for import into Australia (DAWE 2020a).

- There is increasing evidence that *X. fastidiosa* has a capacity to undergo inter-strain recombination, which may produce novel strains with host ranges that differ from their parent strains (Nunney et al. 2014a; Rapicavoli et al. 2018).
- New plant hosts of *Xylella* are likely to be identified in native plant flora that have not previously been exposed to the bacteria due to geographic isolation (Groenteman et al. 2015).
- The number of new host plants being identified has increased in the last 2 decades (see Figure 2.2).

Movement of plants for planting is considered to be the major entry pathway for the pathogen into new areas:

- The 2 major risk pathways for introduction of *X. fastidiosa* to non-infested areas are transport of infected plant material into areas with vector species present, or introduction of new vector species carrying the pathogen (EFSA Panel on Plant Health 2015b).
- Entry of *Xylella* bacteria into the Middle East, Europe, and Asia has been associated with infected plants in the nursery stock trade, as evidenced by geographic tracing of Multi Locus Sequence Typing (MLST) genotypes (Figure 2.6, (EFSA 2018)) and detected incidences in imported nursery stock (Section 2.5.3).
- Pathway risk analysis conducted by the United Kingdom's Department for Environment Food and Rural Affairs (DEFRA 2020) using European records suggests that a significant factor in the long distance/international spread of *Xylella* bacteria has been the introduction of infected germplasm of desirable horticultural crops into new regions (Almeida & Nunney 2015; CABI 2020; EFSA PLH Panel et al. 2019).
- Numerous international interceptions of *Xylella*-infected ornamental nursery stock have been recorded, including species of *Mandevilla sanderi* (Brazilian jasmine), *Pelargonium x hortorum* (zonal geranium), *Juglans* spp. (walnut), *Rubus fruticosus* (blackberry), *Rubus idaeus* (raspberry) and ornamental *Coffea* species (Bergsma-Vlami et al. 2015; Cella et al. 2018; EFSA 2016; EPPO 2020; EUROPHYT 2015, 2016, 2017, 2018b, a; Legendre et al. 2014).
- *Xylella* bacteria inhabit xylem tissue of infected plants (de Mello Varani et al. 2008; Meng et al. 2005), allowing its survival in living plant material during transport and storage (Jacques et al. 2016; Martelli 2016).

Detecting and identifying *Xylella* infection can be difficult, increasing the likelihood that infected plants are imported:

- Disease symptoms may vary across the range of plant hosts (as described in Section 2.7.1) (Almeida & Nunney 2015; Gould & Lashomb 2007; Rathe 2012; Varela, Smith & Phillips 2001).
- Plants may display resistance (Simpson 2017), tolerance or be asymptomatic for months, and for a period as long as 2 to 5 years following infection (Beretta et al. 1996; EFSA Panel on Plant Health et al. 2019a).
- Deciduous nursery stock may be dormant when first inspected, and mild symptoms may not be visually detectable at any time (Zarco-Tejada et al. 2018).
- The non-uniform distribution of *Xylella* bacteria within plants can provide the opportunity for misdiagnosis of infected plant material (Baldi & La Porta 2017; EFSA Panel on Plant Health et al. 2019a; Francis et al. 2006).

Importation of *Xylella* species could occur with insect vectors:

- The 2 major risk pathways for introduction of *X. fastidiosa* to non-infested areas are transport of infected plant material into areas with vector species present, or introduction of new vector species carrying the pathogen (EFSA Panel on Plant Health 2015b).
- More than 75 insect vectors of *Xylella* bacteria are recognised, many of which are known to be polyphagous (Appendix C).
- Insect vectors concealed in imported nursery stock could avoid detection at inspection.
- Vectors that acquire *Xylella* bacteria as adults remain infectious for the remainder of their life (Almeida et al. 2005).
- As discussed in Section 2.6.3, departmental analysis of records of the interceptions of insects capable of vectoring *Xylella* found 25 detections on nursery stock, with 8 of those thought to be associated with nursery stock exported from a *Xylella* country over a 15 year period (2000 to 2015).
- A mitigating factor is that all nursery stock imported to Australia undergoes pre-export inspection and certification, and a mandatory arthropod treatment on arrival—either methyl bromide fumigation or dipping in an insecticide. The vector risk is also not present if plant material is imported as tissue cultures.

Importation could occur with materials from low risk countries in which *Xylella* has established but not been recognised, but this is considered less likely:

- There is evidence that *Xylella* is continuing to spread geographically, as indicated by the 2019 SPS notification by Israel of its presence (EPPO 2019; Plant Protection and Inspection Services 2019).
- Investigations have indicated that *Xylella* was established in the Apulia region of Italy for some years before recognition of its presence (Almeida et al. 2008).
- Among countries from which *Xylella* is not recorded, there are generally high levels of awareness of the risk that is posed, and in most cases, measures intended to exclude its entry are applied (MPI 2020).
- Among countries from which *Xylella* is not recorded, there is increasing recognition of potential host plant susceptibilities and symptoms of infection, and utilisation of diagnostic procedures for its detection is becoming increasingly widespread (FAO 2020; Parkinson & Malumphy 2014).

For the reasons outlined, the likelihood that *Xylella* species will arrive in Australia in a viable state with the importation of nursery stock is assessed as High.

Likelihood of distribution

The likelihood that imported nursery stock infected with bacterial *Xylella* will be distributed within Australia in a viable state, and subsequently transfer to a susceptible part of a host is assessed as: **High**.

The following information provides supporting evidence for this assessment.

Nursery stock imported into Australia is distributed widely across Australia. Human-assisted movement will facilitate this distribution:

- *Xylella* inhabits the xylem tissue of infected plants (de Mello Varani et al. 2008; Meng et al. 2005), allowing for its survival in living plant material during transport and storage (Jacques et al. 2016; Martelli 2016).

- Extensive air and land distribution networks for imported nursery stock provide for routine and long-distant movement of plant material throughout Australian states and territories into commercial orchards, wholesale production nurseries, and for general sale in retail outlets (PHA 2014).
- *Xylella* disease symptoms may be misdiagnosed, allowing imported plants, either symptomatically or asymptomatically infected, to be distributed for propagation and trade.

Xylella bacteria do not readily move to new plant hosts without assistance, either by propagation or through an insect vector:

- The bacteria do not need to move from the import pathway to a suitable host as the pathogen is already within a suitable host.
- Nursery stock is imported into Australia for the specific purpose of propagation. Infected nursery stock is therefore likely to be planted directly into suitable habitats in multiple locations in Australia.
- Imported material is often propagated, by taking cuttings, grafting onto other root stock, dividing plants, multiplication and micro-propagation of tissue cultures. These techniques use the parent plant to create multiple clones, and if the parent plant is infected with *Xylella* bacteria, the clones are also likely to be infected. Where planting material is used to establish large agricultural plantings, distribution of infected plants could occur over large areas.

Vector-assisted spread of *Xylella* is possible:

- No recognised insect vector species are currently present in Australia, but vectors have been known to establish in countries outside their native range. For example, *Homalodisca vitripennis* has a history of incursions and successful establishments, including in California (1998) (Almeida 2007; De Leon, Jones & Morgan 2004) and Hawaii, USA (2004) (Hoover 2004)(Section 2.3.1). *Philaenus spumarius* has spread to Europe, Asia, USA (including Hawaii), north-west Africa and Nigeria (CABI 2020; Ejere & Okpara 2010) and New Zealand (Archibald, Cox & Deitz 1979).
- While vectors have been known to establish outside their native range, there is no known incidence of new vector species arriving and introducing *Xylella* at the same time.
- A single incursion of undetected eggs laid on leaves or stems of host plants imported from England was believed to have allowed entry and then establishment of *P. spumarius* in New Zealand (Archibald, Cox & Deitz 1979; Hamilton 1979; Hamilton & Morales 1992).
- Transmission of *Xylella* species is by xylem-feeding insects in the hemipteran sub-order Auchenorrhyncha. There are more than 360 species of native Australian xylem-feeding insects in this sub-order, and their capacity to vector *Xylella* is unknown.
- High numbers of xylem-feeding insect species in an area is considered to increase the likelihood of at least one species being a suitable insect vector of *Xylella* bacteria (Redak et al. 2004). For this reason, all Australian native Auchenorrhyncha are considered to be potential vectors of *Xylella*.
- Xylem-sap feeding insects are frequently polyphagous and may not be selective for plant species (Coletta-Filho et al. 2017; Nunes et al. 2003; Nunney et al. 2012). Their feeding behaviour may facilitate distribution of *Xylella* bacteria to multiple plant species.
- If an efficient vector is present, there is a strong chance that *Xylella* will persist and spread (Strona, Carstens & Beck 2017), as a high proportion (about 96%) of confirmed plant hosts of *Xylella* are present in Australia (Appendix D).

- Adult Auchenorrhyncha vectors are strong flyers and able to fly relatively long distances, thereby being able to move relatively easily to suitable hosts (Andersen, Mizell & Brodbeck 2016; Conklin & Mizell 2016; Lago et al. 2020a; Strona et al. 2020).

For the reasons outlined, the likelihood that *Xylella* species will be distributed within Australia in association with imported nursery stock, and subsequently transfer to a susceptible part of a host is assessed as High.

Overall likelihood of entry

The overall likelihood of entry is determined by combining the likelihood of importation with the likelihood of distribution using the matrix of rules shown in Table A.2.

The likelihood that *Xylella* species will enter Australia as a result of trade in nursery stock and be distributed in a viable state to a susceptible part of a host is assessed as: **High**.

3.2 *Xylella* spp. associated with seeds for sowing

The risk scenario of biosecurity concern is *Xylella* spp. arriving in Australia through the trade in imported seeds for sowing. As discussed in Section 2.6.4, to date the only positive confirmation of vertical transmission of *Xylella* from seed to the germinated seedling has been in *Carya illinoinensis* (pecan). This likelihood of entry assessment focuses on pecan seeds but could be extrapolated with similar outcomes if other plant species are identified as supporting vertical transmission of *Xylella*.

3.2.1 Likelihood of entry

The likelihood of entry is considered in 2 parts: the likelihood of importation and the likelihood of distribution, which consider pre-border and post-border issues, respectively.

Likelihood of importation

The likelihood that *Xylella* species will arrive in Australia, in association with imported *Carya* seeds for sowing is assessed as **High**.

The following information provides supporting evidence for this assessment.

- Cervantes et al. (2022) showed the ability of *X. fastidiosa* to colonize developing pecan seeds and be transmitted at a rate up to 80% from well-developed pecan seeds to germinated seedlings. Therefore, infected seed may facilitate *Xylella* import.
  - *Xylella fastidiosa* DNA was isolated from mature seeds originating from 7 pecan trees, revealing an infection rate up to 90%. The highest concentrations of *X. fastidiosa* DNA were found in the hilum and outer integument of the seeds and the petioles, respectively.
  - The presence of *X. fastidiosa* in the endosperm of undeveloped pecan seeds was also previously reported (Hilton et al., 2020).
  - Seed-to-seedling transmission was proposed in citrus variegated chlorosis (CVC) of sweet orange where '*X. f.* subsp. *pauca*' was detected in the fruit, seed coat, and embryo (Li et al., 2003; Coletta-Filho et al., 2014; Hartung et al., 2014). Isolates were obtained from symptomatic citrus seedlings germinated from putative *Xylella*-infected seed (Li et al., 2003), but later efforts to isolate or detect the bacterium in seedlings failed (Coletta-Filho et al., 2014; Hartung et al., 2014).

- Pathogen-infected seeds for sowing provide one of the main pathways for the introduction of seed-borne pathogens into new areas (Elmer, 2001). Seed contaminated with *Xylella* would be asymptomatic, facilitating its unimpeded importation.
- Propagation of improved pecan cultivars occurs by grafting clonal scions onto rootstocks that are produced from seedlings (Wells 2017). Pecan seed may be imported into Australia to produce rootstocks.
- The USA is the leading global producer of pecans, but production has expanded to South Africa, Australia, China, Uruguay, Argentina and Brazil, and production is expected to increase over the next 30 years (Wood et al., 1990; Wakeling et al., 2001; Lazarotto et al., 2014; Zhang et al., 2015). It is possible that *Xylella*-infected pecan seed for rootstock production may have been globally distributed and may be present in areas of commercial nut, germplasm, and rootstock production. This may include areas where *Xylella* is not presently recorded.
- A low incidence of infection in the field may enhance a plant pathogen's chances of escaping detection. If the level of infection is very low and/or symptomatic plants are randomly scattered throughout a field, an infection may go undetected for some time.

  – *Xylella* symptoms may vary across the range of plant hosts (Almeida & Nunney 2015; Gould & Lashomb 2007; Rathe 2012; Varela, Smith & Phillips 2001). Plants may display resistance (Simpson 2017), tolerance or be asymptomatic for months, and for a period as long as 2 to 5 years following infection (Beretta et al. 1996; EFSA Panel on Plant Health et al. 2019a).

Likelihood of distribution

The likelihood that imported *Carya* seeds for sowing infected with bacterial *Xylella* will be distributed within Australia in a viable state is assessed as **High**.

The following information provides supporting evidence for this assessment:

- Seed imported for rootstock production could be intended for commercial sale and may be distributed to multiple destinations throughout Australia. Following sale, any contaminated imported seeds will be planted in suitable habitats.
- Commercial pecan production also occurs in multiple locations in Australia, including northern coastal and inland NSW, central and south-eastern QLD, SA and WA (Australian Pecan Association, 2022) and it is possible that these producers use imported seed for rootstock production.
- The pathogen's ability to survive in a seed facilitates its viability en-route to, and during distribution across Australia.
- Cervantes et al. (2022) showed that *X. fastidiosa* can be transmitted from mature seeds to germinated seedlings. This suggests *Xylella* associated with pecan seed is likely to remain viable.

  – Conditions during transport and storage, such as temperature and humidity, are unlikely to affect the viability of *Xylella*.
- Seeds for sowing are imported specifically for the purpose of propagation.

  – The distribution of infected seeds for commercial purposes is likely to facilitate the distribution of the associated pathogens.

  – The distribution of infected imported seeds to commercial seedling nurseries may also facilitate distribution. Asymptomatic seedlings that develop from infected seeds may be overlooked in this setting.

- Propagation of improved pecan cultivars occurs by grafting clonal scions onto rootstocks that are produced from seedlings (Wells 2017). Grafting clonal scions onto infected rootstock would facilitate *Xylella* infection within areas of commercial production.

Overall likelihood of entry

The overall likelihood of entry is determined by combining the likelihood of importation with the likelihood of distribution using a matrix of rules.

The likelihood that *Xylella* species will enter Australia as a result of trade in *Carya* spp. seeds for sowing and be distributed in a viable state to be grown as propagative material, is assessed as **High**.

3.3 Establishment, spread and consequence

3.3.1 Likelihood of establishment

The likelihood that *Xylella* species will establish within Australia based on a comparison of factors in the source and destination areas that affect pest survival and reproduction is assessed as: **High**.

The following information provides supporting evidence for this assessment.

Importing a single infected plant can cause disease outbreaks, as has been observed in the USA and Italy (Almeida & Purcell 2003; Carlucci et al. 2013; Chen et al. 2005; Montero-Astúa et al. 2007; Saponari et al. 2013; Schuenzel et al. 2005).

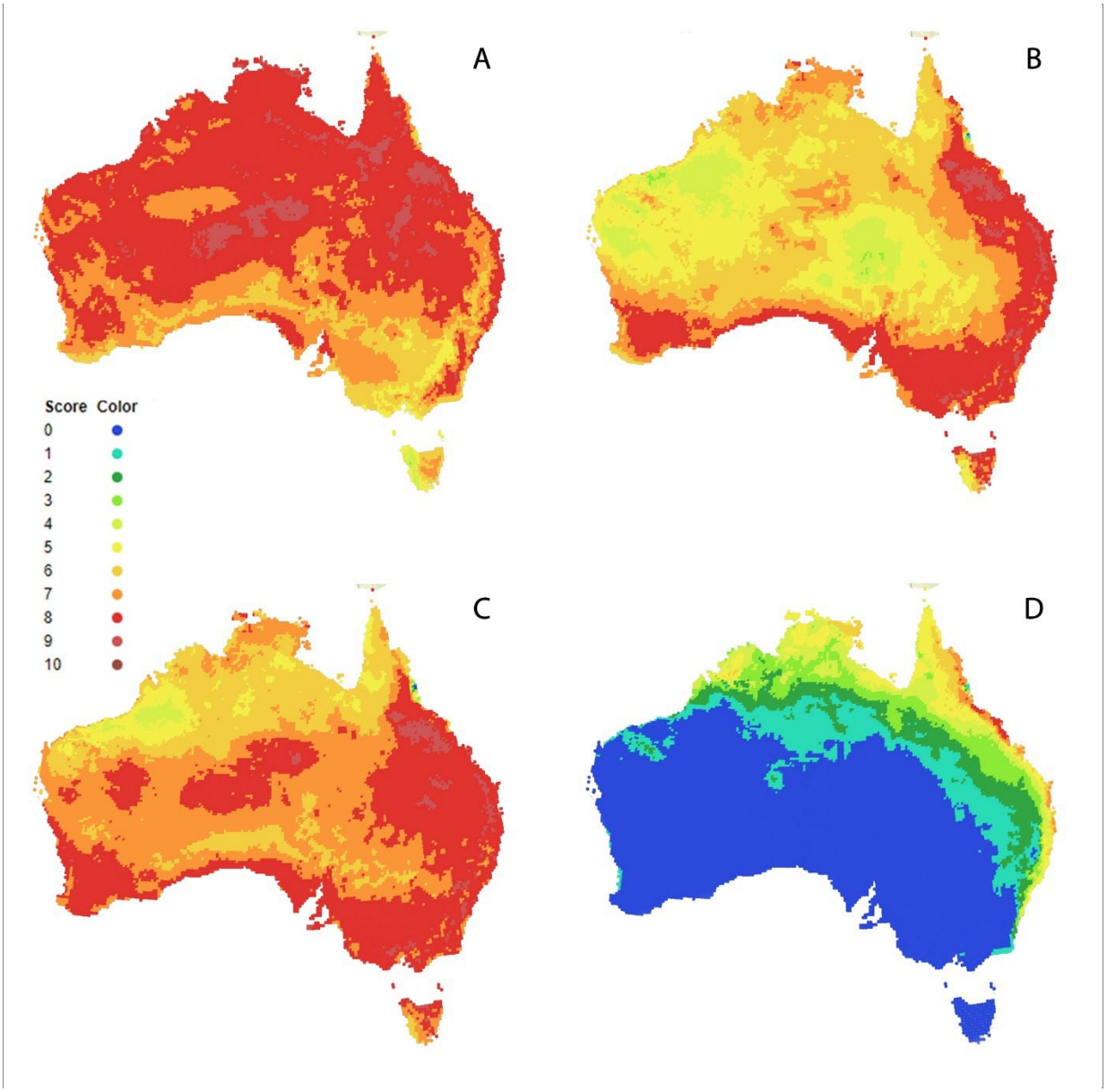
Xylella species have biological characteristics and reproductive strategies suitable for their establishment in Australia:

- Association of *Xylella* species with host internal environments (foreguts of insect vectors and xylem tissues of host plants) provides protection from unfavourable environmental conditions. *Xylella* inhabits the xylem tissue of infected plants (de Mello Varani et al. 2008; Meng et al. 2005), allowing for its survival in living plant material during transport and storage (Jacques et al. 2016; Martelli 2016). In addition, vectors that acquire *Xylella* bacteria as adults remain infectious for the remainder of their life (Almeida et al. 2005).

Xylella infections are known from many different climatic zones:

- *Xylella* has established in countries with climates similar to those in parts of Australia suggesting that the Australian climate is not likely to impede establishment.
- Environmental conditions may affect the spatial and temporal spread of *Xylella* species and the diseases with which they are associated. The department conducted 'Climatch' modelling using temperature and rainfall data from current areas of *Xylella* distribution (Figure 3.1) for *Xylella fastidiosa* subsp. *fastidiosa*, *X. f.* subsp. *multiplex*, '*X. f.* subsp. *pauca*' and *X. taiwanensis*. This modelling shows that much of the Australian environment would be suitable for the establishment of *X. fastidiosa* and its subspecies, while northern and northeastern Australia is predicted to be more suitable for the establishment of *X. taiwanensis*.

Figure 3.1 Predicted environmental suitability for establishment of (A) *Xylella fastidiosa* subsp. *fastidiosa*, (B) '*X. f.* subsp. *pauca*' (C) *X. f.* subsp. *multiplex*, and (D) *X. taiwanensis* in Australia



Source: Modelling conducted by the department, using temperature and rainfall data from known areas of *Xylella* distribution and using Climatch v1.0 (ABARES 2020).
Notes: Climate data used for modelling included arid environments supplemented by irrigation. Lower ratings indicate lesser predicted suitability for bacterial colonisation and persistence (zones with a rating of 5-10 are areas of concern).

Xylella can infect a wide range of host plant species:

- The majority of families containing confirmed plant hosts of *Xylella* (356 genera belonging to 106 plant families—detailed in Appendix D), are present in, and distributed widely throughout Australia. These plant hosts include a range of weeds, grasses, ornamental plants, landscape trees, fruit trees, common and widely grown agricultural, garden and amenity plants, and Australian native plants (listed in Table 2.1). This large plant host range is present in a wide geographic area throughout Australia and provides opportunity for establishment of the bacteria.
- Diagnostic difficulties may delay the detection and identification of *Xylella*–infected plants in the environment, providing opportunity for its persistence and dissemination.

Xylella can utilise a wide range of insect vector species:

- There are currently more than 75 recognised insect vectors of *Xylella* (presented in Appendix C: *Xylella* vectors and preferred plant hosts).
- While the currently recognised insect vectors of *Xylella* are exotic to Australia, the large population of potentially vector-competent xylem-feeding insects present in Australia may facilitate the establishment of *Xylella*, through acquisition and transmission of the bacteria from infected plant hosts.
- The native Australian Auchenorrhyncha fauna is present throughout Australia, with Queensland and New South Wales having the highest representation (see Appendix C).
- Australian environmental conditions are likely to be suitable for exotic insect vectors of *Xylella* to establish, should these pests remain undetected on arrival in Australia and be distributed across the country. Figure 2.1 depicts the department's analysis of predicted environmental suitability for the establishment of 4 well-known insect vectors (discussed in Section 2.3.1) derived from 'Climatch' modelling of temperature and rainfall data. This modelling shows that much of the Australian environment would be suitable for the establishment of one or more of exotic vector species.

Direct evidence from overseas studies indicates that changes in climate, host plant distribution and the ability of insect vectors of *Xylella* to adapt to new areas may have an unpredictable influence on the establishment areas of *Xylella* in Australia:

- For example, Anas et al. (2008) reported an increase in severity of Pierce's disease on grapevines in the southeastern states of the USA because of warmer winter temperatures in the region over the preceding 6 years.
- From predictive modelling studies, there are also indications that climate change may strongly impact the distribution of *Xylella fastidiosa* in Europe (Godefroid et al. 2018). With an increase in winter temperatures, the results have not only predicted a northward expansion for the subspecies *multiplex* by 2070, but also a gradual shift for the bacterium from Southern France, Italy and Portugal towards Northern France, Belgium, and the Netherlands.
- Shih et al. (2013) reported that vectors can adapt to new areas, noting that prior to 1990 *Kolla paulula* were recorded at altitudes of 500 to 1,300 metres, while between 1990 and 2012 were recorded from ground level to 800 metres. These authors suggested that *K. paulula* may have followed their host plants to the lower altitudes, and that the species may have been affected by several environmental factors such as temperature, rainfall and weed abundance.

For the reasons outlined, the likelihood of establishment of *Xylella* spp., in association with imported nursery stock, or an associated infected insect vector, is assessed as High.

3.3.2 Likelihood of spread

The likelihood that *Xylella* species will spread within Australia, based on a comparison of factors in the source and destination areas that affect the expansion of the geographic distribution of the pest is assessed as: **High**.

The following information provides supporting evidence for this assessment. For the purpose of this assessment, it is assumed that a competent vector is present in Australia—either as a recognised exotic vector, or a native Australian species that can become a competent vector. This aligns with assumptions made by Australia's Plant Health Committee in the National *Xylella* Action Plan 2019-2029 (Department of Agriculture 2019b), a documented national approach to

enhance Australia's capacity to prevent the introduction of *Xylella* and prepare for a response should it be detected.

The Australian environment is likely to be suitable for the natural spread of *Xylella* species:

- Spread of *Xylella* species in similar environments and climates to those of Australia has been documented in South America (Coletta-Filho et al. 2017; Nunney et al. 2012), North America (Nunney et al. 2010), Italy (Saponari et al. 2013), France (RSI 2015) and Iran (Amanifar et al. 2014; Amanifar, Taghavi & Salehi 2016).
- Managed environments in Australia, such as fruit and nut orchards, nurseries and private gardens are all favourable for the natural and human-assisted spread of *Xylella* species. Confirmed host plant species are widely distributed, abundantly available, and in geographic areas where potential *Xylella* insect vectors could be expected to occur.
- The Australian natural environment has widespread and common host species for *Xylella*, such as *Acacia* and *Eucalyptus* species (see Table 2.1), and a native Auchenorrhyncha fauna.
- Several of the Australian native plant species identified as hosts of *Xylella* are geographically widespread and commonly cultivated in Australia (ALA 2021; Rathé et al. 2012), providing potentially unbroken tracts of both natural and cultivated vegetation through which the bacterium could spread.

Plant hosts of *Xylella* species are widespread across most parts of Australia:

- Availability of potentially suitable hosts across Australia means that there are no natural barriers that might block the spread of the pathogen.
- Spread of *Xylella* infected plants may also be facilitated by domestic trade of nursery stock.

Potentially vector-competent endemic xylem-feeding insects are present in Australia.

- While all recognised insect vectors of *Xylella* are exotic to Australia, members of the large endemic fauna of polyphagous xylem-feeding insects may facilitate the spread of *Xylella* through acquisition and transmission activities in the event that the pathogen was to establish in the Australian environment.
- Five genera present in Australian (*Kolla*, *Lepyronia*, *Aphrophora*, *Erythroneura*, and *Typhlocyba*) (ABRS 2022a) contain species not present in Australia proven to be vectors (Appendix C). While the known vector species are not present in Australia, the related Australian species may share the ability to vector *Xylella*.
- Knowledge about the feeding habits, host range and other attributes of potential importance for the spread of *Xylella* through potential Australian vectors within Auchenorrhyncha fauna is relatively limited. However, it can be expected that dispersal mechanisms similar to those reported elsewhere (Andersen, Mizell & Brodbeck 2016) will enhance the spread of *Xylella*. It can also be expected that Australian vectors would spread the bacterium to Australian native plant hosts.
- Difficulties associated with diagnosis of *Xylella*, including the requirement for specialised testing, could delay eradication and increase the opportunity for spread among plant hosts and insect vectors.
- Adult Auchenorrhyncha vectors known from overseas are strong flyers and able to fly relatively long distances, thereby being able to move relatively easily to suitable hosts (Andersen, Mizell & Brodbeck 2016; Conklin & Mizell 2016; Lago et al. 2020a; Strona et al. 2020).

For the reasons outlined, the likelihood of spread of *Xylella* species, in association with imported nursery stock, or an associated infected insect vector, is assessed as High.

3.3.3 Overall likelihood of entry, establishment and spread

The overall likelihood of entry, establishment and spread is determined by combining the individual likelihoods of entry, establishment and spread using the matrix of rules in Table A.2.

The overall likelihood that *Xylella* species will enter Australia in association with nursery stock, or an associated infected insect vector, be distributed in a viable state to a susceptible part of a host, establish in Australia and subsequently spread within Australia is assessed as: **High.**

The overall likelihood that *Xylella* species will enter Australia in association with *Carya* spp. seeds for sowing, be distributed in a viable state to a susceptible part of a host, establish in Australia and subsequently spread within Australia is assessed as: **High.**

3.3.4 Consequences

The potential consequences of the establishment of one or more species in the genus *Xylella* have been estimated according to the methods described in Figure A.1.

Based on the decision rules described in Table A.3, that is, where the potential consequences of a pest with respect to a single criterion is rated as 'F', the overall consequences are estimated to be **High.**

Criterion	Estimate and rationale
Direct	
Life of health of plants and plant products	F – Significant at the National level As described in Section 2.4 and Appendix D, more than 500 species of a wide range of important horticultural, commodity and amenity plants in 106 plant families are known to be susceptible to infection and some are severely affected by infection with members of the genus *Xylella*. Horticultural crops affected include citrus, cherry, blueberry, nuts, summerfruit, grape, olive, avocado and pear. These host plants are widely grown across Australia and are some of the highest value horticultural crops grown in Australia (ABARES 2021) with a gross value of production estimated at around $4.7 billion in 2017–18 (ABS 2019a). *Xylella* is also known to affect species of 15 families of Australian native plants (Table 2.1 and Appendix D). The full host range of *Xylella* in Australian native plants is not known but can be expected to increase in a similar pattern to that observed overseas. Groenteman et al. (2015) states that new plant hosts of *Xylella* are likely to be identified in native plant flora that has not previously been exposed to the bacteria due to geographic isolation. The relatively limited number of Australian native plants that have been exposed to *Xylella* in offshore situations have shown susceptibilities to infection. Susceptible taxa include members of iconic genera including *Acacia*, *Eucalyptus* and *Grevillea*. One scenario could involve large numbers of native plants being lost either through the effects of infection or attempts to eradicate or control an incursion (Digiaro & Valentini 2015). ABARES (2021) considered that some tree deaths could occur, resulting in a reduction of tree numbers and weakening of remaining disease affected trees. Specific examples of the effects of *Xylella* on Australian native plants are illustrated in Figure 2.4. Symptoms commonly observed as a consequence of *Xylella* infection include leaf chlorosis, leaf wilting, leaf scorching or scalding, defoliation, stunted growth, reduced fruit size, twig and branch dieback, re-sprouting and decline (Section 2.7.1). Disease symptom expression may be delayed for several months following initial infection by *Xylella*, and plants may appear symptomless but have asymptomatic

Criterion	Estimate and rationale
	unapparent infections (Almeida & Nunney 2015; Jacques et al. 2016). Asymptomatic plant hosts are a possible source of inoculum for xylem sap-feeding insects to transfer and spread *Xylella* bacteria (Jacques et al. 2016; Martelli 2016), and the propagation of such asymptomatically infected nursery stock could provide an unintentional increase in the level of bacterial populations. The breadth of potential consequences is illustrated by the globally documented effects on important horticultural plant species. Worst case scenario estimates of economic losses in olive production in Italy over 50 years range from €1.9 billion to €5.2 billion (Schneider et al. 2020). A 2017 assessment estimated that if *Xylella* were to enter and establish in Australia, the cost to Australian wine grape and wine-making industries would be between A$2.2 billion and A$7.9 billion in aggregate over 50 years (Hafi et al. 2017). Similarly, losses and costs associated with Pierce's Disease in grapevines in California have been estimated at US$104.4 million per year, with US$48.3 million funding Pierce's disease activities undertaken by various government agencies, the nursery and citrus industries, and US$56.1 million being the cost of lost production and vine replacement (Tumber, Alston & Fuller 2014). Recent analysis (Hafi et al. 2021) estimated that a single subspecies of *X. fastidiosa* establishing in Australia could cost between A$1.2 billion and A$8.9 billion over a 50 year period. Establishment of more than one subspecies of *X. fastidiosa* is estimated to cost between A$7.8 and $11.1 billion over the same period. These costs are attributed to production loss, reduced incomes for labour used in fruit processing and marketing, reduced revenue from the food trade industries and environmental impacts. Effects on native Australian plants are difficult to estimate with precision, but for example *Eucalyptus* forest is the most common forest type in Australia covering 101 million hectares, which is 77% of Australia's total native forest area (ABARES 2018). *Eucalyptus* timber is a significant industry in Australia, and in 2016–17 the value of logs harvested from native production forests exceeded A$400 million (ABARES 2019). Any reduction in the survival of *Eucalyptus* forests would have a significant economic impact on timber production. Consequences of impacts that would be sustained in Australia if one or more species of *Xylella* were to establish are difficult to estimate with precision and will depend on factors such as commodity, location, extent and duration of the incursion. It is reasonable to conclude that the impact would be significant at a national level.
Other aspects of the environment	E Significant at the Regional level As discussed above, predicting the susceptibility of Australian native plants to *Xylella* is difficult, and it is likely that more species and taxonomic groups of these plants would be found to be susceptible if *Xylella* were to establish in Australia. Loss of plant diversity and changes in native vegetation types could cause impacts to assemblages of native and feral animals and insects. Some of these impacts (for example, on dipteran, hymenopteran and coleopteran fauna that pollinate (Armstrong 1979)) could have direct effects on pollination efficiencies in field and other crops, as well as in native flora more generally (Arthur et al. 2010; Rader et al. 2014). Loss of pollination services has been discussed in other department publications; for example, it has been estimated that higher costs could be faced by producers of crops such as almonds, apples and cherries if bee pollination was reduced (Department of Agriculture 2019a). *Xylella* is currently known to affect 3 *Eucalyptus* species, and if this host range proves to be wider, the infection of eucalypt native forests would endanger Australia's rich biodiversity and conservation of indigenous Australian's heritage. *Eucalyptus* forests support many forest-dwelling or forest-dependent species of flora and fauna. This includes species endemic to Australia, and species that are listed as threatened under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999 (ABARES 2018). Australia could suffer losses of local and regional cultural value through damage to monumental treescapes, botanical gardens and historically important plantings of native and non-native plants. Similar effects have been recently documented in Italy with the loss of monumental olive trees due to *Xylella* infections(Semeraro et al. 2019)(FAO, 2019), and Washington D.C. in landscape ornamental trees (Harris & Balci 2015).

Criterion	Estimate and rationale
	Where urban forests are infected and damaged by *Xylella*, there may be a decrease of thermal comfort in whole neighbourhoods (Semeraro et al. 2019). Common urban shade trees susceptible to *Xylella* include elm, maple, sycamore, London plane and oak. *Xylella* infection of these plants will likely require development and implementation of replanting policies for the affected urban landscapes to maintain human well-being (Gould & Lashomb 2005; Harris & Balci 2015). Measures such as pruning, weeding and prohibition of planting of susceptible species, such as those conducted in olive plantations in Italy in an effort to control the spread of *Xylella*, have had a negative impact on the beauty of the region (Ali, van der Werf & Lansink 2021). Similar visual detriment to the Australian environment could be expected if such measures were required in areas where Australian native plant species occur. Environmental consequences of *Xylella* would be influenced by the climatic conditions in Australia. The cooler temperatures of southern areas of Australia would be expected to curb the proliferation and spread of the bacterium (Feil & Purcell 2001) and/or suitable insect vectors of the pathogen (Rathé et al. 2011), but the warm northern tropical and sub-tropical temperatures would be expected to provide conditions conducive for the bacteria. Although the potential impacts are likely to be highly context dependent, it is reasonable to conclude that the impact of *Xylella* entry, establishment and spread would be at least significant at a Regional level.
Indirect	
Eradication, control	E Significant at the Regional level Establishment of *Xylella* in Australia could be expected to initiate a complex series of response actions including plant destruction, establishment of quarantine zones, product tracing activities and possible restrictions on trade (PHA 2017). Replacement of susceptible cultivars of crop plants is another longer-term strategy for control (Gould & Lashomb 2007). Control programs generally include prevention and/or containment measures such as the use of disease-free propagating materials, early surveillance and detection, destruction of infected plants, and vector control strategies (IPPC 2017). In Washington D.C., management strategies for suppressing *Xylella* in urban trees involves injections of antibiotics, application of plant growth regulators, and the use of insecticides (Castle et al. 2005; DeStefano et al. 2007; Kostka, Tattar & Sherald 1985; Tubajika et al. 2007). Management of potential host plants and vector species in an affected area would be likely to impose significant economic imposts. For example, management activities (loss of production plants and measures for disease prevention) cost the Californian grape industry an estimated US$104 million per annum and the Brazilian citrus industry US$120 million per annum (IPPC 2017). An eradication campaign for a *Xylella* incursion in Australia is also likely to cause significant economic costs. The eradication campaign for citrus canker from an area with a radius of 50 km in Emerald, QLD was completed in early 2009 and the total cost of the eradication campaign was estimated at $17.6 million (Gambley et al. 2009). In comparison, the cost of eradication of Banana freckle disease from 300 properties in the Northern Territory in Australia over 2015-18 is estimated to have been about A$26 million (Australian Banana Growers Council 2020). The extensive host range of *Xylella* would likely substantially magnify costs of eradication. Activities such as those above would be likely to have significant effects on producer incomes and regional economies. Costs associated with plant destruction, eventual replacement and potential waiting time to resumption of production could flow through the national economy to commodity processes for domestic consumers (Wittwer, McKirdy & Wilson 2006). Many confirmed host species have been imported and established as ornamental and amenity plantings in home garden and landscape settings; many are likely to be susceptible and impacted by disease or deliberate removal. Based on overseas experience, responding to an incursion of *Xylella* would be a challenging and lengthy process, complicated by, and dependent on, the species/subspecies/genotype(s) detected and the plant host-vector-pathogen

Criterion	Estimate and rationale
	interaction. International experience has shown that eradication is likely to be very difficult if detection is delayed (Department of Agriculture 2019b). There are documented circumstances in which eradication has not been feasible, including in areas of Italy where a native vector exists and there are dense and uniform olive plantings (Scortichini 2020). It is reasonable to conclude that the cost of *Xylella* eradication and control would be at least significant at a Regional level.
Domestic trade	E Significant at the Regional level Any incursion/establishment will impact domestic nursery stock trade distribution networks, and this impact will be greater if a vector insect species is also associated with the incursion/establishment. Domestic trade in affected commodities is likely to be paused in the short-term while domestic trading partners assess risks of transfer to other jurisdictions. Domestic trade is likely to recommence with movement restrictions in place. For example, the discovery of *Bactericera cockerelli* (tomato potato psyllid) in Western Australia in 2017 resulted in interstate movement restrictions for fruit, vegetables, nursery stock, cut flowers, used machinery and equipment from Western Australia to other states in Australia (Plant Biosecurity Policy 2018). Similar domestic movement restrictions would be likely if *Xylella* or an exotic vector of *Xylella* were detected in Australia. It is also likely that mandatory insecticidal treatments, specialised packaging/storage and transport requirements to prevent re-infestation, inspection and certification, and accreditation of businesses would be required to reduce the risk of infected vectors travelling with commodities and conveyances. Again, these would be similar to restrictions implemented to control tomato potato psyllid in Western Australia (Plant Biosecurity Policy 2018). Australia's Interstate Certification Assurance (ICA) Scheme (information available at interstatequarantine.org.au/producers/interstate-certification-assurance) would be likely to require greater stringency around host plant movements and freedoms from potential insect vectors.
International trade	D Minor significance at the Regional level The likely impacts are relatively low for international trade in fresh produce; major concerns would relate to presence of plant material contaminates and possibility of insect vector contaminating pests. It is possible that international trade in affected commodities would be impacted in the short-term while trading partners assessed risks of transfer. Consequences would be expected to be more significant for germplasm exports. Trading partners could restrict the taxa of nursery stock that could be exported to exclude *Xylella* hosts. Requirements may also include PCR testing and certification to verify freedom from the bacteria. All trading partners, even those already known to have *Xylella*, could impose these restrictions, to limit the possibility of new genetic diversity of *Xylella* being introduced to their territories. There may however be alternative types of plant material for export available, for example, tissue cultures. In the 5-year period between 2016 and 2020, there were 1,814 consignments of nursery stock exported from Australia valued at $53 million, a proportion of which would be considered *Xylella* hosts. The export trade in *Xylella* host plants may diminish or cease, as importers could preferentially source nursery stock from *Xylella*-free countries. It is reasonable to conclude that the impact on international trade would affect germplasm exports, but that alternative export conditions for *Xylella* host plants would be adopted by trading partners, making this impact of minor significance at a Regional level.
Non-commercial and environmental	E Significant at the Regional level Environmental impacts of *Xylella* establishment could reasonably be expected to be significant at a regional level. Use of insecticides in attempts to suppress any identified exotic and/or native vector(s) would potentially impact native arthropod fauna and create flow-on ecosystem effects to organisms that rely on those arthropods. For example, in the

Criterion	Estimate and rationale
	Puglia region of Italy, twice yearly applications of insecticide have been mandated to control vectors of *Xylella*, resulting in protests from organic farmers and environmentalists (Burdeau 2018). Increased pesticide use required to manage vector species could affect the environment. Spray drift of pesticides can induce soil toxicity, runoff and water system contamination (APVMA 2008; NSW DPI 2012). The Australian Pesticides and Veterinary Medicines Authority (APVMA 2008) defines spray drift as the physical movement of spray droplets (and their dried remnants) through the air from the nozzle to any non- or off-target site at the time of application or soon thereafter. Soil toxicity in agricultural systems is recorded in the US as inhibiting germination and leading to elevated pesticide residues in plants (Dalvi & Salunkhe 1975), possibly leading to issues with MRLs and saleability of crops. Runoff and leaching may affect biodiversity in aquatic ecosystems (NSW DPI 2012). Spray drift has been implicated with the decline of some butterflies in Australia (Sands & New 2002). Drought could cause increased mortality in plants infected with *Xylella.* Australia experiences semi-regular droughts across large regions of the landscape (Bureau of Meteorology 2020). Severe drought combined with *Xylella* infections in Australia's natural landscapes are likely to cause additional levels of plant and tree death. This phenomenon has been reported in California (Smith 2015). This would have flow-on effects to native ecosystems that rely on these landscapes. The requirements for delimitation, containment and eradication would likely necessitate removal of infected and exposed native and exotic plant hosts that are in close proximity to foci of infection. Costs of replanting with tolerant or resistant species – if or when possible – would likely be high, with long term amenity impacts. Species replacement may be difficult in some areas, particularly where the resident species was tolerant of extreme conditions, such as *Acacia* spp. being able to thrive in dry and windy environments where the soils are sandy and of high pH (Griffin et al. 2011)

3.4 Unrestricted risk estimate

Unrestricted risk is the result of combining the overall likelihood of entry, establishment and spread with the outcome of overall consequences. The likelihood and consequences are combined using the risk estimation matrix shown in Table A.4.

Unrestricted risk estimate for members of the genus *Xylella*	
Overall likelihood of entry, establishment and spread	High
Consequences	High
Unrestricted risk	High

The URE for *Xylella* species potentially associated with at-risk nursery stock and/or associated infected insect vector species and seeds for sowing is assessed as **High**, which does not achieve the ALOP for Australia. Therefore, specific risk management measures are required for *Xylella* species on this pathway.

3.5 Pest risk assessment conclusions

Likelihood ratings and consequences estimate for the genus *Xylella* associated with imported nursery stock and seeds for sowing are set out in Table 3.1.

Table 3.1 Summary of unrestricted risk estimates for *Xylella spp.*

Transmission pathway	Likelihood of						Consequences	URE
	Entry			Establishment	Spread	EES		
	Importation	Distribution	Overall					
Imported nursery stock	**High**	High	**High**	High	High	High	High	**High**
Imported seeds for sowing	**High**	High	**High**	High	High	High	High	**High**

4 Pest risk management

This chapter provides information on the proposed risk management of *Xylella* spp. identified in association with imported nursery stock and seeds for sowing. *Xylella* spp. have been assessed to have an unrestricted risk estimate that does not achieve the ALOP for Australia. The objective of the measures discussed in this chapter is to maintain Australian freedom from species of *Xylella*.

Under the IPPC and SPS Agreement, phytosanitary measures to mitigate the risk of introducing new pests, including emergency measures, must be technically justified. In this section of the draft report, the department evaluates the existing standard import conditions and *Xylella* emergency measures to determine whether they are appropriate, and whether they should be maintained or amended. Alternative and additional measures that might manage the risks are also considered.

The history of regulation of *Xylella* plant hosts for Australia is described in Section 1.2.3. Nursery stock and seeds for sowing are subject to standard import conditions (discussed in Sections 4.1.1 and 4.2.1) and to the emergency measures currently in place. A summary of these emergency measures is provided in Table 4.1.

Table 4.1 Summary of emergency measures for plant hosts of *Xylella fastidiosa* (including recognised subspecies) and *X. taiwanensis* imported as nursery stock and seeds for sowing

Plant material type	Import requirements for high risk countries/regions	Import requirements for all other countries/regions
Tissue cultures of species from regulated families	Off-shore testing and certification: Phytosanitary certificate with additional declaration that all tissue cultures of the consignment were derived from mother tissue cultures that were tested by PCR and found free from *Xylella fastidiosa* as indicated on a laboratory test report.	Off-shore certification: Phytosanitary certificate with additional declaration that the tissue cultures in the consignment were derived from plants and tissue cultures that were grown only in 'name of country', which is free from *Xylella fastidiosa*.
In the event of a phytosanitary certificate being deemed unacceptable	On-shore action: Tissue cultures must be de-flasked and grown for a minimum of 12 months in a government PEQ facility before testing by PCR. All regenerated plants must be tested. A positive detection of *Xylella fastidiosa* will result in destruction of all materials from the consignment. All other current conditions for the plant species will apply. Alternative arrangements to those outlined above are export of materials or destruction.	On-shore action: Tissue cultures must be de-flasked and grown for a minimum of 12 months in government or approved PEQ facility before testing by PCR. All regenerated plants must be tested. A positive detection of *Xylella fastidiosa* will result in destruction of all materials from the consignment. All other current conditions for the plant species will apply. Alternative arrangements to those outlined above are re-export of materials or destruction.
Non-tissue culture (cuttings, rooted plants, budwood, corms and bulbs) of species from regulated families	Off-shore approved arrangement and certification: Phytosanitary certificate with additional declaration that plant material in the consignment was produced under an arrangement approved by the exporting country's NPPO in accordance with Australian requirements, and was tested by PCR and found free from *Xylella fastidiosa* as indicated on a laboratory test report.*	Off-shore certification: Phytosanitary certificate with additional declaration that plant material in the consignment and its parent stock were grown only in 'name of country', which is free from *Xylella fastidiosa*.
In the event of an unacceptable Phytosanitary Certificate	On-shore action: Plants must be grown for a minimum 12 months in a government PEQ facility before testing by PCR. All imported plants must be tested. A	On-shore action: Plants must be grown for a minimum 12 months in a government or approved PEQ facility before testing by PCR. All imported plants

	positive detection of *Xylella* will result in destruction of all materials from the consignment. All other current conditions for the plant species will apply. Alternative arrangements include hot-water treatment of the plants at 50˚C for 45 minutes (with all other conditions for the plant species applying following treatment), or re-export or destruction.	must be tested. A positive detection of *Xylella* will result in destruction of all materials from the consignment. All other current conditions for the plant species will apply. Alternative arrangements include hot-water treatment of the plants at 50˚C for 45 minutes (with all other conditions for the plant species applying following treatment), or re-export or destruction.
Seed of *Carya* spp. for sowing	Seed must be grown and disease screened for a minimum of 12 months at a government PEQ facility. Before release from biosecurity control, plants must be tested and found free from *Xylella* species. A positive detection of *Xylella* will result in destruction of all materials from the consignment. All other import conditions will continue to apply, including mandatory phosphine or cold treatment to mitigate the risk of insect pests.	

Source: Table summarised from BICON case alert on emergency quarantine measures for plant pathogen *Xylella fastidiosa* (DAWR 2016) and BICON case alert on emergency measures to manage *Xylella fastidiosa* within *Carya* spp. imported as seeds for sowing (DAFF 2022a).
Note: Certified bulbs in the genera *Narcissus*, *Hyacinthus* and *Hippeastrum* produced under the Bloembollenkeuringsdienst (BKD) scheme from Netherlands are exempt from these emergency measures.
* To date, there are no established arrangements approved by an exporting country's NPPO in accordance with Australian requirements and, therefore, this set of import requirements is currently not available for use.

4.1 Nursery stock

4.1.1 Standard import conditions and their evaluation

Import conditions for nursery stock are determined on the basis of the country of origin, species of plant and the growth form being imported. Under the department's standard import conditions for imported nursery stock:

- All live plant material (apart from orchid tissue cultures imported via airports as accompanied baggage, on the basis that no members of the Orchidaceae are confirmed natural hosts of *Xylella*) requires an import permit issued by the department prior to arrival. Live plant material that requires an import permit, but arrives without one, including where an application is currently under consideration, will be directed for export from Australian territory or required to be destroyed in an approved manner.
- All live plant material (apart from orchid tissue cultures imported via airports as accompanied baggage) must be accompanied by a phytosanitary certificate from the relevant exporting country's government authority (National Plant Protection Organisation) attesting to the general health of the imported nursery stock, or other statements as required by Australia.
- Each shipment must be packed in clean, new packaging and clearly labelled with the full botanical name of the species.
- All plant material must be free from soil, disease symptoms and other extraneous contamination of biosecurity concern.
- All tissue cultures must be free from any bacteria, fungal infection, live insects, nematodes, disease symptoms, or other extraneous contamination of biosecurity concern.
- All nursery stock consignments must be visually inspected by a biosecurity officer on arrival for freedom from bacterial and fungal infection, disease symptoms, live arthropods and other extraneous contamination of biosecurity concern. If pests or disease symptoms are found, samples are to be identified and will be subject to a risk assessment by the department and testing using a range of options. This may result in the consignment

requiring remedial treatment (if an effective treatment is available), export or destruction to ensure that the biosecurity risk is managed.

- All plant material, except for those imported as tissue cultures, must be treated to destroy any potential presence of arthropod pests, either by methyl bromide fumigation, or an insecticidal dip, depending on the type of plant.
- All plant material (apart from some species imported as tissue cultures and some approved high health pathways) require further growth in PEQ for disease screening. The PEQ screening period depends on the species of plant imported, measures applied pre-export and ranges from a minimum of 3 months to 2 years.

It is the importer's responsibility to ensure compliance with all conditions and requirements for entry of the material. This includes ensuring that their suppliers are aware of and comply with Australia's import requirements. Failure to meet the conditions outlined in the department's Biosecurity import conditions system (BICON, available from www.bicon.agriculture.gov.au/BiconWeb4.0) and on the import permit may result in plant material not being permitted entry into Australia.

The type of facility to which imported nursery stock are directed for inspection and post-entry requirements is based on assessed biosecurity risk criteria. Plant material classified as 'high risk' by the department is taken to a government PEQ facility unless other arrangements have been approved by the department. All other live plant material is directed to an appropriate managed facility ('approved arrangement'); approved arrangements may be privately or government owned and are regulated and audited by the department. The duration for which nursery stock must be maintained in a PEQ or approved arrangement facility is dependent on the biosecurity risks associated with the plant species, its form of import, and the species-specific screening/testing requirements.

Mandatory visual inspection of nursery stock prior to export, and on arrival in Australia to verify freedom from material of biosecurity concern

Limitations: only effective if the material of biosecurity concern is visible.

Visual inspection is an adequate detection method for the presence of arthropods in nursery stock consignments, particularly for insects in the hemipteran sub-order Auchenorrhyncha, which are large enough to be seen without magnification.

Nursery stock consignments that are infected with pathogens will not always show visible symptoms. In the case of *Xylella*, disease symptom expression may be delayed for several months following initial infection by *Xylella*, and plants may appear symptomless but have asymptomatic infections (Almeida & Nunney 2015).

Recommendation: visual inspection is inadequate for detecting *Xylella* in nursery stock (including tissue culture). Therefore, additional phytosanitary measures are required to verify freedom, as proposed in Section 4.1.3.

Mandatory treatment of nursery stock for arthropod pests

Limitations: None

Plant material that is contaminated with arthropod pests, particularly those insect species known to have the potential to vector *Xylella*, may provide a pathway for the entry,

establishment and spread of *Xylella* into Australia. The risk of vector entry, although confirmed, is however considered to be low (Rathé et al. 2015; Stanaway et al. 2001) and departmental analysis of insect interceptions (discussed in Section 2.6.3) confirms this opinion.

Recommendation: Arthropods that are plant pests, and that can transmit other plant pests such as pathogens, can be spread by the movement of plant material. This measure provides additional assurance to the visual inspection, and the department proposes that the requirement for a mandatory treatment for arthropod pests remains in place.

Mandatory PEQ screening period for *Xylella* host nursery stock (non-tissue culture)

Limitations: None

Nursery stock infected with pathogens will not always show visible symptoms. This is true for a number of different types of plant pathogens as well as for *Xylella*. A period of growth in PEQ enables specialists to determine whether any visible symptoms of pathogens become evident, and to conduct diagnostic testing for a range of different pathogens to enable release of material free from pathogens of biosecurity concern.

As discussed in Section 2.7.1, *Xylella* disease symptom expression is dependent upon various factors. For this reason, the minimum duration for plants to be held at a PEQ facility prior to testing for *Xylella* spp. will vary, and will be dependent upon factors including the plant species or cultivar, but consideration will also be given to growing conditions and nutritional availability.

Recommendation: The department proposes that a mandatory period of growth in PEQ for *Xylella* screening purposes remain in place for all *Xylella* host nursery stock (non-tissue culture). The minimum duration for plants to be held in PEQ for *Xylella* testing will be 12 months, unless otherwise specified and/or approved by the department.

Some plant species currently categorised as high-risk nursery stock are not affected by the proposed changes because *Xylella* testing requirements are already in place.

4.1.2 Existing emergency measures and their evaluation

Australia's existing emergency measures for *Xylella* are based on the *Xylella* host status of any members of a plant family, country of origin of the material, measures applied offshore and form of the nursery stock (that is, either whole plants, cuttings or plant parts or plant tissue culture) and are summarised in Table 4.1. These measures are discussed and evaluated in this section.

Regulation of all *Xylella* spp.

Limitations: The emergency measures as introduced in 2015 are specific to *Xylella fastidiosa*, but since then a second species of *Xylella* has been described (*X. taiwanensis*).

In setting import conditions, the current emergency measures regulate all *Xylella* species and host plants must be tested using test protocols that target both *X. fastidiosa* and *X. taiwanensis*.

Recommendation: The department proposes that the import conditions are clarified to include all *Xylella* spp. to encompass the 2 currently known species and any others still to be isolated or taxonomically defined. This will mean changes to the wording of additional declarations on phytosanitary certificates (discussed in Section 4.1.3).

Xylella plant host status regulation by plant family

Australia's emergency measures regulate all plant species belonging to a plant family that contains at least one confirmed natural host of *Xylella* spp.

Limitations: It is possible that some plant families containing member species that are confirmed *Xylella* hosts may also contain member species that are not able to host the pathogen.

One of the difficulties in regulating plants to exclude entry of *Xylella* spp. is the number of additional plant hosts that have been identified over time (as discussed in Section 2.4). In addition, there is increasing evidence that *X. fastidiosa* has a capacity to undergo interstrain recombination to produce novel strains, with host ranges that differ from their parental strains (Nunney et al. 2014a; Rapicavoli et al. 2018).

Xylella has a wide host range, and reports of the number of plant host species vary. The department has identified 106 plant families that contain *Xylella* hosts. Host plants may also display resistance (Simpson 2017), tolerance and be asymptomatic for 2 to 5 years following infection (Beretta et al. 1996; EFSA Panel on Plant Health et al. 2019a), and opportunity exists for the misdiagnosis of infected plant material (Baldi & La Porta 2017; EFSA Panel on Plant Health et al. 2019a; Francis et al. 2006). Each of these factors can lead to the slow identification of new plant host species. In the face of such a recognised generality of susceptibilities, Australian emergency measures regulation of *Xylella* was based on the consideration that where one species in a family is a confirmed natural host, all members of the family are likely to share that susceptibility.

The existing family level regulation of plant hosts of *Xylella* does however capture numerous species not known to be *Xylella* hosts. The families known to contain hosts and the number of genera within those families that contain confirmed host species are presented in Appendix D. Twenty plant families contain 3 or more genera of host species; however, in many cases families contain only one or 2 known host species. Some of these plant families contain large numbers of genera not known to contain hosts of *Xylella*, for example the Asteraceae, which contains 1676 genera in total (Royal Botanic Gardens 2022) of which only 38 genera (2.6%) contain confirmed host species of *Xylella* (Appendix D).

The department has estimated the number of genera and species within the 106 currently regulated plant families using a variety of botanical resources. These figures remain estimates because there are differences in opinion about the number of taxa worldwide. The department estimates that more than 10,000 plant genera and 20,000 plant species are currently regulated under the existing family level regulation of plant hosts of *Xylella* spp. For genus level regulation, the number of genera that contain one or more confirmed natural plant hosts of Xylella is 356, with a corresponding reduction in the number of species regulated.

There are associated implications for the department and for industry in regulating plant hosts at family level. For the department, family level regulation:

- increases the number of taxa of plants and number of imported plants that require testing onshore, with subsequent resource implications for PEQ facilities (booking up spaces that could be used for other plant imports) and staffing (both in horticulturists caring for the plants and scientific staff conducting the diagnostic testing). This also created resource implications for the tissue culture pathway and resulted in the need to move the testing

requirement for tissue culture offshore (discussed in more detail in the following subsection).

- creates the need to adjust the genera and species regulated in each family when taxonomic revisions are conducted, and raises the risk of revisions being missed and not regulated soon enough.

For importers and industry, family level regulation:

- can increase the cost of imports because non-tissue culture material requires either mandatory hot water treatment or further growth in PEQ for disease screening. The PEQ screening period depends on the species of plant imported and ranges from a minimum of 12 months to 2 years.

Changing current family level host plant regulation to target genus level also has limitations and benefits. Limitations involve a reduced 'buffer' in host regulation when new plant hosts are identified. The department will need to maintain agility in updating the host plant regulations more frequently to reduce the risk of import of *Xylella* host plants. Benefits include:

- a reduction in the number of plant species requiring *Xylella* testing, potentially introducing resource savings for both the department and industry. Resource savings for the department could be redirected to verification of *Xylella* nursery stock pathways (discussed further in the following subsections).
- a reduction in the frequency with which the department will need to change the plants being regulated due to taxonomic changes at the family level no longer being applicable.
- better adherence to least trade restrictive practices, technical justification and transparency for Australia in meeting obligations under the SPS Agreement.

The department now has more than 7 years' worth of experience with plants imported under family level *Xylella* regulation and no detections of *Xylella* have ever occurred in plants undergoing PEQ testing. This gives confidence that the regulation level can be adjusted. On balance, the reduction in regulation of a number of plant species not known to have *Xylella* hosts within their genera would free up trade for importers and Australian businesses reliant on nursery stock, with a possible trade-off in enabling increased verification for *Xylella* host material imported from low risk countries/regions and tissue culture import pathways.

Recommendation: The department proposes that regulation of plant hosts of *Xylella* be changed from regulating at plant family level (where one or more species within that family is a confirmed natural *Xylella* host) to regulating at genus level (where one or more species within that genus is a confirmed natural *Xylella* host). The 3 genera (*Phlox*: Polemoniaceae, *Simmondsia*: Simmondsiceae and *Linum*: Linaceae) known to be experimental hosts of *Xylella* but with strong associations with the known competent insect vectors of *Xylella* — *Philaneaus spumarius* and/or *Homalodisca vitripennis* — would also be regulated.

This proposed change will enable the department to direct additional resources to an active monitoring program of assurance and verification (discussed later in this chapter) to give additional confidence about the absence of *Xylella* in nursery stock sourced from low risk countries/regions and in tissue cultures originating from high risk countries/regions.

The department will continue to monitor for any changes in *Xylella* host status and adjust the plant host genera list and regulation as appropriate.

Xylella regulation by high and low risk country/region lists

The department currently differentiates nursery stock import conditions based on the definition of *Xylella* spp. presence. High risk countries/regions [that is, the Americas (including the Caribbean), Europe, India, Iran, Israel, Lebanon, Taiwan and Türkiye] were defined as those that had reported *Xylella*, as well as their associated trading blocs (such as the European Union), and areas where *Xylella* is known to be native (the Americas and Caribbean). The low risk country/region list includes all countries/regions not included in the high risk list.

Limitations: The global movement of plant propagative materials contributes to uncertainty over the specific region of origin and the health status of the material. Therefore, the low risk and high risk country/region list may not be completely accurate, and importation of *Xylella* could occur with materials from low risk countries/regions in which *Xylella* has established but not yet been recognised.

Australia's high risk country/region list for *Xylella* includes all countries in the Americas (including the Caribbean), all countries/regions in Europe, and India, Iran, Israel, Lebanon, Taiwan and Türkiye. The reasons for the determination of these countries/regions as high risk include:

- accepted location(s) of origin—*Xylella* is considered to be native to the Americas, with unique subspecies of the bacterium having developed in Central America, North America and South America (see discussion in Section 2.5.1), and movement of competent and infected vectors is possible across country borders. All countries in the Americas, including the Caribbean, are thus considered by the department to be high risk for *Xylella*.
- reports of presence within trading bloc—in the European Union (EU) *Xylella* is known to occur in France, Italy and Spain (see Section 2.5.2). Movement of nursery stock between EU countries has not always been consistently regulated, interceptions of *Xylella* are still occurring in imported nursery stock (see Section 2.5.3), and movement of competent and infected vectors is possible across country borders. The United Kingdom is included in the department's definition of Europe, as prior to Brexit (1 February 2020) unregulated nursery stock movements occurred between those bloc countries.
- reports of presence of *Xylella* spp. in countries/regions—Iran (Amanifar et al. 2014), Israel (EPPO 2019; Plant Protection and Inspection Services 2019) and Taiwan (Su et al. 2013; Su et al. 2016).
- countries/regions where the department considers the presence of *Xylella* to be currently uncertain—India (Gupta & Sharma 1998; Jindal & Sharma 1987; Verma & Sharma 1999), Lebanon (Choueiri 2017; Habib et al. 2016; Temsah, Hanna & Saad 2015) and Türkiye (formerly referred to as Turkey) (Choueiri 2017; Habib et al. 2016; Temsah, Hanna & Saad 2015). The department will continue to regulate these countries as high risk for *Xylella* presence. NPPOSs can prepare a technical submission demonstrating surveillance results over time and the country's own regulation of *Xylella* for consideration by the department.

Among countries from which *Xylella* is not recorded, there are generally high levels of awareness of the risk that is posed, and in most cases, measures intended to exclude its entry are applied (MPI 2020). In addition, among countries from which *Xylella* is not recorded, there is increasing recognition of potential host plant susceptibilities and symptoms of infection, and greater utilisation of diagnostic procedures for its detection (FAO 2020; Parkinson & Malumphy 2014).

Recommendation: The department proposes that differentiated import requirements for nursery stock, dependent on the *Xylella*-status of the country/region of origin, remain in place, and that the high risk and low risk country/region definitions also remain.

The department will continue to monitor for any changes in *Xylella* status in countries/regions and adjust the list and regulation as appropriate. In addition, the department will instigate an active monitoring program of assurance and verification (discussed later in this chapter) to give additional confidence about the absence of *Xylella* in nursery stock sourced from these countries/regions.

Approved offshore sampling and PCR testing for *Xylella* species

Australia's emergency measures for nursery stock imports specify that tissue cultures of *Xylella* host plant material from high risk countries/regions must be certified by the exporting NPPO by presenting the following text on a Phytosanitary Certificate, "*All tissue cultures in this consignment were derived from mother tissue cultures that were tested by PCR and found free of* Xylella fastidiosa *as indicated on laboratory test report number* [insert number/code]*.*"

Limitations: The department does not explicitly specify which PCR tests for *Xylella* are acceptable in permit conditions for imported nursery stock, or that 2 tests are the minimum requirement set by ISPM 27 Annex 25 (FAO 2018). The department also does not specify sampling or pooling (bulking) requirements for plant tissue within permit conditions.

Australian PEQ diagnostics for *Xylella* are based around PCR methods (as discussed in Section 2.7.3), as morphological, serological, or biochemical methods have constraints around timeliness and variability of morphological traits, and are increasingly-less specific than results provided by molecular testing. The routine diagnostic methods for detecting *Xylella* species within a plant host in use by the department's PEQ laboratories are the conventional PCR of Minsavage et al. (1994) and the real-time PCR by Harper, Ward and Clover (2010, erratum 2013). Both PCR diagnostic methods in use by the department align with international diagnostic protocols outlined in ISPM 27 Annex 25, and both tests are used for each host plant, which meets the minimum requirement set by the ISPM. This is discussed in Section 2.7.3. The use of both tests enables detection of both *X*. *fastidiosa* and *X*. *taiwanensis*.

In addition, ISPM 27 Annex 25 sets minimum requirements for identification, being positive results from 2 tests based on different biological principles or from 2 molecular tests that amplify different genetic loci.

ISPM 27 Annex 25 recommended sampling requirements for plant tissue (time period, location of plant tissue and amounts of tissue) is also important, as false negatives can occur during PCR testing, for example, when plant tissue is selected from a plant that has an early infection, or from part of the plant that does not contain the bacteria (EFSA Panel on Plant Health 2015b). ISPM 27 Annex 25 does not specify the amount of plant tissue that can be pooled for testing, and these amounts can differ between plant species (discussed in Section 2.7.4). The department supports pooling of samples, and in the absence of research about appropriate pooling amounts for all plant species has specified that only 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. The most important factor in effective testing for *Xylella* spp. is laboratories having confidence that the

tests being used deliver accurate results if samples are pooled, by determining the limits of each detection test.

Recommendations:
The department proposes specifying the approved offshore testing, sampling and pooling requirements for samples in import permits. The conditions proposed are outlined below.

Testing for tissue culture—for verification of the presence or absence of *Xylella* spp. in the mother plants, the department proposes the following PCR testing protocols:

- the rimM gene sequence real-time PCR test from Harper, Ward and Clover (2010, erratum 2013),

AND

- the conventional PCR from Minsavage et al. (1994).

Alternative PCR testing protocols may be approved by the department, after submission of information by the exporting NPPO.

Sampling requirements for PCR testing for *Xylella* species must align with the sampling protocols of ISPM 27 Annex 25, being:

- sampling must be carried out late in the current growing season when bacterial concentration (titre) is expected to be highest in the xylem tissues. This period is generally from the end of summer until early autumn, as the bacterial concentration is very often low in new spring growth even in plants that have been infected for some time and previously produced disease symptoms.
- Samples must be representative of the entire aerial part of the plant. Selected tissue samples may be from leaf petiole or leaf mid-vein or appropriate vascular tissues with a concentration of xylem vessels.

Pooling of samples is permitted:

- 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.

Record keeping and certification:

- the laboratory must record the identities of plant lots and mother plants that are tested, the number of samples tested and the protocols used, and these details must be included on the laboratory report.
- the exporting country's NPPO must verify the laboratory report to confirm that testing was conducted in accordance with Australian requirements, prior to issuing certification.
- the identifying code or number of the laboratory report must be provided on the Phytosanitary Certificate.
- a copy of the laboratory report must be attached to the Phytosanitary Certificate.

Tissue culture of host plant material from high risk countries/regions

The existing emergency measures allow the import of tissue culture of *Xylella* host plants from high risk countries/regions if the exporting NPPO provides an additional declaration that '*All tissue cultures in this consignment were derived from mother tissue cultures that were tested by*

PCR and found free of Xylella fastidiosa as indicated on laboratory test report number [insert number/code]'. If the imported consignment meets all other requirements, tissue cultures are released with no further biosecurity control

Limitations: This arrangement was introduced with emergency measures in 2015 in consideration of the family level *Xylella* host regulation, which would require an extremely large number of plant species be subject to a testing requirement for *Xylella*. Australia does not have the onshore capabilities to test such anticipated volumes of plants, so offshore testing for tissue culture mother stock was permitted. There are a wide range of countries exporting plants to Australia, and there are a corresponding large number of overseas laboratories used to conduct the testing. Thus, the department has limited oversight of the implementation of laboratory testing protocols used overseas.

Detecting and identifying *Xylella* bacteria can be difficult, and various PCR and other tests for *Xylella* bacteria have been reported in the scientific literature. The non-uniform distribution of *Xylella* bacteria within plants used as source material for tissue culture can provide the opportunity for misdiagnosis of infected plant material (Baldi & La Porta 2017; EFSA Panel on Plant Health et al. 2019a; Francis et al. 2006) (discussed in Section 2.6.1). Growth as whole plants over a 12 month period ensures the bacterium can multiply and spread within the plant, in turn allowing a better chance of detection using molecular methods.

Tissue culture requirements can also be problematic due to the differentiation of mother plants grown in the field as opposed to mother tissue cultures. Commercially important tissue cultures may be propagated from mother tissue cultures grown in laboratory environments where there is limited risk of the plants coming into contact with *Xylella* bacteria and infected insect vectors. There is limited evidence about the efficacy of testing tissue culture material for pathogens, without growing out the material for a period long enough to allow the pathogen load to reach a titre that is detectable.

There are particular types of tissue culture that may pose a lower risk of transmitting *Xylella*, such as meristem cell culture (discussed in Section 2.6.2), however, evidence suggests that *Xylella* has the potential to be inadvertently transferred from the parent plant with the meristem tissue culture propagule. In addition, operationally it is not possible to determine the cell source of the cultured material, so tissue cultures arriving at the Australian border could not be segregated in this way.

At present, the department conducts periodic verification of the NPPO certification of imports but does not conduct any PCR testing for *Xylella* in plants imported in tissue cultured form (except for those species that require growth and testing in government PEQ because of, for example, their importance to Australia's agricultural industries). There is also no requirement for permit holders or NPPOs to present the laboratory reports.

The department has specified the PCR testing protocols that have been approved for *Xylella* detection in this document. In order to verify that these protocols are being used, and the testing conducted using them, the department will need to receive copies of laboratory reports that correspond to the report details endorsed within the accompanying phytosanitary certificate.

Recommendations: The department proposes:

- that the requirement for PCR testing of the mother plants from which the tissue cultures for export to Australia and/or their parent tissue cultures are derived and phytosanitary certification of this testing remain in place. Mother plants are to be maintained in an insect-proof environment while testing for *Xylella* and cell material collection is occurring.
- introducing a requirement for copies of laboratory reports to accompany the phytosanitary certificate. This will assist the department in continuing to conduct periodic verification activities on the laboratory reports, the diagnostic tests used and the results.

Record keeping and certification:

- the laboratory must record the identities of plant lots and mother plants that are tested, the number of samples tested and the protocols used, and these details must be included on the laboratory report.
- the exporting country's NPPO must verify the laboratory report to confirm that testing was conducted in accordance with Australian requirements, prior to issuing certification.
- the identifying code or number of the laboratory report must be provided on the Phytosanitary Certificate.
- a copy of the laboratory report must be attached to the Phytosanitary Certificate.

These proposals necessitate a change in the additional declaration on phytosanitary certificates. The proposed new additional declaration is '*All tissue cultures in this consignment and/or their parent tissue cultures were derived from mother plants that were sampled in accordance with ISPM 27 annex 25 and tested by department-approved PCR tests and found free from* Xylella spp*. as indicated on laboratory report number [insert number/code]. Mother plants were maintained in an insect proof environment while testing and cell collection was performed.'*

Assurance and verification of imported *Xylella* nursery stock

The existing emergency measures allow for offshore certification of country freedom from *Xylella* by the responsible NPPO through a Phytosanitary Certificate with the additional declaration that:

- '*The tissue cultures in the consignment were derived from plants and tissue cultures that were grown only in 'name of country', which is free from* Xylella fastidiosa'.
- Or '*Plant material in this consignment and its parent stock were grown only in 'name of country' which is free from* Xylella fastidiosa*.'*

Limitations: As discussed in Sections 2.7.1 and 2.7.2 and elsewhere in this document, the global movement of plant propagative materials contributes to uncertainty over the specific region of origin and the health status of materials. Therefore, importation of *Xylella* could occur with materials from low risk countries/regions in which *Xylella* has established but not yet been recognised, or from material sourced from unknown geographic regions but shipped through a low risk country/region. While this is a low possibility due to the certification of the responsible NPPO, it could occur.

Recommendation: The department proposes the introduction of assurance and verification processes for *Xylella* presence in host plant nursery stock (including tissue culture), regardless of the country/region of origin. This would not apply to plant material currently categorised as high-risk nursery stock that already has onshore testing requirements in place.

Assurance and verification could include:

- in-country audits of the NPPO's systems for approval of facilities and certification that required PCR testing has been conducted (for tissue cultures originating from high risk countries/regions)
- audits of processes used to establish that plant material and its parent stock were only grown in the country of origin
- onshore testing of arriving consignments
- conducting trace-back exercises, including asking NPPOs to provide evidence of verification of the effectiveness of offshore systems involved in producing the plant material.

The above activities may be subject to departmental fees and charges.

Certification

The existing emergency management measures include:

- offshore certification by an NPPO to confirm freedom from *Xylella* spp., or an NPPO approved arrangement for producing nursery stock that is free from *Xylella;*
- onshore measures where the offshore certification is deemed unacceptable;
- compliance with all other current import conditions for the plant species.

Associated with these requirements are the following elements:

- the export of nursery stock and plant tissue culture must be certified by the NPPO of the exporting country.
- plant consignments must be packaged in such a way that prevents transmission of, and infection by, *Xylella* bacteria. This must incorporate insect vector exclusion.
- where freedom from *Xylella* is being claimed, phytosanitary certificates issued by the exporting country's NPPO must include additional declarations confirming freedom from *Xylella* spp. Information to enable tracing of plant lines being imported to Australia, such as test results and the facility in which the lines were grown, must also be provided.

Limitations: The recognition of *Xylella* presence in a region may be delayed, due to a variety of factors including delayed symptom expression and false testing results. However, among countries from which *Xylella* is not recorded, there are generally high levels of awareness of the risk that is posed, and in most cases, measures intended to exclude its entry are applied (MPI 2020).

Recommendation: The department proposes maintaining the NPPO certification requirements currently in place. Phytosanitary certification for live plant material imported from countries/regions where *Xylella* spp. are not known to occur provides assurance to Australia of area freedom from *Xylella* bacteria. As discussed above in the section on tissue cultures from high risk countries/regions, including a requirement for copies of laboratory reports to accompany the phytosanitary certificate will assist the department in continuing to conduct periodic verification activities on the imported consignments. In addition, inclusion of an assurance and verification step (also discussed above) for imported nursery stock will give the department assurance that processes are working as intended.

Hot water treatment

Hot water treatment is offered as an optional treatment for imported *Xylella* host nursery stock from high risk countries/regions.

Limitations: Hot-water treatment has not been tested across the broad range of *Xylella* hosts, all *X. fastidiosa* subspecies or *X. taiwanensis* so its general application as a phytosanitary treatment may be limited (discussed in Section 2.8.1). Propagation material in poor condition, heat-sensitive cultivars, or plants of generally known sensitivity, may suffer adversely from the treatment. In addition, nursery stock material with stems/trunks greater than 10 cm in diameter or more than 1.5 m in length cannot be adequately treated.

Recommendation: The department proposes removing hot-water treatment as a treatment measure for *Xylella*. The department may assess specific applications for hot water treatment proposed by an NPPO. Evaluation of such measures will require a technical submission from the NPPO that details the proposed measures, including suitable information to support the claimed efficacy, for consideration by the department.

Off-shore approved arrangements and certification

The existing emergency measures contain provision for off-shore approved arrangements and certification for non-tissue culture material from high risk countries/regions (see Table 4.1).

Limitations: To date, there are no established arrangements approved by an exporting country's NPPO in accordance with Australian requirements and, therefore, this set of import requirements is currently not available for use.

Recommendation: The department proposes removing the existing condition for offshore approved arrangements. The department will consider any alternative measure proposed by an NPPO, as specified in Section 4.3 of this document.

4.1.3 Proposed import conditions for nursery stock hosts of *Xylella* species

This draft PRA report proposes that imported nursery stock that belongs to a plant genus known to contain a *Xylella* spp. host should be subject to:

- the department's standard nursery stock import conditions

AND

- the additional measures proposed in Table 4.2.

Table 4.2 Proposed measures for plant hosts of *Xylella* spp. imported as nursery stock

Plant material type	Import requirements for high risk countries/regions	Import requirements for all other countries/regions (low risk)
Tissue cultures of species from regulated genera	Off-shore testing and certification: the mother plants from which tissue cultures and/or their parent tissue cultures are derived must be tested for *Xylella* spp. using the rimM gene sequence real-time PCR test from Harper, Ward and Clover (2010, erratum 2013) and the conventional PCR from Minsavage et al. (1994). Mother plants are to be maintained in an insect proof environment while testing for *Xylella* and cell material collection is occurring. Sampling and sample preparation requirements for PCR testing for *Xylella* species must align with the sampling protocols of ISPM 27 Annex 25, Phytosanitary certification must include the additional declaration *"All tissue cultures in this consignment and/or their parent tissue cultures were derived from mother plants that were sampled in accordance with ISPM 27 Annex 25 and tested by department-approved PCR tests and found free of* Xylella *spp. as indicated on laboratory test report number [insert number/code]. Mother plants were maintained in an insect proof environment while testing and cell collection was performed."* Record keeping and certification: • the laboratory must record the identities of plant lots and mother plants tested, the number of samples tested and details of the protocols used and include these details on a laboratory report. • the exporting country's NPPO must verify the laboratory report to confirm that testing was conducted in line with Australian requirements prior to issuing certification. • the identifying code or number of the laboratory report must be provided on the Phytosanitary Certificate. • a copy of the laboratory report must be attached to the Phytosanitary Certificate.	Off-shore certification: Phytosanitary certificate with additional declaration that *"The tissue cultures in this consignment were derived from plants or tissue cultures that were grown only in [insert name of country], which is free from* Xylella *spp."*
Non-tissue culture (cuttings, rooted plants, budwood, corms and bulbs) of species from regulated genera	Subject to a minimum 12 month PEQ period in a government PEQ facility before testing by PCR.	Off-shore certification: Phytosanitary certificate with additional declaration that "*Plant material in this consignment and its parent stock were grown only in [insert country] which is free from* Xylella *spp."*

In addition, imported consignments of any nursery stock that does not have a mandatory requirement for PEQ grow out and testing may be subject to departmental assurance and verification processes which may be subject to departmental fees and charges.

Where import conditions for any of these import scenarios have not been met, plants must be grown for a minimum 12 months in a government PEQ facility before testing by PCR. All imported plants must be tested. A positive detection of *Xylella* will result in destruction of all materials from the consignment. All other current conditions for the plant species will apply.

The department proposes that when the above-described risk management measures are followed, the restricted risk for *Xylella* spp. in association with imported nursery stock will achieve the ALOP for Australia.

4.2 Seeds for sowing

4.2.1 Standard import conditions and their evaluation

Under Australia's existing policies, all seeds for sowing are subject to the department's standard import conditions. Under these conditions:

- Each shipment must be packed in clean, new packaging and be clearly labelled with the full botanical name of the species.
- Where the seed lot is greater than 10 kg and contains seed of less than 8 mm in diameter, mandatory International Seed Testing Association (ISTA) sampling of each consignment must be used to establish freedom from contamination including weed seeds. This testing may be performed at department approved ISTA laboratories overseas or on arrival in Australia. A biosecurity officer must conduct a visual inspection of each consignment on arrival in Australia to verify the results of the ISTA sampling, or collect a sample for analysis if testing was not conducted overseas.
- Where the seed lot is less than or equal to 10 kg in weight, or contains seed of greater than 8 mm in diameter, a biosecurity officer must conduct a visual inspection of each consignment on arrival in Australia for freedom from live insects, soil, disease symptoms, contaminant seed, other plant material (for example, leaf and stem material, fruit pulp, and/or pod material), animal material (for example, animal faeces and/or feathers) and any other extraneous contamination of biosecurity concern.
- Prior to export the goods must be inspected and found free from evidence of any *Trogoderma* species of biosecurity concern including khapra beetle (*Trogoderma granarium*). To demonstrate compliance, the accompanying Phytosanitary certificate must contain the additional declaration "*Representative samples were inspected and found free from evidence of any species of* Trogoderma *(whether live, dead or exuviae) in Australia's list of* Trogoderma *species of biosecurity concern*.
- Large and/or woody seeds are also subject to a treatment for internal insect infestations, being either fumigation with phosphine or cold treatment.

All consignments imported into Australia regardless of end-use (including seeds for sowing) must meet departmental standards for seed contamination and tolerance.

In evaluating the standard seeds for sowing import conditions, the department considers that there are no conditions that specifically address the risk of *Xylella* spp. transmission from seeds for sowing to seedlings.

4.2.2 Existing emergency measures and their evaluation

On 20 May 2022, the department introduced emergency measures in relation to *Carya* spp. seeds for sowing (discussed in Sections 1.2.3 and 2.6.4). At present, vertical transmission of *Xylella* from seed to the germinated seedling has only been confirmed in *Carya illinoinensis*

(Cervantes et al. 2022). The following discussion focuses on that scenario but will be extrapolated to other plant genera if further evidence confirms transmission.

The emergency measures introduced a mandatory requirement that imported *Carya* spp. seeds for sowing from all countries of origin must be grown for a minimum of 12 months at a government PEQ facility. Before release from biosecurity control, the plants must be tested by PCR and found free from *Xylella* species.

Genus level regulation of *Carya* seeds for sowing

The emergency measures apply to all *Carya* species within the genus.

Limitations: The scientific confirmation of *Xylella* vertical transmission in *Carya illinoinensis* is recent, and to date few studies have focussed on transmission of *Xylella* through seeds, and of those studies conducted, the conclusions have been inconsistent. A number of other *Carya* species are known hosts of *Xylella*, including *Carya cathayensis, C. cordiformis, C. floridana, C. glabra, C. laciniosa, C. pallida, C. palmeri* and *C. tomentosa* (EFSA 2022b). Hilton (2017) found that 9 *Carya* species in addition to pecan and hybrids in the National Collection of Genetic Resources for Pecans and Hickories were infected with *Xylella*. Given the relatedness of these species and assumed similar seed morphology, it is logical to conclude that these other species could also be susceptible to vertical transmission.

Recommendation: Seeds for sowing *Xylella* regulation be retained at the genus level for *Carya* spp. unless specific confirmatory research concludes that transmission only occurs in *Carya illinoinensis*.

Regulation of all countries of origin

The emergency measures apply to *Carya* spp. seeds for sowing from all countries/regions of origin. This contrasts to the nursery stock regulation, which recognises high risk and low risk countries/regions.

Limitations: None. The new scientific evidence of vertical transmission of *Xylella* in *Carya illinoinensis* is a world first, and to date no other country/region has regulated *Carya* seeds for sowing for *Xylella* spp. The commercial propagation of pecans requires propagation of improved cultivars as grafted clonal scions onto rootstocks that are produced from seedlings (Wells 2017). *Carya* species are native to temperate North America (USA, Canada and Northern Mexico). The distribution of pecan scion budwood from the USDA-ARS National Collection of Genetic Resources for Pecans and Hickories (NCGR-Carya) ceased in 2015 due to the endemic nature of *X. fastidiosa* (Grauke, Wood & Harris 2016; Hilton 2017).

A number of countries/regions have established pecan plantations. America is the leading global producer of pecans, but production has expanded to South Africa, Australia, China, Uruguay, Argentina and Brazil, and production is expected to increase over the next 30 years (Lazarotto et al. 2014; Wood, Payne & Grauke 1990; Zhang, Peng & Li 2015) (Wakeling et al., 2001). It is highly likely that the source material for these plantations came from the United States. It is also likely that these countries were not aware of pecan seed transmission of *Xylella* and therefore may not have included import conditions against *Xylella* in their seeds for sowing requirements.

Recommendation *Xylella* regulation for *Carya* spp. seeds for sowing be retained for all countries of origin. The department will assess individual requests from NPPOs for variation to

this, based on provision of appropriate information, including surveillance programs for *Xylella* and results of testing.

Requirement for PEQ grow out and testing

The emergency measures contain a mandatory requirement that imported *Carya* spp. seeds for sowing from all countries of origin must be grown for a minimum of 12 months at a government PEQ facility. Before release from biosecurity control, the plants must be tested by PCR and found free from *Xylella* species.

Limitations: None. Vertical transmission of *Xylella* from seed of *Carya illinoinensis* to seedlings has been proven. As discussed in Section 2.7.1, *Xylella* disease symptom expression is dependent upon various factors. For this reason, the minimum duration for plants to be held at the government PEQ facility prior to testing for *Xylella* spp. will vary, and will be dependent upon various factors, in particular the plant species or cultivar, but consideration will also be given to growing conditions and nutritional availability.

Recommendation: The department proposes that the mandatory period of growth in PEQ for germinated *Carya* spp. seed remain and align with the requirement for imported nursery stock. The minimum period for plants to be held in PEQ for *Xylella* testing will be 12 months, unless otherwise specified and/or approved by the department.

4.2.3 Import conditions for seeds for sowing

This draft PRA report proposes that imported seeds for sowing belonging to the plant genus *Carya* from all countries/regions of origin should be subject to:

- the department's standard seeds for sowing import conditions, AND
- the following additional import conditions:
  - minimum 12 month grow out in a government PEQ facility
  - testing for *Xylella* spp. prior to release.

The department proposes that when these risk management measures are followed, the restricted risk for *Xylella* spp. in association with imported seeds for sowing will achieve the ALOP for Australia.

4.3 Consideration of alternative measures

Consistent with the principle of equivalence detailed in ISPM 11: Pest risk analysis for quarantine pests (FAO 2019b), the department will consider any alternative measure proposed by an NPPO, providing that it demonstrably manages the target pest to achieve the ALOP for Australia. Evaluation of such measures will require a technical submission from the NPPO that details the proposed measures, including suitable information to support the claimed efficacy, for consideration by the department.

4.3.1 NPPO approved arrangement for *Xylella*

Where a high risk *Xylella* country/region wishes to apply for an approved arrangement for export of *Xylella* host plants or seeds for sowing to Australia, potential exporters must contact their country's NPPO to establish appropriate systems. These arrangements will also need to be

assessed (which may include audits) and be approved by the department prior to the arrangement starting.

Such arrangements should include but are not limited to a high health systems approach that incorporates facility containment, sourcing of mother stock, pre-export growth and containment periods, testing, and NPPO approval and management. Consideration must be given to factors such as:

- use of an approved arrangement facility for plant growth that is insect-proofed to exclude all insects (of all life stages) of the suborder Auchenorrhyncha (leafhoppers, froghoppers, sharpshooters, spittlebugs and treehoppers)
- growth of plants intended for import to Australia for their entire life in the approved arrangement facility, regardless of the propagation technique (for example, whether grown from seed, grown vegetatively or grown in tissue culture)
- protection of all nursery stock mother plants within the approved arrangement facility for 12 months prior to testing by the department-approved protocols for species of *Xylella*
- official sampling, prior to export, from the plant lot and testing using the department-approved protocols for species of *Xylella*.

4.4 Review of policy

The department reserves the right to review the import policy as deemed necessary, such as in the event that there is reason to believe that the pest or phytosanitary status in a country has changed, or a host or transmission status of a pathway has changed.

The relevant NPPO must inform the department immediately on recognition of any substantive changes to the status of *Xylella* in its jurisdiction.

5 Conclusion

The IPPC and the SPS Agreement requires emergency phytosanitary measures against the introduction of new pests to be technically justified. The department undertook this PRA to meet Australia's obligations under this convention and agreement by reviewing Australia's existing emergency phytosanitary measures for imported nursery stock and seeds for sowing to manage the risk of *Xylella* spp. entering Australia.

The risk analysis was conducted in accordance with Australia's method for pest risk analysis (Appendix A), which is consistent with the ISPMs, including ISPM 2: *Framework for pest risk analysis* (FAO 2019a) and ISPM 11: *Pest risk analysis for quarantine pests* (FAO 2019b), and the SPS Agreement (WTO 1995).

In conclusion, this draft report proposes that the importation of nursery stock and seeds for sowing to Australia from all countries/regions be permitted, subject to a range of biosecurity requirements outlined in Chapter 4.

The findings of this draft report are based on a comprehensive analysis of scientific literature and other relevant information.

The department considers that the risk management measures proposed in this report will provide an appropriate level of protection against *Xylella* spp. identified as associated with the trade of nursery stock and seeds for sowing from all countries/regions.

All nursery stock and seeds for sowing have been determined by the Director of Biosecurity to be conditionally non-prohibited goods under s174 of the *Biosecurity Act 2015*. Conditionally non-prohibited goods cannot be brought or imported into Australia unless they meet specific import conditions.

This report, upon its finalisation, provides the basis for import conditions for nursery stock and seeds for sowing from all countries/regions. The import conditions will be communicated on BICON.

Appendix A: Method for pest risk analysis

This section sets out the method for the pest risk analysis (PRA) used by the Department of Agriculture, Fisheries and Forestry (the department). This method is consistent with the International Standards for Phytosanitary Measures (ISPMs), including ISPM 2: *Framework for pest risk analysis* (FAO 2019a) and ISPM 11: *Pest risk analysis for quarantine pests* (FAO 2019b) and the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (WTO 1995).

A PRA is 'the process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it' (FAO 2022). A pest is 'any species, strain or biotype of plant, animal, or pathogenic agent, injurious to plants or plant products' (FAO 2022). A 'quarantine pest' is 'a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled' (FAO 2022).

Biosecurity risk consists of 2 major components: the likelihood of a pest entering, establishing and spreading in Australia for a defined import pathway; and the consequences should this happen. These 2 components are combined to give an overall estimate of the pest risk for the defined import pathway.

Unrestricted risk is estimated taking into account, where applicable, the existing commercial production practices of the exporting country and procedures that occur on arrival in Australia. These procedures include verification by the department that the consignment received is as described on the commercial documents and its integrity has been maintained.

Restricted risk is estimated with phytosanitary measure(s) applied. A phytosanitary measure is 'any legislation, regulation or official procedure having the purpose to prevent the introduction or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests' (FAO 2022).

A PRA is conducted in 3 consecutive stages: initiation (A1), pest risk assessment (A2) and pest risk management (A3).

A1 Stage 1: Initiation

Initiation identifies the pest(s) and pathway(s) that are of biosecurity concern and should be considered for risk analysis in relation to the identified PRA area.

A pathway is 'any means that allows the entry or spread of a pest' (FAO 2022). For this risk analysis, the 'pathway' being assessed is defined in Chapter 1 (section 1.2.2).

For this risk analysis, the 'PRA area' is defined as Australia for pests that are absent, or of limited distribution and under official control. For areas with regional freedom from a pest, the 'PRA area' may be defined based on a state or territory of Australia or may be defined as a region of Australia consisting of parts of a state or territory or several states or territories.

According to ISPM 11 (FAO 2019b), the PRA process may be initiated as a result of:

- the identification of a pathway that presents a potential pest hazard. For example, international trade is requested for a commodity not previously imported into the country or a commodity from a new area or new country of origin
- the identification of a pest that may require phytosanitary measures. For example, a new pest risk is identified by scientific research, a pest is repeatedly intercepted, a request is made to import an organism, or an organism is identified as a vector of other pests
- the review or revision of a policy. For example, a country's decision is taken to review phytosanitary regulations, requirements or operations or a new treatment or loss of a treatment system, a new process, or new information impacts on an earlier decision.

The basis for the initiation of this risk analysis is defined in Chapter 1 (section 1.2.1).

The primary elements in the initiation stage are:

- identity of the pests
- potential association of each pest with the pathway being assessed.

The identity of the pests is presented at species level by the species' scientific name in most instances, but a lower taxonomic level may be used where appropriate. Synonyms are provided where the current scientific name differs from that provided by the exporting country's National Plant Protection Organisation (NPPO) or where the cited literature used a different scientific name.

The potential association of each pest with the pathway being assessed considers information on:

  - association of the pest with the host plant/commodity and
  - the presence or absence of the pest in the exporting country/region relevant to the pathway being assessed.

A2 Stage 2: Pest risk assessment

The process for pest risk assessment includes 2 sequential steps:

- pest categorisation (A2.1)
- further pest risk assessment, which includes evaluation of the likelihood of the introduction (entry and establishment) and spread of a pest (A2.2) and evaluation of the magnitude of the associated potential consequences (A2.3).

A2.1 Pest categorisation

Pest categorisation examines the pests identified in the initiation stage (A1) to determine which of these pests meet the definition of a quarantine pest and require further pest risk assessment.

ISPM 11 (FAO 2019b) states that '*The opportunity to eliminate an organism or organisms from consideration before in-depth examination is undertaken is a valuable characteristic of the categorisation process. An advantage of pest categorisation is that it can be done with relatively little information; however information should be sufficient to adequately carry out the categorisation*'. In line with ISPM 11, the department utilises the pest categorisation step to screen out some pests from further consideration where appropriate. For each pest that is not present in Australia, or is present but under official control, the department assesses its potential to enter (importation and distribution) on the pathway being assessed and, if having

potential to enter, its potential to establish and spread in the PRA area. For a pest to cause economic consequences, the pest will need to enter, establish and spread in the PRA area. Therefore, pests that do not have potential to enter on the pathway being assessed, or have potential to enter but do not have potential to establish and spread in the PRA area, are not considered further. The potential for economic consequences is then assessed for pests that have potential to enter, establish and spread in the PRA area. Further pest risk assessments are then undertaken for pests that have potential to cause economic consequences, i.e., pests that meet the criteria for a quarantine pest.

Pest categorisation uses the following primary elements to identify the quarantine pests and to screen out some pests from further consideration where appropriate for the pathway being assessed:

- presence or absence and regulatory status in the PRA area
- potential for entry, establishment and spread in the PRA area
- potential for economic consequences in the PRA area.

A2.2 Assessment of the likelihood of entry, establishment and spread

ISPM 11 (FAO 2019b) provides details of how to assess the 'probability of entry', 'probability of establishment' and 'probability of spread' of a pest. The SPS Agreement (WTO 1995) uses the term 'likelihood' rather than 'probability' for these estimates. In qualitative PRAs, the department uses the term 'likelihood' as the descriptor. The use of the term 'probability' is limited to the direct quotation of ISPM definitions.

A summary of the assessment process is given here, followed by a description of the qualitative methodology used in this risk analysis.

A2.2.1 Likelihood of entry

The likelihood of entry describes the likelihood that a quarantine pest will enter Australia when a given commodity is imported, be distributed in a viable state in the PRA area and subsequently be transferred to a host.

For the purpose of considering the likelihood of entry, the department divides this step into 2 components:

- **Likelihood of importation**—the likelihood that a pest will arrive in Australia in a viable state when a given commodity is imported
- **Likelihood of distribution**— the likelihood that the pest will be distributed in a viable state, as a result of the processing, sale or disposal of the commodity, in the PRA area and subsequently transfer to a susceptible part of a host.

Factors to be considered in the likelihood of importation may include:

- likelihood of the pest being associated with the pathway at origin
  - prevalence of the pest in the source area
  - occurrence of the pest in a life-stage that would be associated with the commodity
  - mode of trade (for example, bulk, packed)
  - volume and frequency of movement along each pathway

  - seasonal timing of imports
  - pest management, cultural and commercial procedures applied at the place of origin (for example, application of plant protection products, handling, culling, and grading)
- likelihood of survival of the pest during transport or storage
  - speed and conditions of transport and duration and conditions of storage compared with the duration of the life cycle of the pest
  - vulnerability of the life-stages of the pest during transport or storage
  - prevalence of the pest likely to be associated with a consignment
  - commercial procedures (for example, refrigeration) applied to consignments during transport and storage in the country of origin, and during transport to Australia
- likelihood of pest surviving existing pest management procedures.

Factors to be considered in the likelihood of distribution may include:

- commercial procedures (for example, refrigeration) applied to consignments during distribution in Australia
- dispersal mechanisms of the pest, including vectors, to allow movement from the pathway to a suitable host
- whether the imported commodity is to be sent to a few or many destination points in the PRA area
- proximity of entry, transit and destination points to suitable hosts
- time of year at which import takes place
- intended use of the commodity (for example, for planting, processing or consumption)
- risks from by-products and waste.

A2.2.2 Likelihood of establishment

Establishment is defined as the 'perpetuation for the foreseeable future, of a pest within an area after entry' (FAO 2022). In order to estimate the likelihood of establishment of a pest, reliable biological information (for example, lifecycle, host range, epidemiology, survival) is obtained from the areas where the pest currently occurs. The situation in the PRA area can then be compared with that in the areas where it currently occurs and expert judgement used to assess the likelihood of establishment.

Factors to be considered in the likelihood of establishment in the PRA area may include:

- availability of suitable hosts, alternate hosts and vectors in the PRA areas
  - prevalence of hosts and alternate hosts in the PRA area
  - whether hosts and alternate hosts occur within sufficient geographic proximity to allow the pest to complete its life cycle
  - whether there are other plant species, which could prove to be suitable hosts in the absence of usual host species
  - whether a vector, if needed for dispersal of the pest, is already present in the PRA area or likely to be introduced
- suitability of environment in the PRA area

  - factors in the environment in the PRA area (for example, suitability of climate, soil, pest and host competition) that are critical to the development of the pest, its host and if applicable its vector, and to their ability to survive periods of climatic stress and complete their life cycles
- cultural practices and control measures in the PRA area that may influence the ability of the pest to establish
- other characteristics of the pest
  - reproductive strategy of the pest and method of pest survival
  - potential for adaptation of the pest
  - minimum population needed for establishment.

A2.2.3 Likelihood of spread

Spread is defined as 'the expansion of the geographical distribution of a pest within an area' (FAO 2022). The likelihood of spread considers the factors relevant to the movement of the pest, after establishment on a host plant or plants, to other susceptible host plants of the same or different species in other areas. In order to estimate the likelihood of spread of the pest, reliable biological information is obtained from areas where the pest currently occurs. The situation in the PRA area is then carefully compared with that in the areas where the pest currently occurs and expert judgement used to assess the likelihood of spread.

Factors to be considered in the likelihood of spread may include:

- suitability of the natural and/or managed environment for natural spread of the pest
- presence of natural barriers
- potential for movement with commodities, conveyances or by vectors
- intended use of the commodity
- potential vectors of the pest in the PRA area
- potential natural enemies of the pest in the PRA area.

A2.2.4 Assigning likelihoods for entry, establishment and spread

Likelihoods are assigned to each step of entry, establishment and spread. Six qualitative likelihood descriptors are used: High; Moderate; Low; Very Low; Extremely Low; and Negligible. Definitions for these descriptors and their indicative ranges are given in Table A.1. The indicative ranges are only provided to illustrate the boundaries of the descriptors and are not used beyond this purpose in qualitative PRAs. These indicative ranges provide guidance to the risk analyst and promote consistency between different pest risk assessments.

Table A.1 Nomenclature of likelihoods

Likelihood	Descriptive definition	Indicative range
High	The event would be very likely to occur	0.7 < to ≤ 1
Moderate	The event would occur with an even likelihood	0.3 < to ≤ 0.7
Low	The event would be unlikely to occur	0.05 < to ≤ 0.3
Very Low	The event would be very unlikely to occur	0.001 < to ≤ 0.05
Extremely Low	The event would be extremely unlikely to occur	0.000001 < to ≤ 0.001
Negligible	The event would almost certainly not occur	0 < to ≤ 0.000001

A2.2.5 Combining likelihoods

The likelihood of entry is determined by combining the likelihood that the pest will be imported into the PRA area and the likelihood that the pest will be distributed within the PRA area, using a matrix of rules (Table A.2). This matrix is then used to combine the likelihood of entry and the likelihood of establishment, and the likelihood of entry and establishment is then combined with the likelihood of spread to determine the overall likelihood of entry, establishment and spread.

For example, if a descriptor of Low is assigned for the likelihood of importation, Moderate for the likelihood of distribution, High for the likelihood of establishment and Very Low for the likelihood of spread, then the likelihood of importation of Low and the likelihood of distribution of Moderate are combined to give a likelihood of Low for entry. The likelihood for entry is then combined with the likelihood assigned for establishment of High to give a likelihood for entry and establishment of Low. The likelihood for entry and establishment is then combined with the likelihood assigned for spread of Very Low to give the overall likelihood for entry, establishment and spread of Very Low. This can be summarised as:

importation x distribution = entry [E]	**Low x Moderate = Low**
entry x establishment = [EE]	**Low x High = Low**
[EE] x spread = [EES]	**Low x Very Low = Very Low**

Table A.2 Matrix of rules for combining likelihoods

	High	Moderate	Low	Very Low	Extremely Low	Negligible
High	High	Moderate	Low	Very Low	Extremely Low	Negligible
Moderate	–	Low	Low	Very Low	Extremely Low	Negligible
Low	–	–	Very Low	Very Low	Extremely Low	Negligible
Very Low	–	–	–	Extremely Low	Extremely Low	Negligible
Extremely Low	–	–	–	–	Negligible	Negligible
Negligible	–	–	–	–	–	Negligible

Time and volume of trade

One factor affecting the likelihood of entry is the volume and duration of trade. If all other conditions remain the same, the overall likelihood of entry will increase as time passes and the overall volume of trade increases.

The department normally considers the likelihood of entry on the basis of the estimated volume of one year's trade. This is a convenient value for the analysis that is relatively easy to estimate and allows for expert consideration of seasonal variations in pest presence, incidence and behaviour to be taken into account. The consideration of the likelihood of entry, establishment and spread and subsequent consequences takes into account events that might happen over a number of years even though only one year's volume of trade is being considered. This

difference reflects biological and ecological facts, for example where a pest or disease may establish in the year of import but spread may take many years.

The use of a one year volume of trade has been taken into account when setting up the matrix that is used to estimate the risk and therefore any policy based on this analysis does not simply apply to one year of trade. Policy decisions that are based on the department's method that uses the estimated volume of one year's trade are consistent with Australia's policy on appropriate level of protection and meet the Australian Government's requirement for ongoing quarantine protection. If there are substantial changes in the volume and nature of the trade in specific commodities then the department will review the risk analysis and, if necessary, provide updated policy advice.

In assessing the volume of trade in this risk analysis, the department assumed that a substantial volume of trade will occur.

A2.3 Assessment of potential consequences

In estimating the potential consequences of a pest if the pest were to enter, establish and spread in Australia, the department uses a 2-step process. In the first step, a qualitative descriptor of the impact is assigned to each of the direct and indirect criteria in terms of the *level of impact* and the *magnitude of impact*. The second step involves combining the impacts for each of the criteria to obtain an 'overall consequences' estimation.

Step 1: Assessing direct and indirect impacts

Direct pest impacts are considered in the context of the impacts on:

- the life or health of plants and plant products

  This may include pest impacts on the life or health of the plants and production effects (yield or quality) either at harvest or during storage.

  - Where applicable, pest impacts on the life or health of humans or of animals and animal products may also be considered.
- other aspects of the environment.

Indirect pest impacts are considered in the context of the impacts on:

- eradication and control

  This may include pest impacts on new or modified eradication, control, surveillance or monitoring and compensation strategies or programs.
- domestic trade

  This may include pest impacts on domestic trade or industry, including changes in domestic consumer demand for a product resulting from quality changes and effects on other industries supplying inputs to, or using outputs from, directly affected industries.
- international trade

  This may include pest impacts on international trade, including loss of markets, meeting new technical requirements to enter or maintain markets and changes in international consumer demand for a product resulting from quality changes.
- non-commercial and environment

This may include pest impacts on the community and environment, including reduced tourism, reduced rural and regional economic viability, loss of social amenity, and any 'side effects' of control measures.

For each of these direct and indirect criteria, the level of impact is estimated over 4 geographic levels, defined as:

- **Local**–an aggregate of households or enterprises (a rural community, a town or a local government area)
- **District**–a geographically or geopolitically associated collection of aggregates (generally a recognised section of a state or territory, such as 'Far North Queensland')
- **Regional**–a geographically or geopolitically associated collection of districts in a geographic area (generally a state or territory, although there may be exceptions with larger states such as Western Australia)
- **National**–Australia wide (Australian mainland states and territories and Tasmania).

For each criterion, the magnitude of impact at each of these geographic levels is described using 4 categories, defined as:

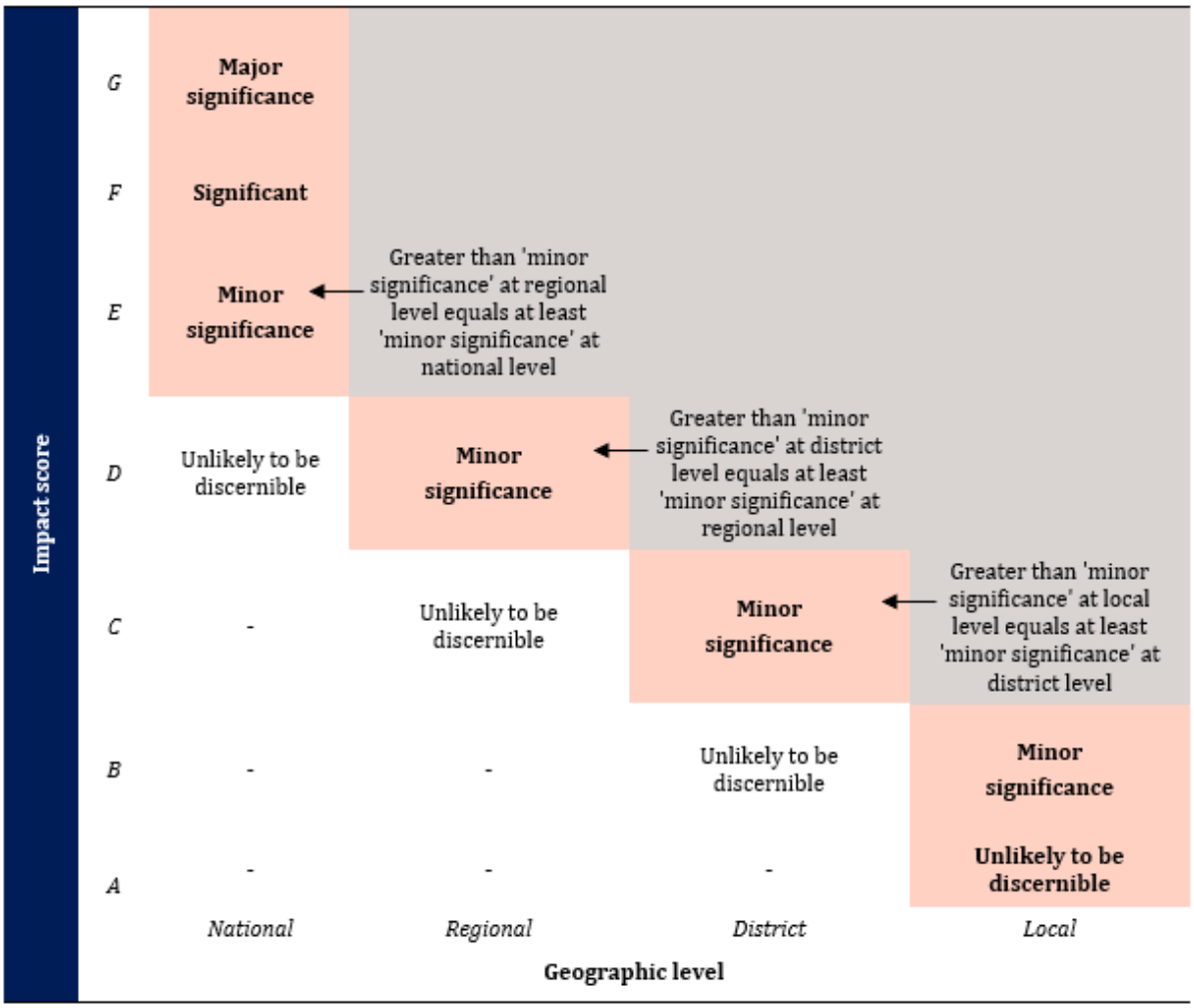
- **Unlikely to be discernible**–pest impact is not usually distinguishable from normal day-to-day variation in the criterion
- **Minor significance**–expected to lead to a minor increase in mortality/morbidity of hosts or a minor decrease in production but not expected to threaten the economic viability of production. Expected to decrease the value of non-commercial criteria but not threaten the criterion's intrinsic value. Effects would generally be reversible.
- **Significant**–expected to threaten the economic viability of production through a moderate increase in mortality/morbidity of hosts, or a moderate decrease in production. Expected to significantly diminish or threaten the intrinsic value of non-commercial criteria. Effects may not be reversible.
- **Major significance**–expected to threaten the economic viability through a large increase in mortality/morbidity of hosts, or a large decrease in production. Expected to severely or irreversibly damage the intrinsic 'value' of non-commercial criteria.

Each individual direct or indirect impact is given an impact score (A–G) using the decision rules in Figure A.1. This is done by determining which of the shaded cells with bold font in Figure A.1 correspond to the level and magnitude of the particular impact.

The following are considered during this process:

- At each geographic level below 'National', an impact more serious than 'Minor significance' is considered at least 'Minor significance' at the level above. For example, a 'Significant' impact at the state or territory level is considered equivalent to at least a 'Minor significance' impact at the national level.
- If the impact of a pest at a given level is in multiple states or territories, districts or regions or local areas, it is considered to represent at least the same magnitude of impact at the next highest geographic level. For example, a 'Minor significance' impact in multiple states or territories represents a 'Minor significance' impact at the national level.
- The geographic distribution of an impact does not necessarily determine the impact. For example, an outbreak could occur on one orchard/farm, but the impact could potentially still be considered at a state or national level.

Figure A.1 Decision rules for determining the impact score for each direct and indirect criterion, based on the *level of impact* and the *magnitude of impact*



For each criterion:

- the *level of impact* is estimated over 4 geographic levels: local, district, regional and national
- the *magnitude of impact* at each of the 4 geographic levels is described using 4 categories: unlikely to be discernible, minor significance, significant and major significance
- an impact score (A–G) is assigned by determining which of the shaded cells with bold font correspond to the level and magnitude of impact.

Step2: Combining direct and indirect impacts

The overall consequence for each pest or each group of pests is achieved by combining the impact scores (A–G) for each direct and indirect criterion using the decision rules in Table A.3. These rules are mutually exclusive, and are assessed in numerical order until one applies. For example, if the first rule does not apply, the second rule is considered, and so on.

Table A.3 Decision rules for determining the overall consequence rating for each pest

Rule	The impact scores for consequences of direct and indirect criteria	Overall consequence rating
1	Any criterion has an impact of 'G'; or more than one criterion has an impact of 'F'; or a single criterion has an impact of 'F' and each remaining criterion an 'E'.	Extreme
2	A single criterion has an impact of 'F'; or all criteria have an impact of 'E'.	High
3	One or more criteria have an impact of 'E'; or all criteria have an impact of 'D'.	Moderate
4	One or more criteria have an impact of 'D'; or all criteria have an impact of 'C'.	Low
5	One or more criteria have an impact of 'C'; or all criteria have an impact of 'B'.	Very Low
6	One or more but not all criteria have an impact of 'B', and all remaining criteria have an impact of 'A'; or all criteria have an impact of 'A'.	Negligible

A2.4 Estimation of the unrestricted risk

Once the assessment of the likelihood of entry, establishment and spread and for potential consequences are completed, the unrestricted risk can be determined for each pest or each group of pests. This is determined by using a risk estimation matrix (Table A.4) to combine the estimates of the likelihood of entry, establishment and spread and the overall consequences of pest establishment and spread.

When interpreting the risk estimation matrix, note the descriptors for each axis are similar (for example, Low, Moderate, High) but the vertical axis refers to likelihood and the horizontal axis refers to consequences. Accordingly, a Low likelihood combined with High consequences, is not the same as a High likelihood combined with Low consequences—the matrix is not symmetrical. For example, the former combination would give an unrestricted risk rating of Moderate, whereas, the latter would give a Low rating.

Table A.4 Risk estimation matrix

Likelihood of pest entry, establishment and spread	Consequences of pest entry, establishment and spread					
	Negligible	Very Low	Low	Moderate	High	Extreme
High	Negligible risk	Very Low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate	Negligible risk	Very Low risk	Low risk	Moderate risk	High risk	Extreme risk
Low	Negligible risk	Negligible risk	Very Low risk	Low risk	Moderate risk	High risk
Very Low	Negligible risk	Negligible risk	Negligible risk	Very Low risk	Low risk	Moderate risk
Extremely Low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very Low risk	Low risk
Negligible	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very Low risk

A2.5 The appropriate level of protection (ALOP) for Australia

The SPS Agreement defines the concept of an 'appropriate level of sanitary or phytosanitary protection (ALOP)' as the level of protection deemed appropriate by the WTO Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory.

Like many other countries, Australia expresses its ALOP in qualitative terms. The ALOP for Australia, which reflects community expectations through government policy, is currently expressed as providing a high level of sanitary or phytosanitary protection aimed at reducing risk to a very low level, but not to zero. The band of cells in Table A.4 marked 'Very Low risk' represents the ALOP for Australia.

A3 Stage 3: Pest risk management

Pest risk management describes the process of identifying and implementing phytosanitary measures to manage risks to achieve the ALOP for Australia, while ensuring that any negative effects on trade are minimised.

The conclusions from pest risk assessment are used to decide whether risk management is required and if so, the appropriate measures to be used. Where the unrestricted risk estimate does not achieve the ALOP for Australia, risk management measures are required to reduce this risk to a very low level. The guiding principle for risk management is to manage risk to achieve the ALOP for Australia. The effectiveness of any proposed/recommended phytosanitary measures (or combination of measures) is evaluated, using the same approach as used to evaluate the unrestricted risk. This ensures the restricted risk for the relevant pest or pests achieves the ALOP for Australia.

ISPM 11 (FAO 2019b) provides details on the identification and selection of appropriate risk management options and notes that the choice of measures should be based on their effectiveness in reducing the likelihood of entry of the pest.

Examples given of measures commonly applied to traded commodities include:

- options for consignments—for example, inspection or testing for freedom from pests, prohibition of parts of the host, a pre-entry or post-entry quarantine system, specified conditions on preparation of the consignment, specified treatment of the consignment, restrictions on end-use, distribution and periods of entry of the commodity
- options preventing or reducing infestation in the crop—for example, treatment of the crop, restriction on the composition of a consignment so it is composed of plants belonging to resistant or less susceptible species, harvesting of plants at a certain age or specified time of the year, production in a certification scheme
- options ensuring that the area, place or site of production or crop is free from the pest—for example, pest-free area, pest-free place of production or pest-free production site
- options for other types of pathways—for example, consider natural spread, measures for human travellers and their baggage, cleaning or disinfestations of contaminated machinery
- options within the importing country—for example, surveillance and eradication programs
- prohibition of commodities—if no satisfactory measure can be found.

Appendix B: Initiation and categorisation for bacterial pathogens in the genus *Xylella*

The steps in the initiation and categorisation process are considered sequentially, with the assessment terminating at 'Yes' for column 3 (except for pests that are present, but under official control and/or pests of regional concern) or the first 'No' for columns 4, 5 or 6.

A detailed description of the method used for a pest risk analysis is provided in Appendix A.

			Potential to enter on pathway				
Pest	**Distribution**	**Present within Australia**	**Potential for importation**	**Potential for distribution**	**Potential for establishment and spread**	**Potential for economic consequences**	**Pest risk assessment required**
BACTERIA							
Xylella spp. (Stål, 1855) [Xanthomonadaceae: Xanthomonadales] *Xylella fastidiosa* Wells et al. 1987 *Xylella taiwanensis* (Su et al. 2016)	North America, Central America, South America, Europe (Italy, France, Spain, Cyprus), Israel, Iran and Taiwan (Su et al. 2016) (see Section 2.5).	No records found.	Yes. *Xylella* spp. are known to have moved to new regions through infected plants for planting and therefore have the potential to be present in host plant species on the nursery stock and seeds for sowing pathway. Infected xylem feeding vectors from the Hemipteran subfamily Cicadellinae (sharpshooters) and the superfamily Cercopoidea (spittlebugs) also have the potential to be on this pathway, associated with nursery stock (see Section 2.6).	Nursery stock and seeds for sowing are imported into Australia for the specific purpose of propagation and are distributed widely across Australia.	Yes. *Xylella* spp. have established and spread outside their native range (see Section 2.5.2).	Yes. *Xylella* spp. are known to cause economic damage to a wide range of plant hosts including commercial fruits, forest and amenity trees and Australian native plant species (see Section 2.4).	Yes

Appendix C: *Xylella* vectors and their preferred plant hosts

For the department's detailed list of recorded *Xylella* insect vectors, their host plants, and the references that recorded these associations, please refer to the Appendix D Excel spreadsheet, which is available from the department's website at agriculture.gov.au/biosecurity/risk-analysis/plant/Xylella.

This spreadsheet records the department's research of confirmed *Xylella* vector species, the host plants that these vectors feed on and the scientific reference that records that insect/plant association. This is not a complete list of all insect species capable of vectoring *Xylella*, as not all insect/plant associations are known or have been documented.

All web links in references were accessible and active on week of 21 November 2022.

Appendix D: *Xylella* plant hosts

For the department's detailed list of all recorded natural *Xylella* plant hosts, and the references that record these associations, please refer to the Appendix D Excel spreadsheet, which is available from the department's website at agriculture.gov.au/biosecurity/risk-analysis/plant/Xylella.

This spreadsheet records the department's determination of plant species confirmed as natural hosts of *Xylella* spp., their plant family taxonomic placement and the scientific reference that records that natural *Xylella* host association. An additional 3 of the plant families contained in this spreadsheet (Polemoniaceae, Simmondsiaceae and Linaceae) are experimental hosts of *Xylella* and are regulated by Australia because they have strong associations with the known competent insect vectors of *Xylella—Philaneaus spumarius* and/or *Homalodisca vitripennis* (Black 2010; Wistrom & Purcell 2005). Those vector associations are documented in Appendix C.

All web links in references were accessible and active on week of 21 November 2022 unless otherwise noted.

Glossary, acronyms and abbreviations

Term or abbreviation	Definition
Additional declaration	A statement that is required by an importing country to be entered on a phytosanitary certificate and which provides specific additional information on a consignment in relation to regulated pests or regulated articles (FAO 2022).
Appropriate level of protection (ALOP)	The level of protection deemed appropriate by the Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory (WTO 1995).
Appropriate level of protection (ALOP) for Australia	The *Biosecurity Act 2015* defines the appropriate level of protection (or ALOP) for Australia as a high level of sanitary and phytosanitary protection aimed at reducing biosecurity risks to very low, but not to zero.
Area	An officially defined country, part of a country or all or parts of several countries (FAO 2022).
Area of low pest prevalence	An area, whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific pest is present at low levels and which is subject to effective surveillance or control (FAO 2022).
Arthropod	The largest phylum of animals, including the insects, arachnids and crustaceans.
Australian territory	Australian territory as referenced in the *Biosecurity Act 2015* refers to Australia, Christmas Island and Cocos (Keeling) Islands and any external Territory to which that provision extends.
BA	Biosecurity Advice
BICON	Australia's Biosecurity Import Conditions system bicon.agriculture.gov.au/BiconWeb4.0
Biosecurity	The prevention of the entry, establishment or spread of unwanted pests and infectious disease agents to protect human, animal or plant health or life, and the environment.
Biosecurity import risk analysis (BIRA)	The *Biosecurity Act 2015* defines a BIRA as an evaluation of the level of biosecurity risk associated with particular goods, or a particular class of goods, that may be imported, or proposed to be imported, into Australian territory, including, if necessary, the identification of conditions that must be met to manage the level of biosecurity risk associated with the goods, or the class of goods, to a level that achieves the ALOP for Australia. The risk analysis process is regulated under legislation.
Biosecurity measures	The *Biosecurity Act 2015* defines biosecurity measures as measures to manage any of the following: biosecurity risk, the risk of contagion of a listed human disease, the risk of listed human diseases entering, emerging, establishing themselves or spreading in Australian territory, and biosecurity emergencies and human biosecurity emergencies.
Biosecurity risk	The *Biosecurity Act 2015* refers to biosecurity risk as the likelihood of a disease or pest entering, establishing or spreading in Australian territory, and the potential for the disease or pest causing harm to human, animal or plant health, the environment, economic or community activities.
Consignment	A quantity of plants, plant products or other articles being moved from one country to another and covered, when required, by a single phytosanitary certificate (a consignment may be composed of one or more commodities or lots) (FAO 2022).
Control (of a pest)	Suppression, containment or eradication of a pest population (FAO 2022).
Endangered area	An area where ecological factors favour the establishment of a pest whose presence in the area will result in economically important loss (FAO 2022).
Endemic	Belonging to, native to, or prevalent in a particular geography, area or environment.

Term or abbreviation	Definition
Entry (of a pest)	Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled (FAO 2022).
Establishment (of a pest)	Perpetuation, for the foreseeable future, of a pest within an area after entry (FAO 2022).
FAO	Food and Agriculture Organization of the United Nations
Fresh	Living; not dried, deep-frozen or otherwise conserved (FAO 2022).
Fumigation	A method of pest control that completely fills an area with gaseous pesticides to suffocate or poison the pests within.
Genus	A taxonomic category ranking below a family and above a species and generally consisting of a group of species exhibiting similar characteristics. In taxonomic nomenclature the genus name is used, either alone or followed by a Latin adjective or epithet, to form the name of a species.
Goods	The *Biosecurity Act 2015* defines goods as an animal, a plant (whether moveable or not), a sample or specimen of a disease agent, a pest, mail or any other article, substance or thing (including, but not limited to, any kind of moveable property).
Host	An organism that harbours a parasite, mutual partner, or commensal partner, typically providing nourishment and shelter.
Host range	Species capable, under natural conditions, of sustaining a specific pest or other organism (FAO 2022).
Import permit	Official document authorising importation of a commodity in accordance with specified phytosanitary import requirements (FAO 2022).
Infection	The internal 'endophytic' colonisation of a plant, or plant organ, and is generally associated with the development of disease symptoms as the integrity of cells and/or biological processes are disrupted.
Infestation (of a commodity)	Presence in a commodity of a living pest of the plant or plant product concerned. Infestation includes infection (FAO 2022).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations (FAO 2022).
Intended use	Declared purpose for which plants, plant products or other articles are imported, produced or used (FAO 2022).
Interception (of a pest)	The detection of a pest during inspection or testing of an imported consignment (FAO 2022).
International Plant Protection Convention (IPPC)	The IPPC is an international plant health agreement, established in 1952, that aims to protect cultivated and wild plants by preventing the introduction and spread of pests. The IPPC provides an international framework for plant protection that includes developing International Standards for Phytosanitary Measures (ISPMs) for safeguarding plant resources.
International Standard for Phytosanitary Measures (ISPM)	An international standard adopted by the Conference of the Food and Agriculture Organization, the Interim Commission on Phytosanitary Measures or the Commission on Phytosanitary Measures, established under the IPPC (FAO 2022).
Introduction (of a pest)	The entry of a pest resulting in its establishment (FAO 2022).
Lot	A number of units of a single commodity, identifiable by its homogeneity of composition, origin et cetera, forming part of a consignment (FAO 2022). Within this report a 'lot' refers to a quantity of fruit of a single variety, harvested from a single production site during a single pick and packed at one time.
National Plant Protection Organization (NPPO)	Official service established by a government to discharge the functions specified by the IPPC (FAO 2022).

Term or abbreviation	Definition
Nymph	The immature form of some insect species that undergoes incomplete metamorphosis. It is not to be confused with larva, as its overall form is already that of the adult.
Official control	The active enforcement of mandatory phytosanitary regulations and the application of mandatory phytosanitary procedures with the objective of eradication or containment of quarantine pests or for the management of regulated non-quarantine pests (FAO 2022).
Pathogen	A biological agent that can cause disease to its host.
Pathway	Any means that allows the entry or spread of a pest (FAO 2022).
Pest	Any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products (FAO 2022).
Pest categorisation	The process for determining whether a pest has or has not the characteristics of a quarantine pest or those of a regulated non-quarantine pest (FAO 2022).
Pest free area (PFA)	An area in which a specific pest is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (FAO 2022).
Pest free place of production (PFPP)	Place of production in which a specific pest is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period (FAO 2022).
Pest free production site (PFPS)	A production site in which a specific pest is absent, as demonstrated by scientific evidence, and in which, where appropriate, this condition is being officially maintained for a defined period (FAO 2022).
Pest risk analysis (PRA)	The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it (FAO 2022).
Pest risk assessment (for quarantine pests)	Evaluation of the probability of the introduction and spread of a pest and of the magnitude of the associated potential economic consequences (FAO 2022).
Pest risk assessment (for regulated non-quarantine pests)	Evaluation of the probability that a pest in plants for planting affects the intended use of those plants with an economically unacceptable impact (FAO 2022).
Pest risk management (for quarantine pests)	Evaluation and selection of options to reduce the risk of introduction and spread of a pest (FAO 2022).
Pest risk management (for regulated non-quarantine pests)	Evaluation and selection of options to reduce the risk that a pest in plants for planting causes an economically unacceptable impact on the intended use of those plants (FAO 2022).
Pest status (in an area)	Presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgement on the basis of current and historical pest records and other information (FAO 2022).
Phytosanitary certificate	An official paper document or its official electronic equivalent, consistent with the model certificates of the IPPC, attesting that a consignment meets phytosanitary import requirements (FAO 2022).
Phytosanitary certification	Use of phytosanitary procedures leading to the issue of a phytosanitary certificate (FAO 2022).
Phytosanitary measure	Phytosanitary relates to the health of plants. Any legislation, regulation or official procedure having the purpose to prevent the introduction or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests (FAO 2022). In this risk analysis the term 'phytosanitary measure' and 'risk management measure' may be used interchangeably.

Term or abbreviation	Definition
Phytosanitary procedure	Any official method for implementing phytosanitary measures including the performance of inspections, tests, surveillance or treatments in connection with regulated pests (FAO 2022).
Phytosanitary regulation	Official rule to prevent the introduction or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests, including establishment of procedures for phytosanitary certification (FAO 2022).
Polyphagous	Feeding on a relatively large number of hosts from different plant family and/or genera.
PRA area	Area in relation to which a pest risk analysis is conducted (FAO 2022).
Production site	In this report, a production site is a continuous planting of nursery stock treated as a single unit for pest management purposes. If a property is subdivided into one or more units for pest management purposes, then each unit is a production site.
Quarantine	Official confinement of regulated articles, pests or beneficial organisms for inspection, testing, treatment, observation or research (FAO 2022).
Quarantine pest	A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (FAO 2022).
Regulated article (RA)	Any plant, plant product, storage place, packaging, conveyance, container, soil and any other organism, object or material capable of harbouring or spreading pests, deemed to require phytosanitary measures, particularly where international transportation is involved (FAO 2022).
Regulated non-quarantine pest	A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party (FAO 2022).
Regulated pest	A quarantine pest or a regulated non-quarantine pest (FAO 2022).
Restricted risk	Restricted risk is the risk estimate when risk management measures are applied.
Risk analysis	Refers to the technical or scientific process for assessing the level of biosecurity risk associated with the goods, or the class of goods, and if necessary, the identification of conditions that must be met to manage the level of biosecurity risk associated with the goods, or class of goods to a level that achieves the ALOP for Australia.
Risk management measure	Conditions that must be met to manage the level of biosecurity risk associated with the goods or the class of goods, to a level that achieves the ALOP for Australia. In this risk analysis, the term 'risk management measure' and 'phytosanitary measure' may be used interchangeably.
Spread (of a pest)	Expansion of the geographical distribution of a pest within an area (FAO 2022).
SPS Agreement	WTO Agreement on the Application of Sanitary and Phytosanitary Measures.
Stakeholders	Government agencies, individuals, community or industry groups or organizations, whether in Australia or overseas, including the proponent/applicant for a specific proposal, who have an interest in the policy issues.
Surveillance	An official process which collects and records data on pest presence or absence by survey, monitoring or other procedures (FAO 2022).
Systems approach(es)	The integration of different risk management measures, at least 2 of which act independently, and which cumulatively achieve the appropriate level of protection against regulated pests.
Trash	Soil, splinters, twigs, leaves and other plant material, other than fruit as defined in the scope of this risk analysis.

Term or abbreviation	Definition
	For example, stem and leaf material, seeds, soil, animal matter/parts or other extraneous material
Treatment (as a phytosanitary measure)	Official procedure for killing, inactivating, removing, rendering infertile or devitalising regulated pests (FAO 2022).
Unrestricted risk	Unrestricted risk estimates apply in the absence of risk management measures.
Vector	In this report, a vector is an organism that is capable of harbouring and spreading a pest from one host to another.
Viable	Alive, able to germinate or capable of growth and/or development.
WTO	World Trade Organization

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